Biostatistics: Data and Models

John D. Reeve
Southern Illinois University Carbondale
Carbondale, IL 62901

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Chapter 1

Introduction

1.1 Why this textbook?

Welcome to **Biostatistics: Data and Models**! This textbook and course provides a survey of statistical methods commonly used in biology and ecology, an introduction to statistical theory, and significant exposure to the statistical software package SAS 9.4 (©2012 SAS Institute Inc.). The course is designed for graduate students in the life sciences. It assumes some familiarity with mathematical notation, functions, and algebra. A review of these topics is also presented early in the course. Knowledge of calculus is helpful but not essential. No previous courses in statistics are needed.

There are many useful introductory statistical textbooks (e.g., Sokal & Rohlf 1995, Steel et al. 1997, Schork & Remington 2000), so what is different about this one? One is the close integration of the text with SAS programs and output. Many texts do not discuss a particular software package, provide only abbreviated examples, or present them under separate cover. However, these packages play an essential role in modern statistical analyses, and fluency in a statistical language is a basic tool for the practicing scientist. I selected SAS as the statistical package for this course because of its popularity, extensive documentation, and unequaled support of mixed models, a common statistical procedure. An alternative is the free software package called R (R Core Team 2016). For those interested in learning this software, R programs similar in function to the SAS code can be downloaded at the website for this text.

Another difference in this textbook is the integration of statistical proce-

dures and theory. Most introductory textbooks present the statistical procedures and a mechanistic explanation of how they work, without discussing the underlying theory. The theory is typically presented in advanced courses to a more mathematically inclined audience. However, I feel that some knowledge of the theory is essential to graduate students and scientists in the life sciences, and so some theoretical concepts are included in this text. For example, likelihood is used throughout the text to explain how parameters are estimated and statistical tests derived. Besides many basic statistical procedures, likelihood theory also plays a role in model building and selection using information criteria, as well as Bayesian statistics, two expanding fields of statistical analysis.

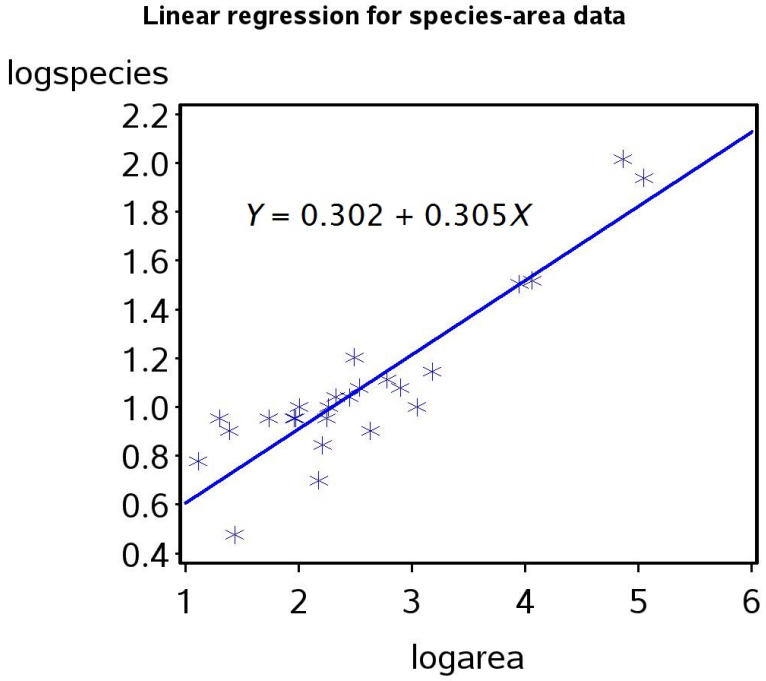
As part of this integration of theory, statistical models are presented throughout the text. What is a statistical model? Suppose we are interested in fitting a line through some data points, which are in the form of (Y, X) pairs. A standard statistical model for fitting a line through such data is the linear regression model:

$$Y = \alpha + \beta X + \epsilon, \tag{1.1}$$

where α is the intercept of the line, β is the slope, and ϵ represents random variation of the data around the line. **There is always some random variation around the line, especially with biological data.** If there were no random variation, a statistical approach would not be needed – one could simply draw a line through the data.

Fig. 1.1 shows an example of this model, fitted to data on the number of reptile species on islands of varying size in the West Indies (Wright 1981). We will examine how the parameters of such models (α and β) can be estimated using likelihood theory, and how to test whether there is indeed a relationship between Y and X (as it appears in Fig. 1.1). It is also possible to make predictions from statistical models. For example, we could use this model to potentially predict the number of reptile species expected on other islands, ones not included in this data set.

Figure 1.1: Number of reptile species vs. island area in the West Indies (Wright 1981). The number of species and island area were log-transformed before analysis. The fitted line and model equation are also shown.



1.2 Types of data

The first step faced by the statistical analyst is determining the form of the data. There are four types of data frequently encountered by scientists and statisticians: continuous, discrete, rank, and categorical data. **Continuous data** are quantities like the length and weight of an organism, concentrations of chemicals in the environment, or the growth rate of a population. The distinguishing feature of continuous data is that the observations can be described using real numbers. For example, the length of an organism might be 4.53 cm, while its weight 1.23 g. In contrast, **discrete data** always take integer values. They can be counts of organisms in a location, the number of vertebra in the spine, or quantities like the number of disease cases in a month. Typically, discrete data are non-negative integers, i.e., $0, 1, 2, 3, 4$ and so forth. The number of species in Fig. 1.1 could be treated as either continuous or discrete - although they are integer values, they are large enough to take many potential values and approximated as continuous data.

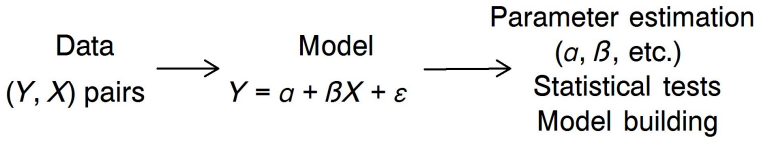
Rank or ordinal data are observations that indicate the relative ordering of the data. For example, suppose an entomologist wants to rapidly assess the level of damage caused by caterpillars to their host plants. It may be easy to quickly assess whether the plants have no damage (a rank of 1), or light (2), medium (3), and heavy damage (4), but finer gradations would be difficult. Rank data also play an important role in a set of procedures called nonparametric statistics, because these procedures often convert continuous or discrete data to rank data. **Categorical data** are observations that fall into separate categories. For example, we might classify specimens of an animal as male, female, or juvenile. No numbers are associated with these categories, although we would likely be interested in how many animals occur in each category, i.e., their frequencies.

1.3 Data and models

Once the data are classified into one of the above types, this determines to a large extent the statistical analysis. For example, suppose the data are (Y, X) pairs as in Fig. 1.1. A linear regression model like Eq. 1.1 would seem appropriate, because the data lie near a straight line. How could we model the random variation around the line? One common choice is

to assume that ϵ has a normal or bell-shaped distribution, which we later examine in detail. Once a statistical model is chosen, this largely determines the analysis including how model parameters (α, β, and parameters for ϵ) are estimated and statistical tests conducted, often using likelihood theory. Another important task in statistics is model building, in which a number of different models are fitted to the data and the best-fitting one selected (there are various criteria for determining which is best). Fig. 1.2 shows this general process.

Figure 1.2: Sequence of analysis for many statistical problems.



1.4 Sequence of topics

The next chapter in this text is a brief review of the mathematics useful in statistics, and an introduction to SAS programming (Chapter 2). We then introduce descriptive statistics, which are quantities like the mean or average, designed to summarize the properties of a data set (Chapter 3). The next topic is probability theory, which provides an explanation for many natural processes that apparently have random components, and provides a foundation for statistics (Chapter 4). We then turn to probability distributions for both discrete and continuous data, which are essentially models for random processes (Chapter 5 and 6), and how means and other quantities are defined for these distribution (Chapter 7). We then examine how parameters for these distributions are estimated using likelihood, along with a measure of the reliability of these estimates (Chapter 8, 9), and how hypotheses concerning the parameters are tested (Chapter 10).

Several chapters are devoted to analysis of variance, or ANOVA, used to compare the means of different groups (Chapter 11-15). These groups are often generated by different experimental treatments, and ANOVA and related

techniques provide a way of examining whether the treatments produces differences among these groups. Nonparametric alternatives to ANOVA are also considered (Chapter 16). We then examine linear regression and correlation, which are alternate methods of examining the relationship between two variables (Chapter 17, Chapter 18). These methods are designed for continuous variables, but can be adapted to discrete ones. Chapter 19 presents more complicated designs including three-way and nested ANOVA, and analysis of covariance (ANCOVA). In Chapter 20, we examine several techniques useful for analyzing categorical data. Several large data sets used as examples are listed in Chapter 21, while Chapter 22 contains statistical tables used throughout the text.

1.5 References

R Core Team (2016). *R: A Language and Environment for Statistical Computing*. The R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Schork, M. A. & Remington, R. D. (2000) *Statistics with Applications to the Biological and Health Sciences, Third Edition*. Prentice Hall, Upper Saddle River, New Jersey, NJ.

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Chapter 2

Review of Mathematics

In this chapter, we will briefly review some of the mathematical concepts used in this textbook. Knowing these concepts will make it much easier to understand the mathematical underpinnings of statistics, especially the formulas used in statistics as well as their derivations. A particularly important concept is that of a function. We will commonly encounter several types of functions in statistics, including probability densities or distributions, likelihood functions, the functions used in statistical models, and ones used to transform the observations before statistical analysis.

2.1 Exponents

This section provides a brief summary of useful rules concerning exponents that often appear in statistical functions. Let a and b be two real numbers (numbers of any kind between $-\infty$ and ∞) that form the base of the exponent. This includes the special numbers $e \approx 2.71828$ and $\pi \approx 3.14159$ that often occur in statistics. As exponents or powers, let m and n be any positive

integers $(1, 2, 3, ...)$. We then have

$$a^m a^n = a^{m+n} \tag{2.1}$$
$$(a^m)^n = a^{mn} \tag{2.2}$$
$$\frac{a^m}{a^n} = a^{m-n} \tag{2.3}$$
$$(a \times b)^n = a^n b^n \tag{2.4}$$
$$\left(\frac{a}{b}\right)^n = \frac{a^n}{b^n} \tag{2.5}$$

(Schmidt & Ayres 2003). For example, suppose that $a = 2$, $b = 3$, $m = 5$, and $n = 4$. We have

$$a^m a^n = a^{m+n} \tag{2.6}$$
$$2^5 2^4 = 2^{5+4} = 2^9 = 512 \tag{2.7}$$

$$(a^m)^n = a^{mn} \tag{2.8}$$
$$(2^5)^4 = 2^{5\times 4} = 2^{20} = 1048576 \tag{2.9}$$

$$\frac{a^m}{a^n} = a^{m-n} \tag{2.10}$$
$$\frac{2^5}{2^4} = 2^{5-4} = 2^1 = 2 \tag{2.11}$$

$$(a \times b)^n = a^n b^n \tag{2.12}$$
$$(2 \times 3)^5 = 2^5 3^5 = 32 \times 243 = 7776 \tag{2.13}$$

$$\left(\frac{a}{b}\right)^n = \frac{a^n}{b^n} \tag{2.14}$$
$$\left(\frac{2}{3}\right)^5 = \frac{2^5}{3^5} = \frac{32}{243} = 0.132 \tag{2.15}$$

These rules also hold for m and n any real number provided a and b are positive. Some special cases of the above rules are also commonly encountered

in statistics. We have

$$a^0 = 1, a \neq 0 \tag{2.16}$$
$$0^0 = 0 \tag{2.17}$$
$$a^{1/2} = \sqrt{a} \tag{2.18}$$
$$a^{-m} = \frac{1}{a^m}, a \neq 0 \tag{2.19}$$

Now suppose that $a = 4$ and $m = 2$. We have

$$4^0 = 1 \tag{2.20}$$
$$4^{1/2} = \sqrt{4} = 2 \tag{2.21}$$
$$4^{-2} = \frac{1}{4^2} = \frac{1}{16} = 0.0625 \tag{2.22}$$

2.2 Inequalities

Statistical statements often involve the use of inequalities. For example, suppose that you are interested in the size distribution of a fish population. You might be interested in estimating the probability or proportion of fish that equal or exceed the legal catch size, say 12 inches. If y stands for fish size, then you would be interested in estimating the probability of fish for which $y \geq 12$ inches. You might also be interested in fish which lie within a certain range of size, say 6 to 12 inches. This could be written as $6 < y < 12$ inches using inequalities. The results of statistical tests are often reported using inequalities as well. You will commonly encounter statements of the form '$P < 0.05$' in scientific papers, which says that the probability P of a certain event occurring is less than 5%, or 1 chance in 20.

Inequalities can be manipulated much like equalities in algebra, with some exceptions. Let x and y stand for any two numbers, or more complex mathematical quantities. If $x < y$, then

$$x + b < y + b \tag{2.23}$$

where b is another number or quantity, and

$$ax < ay \tag{2.24}$$

where a is a **positive** number or other quantity. If a is **negative**, then

$$ax > ay. \tag{2.25}$$

Thus, multiplying an inequality by a negative number flips the direction of the inequality. For example, let $x = 5$, $y = 6$, and $a = -2$. We have $x < y$, but clearly $-2(5) = -10$ is greater than $-2(6) = -12$.

Another exception involves the inverse or reciprocal of an inequality. If $x < y$ and both are positive (or both negative), then

$$\frac{1}{x} > \frac{1}{y}. \tag{2.26}$$

Note the changed direction of the inequality. For example, if $x = 5$ and $y = 6$ so that $x < y$, the inequality is reversed because we have $1/5 > 1/6$. However, if $x < y$ and x is negative, then

$$\frac{1}{x} < \frac{1}{y}. \tag{2.27}$$

For example, if $x = -5$ and $y = 6$ then we have $1/-5 < 1/6$, or $-1/5 < 1/6$. These results can also be obtained through direct application of Eq. 2.24 and 2.25.

2.3 Functions

A variable is a symbol such as x or y chosen to represent a set of numbers, typically real numbers. A function is a relationship between x and y such that each value of x generates a single value of y (Schmidt & Ayres 2003). When such a relationship holds, it is customary to say that y is a function of x. An example of a function is the equation

$$y = 2x + 1 \tag{2.28}$$

This happens to be the equation of a line with a slope of 2 and an intercept of 1. In general, we can write a function using the notation

$$y = f(x) \tag{2.29}$$

where $f(x)$ stands for any possible function of x. In this context, x is often called the independent variable and y the dependent variable.

2.3.1 Functions in Statistics

One commonly used function in statistics is the equation for a line, namely

$$y = ax + b \tag{2.30}$$

where a is the slope and b is the intercept of the line. This function plays an important role in linear regression, a statistical procedure that fits a line to a series of points of the form (x, y) (see Chapter 17). Also common are quadratic functions of the form

$$y = ax^2 + bx + c \tag{2.31}$$

where a, b, and c are constants. Rather than a straight line, quadratic functions are shaped like a parabola.

Exponential and log functions are also commonly used in statistics. Examples of exponential functions are

$$y = 10^x \tag{2.32}$$

and

$$y = e^x, \tag{2.33}$$

where $e = 2.71828\ldots$, also written as

$$y = \exp(x). \tag{2.34}$$

Examples of log functions are the natural log and base 10 log, written as

$$y = \ln(x) \tag{2.35}$$

and

$$y = \log(x). \tag{2.36}$$

Confusingly, the natural log is sometimes written as $\log(x)$, while base 10 log is written as $\log_{10}(x)$. SAS uses this notation for log functions. The log functions are only defined for $x > 0$.

The exponential and log functions are inverses, meaning they reverse the action of each other. For example, we have

$$\exp(\ln(x)) = x \tag{2.37}$$

and

$$\ln(\exp(x)) = x. \tag{2.38}$$

For example, if you find $\ln(x)$ for some value of x, then apply the exp function to $\ln(x)$, you get the original value of x as the answer. Suppose that $x = 2$. We have $\ln(x) = \ln(2) = 0.693$, and then $\exp(\ln(2)) = \exp(0.693) = 2$. The same thing happens for the functions 10^x and $\log(x)$.

Another common function in statistics is the absolute value function, written as

$$y = |x|. \tag{2.39}$$

It is defined as follows. If x is positive or zero then $|x|$ is simply equal to x, while if x is negative then $|x| = -x$. For example, if $x = -2$ then $y = |-2| = -(-2) = 2$. A common use of the absolute value in statistics is to define a symmetric interval around zero. For example, the inequality $-3 < x < 3$ can also be written as $|x| < 3$.

The most commonly used distribution in statistics is the normal distribution, which can be written as a combination of several simpler functions:

$$y = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \tag{2.40}$$

Here μ and σ^2 are two parameters that govern the shape of the normal distribution, in particular its mean and variance (see Chapter 6).

2.3.2 Plotting functions using SAS - SAS demo

It can be difficult to discern the shape of a function without a graph. For example, the function describing the normal distribution gives you the famous bell-shaped curve, but this is not obvious from the equation. We will develop a SAS program that will plot any function, given its mathematical form, the values of any constants, and the range of x values for which a plot is needed. We will examine this plotting program in some detail, because it illustrates the structure of the programs used throughout this textbook.

SAS programs consist of a series of steps or instructions that enable you to input and manipulate data and then generate statistical results and graphs. Data are entered and manipulated using SAS `data` steps, while statistical results and graphs are generated using SAS procedures or `proc` steps. Note that SAS is not case-sensitive, so programs can be in either upper or lower case.

The first line of the program is a comment, used here to give the file name of the program. Any line of a SAS program beginning with an asterisk (*) is a comment, which are used to describe the program and its actions but are not executed by SAS.

```
* fplot.sas;
```

The next three lines consist of the instructions

```
options pageno=1 linesize=80;
title "Plot a function y = f(x)";
title2 "Linear function";
```

The `options` line tells SAS to start numbering the pages of output at page one, then sets the page width to 80 characters. This isn't essential but makes the output easier to read. The two `title` lines add a main title and subtitle to the output. Note that each of the lines ends with a semicolon (;). This is absolutely critical in SAS programming, because it tells SAS where a particular statement or command ends. A misplaced or absent semicolon will typically cause errors when running the program.

The next part of the SAS code is a `data` step (SAS Institute Inc. 2014a). The idea here is to generate a data set with a sequence of x and $y = f(x)$ values that will later be plotted. The minimum and maximum values of x are set by specifying values for `xmin` and `xmax`, while the number of divisions is set by `xdiv` (the more divisions the finer the x scale and the smoother the graph). The program then calculates the step length between x values (`xlength`) using these quantities. The values of x and $y = f(x)$ are calculated in a programming loop using a `do` statement . Each pass through the loop calculates a new value of x, then finds $y = f(x)$ for that value of x. The results are then sent to a SAS data file using an `output` statement. You can set the name of the data file in the first line of the `data` step, which in this case is `fplot`. Note that six different functions are listed in this `data` step, but only one would be active (the line function) because the remainder are comments. This is a useful programming trick to deactivate sections of code.

```
data fplot;
    * Minimum and maximum values of x;
    xmin = -5;
    * Use for ln function, must have x > 0;
    *xmin = 0.001;
    xmax = 5;
    * Divisions between xmin and xmax (more = smoother graph);
    xdiv = 100;
    * Calculate step length;
    xlength = (xmax-xmin)/xdiv;
    * Find x and y = f(x) values for the plot;
    do i=0 to xdiv;
        x = xmin + i*xlength;
        * Insert f(x) formula here;
        * line function;
        y = 2*x + 1;
        * quadratic function;
        *y = -x**2 + 2*x + 5;
        * exponential function;
        *y = exp(x);
        * ln function;
        *y = log(x);
        * absolute value function;
        *y = abs(x);
        * normal distribution;
        *mu = 1;
        *sig2 = 1;
        *y = (1/sqrt(2*3.14159*sig2))*exp(-((x-mu)**2)/(2*sig2));
        * Output x and y to SAS data file;
        output;
    end;
run;
```

The resulting data are then printed using the SAS `print` procedure (SAS Institute Inc. 2014b), using the syntax below. The option `data=fplot` tells the `print` procedure to use this particular data file. If this option were omitted, the last data file created would automatically be used. The `run` statement tells SAS that the `proc print` command is complete and that it should get busy printing the data file.

```
* Print data;
proc print data=fplot;
run;
```

The `gplot` procedure is used to plot the function using the new data set (see below) (SAS Institute Inc. 2014c). The `plot` statement tells SAS which of your SAS variables are the x and y variables - the variable before the asterisk (`*`) is the y variable, after it the x variable (it is the position that is important, not the name of the variable). The `href = 0` and `vref = 0` options make SAS draw vertical and horizontal lines through the origin $(0, 0)$. The `symbol1` statement tells SAS to join the points with a line (`i=join`), draw no symbol for each data point (`v=none`), and make the line connecting the points red (`c=red`). The remainder of the options listed in the program are intended to make the graph more legible by increasing the thickness of the lines and size of the axes labels. If you are curious how they work, try experimenting with the numbers given in the options. The `quit` statement returns control to SAS after running the program.

```
* Plot y = f(x);
proc gplot data=fplot;
    plot y*x=1 / href=0 vref=0 whref=3 wvref=3 vaxis=axis1 haxis=axis1;
    symbol1 i=join v=none c=red width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

See the full program listing below, a portion of the printed output, and graphs for the various functions included in the program.

SAS program

```
* fplot.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Plot a function y = f(x)";
title2 "Linear function";
data fplot;
    * Minimum and maximum values of x;
    xmin = -5;
    * Use for ln function, must have x > 0;
    *xmin = 0.001;
    xmax = 5;
    * Divisions between xmin and xmax (more = smoother graph);
    xdiv = 100;
    * Calculate step length;
    xlength = (xmax-xmin)/xdiv;
    * Find x and y = f(x) values for the plot;
    do i=0 to xdiv;
        x = xmin + i*xlength;
        * Insert f(x) formula here;
        * line function;
        y = 2*x + 1;
        * quadratic function;
        *y = -x**2 + 2*x + 5;
        * exponential function;
        *y = exp(x);
        * ln function;
        *y = log(x);
        * absolute value function;
        *y = abs(x);
        * normal distribution;
        *mu = 1;
        *sig2 = 1;
        *y = (1/sqrt(2*3.14159*sig2))*exp(-((x-mu)**2)/(2*sig2));
        * Output x and y to SAS data file;
        output;
    end;
run;
* Print data;
proc print data=fplot;
run;
* Plot y = f(x);
proc gplot data=fplot;
    plot y*x=1 / href=0 vref=0 whref=3 wvref=3 vaxis=axis1 haxis=axis1;
```

```
    symbol1 i=join v=none c=red width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

SAS output

Plot a function y = f(x) 1
Linear function 09:07 Friday, January 29, 2010

Obs	xmin	xmax	xdiv	xlength	i	x	y
1	-5	5	100	0.1	0	-5.0	-9.0
2	-5	5	100	0.1	1	-4.9	-8.8
3	-5	5	100	0.1	2	-4.8	-8.6
4	-5	5	100	0.1	3	-4.7	-8.4
5	-5	5	100	0.1	4	-4.6	-8.2
6	-5	5	100	0.1	5	-4.5	-8.0
7	-5	5	100	0.1	6	-4.4	-7.8
8	-5	5	100	0.1	7	-4.3	-7.6
9	-5	5	100	0.1	8	-4.2	-7.4
10	-5	5	100	0.1	9	-4.1	-7.2
11	-5	5	100	0.1	10	-4.0	-7.0

etc.

Figure 2.1: Plot of $y = 2x + 1$

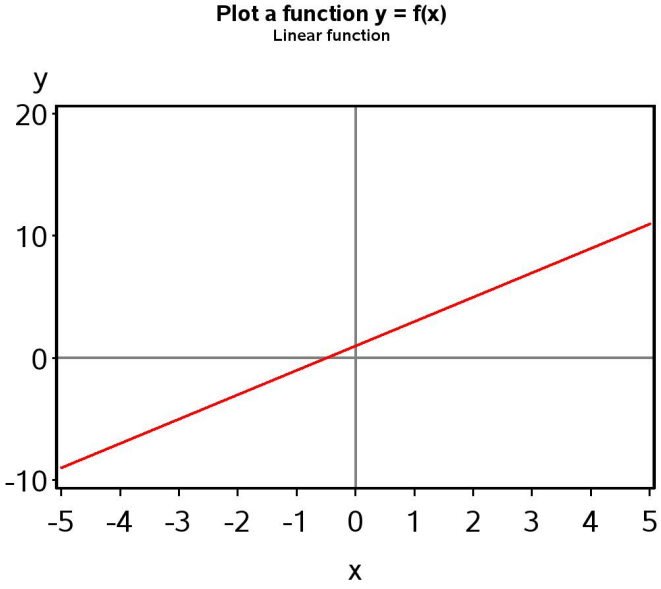


Figure 2.2: Plot of $y = -x^2 + 2x + 5$

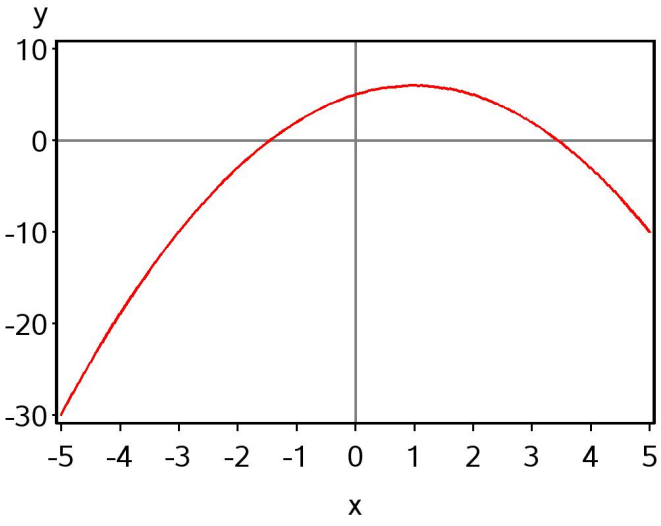


Figure 2.3: Plot of $y = e^x = \exp(x)$

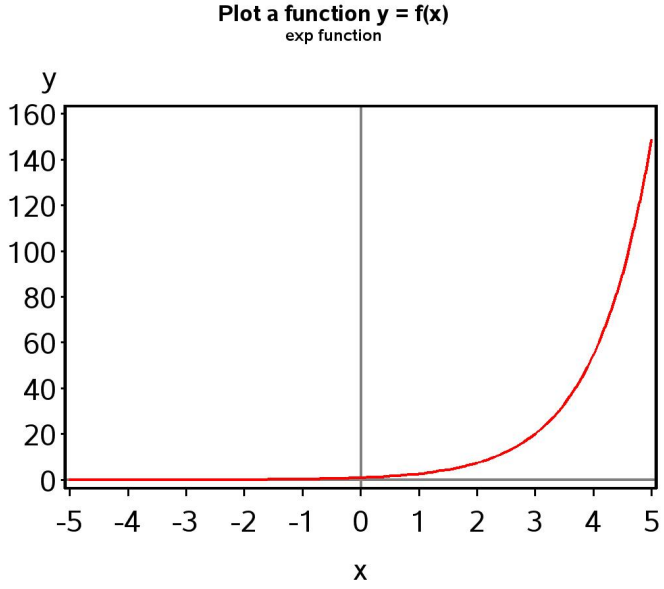


Figure 2.4: Plot of $y = \ln(x)$

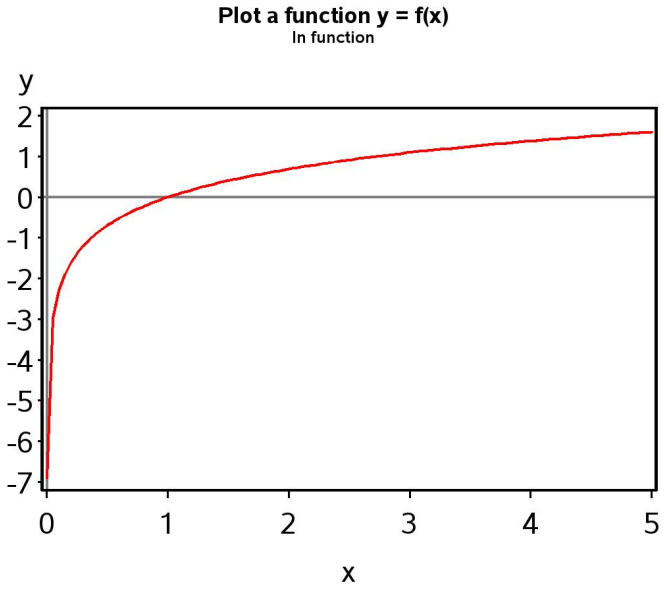


Figure 2.5: Plot of $y = |x|$

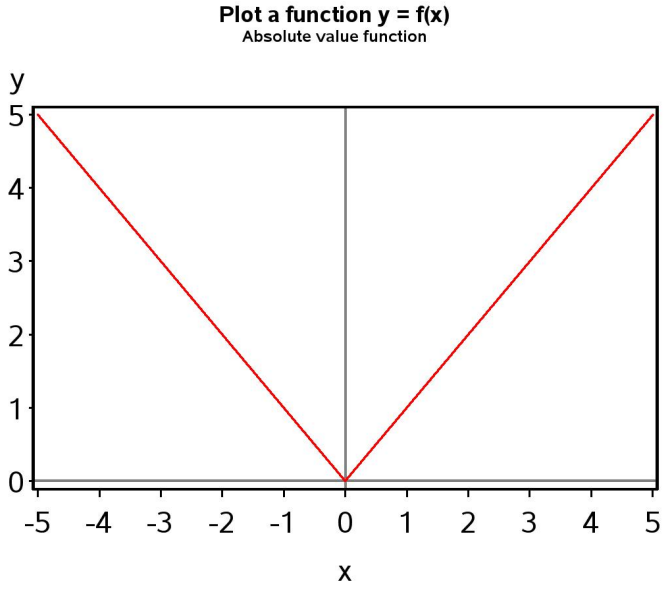
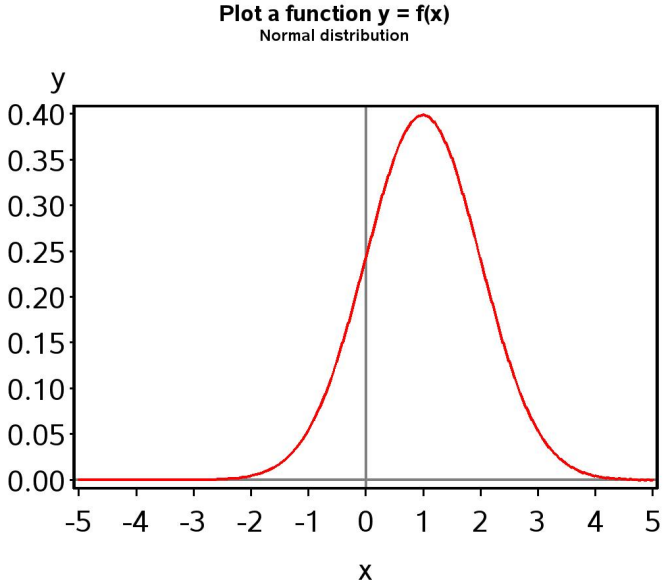


Figure 2.6: Plot of $y = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$, for $\mu = 1$ and $\sigma^2 = 1$



2.4 Solving linear equations

We next review how to solve a linear equation for x, a procedure that will be useful in later developments. A linear equation has the general form

$$ax + b = cx + d \tag{2.41}$$

where a, b, c, and d are constants that are possibly zero, while x is a variable. We want to find a value of x that makes this equation true, meaning the two sides of the equation are equal. To solve this problem, you perform the same operations on both sides of the equation until you have x alone on one side of the equation. The other side is then the answer to this problem (Schmidt & Ayres 2003). More generally, a-d and x could also be more complicated expressions that one manipulates to obtain an expression for x.

To illustrate this procedure, suppose we have the equation

$$5x - 4 = 3x - 3. \tag{2.42}$$

Subtracting $3x$ from both sides of the equation, we get

$$2x - 4 = -3. \tag{2.43}$$

We next add 4 to both sides to obtain

$$2x = 1. \tag{2.44}$$

Dividing both sides by 2 we obtain the solution

$$x = 1/2. \tag{2.45}$$

If you want to check if the solution is correct, you can always substitute it back into the original equation. We have

$$5(1/2) - 4 = 3(1/2) - 3 \tag{2.46}$$
$$2.5 - 4 = 1.5 - 3 \tag{2.47}$$
$$-1.5 = -1.5. \tag{2.48}$$

So $x = 1/2$ is in fact the correct solution.

2.5 Roots of equations

For a particular function $y = f(x)$, it is often useful to find the values of x for which $y = f(x) = 0$. Values of x for which this is true are called the roots of the equation $f(x) = 0$ (Schmidt & Ayres 2003). Graphically, the roots are the values of x where the function crosses the x-axis, i.e., the function is equal to zero. It is possible to find the roots for many functions algebraically, but not every function has roots, and for some functions they can only be found numerically using software and a computer.

Roots are easy to find for linear functions. Recall that a linear function takes the general form

$$y = a + bx \tag{2.49}$$

where a and b are constants. We want to find values of x for which

$$a + bx = 0 \tag{2.50}$$

We then use the rules for solving linear equations to find x. Subtracting a from both sides and dividing by b, we obtain

$$x = \frac{-a}{b} \tag{2.51}$$

Suppose that $a = 1$ and $b = 2$, so that our function is

$$y = 1 + 2x. \tag{2.52}$$

It follows that the root of this function is $x = -a/b = -1/2$. If we examine the graph generated earlier for this function, we see that the function indeed crosses the x-axis at $x = -1/2$.

We can also find the roots for quadratic functions using, logically enough, the quadratic formula. Recall that a quadratic function takes the general form

$$y = ax^2 + bx + c \tag{2.53}$$

We want to find values of x for which

$$ax^2 + bx + c = 0. \tag{2.54}$$

The quadratic formula says that the roots are given by the equation

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \tag{2.55}$$

We previously plotted a quadratic function of the form

$$y = -x^2 + 2x + 5 \tag{2.56}$$

To find the roots, we need to solve the equation

$$-x^2 + 2x + 5 = 0 \tag{2.57}$$

Inspecting this equation, we see that $a = -1$, $b = 2$, and $c = 5$. Inserting these values in the quadratic formula, we obtain

$$x = \frac{-2 \pm \sqrt{2^2 - 4(-1)5}}{2(-1)} = \frac{-2 \pm \sqrt{24}}{-2} \tag{2.58}$$

$$= \frac{-2 \pm 4.90}{-2} = \frac{-6.90}{-2}, \frac{2.90}{-2} = 3.45, -1.45 \tag{2.59}$$

The roots of this quadratic equation are therefore equal to 3.45, 1.45. This result agrees with the graph drawn earlier.

2.6 Calculus

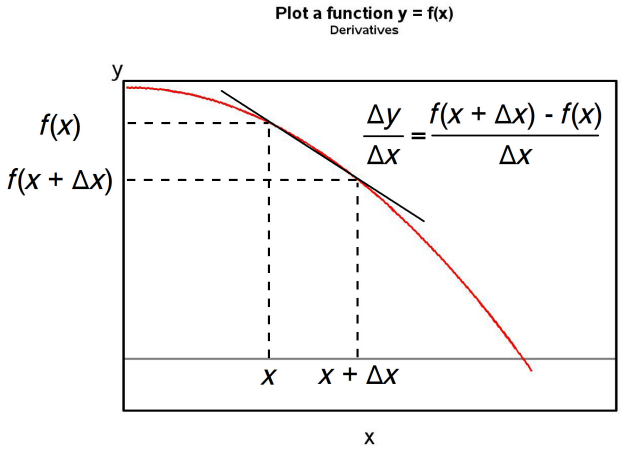
We will make only limited use of calculus in this course, but it is useful to review the concepts of derivatives and integrals. Derivatives are often used in estimating the parameters of statistical models through a method called maximum likelihood (Chapter 8). Integrals are used to generate the probabilities associated with confidence intervals, statistical tests, and other procedures. For example, the statistical tables given in Chapter 22 were all generated using integrals.

2.6.1 Derivatives

A derivative of a function $y = f(x)$ is defined to be the slope of the function at a particular value of x. Recall that the slope is defined as the change in y divided by the change in x. The mathematical definition of a derivative is given by the equation

$$\lim_{\Delta x \to 0} \frac{\Delta y}{\Delta x} = \lim_{\Delta x \to 0} \frac{f(x + \Delta x) - f(x)}{\Delta x} \tag{2.60}$$

Figure 2.7: Definition of a derivative



where Δx is the change in x, while Δy is the change in y, defined as $f(x + \Delta x) - f(x)$ (Schmidt & Ayres 2003). This equation says that the derivative is given by the limit, as Δx goes to zero, of the slope $\Delta y/\Delta x$. See also Fig. 2.7. The derivative of a function may be written as $\frac{dy}{dx}$ or $f'(x)$.

Now suppose we have a linear function like

$$y = ax + b. \tag{2.61}$$

The derivative of this function is simply a, the slope of the line. It is equal to a regardless of the value of x, because a line has the same slope everywhere. We would write this as $\frac{dy}{dx} = a$ or $f'(x) = a$.

Assume now that we have a quadratic function. There is a formula for the derivative of a power of x that is often useful. If $y = f(x) = kx^n$, where k and n are any constants, then

$$\frac{dy}{dx} = knx^{n-1}. \tag{2.62}$$

We can use this formula to find the derivative of a quadratic function of the form

$$y = ax^2 + bx + c. \tag{2.63}$$

We have

$$\frac{dy}{dx} = a(2)x^{2-1} + b(1)x^{1-1} + 0 = 2ax + b. \tag{2.64}$$

To obtain this result, we also made use of the fact that the derivative of a constant (c in this case) is always zero (because it is unchanging), and that the derivative of a sum of functions is the sum of the derivatives.

One important application of the derivative in statistics is to find the maximum or minimum of a function. In particular, the derivative of a function is equal to zero at the maximum or minimum. This follows because a function that has a maximum must eventually stop rising and begin to fall, and at that point the slope is equal to zero. The same reasoning applies to a minimum.

To find the maximum or minimum for our general quadratic function, we set $dy/dx = 0$ and solve for x. We have

$$\frac{dy}{dx} = 2ax + b = 0. \tag{2.65}$$

Solving this linear equation for x, we find that the maximum or minimum will occur at $x = \frac{-b}{2a}$.

2.6.2 Function plot with derivative - SAS demo

We will plot a quadratic function and its derivative to observe the relationship between the two. Suppose that we have the following quadratic function:

$$y = -x^2 + 2x + 5. \tag{2.66}$$

The derivative of this function is

$$\frac{dy}{dx} = -2x^{2-1} + 2(1)x^{1-1} + 0 = -2x + 2. \tag{2.67}$$

We can find the minimum or maximum of this function by setting the derivative equal to zero and solving for x. We have

$$-2x + 2 = 0 \tag{2.68}$$

for which the solution is $x = 1$.

We will now plot both y and dy/dx using a revised version of our plotting program. This program calculates both y and dy/dx within the `do` loop,

then plots both sets of points on the same graph using the overlay option in `proc gplot`. See SAS program and output below.

Note that the derivative of this quadratic function is a straight line with a slope of -2 and an intercept of 2. It equals zero at the point where it intercepts the x-axis, which also corresponds to the maximum of the quadratic function. Our calculation above shows this occurs at $x = 1$.

SAS program

```
* fplot_deriv.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Plot a function and its derivative";
title2 "Quadratic function";
data fplot2;
    * Minimum and maximum values of x;
    xmin = -5;
    xmax = 5;
    * Divisions between xmin and xmax (more = smoother graph);
    xdiv = 100;
    * Calculate step length;
    xlength = (xmax-xmin)/xdiv;
    * Find x, y = f(x), and dy/dx values for the plot;
    do i=0 to xdiv;
        x = xmin + i*xlength;
        * quadratic function;
        y = -x**2 + 2*x + 5;
        * derivative of this function;
        dydx = -2*x + 2;
        * Output x, y, and dydx to SAS data file;
        output;
    end;
run;
* Print data;
proc print data=fplot2;
run;
* Plot y = f(x) and dydx;
proc gplot data=fplot2;
    plot y*x=1 dydx*x=2 / href=0 vref=0 overlay whref=3 wvref=3 vaxis=axis1
    haxis=axis1;
    symbol1 i=join v=none c=red width=3;
    symbol2 i=join v=none c=blue width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

SAS output

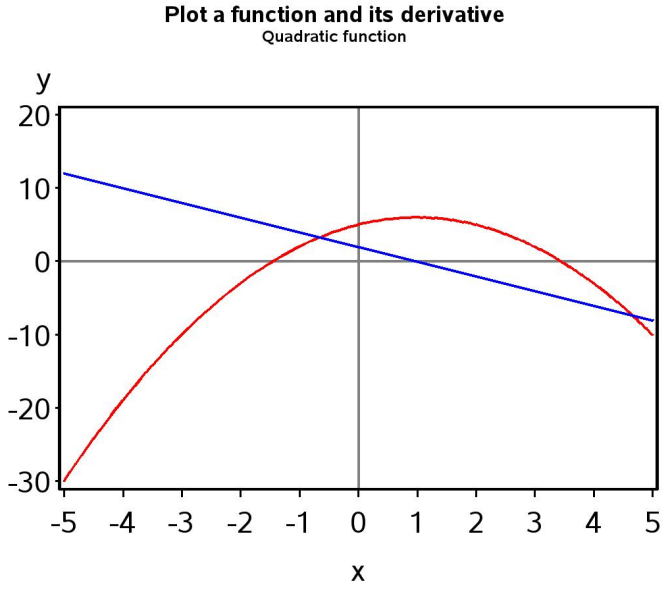
```
                    Plot a function and its derivative                    1
                            Quadratic function 09:07 Friday, January 29, 2010

    Obs    xmin    xmax    xdiv    xlength     i       x         y      dydx

     1      -5       5      100      0.1       0     -5.0     -30.00    12.0
     2      -5       5      100      0.1       1     -4.9     -28.81    11.8
     3      -5       5      100      0.1       2     -4.8     -27.64    11.6
     4      -5       5      100      0.1       3     -4.7     -26.49    11.4
     5      -5       5      100      0.1       4     -4.6     -25.36    11.2
     6      -5       5      100      0.1       5     -4.5     -24.25    11.0
     7      -5       5      100      0.1       6     -4.4     -23.16    10.8
     8      -5       5      100      0.1       7     -4.3     -22.09    10.6
     9      -5       5      100      0.1       8     -4.2     -21.04    10.4
    10      -5       5      100      0.1       9     -4.1     -20.01    10.2
    11      -5       5      100      0.1      10     -4.0     -19.00    10.0

etc.
```

Figure 2.8: Plot of $y = -x^2 + 2x + 5$ and $dy/dx = -2x + 2$



2.6.3 Integrals

Statistics makes heavy use of integrals in working with the normal and other statistical distributions, although statistical tables or software typically do the work for the end user. For example, tables of the normal distribution provide probabilities for certain intervals - these probabilities are actually areas under the bell-shaped curve and are calculated by integration.

One kind of integral often encountered in statistics is a called a definite integral. It is basically the area A under a function $f(x)$ over some range of x values, say $a < x < b$. It is written mathematically as the equation

$$A = \int_a^b f(x)dx. \tag{2.69}$$

Here the symbol $\int$ is the integral sign, with the range of x values ($a < x < b$) shown as sub- and superscripts of the integral sign.

To make things more concrete, we will illustrate definite integrals using the normal distribution function. Consider this function for $\mu = 5$ and $\sigma^2 = 1$, and the area A under it from $x = 5$ to $x = 6$ (Fig. 2.9). If we were modeling the behavior of some biological variable (say body mass of a small animal) using this distribution, the area A would be the probability that an animal falls within this range of x values. It would be expressed in mathematical terms as the integral

$$A = \int_a^b \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} dx = \int_5^6 \frac{1}{\sqrt{2\pi}} e^{-\frac{(x-5)^2}{2}} dx. \tag{2.70}$$

How is the area A actually calculated through integration? We can approximate this area by dividing it into strips of width $\Delta x = 0.25$ and height $f(x)$ given by the normal distribution function (Fig. 2.10). Adding the areas of these strip, we obtain $A \approx 0.099 + 0.093 + 0.080 + 0.068 = 0.340$. If we increased the number of strips while simultaneously decreasing the width of the strips Δx, we would get an even more accurate approximation to A. The integral is defined as the limit of this process, as the number of strips approaches infinity and their width $\Delta x \to 0$ (Schmidt & Ayres 2003). The exact value of the area obtained through this process is $A = 0.341$.

Figure 2.9: Plot of $y = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$, for $\mu = 5$ and $\sigma^2 = 1$

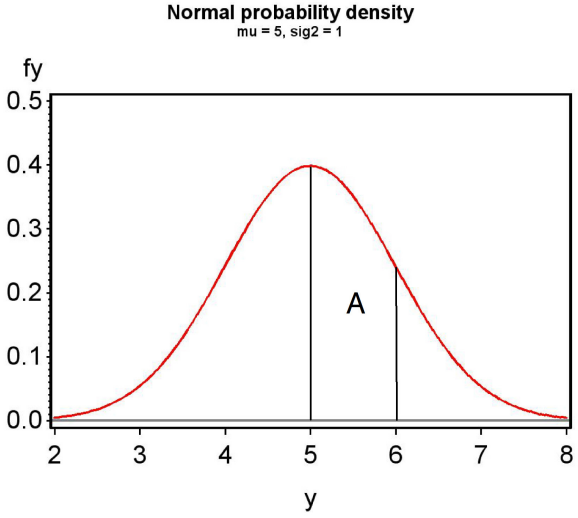
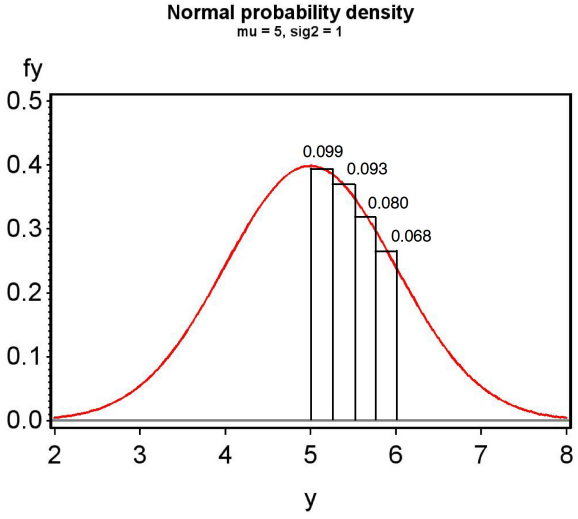


Figure 2.10: Plot of $y = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$, for $\mu = 5$ and $\sigma^2 = 1$



2.7 References

SAS Institute Inc. (2014a) *SAS 9.4 Language Reference: Concepts, Third Edition.* SAS Institute Inc., Cary, NC.

SAS Institute Inc. (2014b) *Base SAS 9.4 Procedures Guide, Third Edition.* SAS Institute Inc., Cary, NC.

SAS Institute Inc. (2014b) *SAS/GRAPH 9.4: Reference, Third Edition.* SAS Institute Inc., Cary, NC.

Schmidt, P. A. & Ayres, F. Jr. (2003) *Schaums Outline of Theory and Problems of College Mathematics, 3rd Edition.* The McGraw-Hill Companies, Inc., New York, NY.

2.8 Problems

1. Suppose that you have the quadratic function $y = x^2 - 2x - 8$. Find the roots of this function, then determine the value of x that minimizes it. Plot the function using SAS, attaching your program, output, and graph.

2. Consider the quadratic function $y = -2x^2 + 5x + 5$. Find the roots of this function, then determine the value of x that maximizes it. Plot the function and its derivative dy/dx using SAS, attaching your program, output, and graph.

3. Plot the function $y = 0.5\lambda^3 x^2 \exp(-\lambda x)$ for $\lambda = 2$ and $0 \leq x \leq 5$ using SAS. Attach your program, output, and graph. This function is a special case of the gamma distribution, a probability distribution often used to model continuous data (see Chapter 6).

Chapter 3

Populations and Statistics

This chapter covers two topics that are fundamental in statistics. The first is the concept of a statistical population, which is the basic unit on which statistics are conducted and inferences made. We then examine descriptive statistics and frequency distributions, which are used quantify the properties of samples from a statistical population.

3.1 Statistical populations

Suppose we want to estimate the body length of an insect species in a particular location, say a forest stand. We sample the insects in some way (traps, sweep nets, locate them visually, etc.), and average their lengths to obtain an estimate of insect length. We can therefore make some inference about insect lengths in this particular forest stand, which we can call a **statistical population**. A statistical population is defined by both the question of interest (insect length) as well as the sampling method. If we sample insects in only a single forest stand, then the statistical population is length in that stand, not other stands. This is commonly called the **scope of inference** of the study. If we sampled within multiple stands in a forest, then we could potentially examine length for the forest as whole, which would be a different statistical population and the scope of inference would be broader. The sampling technique itself can also affect the statistical population. For example, only a subset of insects might be caught with sweep nets (maybe slower, smaller ones) and this would be a different set than those found visually. The two sampling techniques might therefore define different statistical populations.

Biologists are continually searching for better methods of sampling organisms, ones that better represent their true properties. In many cases the idea is to approximate what is known as **random sample** of the statistical population (see Chapter 8).

In the insect length example above, the statistical population coincides with individual insects in a location. However, the observations comprising a statistical population can be other quantities. For example, suppose we want to estimate the abundance of these insects using traps. We could deploy several traps in the stand, and then average the number of insects caught to estimate their abundance. The statistical population in this case would consist of number of insects caught in traps deployed at that location, rather than individual insects. Or one might be interested in soil nitrogen levels in the stand, estimated using core samples. In this case, the statistical population would be the nitrogen levels in core samples at this location.

Another type of statistical population involves experiments. Suppose we are interested in trapping the same insects in the forest stand, but now have traps baited with different attractants, say A, B, and C. Several traps are baited with each attractant, and the number of insects caught observed for each trap. We are interested in whether the number of insects caught varies with the attractant used. In this case, the statistical population would be trap catches for the different attractants. Similarly, suppose we were interested in the effect of different commercial diets on the growth rate of fish. Different fish would be fed the various diets and their growth rate observed. Here the statistical population would be the growth rate of individual fish for the different diets. Experiments also have a scope of inference. If we use four particular diets to grow fish, our conclusions are restricted to these four diets and not other diets. If the experiment used a particular strain of fish, our inferences would also be restricted to this strain.

3.2 Descriptive statistics and frequency

Given a sample from a statistical population, the first step in understanding its properties is to calculate a number of descriptive statistics. Some statistics give you an idea of the overall magnitude or location of the data, and are traditionally called **statistics of location**. We will examine two such statistics, the sample mean and the median. Other statistics give an indication of the scatter or spread of the data, and are called **statistics of**

dispersion. These include the sample variance, standard deviation, the coefficient of variation, and range of the data. Another important tool is the **frequency distribution** of the sample, often plotted as a histogram indicating the frequency of different values in the sample. Three other statistics, the mode, skewness, and kurtosis, provide information on the shape of this frequency distribution.

To illustrate how the various descriptive statistics are calculated, we will use a small subset of a larger data set on the elytra length for a predatory beetle, *Thanasimus dubius* (Coleoptera: Cleridae). This predator attacks insects known as bark beetles, some species of which are serious pests of coniferous forests (Berryman 1988). Beetles have two pairs of wings. The first pair, the elytra, act as covers for a membraneous second pair that are used in flight. The data are drawn from a rearing study of *T. dubius*, in which elytra length (mm) was used as a overall index of body size (Reeve et al. 2003). The subset data are for eight female *T. dubius* and are listed below:

```
5.2 4.2 5.7 5.4 4.0 4.5 5.2 4.2
```

We will later examine the full data set consisting of 130 individuals using SAS programs.

3.2.1 Sample mean

The sample mean is the average of the values in the sample, and is symbolized as $\bar{Y}$. It is commonly used as a measure of the location or center of the observations. If $Y_1, Y_2, \ldots, Y_n$ represent the observations in a sample from a statistical population, where n is the sample size, the sample mean is calculated using the formula

$$\bar{Y} = \frac{Y_1 + Y_2 + \ldots + Y_n}{n} = \frac{\sum_{i=1}^{n} Y_i}{n}. \tag{3.1}$$

The symbol $\sum_{i=1}^{n}$ stands for summing the observations, beginning with $i = 1$ and ending with $i = n$. The units of $\bar{Y}$ are the same as those for the Y_i values.

For our sample data set involving $n = 8$ elytra from female *T. dubius* beetles, we have

$$\bar{Y} = \frac{5.2 + 4.2 + 5.7 + 5.4 + 4.0 + 4.5 + 5.2 + 4.2}{8} = \frac{38.4}{8} = 4.8 \text{ mm}. \tag{3.2}$$

3.2.2 Median

The median is defined as the middle value of the sample, after ordering the sample from the smallest to the largest value. Suppose that $Y_{[j]}$ is the jth value in the ordered data set, with $Y_{[1]}$ the smallest value and $Y_{[n]}$ the largest. If n is odd, the median is equal to the middle value in the ordered data set, or $Y_{[n/2+1/2]}$. If n is even then the median is the average of the two middle values, or $(Y_{[n/2]} + Y_{[n/2+1]})/2$.

To find the median for the elytra data set, we first order the observations from smallest to largest. We have

j (order):	1	2	3	4	5	6	7	8
$Y_{[j]}$:	4.0	4.2	4.2	4.5	5.2	5.2	5.4	5.7

Because $n = 8$ is even, the median is the average of the middle two observations, or $(Y_{[n/2]} + Y_{[n/2+1]})/2 = (Y_{[8/2]} + Y_{[8/2+1]})/2 = (Y_{[4]} + Y_{[5]})/2 = (4.5 + 5.2)/2 = 4.85$.

Suppose now we had only $n = 7$ observations, with the ordered data set equal to

j (order):	1	2	3	4	5	6	7
$Y_{[j]}$:	4.0	4.2	4.2	4.5	5.2	5.2	5.4

Because $n = 7$ is odd, the median is the middle observation, or $Y_{[n/2+1/2]} = Y_{[7/2+1/2]} = Y_{[4]} = 4.5$ mm.

The median is also a measure of the location of the data, like the sample mean $\bar{Y}$, but is less sensitive to very large or small values in the sample. For example, suppose that the largest observation in the elytra data set was 100.0. The median would be unchanged because the ordering of the observations is unchanged, but now $\bar{Y} = 16.8$ mm, much larger than before.

The median represents a value that essentially divides the data in half, with 50% of the observations lying above or below it. This is an example of a statistic generically called **quantiles** or **percentiles**, with the median a 50% quantile. Other commonly used quantiles are the 25% and 75% quantiles. They and the median are sometime called **quartiles** because they divide the data into four quarters.

3.2.3 Sample variance

The sample variance, written as s^2, is a measure of the dispersion or scatter in the data around the sample mean. It is calculated using the formula

$$s^2 = \frac{\sum_{i=1}^{n}(Y_i - \bar{Y})^2}{n-1} \tag{3.3}$$

The sample variance s^2 will be small if the observations cluster tightly around $\bar{Y}$, because this makes $(Y_i - \bar{Y})^2$ small. Conversely, if the observations are widely scattered these terms will be large, making s^2 large. The units of s^2 are those of Y_i, but squared.

To find s^2 for the elytra data set, we first need to calculate the sample mean. We previously found that $\bar{Y} = 4.8$ mm. We then calculate s^2 using the above formula. We have

$$s^2 = \frac{(5.2-4.8)^2 + (4.2-4.8)^2 + \ldots + (4.2-4.8)^2}{8-1} \tag{3.4}$$

$$= \frac{0.16 + 0.36 + 0.81 + 0.36 + 0.64 + 0.09 + 0.16 + 0.36}{7} \tag{3.5}$$

$$= \frac{2.94}{7} = 0.42 \text{ mm}^2. \tag{3.6}$$

3.2.4 Standard deviation

The sample standard deviation, written as s, is simply the square root of s^2. We have

$$s = \sqrt{s^2} \tag{3.7}$$

For the elytra example, we have $s = \sqrt{s^2} = \sqrt{0.42} = 0.645$ mm. The units of s are the same as those of Y_i, which makes it more comparable to statistics of location like $\bar{Y}$.

3.2.5 Coefficient of variation

The coefficient of variation, or CV, provides a measure of the variability of the observations expressed as a percentage of the sample mean. It is calculated using the formula

$$CV = 100\% \times \frac{s}{\bar{Y}}. \tag{3.8}$$

The CV allows one to compare the variability of observations on variables that have different means. For example, suppose that we want to compare variability in *T. dubius* elytra length with variability in another predator that has a longer overall length. For biological variables like length, the standard deviation s often seems proportional to the sample mean $\bar{Y}$. If we divide s by $\bar{Y}$, as in the CV, we can control to some extent the influence of $\bar{Y}$ on variability. This allows us to compare variability in length across the two predators on a more even basis.

3.2.6 Range

The range is defined as the difference between the largest and smallest observations, i.e.,

$$\text{range} = Y_{\max} - Y_{\min}, \tag{3.9}$$

where $Y_{\max}$ is the largest observation and $Y_{\min}$ is the smallest. For the elytra data, we have $Y_{\max} = 5.7$ and $Y_{\min} = 4.0$, so

$$\text{range} = 5.7 - 4.0 = 1.7. \tag{3.10}$$

The range is another statistic of dispersion, but has some problems. The range tends to increase in size as the sample size n increases, because larger samples are more likely to yield very small or large observations. This is not the case for s^2 or s.

3.2.7 Frequency distributions - SAS demo

Frequency distributions are another way of summarizing and describing a sample from a statistical population. They typically take the form of a histogram showing the frequency of different observations in the sample. We will use SAS to construct frequency distributions as well as calculate descriptive statistics like $\bar{Y}$, s^2, and so forth. We will use the full elytra data set for *T. dubius* (Reeve et al. 2003) to illustrate these calculations. This data set contains both male and female beetles, and we will conduct separate analyses for each sex. See also Chapter 21.

The program first uses a `data` step to read in the observations and make a data file (SAS Institute Inc. 2014a). The line

```
data elytra;
```

tells SAS to set up a data file named `elytra`. If you omit a name from this statement, SAS will automatically generate one for you. The line

```
input sex $ length;
```

tells SAS to read in two variables and give them the names `sex` and `length`. It also tells SAS to expect the data in the form of two columns. The `$` symbol after `sex` tells SAS that it is a character variable, consisting of a word or letters rather than a number. The default is for a numeric variable. The line

```
datalines;
```

tells SAS that following lines in the program are the actual data. The program then lists the data, followed by another semicolon and then a `run` statement (see below). The full data set is not listed here because it is extensive (see Chapter 21, Section 21.1). The `run` statement tells SAS the `data` step is over, and also that it should process the data and generate a SAS data file.

```
M   4.9
F   5.2
M   4.9
F   4.2
F   5.7

etc.

M   5.1
F   4.4
M   4.8
M   4.6
F   3.7
;
run;
```

We are now ready to do something with our newly minted SAS data file, named `elytra`. It is usually a good idea just to print the data file to make sure SAS correctly read the data. This is accomplished using the `proc print` code listed below.

```
* Print data set;
proc print data=elytra;
run;
```

The final lines of the SAS program invoke `proc univariate` to generate the histogram and calculate a number of descriptive statistics (SAS Institute Inc. 2014b). The first and third lines are comments. The second line tells SAS to call `proc univariate` and requests that certain plots be made using the `plots` option. The `class` statement tells the procedure to conduct a separate analysis for each sex in the data set, while the `var` statements tells it which variable to analyze, in this case the variable `length`. The `histogram` statement asks for a histogram of `length`, with the statements after the forward slash (`/`) being options for the graph. The option `vscale=count` tells SAS to make the vertical axis using counts of the observations (the default uses percentages). The remaining options control the width of the lines in the graph as well as text height. The program would work without these options but would generate a different-looking histogram.

```
* Descriptive statistics and histograms;
proc univariate plots data=elytra;
    * Separate analyses for each sex;
    class sex;
    var length;
    histogram length / vscale=count wbarline=3 waxis=3 height=4;
run;
quit;
```

After running the program, we obtain output with various statistics of location and dispersion, including the sample mean, median range, variance, and standard deviation, as well as a graph showing the frequency distribution. A separate analysis is generated for each sex (`M` or `F`) of the beetles. We see that females have somewhat longer elytra than males ($\bar{Y} = 4.940$ mm vs. 4.713 mm), and there are small differences in other statistics. See a complete program listing below, and SAS output with some editing to reduce its length.

SAS Program

```
* descriptive.sas;
options pageno=1 linesize=80;
title 'Descriptive statistics for the elytra data';
data elytra;
    input sex $ length;
    datalines;
M   4.9
F   5.2
M   4.9
F   4.2
F   5.7

etc.

M   5.1
F   4.4
M   4.8
M   4.6
F   3.7
;
run;
* Print data set;
proc print data=elytra;
run;
* Descriptive statistics and histograms;
proc univariate plots data=elytra;
    * Separate analyses for each sex;
    class sex;
    var length;
    histogram length / vscale=count wbarline=3 waxis=3 height=4;
run;
quit;
```

SAS Output

Descriptive statistics for the elytra data 1
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Obs	sex	length
1	M	4.9
2	F	5.2
3	M	4.9
4	F	4.2
5	F	5.7

etc.

Descriptive statistics for the elytra data 4
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The UNIVARIATE Procedure
Variable: length
sex = F

Moments

N	60	Sum Weights	60
Mean	4.94	Sum Observations	296.4
Std Deviation	0.48544929	Variance	0.23566102
Skewness	-0.521146	Kurtosis	0.16125847
Uncorrected SS	1478.12	Corrected SS	13.904
Coeff Variation	9.82690878	Std Error Mean	0.06267123

Basic Statistical Measures

Location		Variability	
Mean	4.940000	Std Deviation	0.48545
Median	5.000000	Variance	0.23566
Mode	5.200000	Range	2.20000
		Interquartile Range	0.70000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	78.82404	Pr > |t|	<.0001
Sign	M	30	Pr >= |M|	<.0001
Signed Rank	S	915	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	5.9
99%	5.9
95%	5.7
90%	5.5
75% Q3	5.3
50% Median	5.0
25% Q1	4.6
10%	4.3
5%	4.0
1%	3.7
0% Min	3.7

Descriptive statistics for the elytra data 7
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The UNIVARIATE Procedure
Variable: length
sex = M

Moments

N	70	Sum Weights	70
Mean	4.71285714	Sum Observations	329.9
Std Deviation	0.44977335	Variance	0.20229607
Skewness	-0.896502	Kurtosis	1.00307174
Uncorrected SS	1568.73	Corrected SS	13.9584286
Coeff Variation	9.5435388	Std Error Mean	0.0537582

Basic Statistical Measures

Location Variability

Mean	4.712857	Std Deviation	0.44977
Median	4.800000	Variance	0.20230
Mode	5.000000	Range	2.40000
		Interquartile Range	0.50000

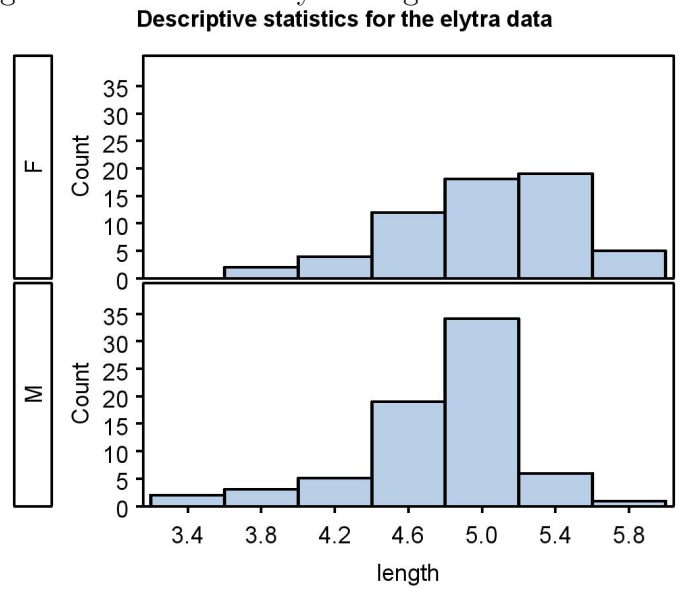
Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	87.66769	Pr > |t|	<.0001
Sign	M	35	Pr >= |M|	<.0001
Signed Rank	S	1242.5	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	5.80
99%	5.80
95%	5.20
90%	5.15
75% Q3	5.00
50% Median	4.80
25% Q1	4.50
10%	4.00
5%	3.80
1%	3.40
0% Min	3.40

Figure 3.1: *T. dubius* elytra length - females and males



3.2.8 Mode

The mode is defined to be the most frequent value in the data set, and is another statistic of location. The mode in itself does not have many applications in biology, but is commonly used to describe the shape of a frequency distribution for the sample (see above). For example, we describe a frequency distribution as being unimodal if it has a single peak, and bimodal if there are two peaks. Examining the SAS output listed above, we see that female *T. dubius* beetles have a mode of 5.2 mm, while the mode for males is 5.0 mm. Both distributions appear to be unimodal.

3.2.9 Skewness

Skewness is a measure of the symmetry of the frequency distribution. Distributions that show an extended left tail to the frequency distribution, as well as the pattern mode $>$ median $>$ mean, are said to be skewed to the left. Fig. 3.2 shows an example of a left-skewed frequency distribution for some

variable `y`. Conversely, distributions with an extended right tail and the pattern mean > median > mode are skewed to the right (Fig. 3.3). Skewness can be quantified by calculating the statistic g_1, given by the formula

$$g_1 = \frac{n}{(n-1)(n-2)} \sum_{i=1}^{n} \left(\frac{Y_i - \bar{Y}}{s} \right)^3 . \tag{3.11}$$

The cubic terms here measure the asymmetry of the distribution. If the distribution is skewed to the left, with more values farther to the left than the right of $\bar{Y}$, there will tend to be large negative cubic terms, making $g_1 < 0$. Conversely, distributions skewed to the right will have large positive cubic terms and $g_1 > 0$. For distributions that are symmetrical we have $g_1 \approx 0$. For example, a frequency distribution for normally-distributed data would be symmetrical with $g_1 \approx 0$ (Fig. 3.4). For the elytra example, both male and female *T. dubius* have frequency distributions that appear skewed to the left, and also have negative g_1 values. Skewness is most often used as a description of the general shape of a distribution.

Figure 3.2: Frequency distribution that is skewed left ($g_1 < 0$).

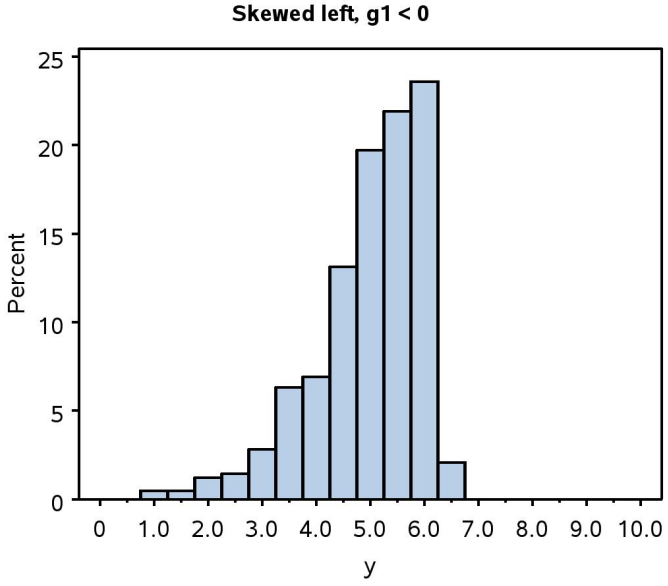


Figure 3.3: Frequency distribution that is skewed right ($g_1 > 0$).

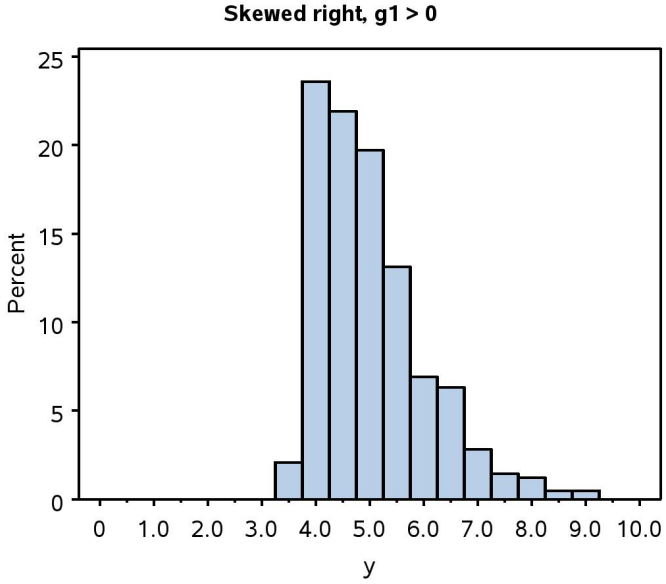
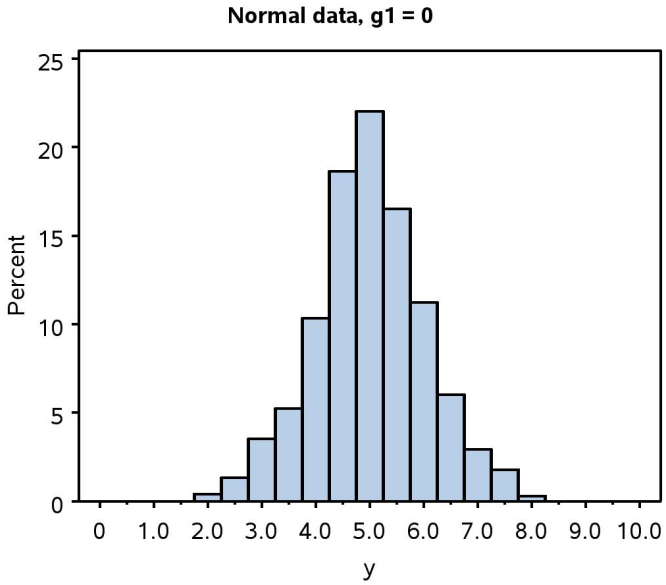


Figure 3.4: Frequency distribution for normal data ($g_1 \approx 0$).



3.2.10 Kurtosis

Kurtosis is a measure of how peaked or flat is a frequency distribution relative to the normal distribution. Distributions with a stronger central peak than the normal, and heavier left and right tails, are called leptokurtic (compare Fig. 3.5 and 3.6). Conversely, distributions with a weak peak and tails are called platykurtic (see Fig. 3.7 vs. 3.6). Kurtosis is quantified by calculating the statistic g_2:

$$g_2 = \frac{n(n+1)}{(n-1)(n-2)(n-3)} \sum_{i=1}^{n} \left(\frac{Y_i - \bar{Y}}{s} \right)^4 - \frac{3(n-1)^2}{(n-2)(n-3)}. \qquad (3.12)$$

The behavior of the terms in g_2 is less intuitive than those in the skewness statistic g_1. In any event, distributions that are leptokurtic have values of $g_2 > 0$, while platykurtic ones have $g_2 < 0$, with $g_2 \approx 0$ for distributions resembling the normal. For the elytra example, male *T. dubius* have a leptokurtic distribution with $g_2 = 1.003$, and the frequency distribution shows a strong central peak with heavy tails. The value of $g_2 = 0.161$ is smaller for female *T. dubius*, suggesting a shape more similar to the normal distribution. Like skewness, kurtosis is used to describe the general shape of the distribution.

Figure 3.5: Frequency distribution that is leptokurtic ($g_2 > 0$).

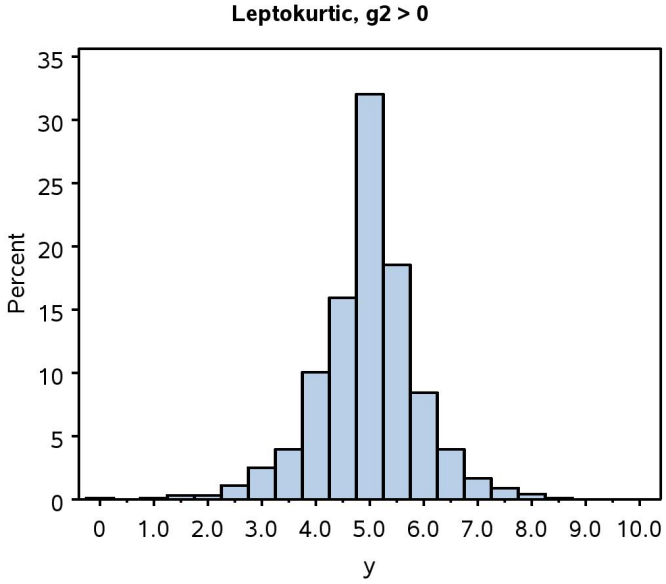


Figure 3.6: Frequency distribution for normal data ($g_2 \approx 0$).

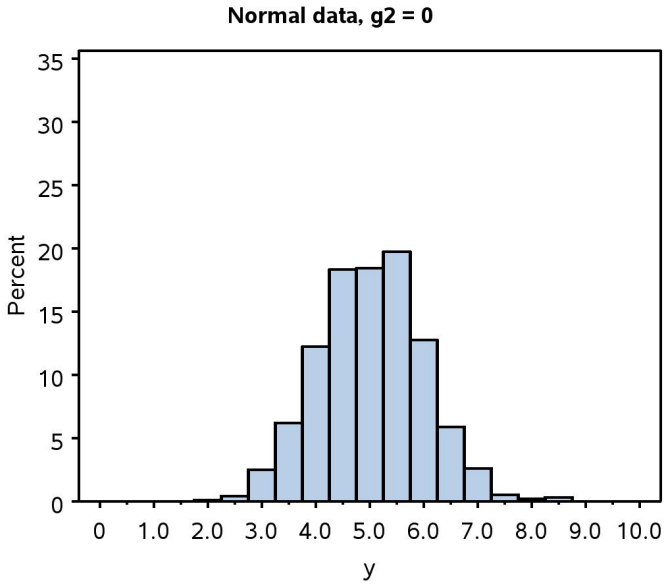
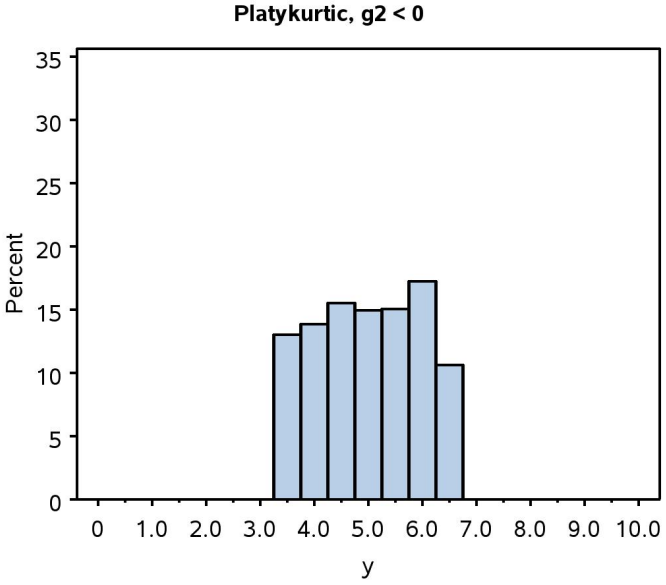


Figure 3.7: Frequency distribution that is platykurtic ($g_2 < 0$).



3.2.11 Development time - SAS demo

We now examine another data set involving the development time of *T. dubius* reared under laboratory conditions (Reeve et al. 2003). Two different development times were measured, the time from the first larval stage until the prepupal stage, and the prepupal to adult stage. The program used to analyze these data is listed below. The `input` line is different than our previous program, because there are two variables (`time_pp` and `time_adult`) to analyze for each insect listed, which occur in two columns. The `var` and `histogram` statements in `proc univariate` are similar, listing the two variables so that descriptive statistics and frequency distributions are generated for both.

Note the periods (. values) given in the data set - these indicate missing values to SAS. In this study, observations were missing usually because the insect died before reaching the adult stage, but missing values can also be used to indicate lost data. The full data set for this example is listed in Chapter 21, Section 21.2.

After running the program, we obtain output with statistics of location and dispersion as well as a frequency distribution, with a separate analysis for each variable. Clearly the larval-prepupal development time (`time_pp`) is shorter than the prepupal adult (`time_adult`) one ($\bar{Y} = 31.354$ vs. 75.353 days), and also shows less variability as indicated by the sample standard deviation ($s = 3.328$ vs. 26.347 days). Both variables appear to be skewed to the right, as indicated by positive values of g_1 as well as the result that mean > median > mode. Larval-prepupal development time shows little kurtosis ($g_2 = 0.047$), while prepupal-adult time apparently has a platykurtic distribution ($g_2 = -0.624$). This can also be observed in the frequency distribution for this variable, which is relatively flat in shape.

SAS Program

```
* descriptive_2.sas;
options pageno=1 linesize=80;
title 'Descriptive statistics for the development data';
data devel_time;
    input time_pp time_adult;
    datalines;
34  65
31  48
29   .
30  55
32  62

etc.

29   .
29 108
31 103
33   .
29  92
;
run;
* Print data set;
proc print data=devel_time;
run;
* Descriptive statistics, histograms, and normal quantile plots;
proc univariate plots data=devel_time;
    var time_pp time_adult;
    histogram time_pp time_adult / vscale=count wbarline=3 waxis=3 height=4;
run;
quit;
```

SAS Output

Descriptive statistics for the development data 1
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Obs	time_pp	time_adult
1	34	65
2	31	48
3	29	.
4	30	55
5	32	62

etc.

Descriptive statistics for the development data 3
13:44 Tuesday, May 18, 2010

The UNIVARIATE Procedure
Variable: time_pp

Moments

N	96	Sum Weights	96
Mean	31.3541667	Sum Observations	3010
Std Deviation	3.32764866	Variance	11.0732456
Skewness	0.75038358	Kurtosis	0.04666776
Uncorrected SS	95428	Corrected SS	1051.95833
Coeff Variation	10.6130987	Std Error Mean	0.33962672

Basic Statistical Measures

Location		Variability	
Mean	31.35417	Std Deviation	3.32765
Median	31.00000	Variance	11.07325
Mode	30.00000	Range	14.00000
		Interquartile Range	5.00000

Tests for Location: Mu0=0

Test	-Statistic-	-----p Value------

Student's t	t	92.31949	Pr > |t|	<.0001
Sign	M	48	Pr >= |M|	<.0001
Signed Rank	S	2328	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	41
99%	41
95%	39
90%	36
75% Q3	34
50% Median	31
25% Q1	29
10%	27
5%	27
1%	27
0% Min	27

Descriptive statistics for the development data 6
13:44 Tuesday, May 18, 2010

The UNIVARIATE Procedure
Variable: time_adult

Moments

N	68	Sum Weights	68
Mean	75.3529412	Sum Observations	5124
Std Deviation	26.3465791	Variance	694.14223
Skewness	0.51461555	Kurtosis	-0.6244048
Uncorrected SS	432616	Corrected SS	46507.5294
Coeff Variation	34.9642346	Std Error Mean	3.19499201

Basic Statistical Measures

Location		Variability	
Mean	75.35294	Std Deviation	26.34658
Median	68.00000	Variance	694.14223

Mode	42.00000	Range	105.00000
		Interquartile Range	46.50000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	23.5847	Pr > |t|	<.0001
Sign	M	34	Pr >= |M|	<.0001
Signed Rank	S	1173	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	147.0
99%	147.0
95%	116.0
90%	110.0
75% Q3	99.0
50% Median	68.0
25% Q1	52.5
10%	43.0
5%	42.0
1%	42.0
0% Min	42.0

Figure 3.8: Development time - larval to prepupal stage

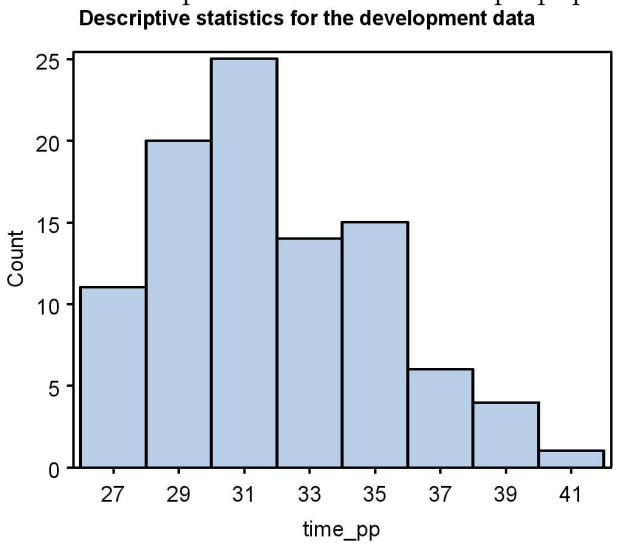
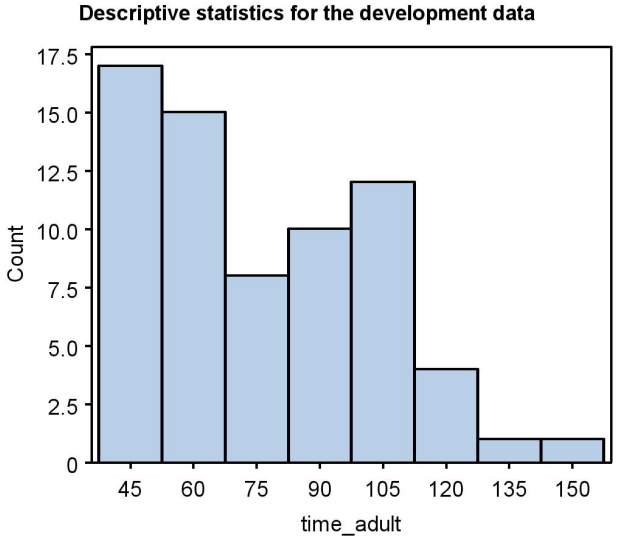


Figure 3.9: Development time - prepupal to adult stage



3.2.12 Frequency distributions for categorical data - SAS demo

The descriptive statistics we have developed so far are appropriate for continuous or discrete data. What about categorical data? One common way of summarizing categorical data is a frequency distribution, showing the number of occurrences in each category and possibly also their percentages. We can illustrate this process using the elytra data. There is one categorical variable in this data set, the sex of the beetle, and we might be interested in whether there were equal numbers of males and females. It also possible to derive categorical variables from the observations themselves. Suppose we classify a beetle as being 'small' if `length` is less than 5.0 mm, and 'large' otherwise. We can define this new variable within the SAS `data` set using an `if-then-else` statement. The code necessary to generate this new variable for the elytra data is shown below. It generates a new variable called `size` that takes the value `small` or `large` depending on the value of `length`.

```
* descriptive_freq.sas;
options pageno=1 linesize=80;
title 'Frequency distribution for the elytra data';
data elytra;
        input sex $ length;
        * Classify insects into two groups by size;
        if length < 5.0 then size="small"; else size="large";
        datalines;
M        4.9
F        5.2
M        4.9
F        4.2
F        5.7

etc.

M   5.1
F   4.4
M   4.8
M   4.6
F   3.7
;
run;
```

We can then generate a frequency distribution for both `sex` and `size` using `proc freq` (SAS Institute Inc. 2014b). The `tables sex*size` statement will generate a two-way table of frequencies, classifying each observation into one of four categories (female-large, female-small, male-large, male-small). See below.

```
* Frequency distribution;
proc freq data=elytra;
        table sex*size;
run;
```

The complete program and output are listed below. From the frequency table generated by `proc freq`, we see that there are more males than females in the data set, and more small vs. large insects. Female beetles have a greater proportion of large insects than males.

SAS Program

```
* descriptive_freq.sas;
options pageno=1 linesize=80;
title 'Frequency distribution for the elytra data';
data elytra;
        input sex $ length;
        * Classify insects into two groups by size;
        if length < 5.0 then size="small"; else size="large";
        datalines;
M        4.9
F        5.2
M        4.9
F        4.2
F        5.7

etc.

M        5.1
F        4.4
M        4.8
M        4.6
F        3.7
;
run;
* Print data set;
proc print data=elytra;
run;
* Frequency distribution;
proc freq data=elytra;
        table sex*size;
run;
quit;
```

SAS Output

Frequency distribution for the elytra data 1
09:37 Wednesday, August 18, 2010

Obs	sex	length	size
1	M	4.9	small
2	F	5.2	large
3	M	4.9	small
4	F	4.2	small
5	F	5.7	large

etc.

Frequency distribution for the elytra data 4
09:37 Wednesday, August 18, 2010

The FREQ Procedure

Table of sex by size

sex / size	large	small	Total
F	31	29	60
	23.85	22.31	46.15
	51.67	48.33	
	56.36	38.67	
M	24	46	70
	18.46	35.38	53.85
	34.29	65.71	
	43.64	61.33	
Total	55	75	130
	42.31	57.69	100.00

3.3 References

Berryman, A. A. (1988) *Dynamics of Forest Insect Populations: Patterns, Causes, Implications*. Plenum Press, New York, NY.

Lei, C.-H. & Armitage, K. B. (1980) Growth, development and body size of field and laboratory population of *Daphnia ambigua*. *Oikos* 35: 31-48.

Reeve, J. D., Rojas, M. G. & Morales-Ramos, J. A. (2003) Artificial diet and rearing methods for *Thanasimus dubius* (Coleoptera: Cleridae), a predator of bark beetles (Coleoptera: Scolytidae). *Biological Control* 27: 315-322.

SAS Institute Inc. (2014a) *SAS 9.4 Language Reference: Concepts, Third Edition.* SAS Institute Inc., Cary, NC, USA.

SAS Institute Inc. (2014b) *Base SAS 9.4 Procedures Guide: Statistical Procedures, Third Edition.* SAS Institute Inc., Cary, NC, USA.

3.4 Problems

1. For the data below, find the mean, median, variance, standard deviation and CV using the formulas for these quantities and a calculator. Show the steps in your calculations. Feel free to check your answers using SAS.

```
88.6  88.0  89.8  92.0  108.1  113.6  103.4  109.9  94.5  96.7  101.7
```

2. Ten adult females of the zooplankton species *Daphnia ambigua* were selected and their carapace length measured (μm) (Lei & Armitage 1980). The following data were obtained:

```
487 429 428 378 410 401 358 392 414 480
```

   Calculate the mean, median, variance, standard deviation, and CV for these data by hand. Show all your calculations. Check your answers using SAS.

3. A laboratory study was conducted on the development time of another bark beetle predator, *Temnochila virescens* (Coleoptera: Trogositidae). The numbers listed below are the larval development time (days) of 35 insects.

```
 73  65  58  54  78  57  90
103  59  52  73  67  67  53
 59  55  58  78  64  60  52
 96  68  81  76  77  57  79
 71  74  65  65  64  56  62
```

   (a) Use SAS to find the mean, median, mode, variance, standard deviation, and CV of these data, then plot a frequency distribution. Attach your program, output, and graph.

   (b) Examine the frequency distribution and skewness value (g_1) for these data. Do the data appear to be skewed, and if so in what direction? Explain your answer.

Chapter 4

Probability Theory

Probability theory is a branch of mathematics that is an essential component of statistics. It originally evolved from efforts to understand the odds and probabilities involved in games of chance, called classical probability theory (Weatherford 1982). The modern theory is developed from a small number of *a priori* axioms (like other mathematical theories) from which the rest the theory is deduced, including the behavior of probabilities and various rules for calculating them (Kolmogorov 1951, Weatherford 1982). While theoretical in origin, probability theory has proven to be spectacularly useful because it provides explanations for many natural processes, as well as the mathematical underpinnings for an enormous range of statistical procedures in the sciences.

4.1 Probability theory

4.1.1 Events

We can develop many elements of probability theory using a simple example, a single throw of a dice cube. If we throw the cube once, there are six possible outcomes corresponding to $1, 2, \ldots$, or 6 spots appearing on the cube. We call the possible outcomes of this single throw of a dice cube a **sample space** S, commonly written using set notation as

$$S = \{1, 2, 3, 4, 5, 6\} \tag{4.1}$$

We now define as **events** various subsets of the elements in S. **Simple events** contain exactly one element of S. For S defined above, the simple

events are $\{1\}, \{2\}, \{3\}, \{4\}, \{5\}$, and $\{6\}$. More specifically, the event $\{2\}$ signifies that a single throw of a dice cube showed two spots. More complex events contain more than one element of S. For example, consider the event A that the number of spots is odd, meaning that either one, three, or five spots showed on the dice cube after a single throw. We would write this event as the set $A = \{1, 3, 5\}$. Another possible event B is that the number of spots is less than or equal to three, or $B = \{1, 2, 3\}$. An event C such that number of spots is even would be written as $C = \{2, 4, 6\}$. Technically, both S itself and the empty set $\phi = \{\}$ are also possible events. S would always happen no matter the outcome of the throw, because some number of spots always occurs. The event corresponding to the empty set $\phi = \{\}$ would never happen because some number of spots always occurs after a throw. Sometimes certain events are subsets of other ones. For example, the event A defined above is a subset of S because every element of A is contained in S. This is written as $A \subset S$ using set notation.

4.1.2 Union, intersection, and complement of events

We now consider various combinations of events, again using our dice example. The **union** of two events A and B is defined to be the set containing all the simple events in A and B. The union is written using the notation $A \cup B$. For example, consider two of the events defined above for the dice example, $A = \{1, 3, 5\}$ (the number of spots is odd) and $B = \{1, 2, 3\}$ (the number of spots is less than or equal to three). We have

$$A \cup B = \{1, 3, 5\} \cup \{1, 2, 3\} = \{1, 2, 3, 5\}. \tag{4.2}$$

The union of two events can also be visualized using Venn diagrams, with the events A and B represented by circles and the shaded area their union (Fig. 4.1). The rectangle labeled S represents the entire sample space.

The **intersection** of two events A and B is defined to be the set containing simple events present in both A and B. The intersection is written using the notation $A \cap B$ or just AB. For example, consider the events A and B from the dice example. We have

$$A \cap B = \{1, 3, 5\} \cap \{1, 2, 3\} = \{1, 3\}. \tag{4.3}$$

The intersection of these two events is shown by the shaded area in Fig. 4.2. It is possible to have the intersection of two events be the empty set ϕ.

Consider the events A (spots is odd) and C (spots even) for the dice example. We have

$$A \cap C = \{1, 3, 5\} \cap \{2, 4, 6\} = \{\} = \phi. \tag{4.4}$$

Fig. 4.3 shows this outcome, with no shaded area because the intersection is empty. When the intersection of two events is the empty set, we say the two events are **mutually exclusive**. This means either one or the other event has occurred – it is impossible for them to happen at the same time.

The **complement** of an event A is the set of simple events in S remaining after we subtract those in A, typically written as A^c. For the event $A = \{1, 3, 5\}$ from the dice example, we have $A^c = \{2, 4, 6\}$, the simple events remaining in S after we substract those in A. Using set notation, $A^c = S - A = \{1, 2, 3, 4, 5, 6\} - \{1, 3, 5\} = \{2, 4, 6\}$. We can also represent A^c in a diagram with the shaded area representing all outcomes outside of A (Fig. 4.4). Complements of events frequently arise in the use of statistical tables.

Figure 4.1: $A \cup B = \{1, 2, 3, 5\}$.

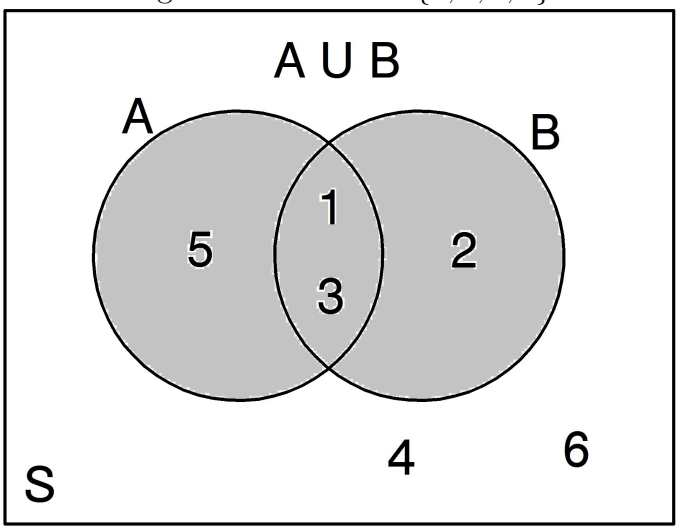


Figure 4.2: $A \cap B = \{1, 3\}$.

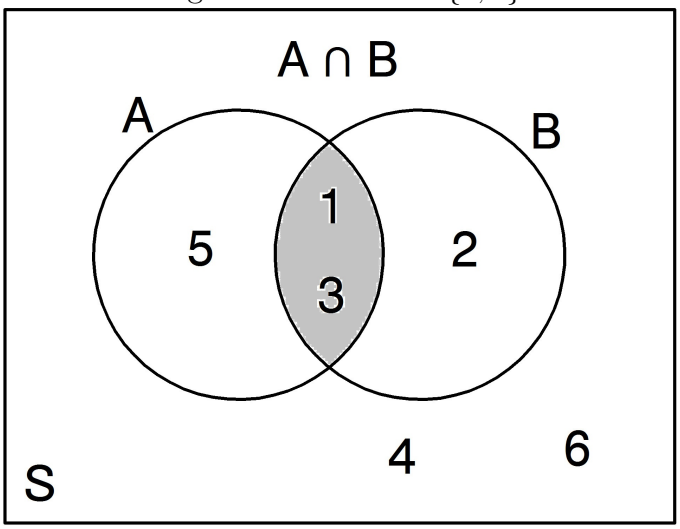


Figure 4.3: $A \cap C = \phi$.

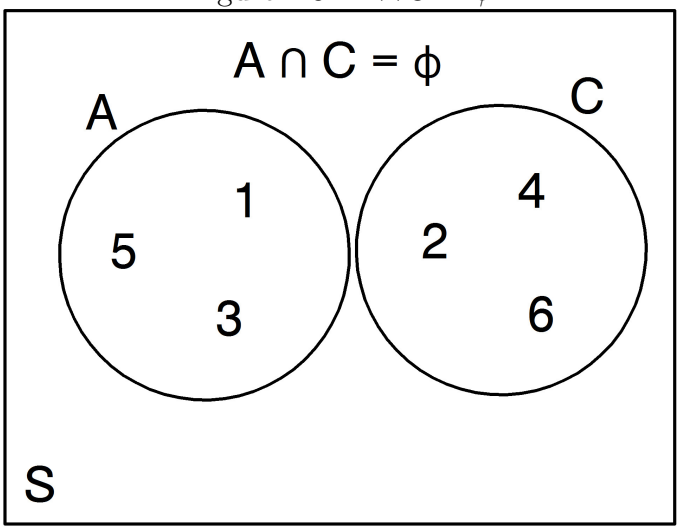
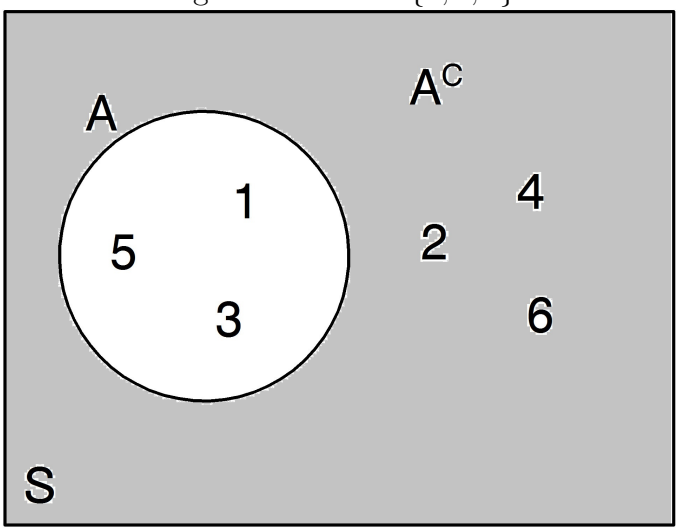


Figure 4.4: $A^C = \{2, 4, 6\}$.



4.1.3 Probability distributions

We now describe how probabilities are attached to events using a probability distribution, which can be mathematically defined based on certain axioms (Mood et al. 1974). Here, we simply list a number of properties of probability distributions that are useful to the practicing statistician. For a sample space S and any two events A and B, we have

1. $P[A] \geq 0$.
2. $P[S] = 1$.
3. $P[\phi] = 0$, where ϕ is the empty set.
4. $P[A \cup B] = P[A] + P[B] - P[A \cap B]$.
5. $P[A^c] = 1 - P[A]$.

Here the notation $P[A]$ stands for the probability of some event A.

While we have listed some of the properties of a probability distribution, we have not actually defined one yet. Recall the dice example, in which a single dice cube is thrown and the number of spots observed. If the cube is fair, then it is reasonable to assume that each number is equally likely to occur, and there are six possible numbers, so we assign a probability of 1/6 to each number. In particular, we have

$$P[\{1\}] = P[\{2\}] = P[\{3\}] = P[\{4\}] = P[\{5\}] = P[\{6\}] = 1/6 \tag{4.5}$$

How should we assign probabilities to events like $A = \{1, 3, 5\}$? We define these events to have a probability equal to the sum of the probabilities for each simple event within them. For example, we have

$$P[A] = P[\{1, 3, 5\}] = P[\{1\}] + P[\{3\}] + P[\{5\}] \tag{4.6}$$

$$= 1/6 + 1/6 + 1/6 = 3/6 = 1/2. \tag{4.7}$$

This result also makes intuitive sense for the event A, because we would expect the dice cube to produce an odd number of spots half of the time. We can view this probability distribution as a model of the dice cube's behavior, which would be accurate if the dice cube is fair. This is a common task faced by a statistician in analyzing a problem – determine an appropriate probability distribution to describe a particular type of data.

We will now calculate the probabilities for certain events to illustrate how this probability distribution can be used. Recall that the sample space for this distribution is $S = \{1, 2, 3, 4, 5, 6\}$. Suppose we have three events, namely $A = \{1, 3, 5\}$ (an odd number of spots), $B = \{1, 2, 3\}$ (less than or equal to three spots), and $C = \{2, 4, 6\}$ (an even number of spots).

We have already illustrated how to find the probability for A. For B, we have

$$P[B] = P[\{1, 2, 3\}] = P[\{1\}] + P[\{2\}] + P[\{3\}] \tag{4.8}$$
$$= 1/6 + 1/6 + 1/6 = 3/6 = 1/2. \tag{4.9}$$

For C the probability is

$$P[C] = P[\{2, 4, 6\}] = P[\{2\}] + P[\{4\}] + P[\{6\}] \tag{4.10}$$
$$= 1/6 + 1/6 + 1/6 = 3/6 = 1/2. \tag{4.11}$$

For the sample space S, which is also an event, we have

$$P[S] = P[\{1, 2, 3, 4, 5, 6\}] \tag{4.12}$$
$$= P[\{1\}] + P[\{2\}] + P[\{3\}] + P[\{4\}] + P[\{5\}] + P[\{6\}] \tag{4.13}$$
$$= 1/6 + 1/6 + 1/6 + 1/6 + 1/6 + 1/6 = 6/6 = 1. \tag{4.14}$$

We also have $P[\{\}] = P[\phi] = 0$ because it is impossible to have no spots showing on the dice cube.

What is the probability for $A \cap B$? We have

$$P[A \cap B] = P[\{1, 3, 5\} \cap \{1, 2, 3\}] = P[\{1, 3\}] \tag{4.15}$$
$$= P[\{1\}] + P[\{3\}] = 1/6 + 1/6 = 1/3. \tag{4.16}$$

For $A \cup B$ we can calculate the probability in two ways. We can directly find it as follows. We have

$$P[A \cup B] = P[\{1, 3, 5\} \cup \{1, 2, 3\}] = P[\{1, 2, 3, 5\}] \tag{4.17}$$
$$= P[\{1\}] + P[\{2\}] + P[\{3\}] + P[\{5\}] \tag{4.18}$$
$$= 1/6 + 1/6 + 1/6 + 1/6 = 2/3. \tag{4.19}$$

We can also use the formula listed in Property 4 to find this probability. We have

$$P[A \cup B] = P[A] + P[B] - P[A \cap B] \tag{4.20}$$
$$= 1/2 + 1/2 - 1/3 = 2/3. \tag{4.21}$$

We obtain the same answer as by direct calculation.

We can understand how the Property 4 formula works by considering the diagram for $A \cup B$ (Fig. 4.1). Suppose that the shaded area for event A represents the probability for A, and similarly for event B. If we add $P[A]$ and $P[B]$ together, this would actually be greater than $P[A \cup B]$ because it counts the area of the intersection $(A \cap B)$ twice. This explains why we need to subtract $P[A \cap B]$ in Property 4 to obtain $P[A \cup B]$.

We now find the probability for A^c. We can directly calculate it by finding the probability for $A^c = S - A = \{1, 2, 3, 4, 5, 6\} - \{1, 3, 5\} = \{2, 4, 6\}$, so $P[A^c] = P[\{2, 4, 6\}] = 1/2$. Alternately, by Property 5 above,

$$P[A^c] = 1 - P[A] = 1 - 1/2 = 1/2. \tag{4.22}$$

Property 5 can also be explained by a diagram. The rectangle in Fig. 4.4 represents the sample space S, and by Property 2 we have $P[S] = 1$. If the circle for event A represents $P[A]$, then clearly $P[A^c] = 1 - P[A]$.

4.1.4 Probability spaces

The combination of a sample space S, a collection of all possible events on the sample space (A, B, S, ϕ, etc.), and a probability distribution is called a **probability space**.

4.1.5 Independence of events

Independence of events is an important concept in statistics, and basically implies that an event A has no effect on whether B occurs, and vice versa. In terms of probabilities, two events A and B are defined to be independent if

$$P[A \cap B] = P[A]P[B]. \tag{4.23}$$

Are the events $A = \{1, 3, 5\}$ and $B = \{1, 2, 3\}$ defined for the dice cube example independent? We have

$$P[A \cap B] = P[\{1, 3, 5\} \cap \{1, 2, 3\}] = P[\{1, 3\}] = 1/3. \tag{4.24}$$

However,

$$P[A]P[B] = 1/2 \times 1/2 = 1/4. \tag{4.25}$$

This implies that A and B are not independent because $P[A \cap B] \neq P[A]P[B]$. To see why this happens, observe that when the number of spots is less than

or equal to three (B occurs), the number of spots is more likely to be odd (A occurs) because two of the three outcomes in B are odd.

We now work an example where the two events are independent. Suppose that $D = \{1, 2, 3, 4\}$, the event that the number of spots is less than or equal to four. Are A and D independent? We have

$$P[A \cap D] = P[\{1, 3, 5\} \cap \{1, 2, 3, 4\}] \tag{4.26}$$
$$= P[\{1, 3\}] = 1/6 + 1/6 = 1/3, \tag{4.27}$$

and

$$P[A]P[D] = 1/2 \times P[\{1, 2, 3, 4\}] \tag{4.28}$$
$$= 1/2 \times (1/6 + 1/6 + 1/6 + 1/6 \tag{4.29}$$
$$= 1/2 \times 2/3 = 1/3. \tag{4.30}$$

This implies that A and D are independent because $P[A \cap D] = P[A]P[D]$. This outcome seems reasonable – when the number of spots is less than or equal to four (D occurs), the probability of the number of spots being odd is still equal to $1/2$ because half of the outcomes in D are odd.

4.1.6 Conditional probability

Suppose that an event B has already happened, so that we have some information on a particular system or situation. Could this affect the probability that some other event A would occur? This is the idea behind **conditional probability**, an important concept in statistics that is related to independence. The conditional probability of an event A, given that B has occurred, is given by the formula

$$P[A|B] = \frac{P[A \cap B]}{P[B]}. \tag{4.31}$$

The notation '$A|B$' is read as A given B. For the dice cube example, what is the conditional probability of $A = \{1, 3, 5\}$ given that $B = \{1, 2, 3\}$ has occurred? We have

$$P[A|B] = \frac{P[A \cap B]}{P[B]} = \frac{1/3}{1/2} = 2/3. \tag{4.32}$$

Note that the $P[A|B] > P[A]$ because $2/3 > 1/2$. Thus, if B has occurred it is more likely that A occurs, because two of three outcomes in B are odd.

If two events are independent, implying that $P[A \cap B] = P[A]P[B]$, then we have

$$P[A|B] = \frac{P[A \cap B]}{P[B]} = \frac{P[A]P[B]}{P[B]} = P[A]. \tag{4.33}$$

Thus, if two events are independent then the fact that B has occurred does not alter the probability for A. We can illustrate this for the dice cube example using the events $A = \{1, 3, 5\}$ and $D = \{1, 2, 3, 4\}$, which we earlier showed to be independent. We have

$$P[A|D] = \frac{P[A \cap D]}{P[D]} = \frac{P[A]P[D]}{P[D]} \tag{4.34}$$

$$= \frac{1/3}{2/3} = 1/2 = P[A]. \tag{4.35}$$

Thus, if D has occurred it has no effect on the probability of A occurring. This follows because half the events in D are odd, and so the probability of obtaining an odd number (1/2) is exactly the same as the original probability.

4.1.7 A biological probability distribution

We now examine a more biological example involving the infection of amphibians by the chytrid fungus *Batrachochytrium dendrobatidis*, which appears responsible for the decline of amphibians in some regions (Lips et al. 2006). Certain amphibian species appear less susceptible than others by virtue of natural immunity or their ecological traits (Lips et al. 2003), and we would expect infection rates to therefore vary among species. Suppose we know that at a particular location the amphibians can be classified into three common species (A, B, and C) that can also be divided into infected and uninfected individuals, with the frequency of individuals in each category having the distribution given in Table 4.1. In practice, we would need to estimate these proportions, but we will assume they are already known.

Table 4.1: Proportions of individuals from three amphibian species (A, B, and C), classified as infected (Yes) or free of chytrid fungus (No).

		Species		
		A	B	C
Infected	No	0.25	0.2	0.15
	Yes	0.25	0.1	0.05

Suppose we now sample a single individual from this location. The sample space would be $S = \{$A-Yes, A-No, B-Yes, B-No, C-Yes, C-No$\}$. Here 'A-No' stands for an amphibian of species A that is free of fungus, and is one of six simple events. The probability of sampling an A-No individual would be $P[\text{A-No}] = 0.25$, with the probabilities for other simple events given by the entries in Table 4.1. Note that $P[S] = P[\{$A-No, A-Yes, B-No, B-Yes, C-No, C-Yes$\} = 0.25 + 0.25 + 0.2 + 0.1 + 0.15 + 0.05 = 1$ as is necessary for a probability distribution.

We now calculate the probabilities for certain events. Suppose that A is the event that species A is sampled, implying that $A = \{$A-No, A-Yes$\}$. We have

$$P[A] = P[\{\text{A-No}, \text{A-Yes}\}] \tag{4.36}$$
$$= P[\{\text{A-No}\}] + \text{P}[\{\text{A-Yes}\}] \tag{4.37}$$
$$= 0.25 + 0.25 = 0.5 \tag{4.38}$$

Thus, we would expect half the amphibians sampled to be species A. Suppose we also want to find the probability for A^c. By Property 5 above, we have

$$P[A^c] = 1 - P[A] = 1 - 0.5 = 0.5 \tag{4.39}$$

Now let B be the event that species B is sampled, so that $B = \{$B-No, B-Yes$\}$ and $P[B] = P[\{\text{B-No}, \text{B-Yes}\}] = \text{P}[\{\text{B-No}\}] + \text{P}[\{\text{B-Yes}\}] = 0.2 + 0.1 = 0.3$. What is the probability for $A \cap B$? We see that A and B share no simple events, so $P[A \cap B] = P[\{\}] = P[\phi] = 0$. The two events are therefore mutually exclusive, which is not surprising because the sampled amphibian can only be species A or B, not both.

What happens for $A \cup B$? We can directly calculate this probability by

finding the simple events in $A \cup B$. We have

$$\begin{aligned} P[A \cup B] &= P[\{\text{A-No, A-Yes}\} \cup \{\text{B-No, B-Yes}\}] && (4.40)\\ &= P[\{\text{A-No, A-Yes, B-No, B-Yes}\}] && (4.41)\\ &= P[\{\text{A-No}\}] + \text{P}[\{\text{A-Yes}\}] + \text{P}[\{\text{B-No}\}] + \text{P}[\{\text{B-Yes}\}] && (4.42)\\ &= 0.25 + 0.25 + 0.20 + 0.10 = 0.80. && (4.43) \end{aligned}$$

An alternate way to calculate this probability uses Property 4 listed above. In particular,

$$\begin{aligned} P[A \cup B] &= P[A] + P[B] - P[A \cap B] && (4.44)\\ &= 0.5 + 0.3 - 0 = 0.8, && (4.45) \end{aligned}$$

the same answer as before.

We now define an event I which stands for infected amphibians, meaning $I = \{\text{A-Yes, B-Yes, C-Yes}\}$. We have

$$\begin{aligned} P[I] &= P[\{\text{A-Yes, B-Yes, C-Yes}\}] && (4.46)\\ &= P[\{\text{A-Yes}\}] + \text{P}[\{\text{B-Yes}\}] + \text{P}[\{\text{C-Yes}\}] && (4.47)\\ &= 0.25 + 0.1 + 0.05 = 0.4 && (4.48) \end{aligned}$$

This means that the overall probability of sampling an infected animal is 0.4. Suppose that we already know the sampled amphibian is species C. What is the probability that it is infected given it is species C, or $P[I|C]$? We have

$$\begin{aligned} P[I|C] &= \frac{P[I \cap C]}{P[C]} = \frac{P[\{\text{A-Yes, B-Yes, C-Yes}\} \cap \{\text{C-No, C-Yes}\}]}{P[\{\text{C-No, C-Yes}\}]} && (4.49)\\ &= \frac{P[\{\text{C-Yes}\}]}{P[\{\text{C-No, C-Yes}\}]} && (4.50)\\ &= \frac{0.05}{0.2} = 0.25. && (4.51) \end{aligned}$$

Thus, if an individual of species C has been sampled the probability of it being infected is 0.25. We can also see this by examining the column for species C in Table 4.1, where the proportion of infected animals is $0.05/(0.15+0.05) = 0.25$.

4.1.8 Bayes theorem

Another use of conditional probability involves Bayes Theorem, named for the Reverend Thomas Bayes, an eighteenth century clergyman who first derived the theorem. The theorem is often used in the interpretation of medical tests as well as the field of Bayesian statistics (Ellison 1996).

Recall the example above involving amphibians and their infection by chytrid fungus. Let D be the event an amphibian actually has the disease while D^C implies they are disease-free. Now suppose a particular test is used to determine if a sampled amphibian has the disease. Let T be the event the amphibian tests positive for the disease, while T^c means the amphibian tests negative. The test is less than perfect, however, and sometimes gives a positive result when the amphibian is disease-free (a false positive) and a negative one when it is diseased (a false negative). What we would like to calculate is the probability that an amphibian actually has the disease given that it tests positive, or $P[D|T]$. This is called the **positive predictive value** of the test.

What is known for the test is the probability of testing positive for amphibians with the disease, $P[T|D]$, called the **sensitivity** of the test. This would be determined by testing a large number of amphibians that are known to have the disease by other means, and finding the proportion that test positive. Also known is the probability of testing negative for amphibians that are disease-free, $P[T^c|D^c]$, called the **specificity** of the test. We will also need an estimate of the probability that an amphibian has the disease in the population as a whole, $P[D]$, called the **prevalence** of the disease.

To find $P[D|T]$, we begin by using the definition of conditional probability:

$$P[D|T] = \frac{P[D \cap T]}{P[T]} = \frac{P[T \cap D]}{P[T]} \tag{4.52}$$

We can also write

$$P[T|D] = \frac{P[T \cap D]}{P[D]}, \tag{4.53}$$

which implies that $P[T \cap D] = P[T|D]P[D]$. Inserting this result into Eq. 4.52, we obtain

$$P[D|T] = \frac{P[T|D]P[D]}{P[T]}. \tag{4.54}$$

We are nearly there, except that we need to express $P[T]$ in terms of known quantities. The event T is made up of two mutually exclusive groups, am-

phibians that test positive and have the disease ($T \cap D$), and ones that test positive that are disease-free ($T \cap D^c$). From above, we have $P[T \cap D] = P[T|D]P[D]$ and can similarly show that $P[T \cap D^c] = P[T|D^c]P[D^c]$. Because the two groups are mutually exclusive, we can write $P[T]$ as the sum of the probabilities for each group:

$$P[T] = P[T \cap D] + P[T \cap D^c] = P[T|D]P[D] + P[T|D^c]P[D^c]. \quad (4.55)$$

Substituting this quantity into Eq. 4.52, we obtain Bayes' theorem:

$$P[D|T] = \frac{P[T|D]P[D]}{P[T|D]P[D] + P[T|D^c]P[D^c]}. \quad (4.56)$$

Because $P[T|D^c] = 1 - P[T^c|D^c]$ and $P[D^c] = 1 - P[D]$, we can also write Bayes' theorem as

$$P[D|T] = \frac{P[T|D]P[D]}{P[T|D]P[D] + (1 - P[T^c|D^c])(1 - P[D])}. \quad (4.57)$$

or

$$P[D|T] = \frac{\text{sensitivity} \times \text{prevalence}}{\text{sensitivity} \times \text{prevalence} + (1 - \text{specificity}) \times (1 - \text{prevalence})}. \quad (4.58)$$

We can thus express the theorem in terms of the sensitivity and specificity of the test, and the overall prevalence of the disease, which are known quantities.

Bayes theorem – sample calculation

Suppose that the test for amphibian disease has a high sensitivity ($P[T|D] = 0.95$) as well as a high specificity ($P[T^c|D^c] = 0.90$). A particular amphibian population has a fairly high prevalence of the disease ($P[D] = 0.25$, implying 25% are infected). What is the probability that an animal that tests positive from this population has the disease, $P[D|T]$? Inserting these quantities in Bayes theorem (Eq. 4.57 or 4.58), we obtain

$$P[D|T] = \frac{P[T|D]P[D]}{P[T|D]P[D] + (1 - P[T^c|D^c])(1 - P[D])} \quad (4.59)$$

$$= \frac{0.95 \times 0.25}{0.95 \times 0.25 + (1 - 0.9) \times (1 - 0.25)} \quad (4.60)$$

$$= \frac{0.2375}{0.2375 + 0.075} \quad (4.61)$$

$$= 0.76 \quad (4.62)$$

So, the probability that an animal that test positive actually has the disease is 0.76. We now examine what happens if the prevalence of the disease is lower, say $P[D = 0.05]$, implying only 5% are infected. We have

$$P[D|T] = \frac{P[T|D]P[D]}{P[T|D]P[D] + (1 - P[T^c|D^c])(1 - P[D])} \tag{4.63}$$

$$= \frac{0.95 \times 0.05}{0.95 \times 0.05 + (1 - 0.9) \times (1 - 0.05)} \tag{4.64}$$

$$= \frac{0.0475}{0.0475 + 0.095} \tag{4.65}$$

$$= 0.1425 \tag{4.66}$$

Now the probability that the animal has the disease is only 0.1425, despite using exactly the same sensitivity and specificity values for the test. What has happened here?

The explanation is that when prevalence is low, the majority of positive test results are actually false positives, in which disease-free animals test positive. This is reflected in the denominator of Eq. 4.63, where the term 0.095 (the probability of testing positive and being disease-free) is actually larger than the term .0475 (the probability of testing positive and having the disease). To fix this problem it would be helpful to have a test with higher specificity to reduce the incidence of false positives.

Bayesian statistics

Another type of probability theory, called subjective or Bayesian probability theory, equates probability with a degree of belief on the part of the analyst (Weatherford 1982). This theory makes use of Bayes theorem but with a different interpretation of the probabilities. Suppose that $P[D]$ is the belief by an investigator that a particular animal has the disease before the test, rather than the prevalence (frequency of the disease) in the amphibian population. The value of $P[D|T]$ calculated using Bayes' theorem now represents the investigator's belief that the animal has the disease after observing a positive test result. See Ellison et al. (1996) for a summary of arguments for Bayesian statistics, which is based on this interpretation of probability as belief. Dennis (1996) provides arguments against Bayesian statistics and in favor of 'frequentist' statistics, the kind of statistics based on the form of probability developed in this chapter. Perhaps his most telling argument is

that while frequentist statistics has its problems, its contribution to scientific progress is unquestionable.

4.2 References

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4.3 Problems

1. Suppose you have a loaded dice cube, such that $P[\{1\}] = 0.1, P[\{2\}] = 0.1, P[\{3\}] = 0.1, P[\{4\}] = 0.2, P[\{5\}] = 0.2$, and $P[\{6\}] = 0.3$. The cube is tossed a single time and the number of spots observed. Answer the following questions. Note that 'and' denotes an intersection of events, 'or' the union of events, and 'given' a conditional probability.

   (a) What is the probability that the number is even?

   (b) What is the probability that the number is odd and ≥ 3?

   (c) Are the events odd and ≥ 3 independent?

   (d) What is the probability that the number is odd or ≥ 3?

   (e) What is the probability that the number is odd, given that it is ≥ 3?

2. The PSA (prostate specific antigen) test is used to screen older men for prostate cancer. This test has a sensitivity of 0.90 and specificity of 0.719 (Mettlin et al. 1994). Assuming a prevalence of 0.1, find the probability that an individual with a positive test has cancer. Show your calculations.

3. Suppose you know that a particular animal population consists of 40% juveniles and 60% adults, and have a sample of two animals selected at random from the population.

   (a) What is the sample space for this scenario?

   (b) In a sample of two animals, what is the probability of obtaining two juveniles in a row?

   (c) What is the probability of obtaining one adult and one juvenile, in that order?

   (d) What is the probability of obtaining one juvenile and one adult, in that order?

   (e) What is the probability of obtaining two adults in a row?

Chapter 5

Discrete Random Variables

Random variables and their associated probability distributions are a basic component of statistical analyses. A statistician will examine the experiment or study and determine the type of observations or data it produces (continuous, discrete, or categorical) and then select a random variable and its distribution to model these data. We examine here three discrete random variables, the binomial, Poisson, and negative binomial, and their probability distributions. There are other discrete random variables but these three are the most commonly encountered in practice. These variables only take integer values and are typically used to model discrete or count data. We will also see how to calculate the mean and variance for a discrete random variable, using its probability distribution and a quantity called the **expected value**.

The basic concept of a **random variable** is to map the outcome of some random event into a number. For example, consider the dice cube example from Chapter 4. Define a number Y that is the number of spots showing on the dice – Y is a random variable. The sample space for Y would be $S = 1, 2, 3, 4, 5, 6$ and the events any combination of these values. One requirement for Y to be a random variable is that events of the form $Y \leq y$ for any real number y are events in the probability space (Mood et al. 1974). For example, suppose that $y = 3.5$ for the dice cube example. The set defined by $Y \leq 3.5$ corresponds to the event $A = \{1, 2, 3\}$ and so is a member of the probability space for this example. This requirement is necessary in order to calculate probabilities for the random variable, and there is always a probability distribution associated with a particular random variable.

5.1 Binomial distribution

Binomial random variables are commonly used to model categorical observations or data that have two outcomes or states. For example, suppose we are sampling animals and classifying them into two age classes, say either adult (an event A) or juvenile (J). If we sample a single individual and classify it, the sample space would be $S = \{A, J\}$. We could then define a probability distribution such that $P[\{A\}] = p$ and $P[\{J\}] = 1 - p$, where p is the probability of observing an adult. Then, a random variable Y equal to the **number** of adults would be a binomial random variable. The random variable Y would have a sample space $S = \{0, 1\}$ corresponding to the number of adults. We could write the probability distribution for these two events as

$$P[Y = y] = p^y(1 - p)^{1-y}, \tag{5.1}$$

where $y = 0$ or 1. To see how this formula works, suppose we want the probability for $Y = 1$, so that $y = 1$. Inserting $y = 1$ in the above formula, we obtain

$$P[Y = 1] = p^1(1 - p)^{1-1} = p^1(1 - p)^0 = p. \tag{5.2}$$

To find the probability for $Y = 0$, we insert $y = 0$ in the formula to find

$$P[Y = 0] = p^0(1 - p)^{1-0} = p^0(1 - p)^1 = 1 - p. \tag{5.3}$$

Suppose that we now sample two animals and let Y again be the number of adults. The sample space for Y would now be $S = \{0, 1, 2\}$. What would be the probability distribution for this random variable? Assuming the two animals sampled are independent events, the probability of seeing two adults ($Y = 2$) in a row would be $p \times p = p^2$, while two juveniles ($Y = 0$) would be $(1 - p) \times (1 - p) = (1 - p)^2$. There are two ways of having one adult and one juvenile, a adult first and a juvenile second, or vice versa. The probability for each is $p \times (1 - p)$, so the probability of seeing one adult would be twice that, or $2p(1 - p)$. A general formula describing the probability distribution for this variable would be

$$P[Y = y] = \binom{2}{y} p^y(1 - p)^{2-y}. \tag{5.4}$$

where

$$\binom{2}{y} = \frac{2!}{y!(2 - y)!}. \tag{5.5}$$

The quantity $\binom{2}{y}$, known as a binomial coefficient, provides a way of calculating the number of ways y adults can occur among 2 sampled animals. It is often read as '2 choose y'. It makes use of factorials, which are defined for an integer j as the product $j \times (j-1) \times (j-2)... \times 1$. For example, $4! = 4 \times 3 \times 2 \times 1$. By convention, $0! = 1$.

To see how this distribution works, we will calculate the probability for different values of y. We have

$$P[Y=0] = \binom{2}{0} p^0 (1-p)^{2-0} = \frac{2!}{0!(2-0)!}(1-p)^2 \tag{5.6}$$

$$= \frac{2 \times 1}{1(2 \times 1)}(1-p)^2 \tag{5.7}$$

$$= \frac{2}{2}(1-p)^2 = (1-p)^2 \tag{5.8}$$

and

$$P[Y=1] = \binom{2}{1} p^1 (1-p)^{2-1} = \frac{2!}{1!(2-1)!} p(1-p) \tag{5.9}$$

$$= \frac{2 \times 1}{1(1)} p(1-p) \tag{5.10}$$

$$= \frac{2}{1} p(1-p) = 2p(1-p). \tag{5.11}$$

Finally, we have

$$P[Y=2] = \binom{2}{2} p^2 (1-p)^{2-2} = \frac{2!}{2!(2-2)!} p^2 \tag{5.12}$$

$$= \frac{2 \times 1}{(2 \times 1)1} p^2 \tag{5.13}$$

$$= \frac{2}{2} p^2 = p^2. \tag{5.14}$$

Do these probabilities sum to 1, satisfying this requirement for a probability distribution? We have $(1-p)^2 + 2p(1-p) + p^2 = (1-p)(1-p) + 2p - 2p^2 + p^2 = 1 - 2p + p^2 + 2p - 2p^2 + p^2 = 1$.

Suppose that we continue to sample l different animals, and let Y be the number of adults. The sample space for this binomial random variable would be $S = \{0, 1, 2, ..., l\}$. The probability distribution for this random variable

is called the **binomial distribution**, and can be written using the formula

$$P[Y = y] = f(y) = \binom{l}{y} p^y (1-p)^{l-y} \tag{5.15}$$

where $y = 0, 1, 2, ..., l$ (Mood et al. 1974). The notation $f(y)$ is often used to denote a probability distribution, which is a function of y given the parameter values.

5.1.1 Binomial distribution - SAS demo

The SAS program below calculates and plots the binomial probabilities for different values of y using the SAS function `pdf`, given the values of the binomial parameters l and p. The probabilities are plotted for three different values of p, with $l = 10$. We see that for $p = 0.5$ the probability distribution has a peak at $y = 5$ (Fig. 5.1), indicating that five adults is the most likely outcome in 10 sampled animals. For $p = 0.25$ an adult occurs only 25% of the time, and so the probability distribution shifts to the left, with $y = 2$ having the highest probability (Fig. 5.2). For an adult almost certain, $p = 0.9$, then the probability distribution is shifted to the right with the peak at $y = 9$ (Fig. 5.3).

SAS Program

```
* binom_plot.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Plot probabilities for the binomial distribution";
title2 "k = 10, p = 0.5";
data binom_plot;
    * Binomial parameters here;
    l = 10;
    p = 0.5;
    do y=0 to l;
        * Binomial distribution function;
        proby = pdf('binomial',y,p,l);
        * Output y and proby to SAS data file;
        output;
    end;
run;
* Print data;
proc print data=binom_plot;
```

```
run;
* Plot probabilities;
proc gplot data=binom_plot;
    plot proby*y=1 / vref=0 wvref=3 vaxis=axis1 haxis=axis1;
    symbol1 i=needle v=dot c=red width=3 height=2;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

SAS Output

```
Plot probabilities for the binomial distribution                1
                    l = 10, p = 0.5  09:04 Wednesday, March 17, 2010

 Obs     l      p       y      proby

   1    10     0.5      0     0.00098
   2    10     0.5      1     0.00977
   3    10     0.5      2     0.04395
   4    10     0.5      3     0.11719
   5    10     0.5      4     0.20508
   6    10     0.5      5     0.24609
   7    10     0.5      6     0.20508
   8    10     0.5      7     0.11719
   9    10     0.5      8     0.04395
  10    10     0.5      9     0.00977
  11    10     0.5     10     0.00098
```

Figure 5.1: Binomial distribution for $l = 10, p = 0.5$

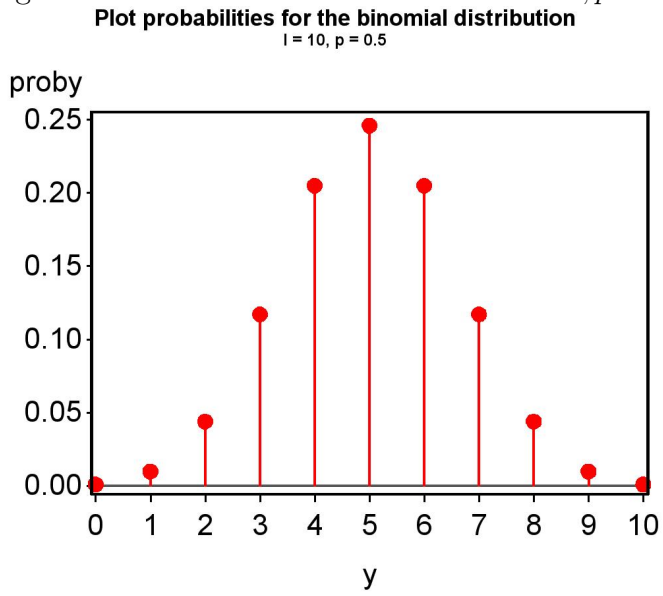


Figure 5.2: Binomial distribution for $l = 10, p = 0.25$

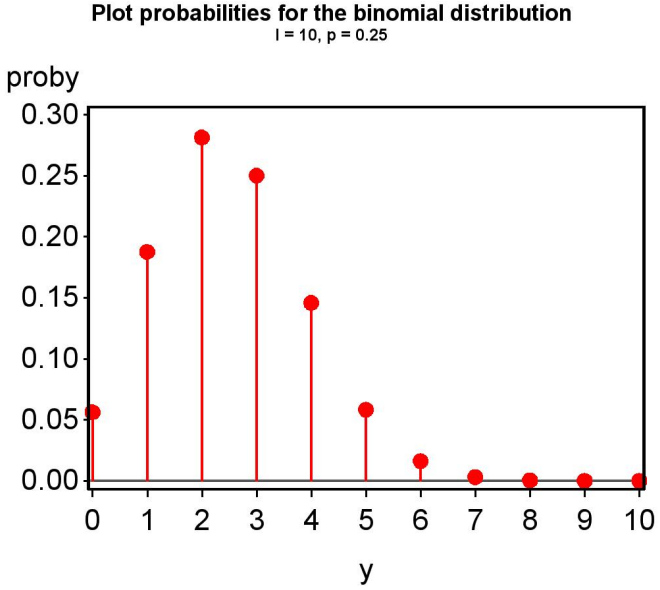
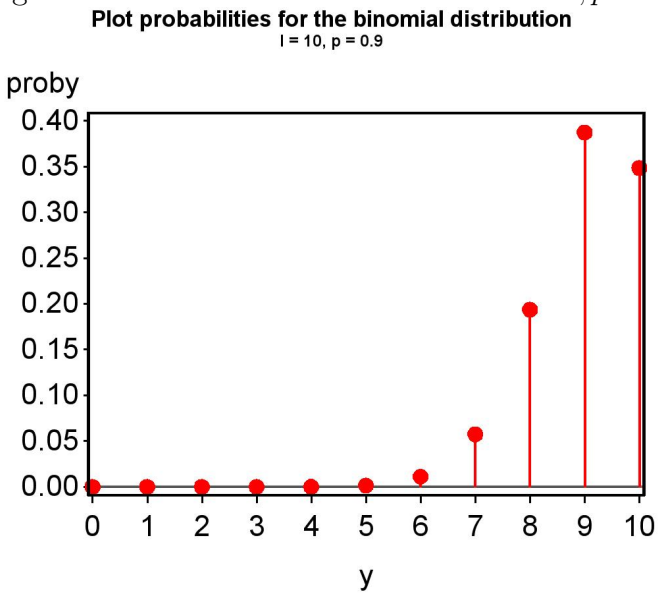


Figure 5.3: Binomial distribution for $l = 10, p = 0.9$



5.2 Poisson distribution

Poisson random variables are commonly used to model counts of organisms or events in either space or time. For example, a Poisson random variable could be used to model the number of organisms in a sampling quadrat, or the number of flu infections per week in a city. The sample space for a Poisson random variable Y is $S = \{0, 1, 2, ..., \infty\}$, implying there is no upper limit on the counts. The Poisson distribution is given by the formula

$$P[Y = y] = f(y) = \frac{e^{-\lambda}\lambda^y}{y!} \tag{5.16}$$

where $y = 0, 1, 2, ..., \infty$. The parameter λ controls the shape of the distribution and is equal to the mean value of Y. For example, suppose the $\lambda = 2$. We have

$$P[Y = 0] = f(0) = \frac{e^{-2}2^0}{0!} = \frac{0.13534(1)}{1} = 0.13534, \tag{5.17}$$

$$P[Y = 1] = f(1) = \frac{e^{-2}2^1}{1!} = \frac{0.13534(2)}{1} = 0.27068, \tag{5.18}$$

$$P[Y = 2] = f(2) = \frac{e^{-2}2^2}{2!} = \frac{0.13534(4)}{2} = 0.27068, \tag{5.19}$$

$$P[Y = 3] = f(3) = \frac{e^{-2}2^3}{3!} = \frac{0.13534(8)}{6} = 0.18045, \tag{5.20}$$

$$P[Y = 4] = f(4) = \frac{e^{-2}2^4}{4!} = \frac{0.13534(16)}{24} = 0.09023 \tag{5.21}$$

and so forth.

The Poisson distribution can arise in nature if certain assumptions hold true about the underlying process generating the data or observations (Mood et al. 1974, Snyder & Miller 1991). Suppose that we define an occurrence as a plant being present in a quadrat, or a case of disease occurring in a particular interval of time. **For the distribution of occurrences to be Poisson, we first need the probability of more than one occurrence to be small relative to the probability of exactly one occurrence, for a sufficiently small area of space (or short period of time).** In other words, two events are unlikely to occur in a small area or period of time. **Second, the number of occurrences in different areas of space (or time intervals) should be independent.** Another way of obtaining

the Poisson distribution is as a limiting case of the binomial distribution. It can be shown that if lp is held constant (by making p small) while $l \to \infty$, the binomial distribution approaches a Poisson with $\lambda = lp$.

5.2.1 Poisson distribution - SAS demo

The following SAS program illustrates how the Poisson distribution varies for different values of λ. It is similar to the binomial distribution program, using the SAS function `pdf` to again find the probabilities (see below). We see that as λ increases, the Poisson distribution shifts to the right (Fig. 5.4, 5.5).

SAS Program

```
* Poisson_plot.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Plot probabilities for the Poisson distribution";
title2 "lambda = 2";
data poisson_plot;
    * Poisson parameter here;
    lambda = 2;
    * Maximum value of y for plot;
    ymax = 20;
    do y=0 to ymax;
        * Poisson distribution function;
        proby = pdf('poisson',y,lambda);
        * Output y and proby to SAS data file;
        output;
    end;
run;
* Print data;
proc print data=poisson_plot;
run;
* Plot probabilities;
proc gplot data=poisson_plot;
    plot proby*y=1 / vref=0 wvref=3 vaxis=axis1 haxis=axis1;
    symbol1 i=needle v=dot c=red width=3 height=2;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

SAS Output

Plot probabilities for the Poisson distribution 1
lambda = 2 09:04 Wednesday, March 17, 2010

Obs	lambda	ymax	y	proby
1	2	20	0	0.13534
2	2	20	1	0.27067
3	2	20	2	0.27067
4	2	20	3	0.18045
5	2	20	4	0.09022
6	2	20	5	0.03609
7	2	20	6	0.01203
8	2	20	7	0.00344
9	2	20	8	0.00086
10	2	20	9	0.00019
11	2	20	10	0.00004
12	2	20	11	0.00001
13	2	20	12	0.00000
14	2	20	13	0.00000
15	2	20	14	0.00000
16	2	20	15	0.00000
17	2	20	16	0.00000
18	2	20	17	0.00000
19	2	20	18	0.00000
20	2	20	19	0.00000
21	2	20	20	0.00000

Figure 5.4: Poisson distribution for $\lambda = 2$

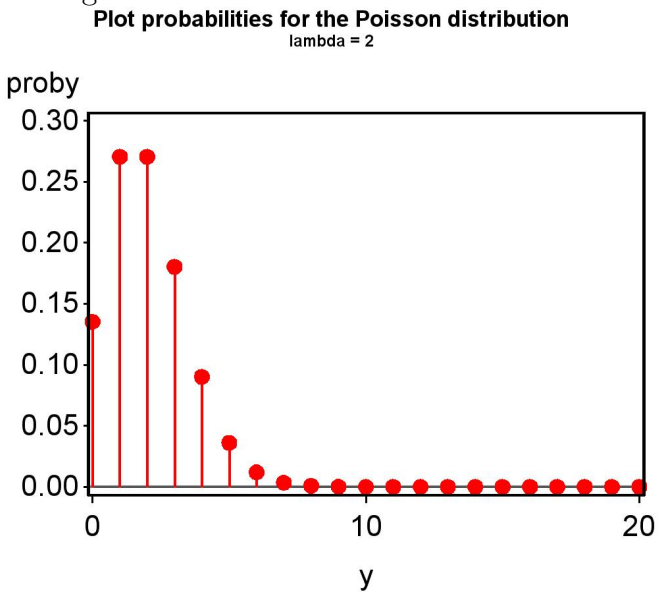
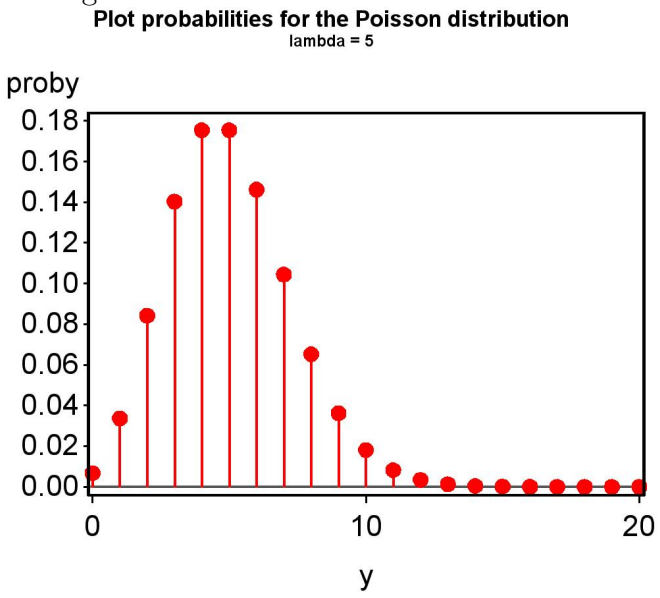


Figure 5.5: Poisson distribution for $\lambda = 5$



5.3 Negative binomial distribution

Another useful tool for modeling count data is the negative binomial distribution. **It can be thought of as a mixture of Poisson distributions, each with a different value of λ.** For example, suppose that we are sampling insects in a forest across a number of locations. At the ith location the distribution of insects might be Poisson with parameter λ_i, but λ_i also differs among locations. Then the distribution of insects, considered across all locations, may have a negative binomial distribution. Because the density of most organisms typically varies in space, the negative binomial distribution often provides a better description of count data than the Poisson. The sample space for a negative binomial random variable Y is $S = \{0, 1, 2, ..., \infty\}$, the same as the Poisson. The probability distribution for the negative binomial is given by the formula

$$P[Y = y] = f(y) = \frac{\Gamma(k+y)}{\Gamma(y+1)\Gamma(k)} \frac{(m/(k+m))^y}{(1+m/k)^k} \tag{5.22}$$

where $y = 0, 1, 2, ..., \infty$. The Γ symbol stands for the gamma function, which behaves like the factorial function but can be applied to non-integer quantities. The negative binomial distribution has two parameters, m and k, with m the mean of the distribution and k controlling its shape. For large values of k the negative binomial distribution approaches the Poisson distribution, while for small k the distribution becomes increasingly skewed to the right. See Bliss and Fisher (1953) for further information on this distribution.

5.3.1 Negative binomial distribution - SAS demo

The SAS program below shows how the shape of the negative binomial distribution varies with the parameter k. The program directly calculates the probabilities using the formula above, rather than the SAS `pdf` function, because we are using a different parameterization of the distribution than SAS. We see that distribution becomes more skewed to the right as k decreases (Fig. 5.6, 5.7).

SAS Program

```
* negbin_plot.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Plot probabilities for the negative binomial distribution";
title2 "m = 5, k = 5";
data negbin_plot;
    * negative binomial parameters here;
    m = 5; k = 5;
    * Maximum value of y for plot;
    ymax = 20;
    do y=0 to ymax;
        * Negative binomial distribution function;
        proby = (gamma(k+y)/(gamma(y+1)*gamma(k)))*((m/(k+m))**y/(1+m/k)**k);
        * Output y and proby to SAS data file;
        output;
    end;
run;
* Print data;
proc print data=negbin_plot;
run;
* Plot probabilities;
proc gplot data=negbin_plot;
    plot proby*y=1 / vref=0 wvref=3 vaxis=axis1 haxis=axis1;
    symbol1 i=needle v=dot c=red width=3 height=2;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

SAS Output

Plot probabilities for the negative binomial distribution 1
m = 5, k = 5 15:36 Sunday, March 21, 2010

Obs	m	k	ymax	y	proby
1	5	5	20	0	0.03125
2	5	5	20	1	0.07813
3	5	5	20	2	0.11719
4	5	5	20	3	0.13672
5	5	5	20	4	0.13672
6	5	5	20	5	0.12305
7	5	5	20	6	0.10254
8	5	5	20	7	0.08057
9	5	5	20	8	0.06042
10	5	5	20	9	0.04364
11	5	5	20	10	0.03055
12	5	5	20	11	0.02083
13	5	5	20	12	0.01389
14	5	5	20	13	0.00908
15	5	5	20	14	0.00584
16	5	5	20	15	0.00370
17	5	5	20	16	0.00231
18	5	5	20	17	0.00143
19	5	5	20	18	0.00087
20	5	5	20	19	0.00053
21	5	5	20	20	0.00032

Figure 5.6: Negative binomial distribution for $m = 5, k = 5$

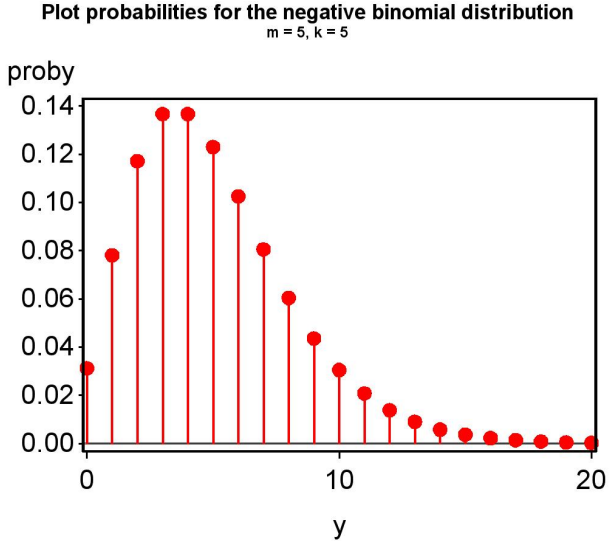
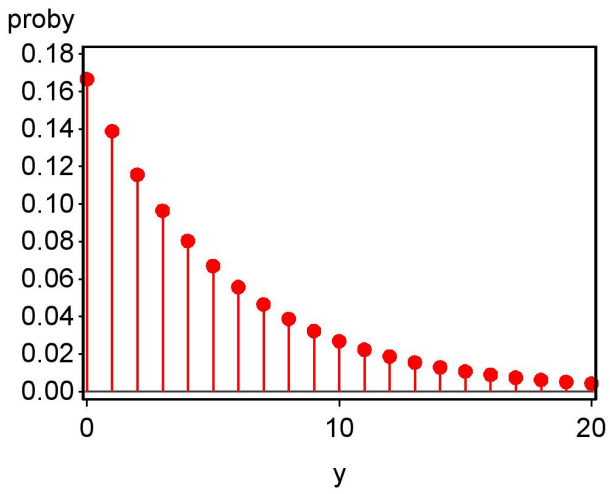


Figure 5.7: Negative binomial distribution for $m = 5, k = 1$



5.4 Expected values for discrete distributions

We have already seen how to calculate the mean, variance, and standard deviation for a set of observations (see Chapter 3). It is possible to calculate analogous quantities for probability distributions, such as the binomial, using the concept of an **expected value**.

Let Y be a random variable with some discrete probability distribution, such as the binomial, Poisson, or other distribution. The expected value or theoretical mean of Y, denoted by the expression $E[Y]$, is defined by the equation

$$E[Y] = \sum_y yP[Y = y] = \sum_y yf(y). \tag{5.23}$$

Here the summation is taken over all possible values of y for the probability distribution. **The expected value is a weighted average of each possible value of y, with the weights being the probability associated with each y.** It is a measure of the central location of the distribution of Y, in analogy to the sample mean $\bar{Y}$ for a data set. The expected value of Y can also be thought of as the sample mean $\bar{Y}$ of an infinitely large number of observations of Y.

For example, let Y have a binomial distribution with $l = 5$ and $p = 0.2$. We will first calculate some probabilities for the binomial distribution, then use them to calculate the expected value of Y, or $E[Y]$. We have

$$P[Y = 0] = f(0) = \binom{5}{0} 0.2^0(1 - 0.2)^{5-0} \tag{5.24}$$

$$= \frac{5!}{0!(5-0)!} 1(0.8^5) \tag{5.25}$$

$$= \frac{120}{1(120)} 0.32768 \tag{5.26}$$

$$= 0.32768. \tag{5.27}$$

$$P[Y=1] = f(1) = \binom{5}{1} 0.2^1 (1-0.2)^{5-1} \tag{5.28}$$
$$= \frac{5!}{1!(5-1)!} 0.2(0.8^4) \tag{5.29}$$
$$= \frac{120}{1(24)} 0.08192 \tag{5.30}$$
$$= 0.40960. \tag{5.31}$$

$$P[Y=2] = f(2) = \binom{5}{2} 0.2^2 (1-0.2)^{5-2} \tag{5.32}$$
$$= \frac{5!}{2!(5-2)!} 0.04(0.8^3) \tag{5.33}$$
$$= \frac{120}{2(6)} 0.02048 \tag{5.34}$$
$$= 0.20480. \tag{5.35}$$

$$P[Y=3] = f(3) = \binom{5}{3} 0.2^3 (1-0.2)^{5-3} \tag{5.36}$$
$$= \frac{5!}{2!(5-2)!} 0.008(0.8^2) \tag{5.37}$$
$$= \frac{120}{2(6)} 0.00512 \tag{5.38}$$
$$= 0.05120. \tag{5.39}$$

$$P[Y=4] = f(4) = \binom{5}{4} 0.2^4 (1-0.2)^{5-4} \tag{5.40}$$
$$= \frac{5!}{4!(5-4)!} 0.0016(0.8^1) \tag{5.41}$$
$$= \frac{120}{24(1)} 0.00128 \tag{5.42}$$
$$= 0.00640. \tag{5.43}$$

$$P[Y=5] = f(5) = \binom{5}{5} 0.2^5 (1-0.2)^{5-5} \tag{5.44}$$

$$= \frac{5!}{5!(5-5)!} 0.00032(0.8^0) \tag{5.45}$$

$$= \frac{120}{120(1)} 0.00032 \tag{5.46}$$

$$= 0.00032. \tag{5.47}$$

These probabilities sum to 1, indicating our calculations are correct. Alternately, we could use the SAS program `binom_plot.sas` to find these probabilities.

We will now calculate $E[Y]$ using these probabilities and the formula for $E[Y]$ given above. We have

$$E[Y] = \sum_y y f(y) = 0(0.32768) + 1(0.40960) + 2(0.20480) \tag{5.48}$$

$$+ 3(0.05120) + 4(0.00640) + 5(0.00032) \tag{5.49}$$

$$= 0 + 0.40960 + 0.40960 \tag{5.50}$$

$$+ 0.15360 + 0.02560 + 0.00160 \tag{5.51}$$

$$= 1.00000 \tag{5.52}$$

So, $E[Y] = 1$ for the binomial distribution with $l = 5$ and $p = 0.2$.

For the binomial distribution in general, it can be shown that

$$E[Y] = lp \tag{5.53}$$

for any value of l and p. Thus, the expected value or theoretical mean for the binomial distribution can be easily calculated given the parameters of this distribution. Plugging $l = 5$ and $p = 0.2$ into this equation, we obtain $E[Y] = 5 \times 0.2 = 1.0$, the same value as obtained using the expected value formula.

Other probability distributions would have a different formula for the expected value or theoretical mean, but the formula always involves the parameters of the distribution. For the Poisson distribution it can be shown that $E[Y] = \lambda$, while for the negative binomial distribution $E[Y] = m$.

5.4.1 Variance for discrete distributions

We can also define the theoretical variance for a random variable Y using expected values. This variance measures the dispersion of Y, and can also be

thought of as the sample variance s^2 of an infinite number of observations. The variance of a discrete random variable Y, denoted by $Var[Y]$, is defined as

$$Var[Y] = E[(Y - E[Y])^2] = \sum_y (y - E[Y])^2 P[Y = y] \tag{5.54}$$

$$= \sum_y (y - E[Y])^2 f(y). \tag{5.55}$$

Note that this formula makes use of $E[Y]$, so it must be calculated first. As an example, let Y have the same binomial distribution as before, with $l = 5$ and $p = 0.2$, for which $E[Y] = 1$. Using the probabilities calculated above, we have

$$Var[Y] = \sum_y (y - E[Y])^2 f(y) \tag{5.56}$$

$$= (0-1)^2(0.32768) + (1-1)^2(0.40960) + (2-1)^2(0.20480) \tag{5.57}$$

$$+ (3-1)^2(0.05120) + (4-1)^2(0.00640) + (5-1)^2(0.00032) \tag{5.58}$$

$$= 1(0.32768) + 0(0.40960) + (1)0.20480 \tag{5.59}$$

$$+ 4(0.05120) + 9(0.00640) + (16)0.00032 \tag{5.60}$$

$$= 0.32768 + 0 + 0.20480 + 0.20480 + 0.05760 + 0.00512 \tag{5.61}$$

$$= 0.8. \tag{5.62}$$

For the binomial distribution, it can be mathematically shown that for any value of l and p, we have

$$Var[Y] = lp(1 - p). \tag{5.63}$$

Thus, the theoretical variance for the binomial distribution can also be calculated using the parameters of this distribution. Plugging $l = 5$ and $p = 0.2$ into this equation, we obtain $Var[Y] = 5(0.2)(1 - 0.2) = 0.8$, the same value as obtained using the variance formula.

Other probability distributions would have a different formula for the theoretical variance. For the Poisson distribution it can be shown that $Var[Y] = \lambda$. Because $E[Y] = \lambda$ for the Poisson, this implies the mean and variance of a Poisson random variable are equal. For the negative binomial distribution, $Var[Y] = m + m^2/k$, while $E[Y] = m$. This implies the variance of the negative binomial is always greater than its mean. The theoretical standard deviation is simply $\sqrt{Var[Y]}$.

5.5 Discrete random variables and samples

Discrete random variables like the binomial and Poisson are used to model real observations that are counts. But how well do these mathematical quantities match the behavior of the observations? We will now develop a graphical method of comparing the observed data with the pattern expected for discrete random variables, in particular the Poisson and negative binomial distributions. There are also statistical procedures called goodness-of-fit tests that are used for this purpose, but we defer this to Chapter 20.

5.5.1 Parasitic wasps - SAS demo

Small insects are often sampled using sticky-traps, which are small cards covered with a substance called Tanglefoot®(The Tanglefoot Company, Grand Rapids, MI). For example, Reeve & Cronin (2010) used this method to sample populations of the parasitic wasp *Anagrus columbi*, which attacks eggs of the planthopper *Prokelisia crocea*. Suppose $n = 100$ traps are deployed for some period of time, then the traps collected and the wasps counted. If individual wasps are randomly and independently distributed across the field, we would expect the number of wasps per trap to have a Poisson distribution. We can then compare the observed distribution with the expected one for the Poisson distribution, to see if they resemble one another. If so, we can say the Poisson distribution provides an adequate description of these observations.

The first step in this procedure is simply to tabulate the number of traps with $0, 1, 2, 3, ...$ wasps, which is the observed frequency distribution. We can use `proc freq` in SAS to accomplish this task as in the following program. The numbers listed as data here are the number of wasps for each of the $n = 100$ sticky-traps. The statement `tables y` tells `proc freq` to count the number of observations for each value of `y` in the data set. The output generated is a table of these frequencies.

SAS Program

```
* poisson_freq.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Tabulate Poisson data';
data poisson;
    input y @@;
    datalines;
4 6 3 5 3 1 3 3 4 2
4 0 2 3 1 3 4 6 5 1
3 3 4 3 2 3 7 4 3 3
4 3 4 3 4 0 3 0 3 3
4 8 2 2 4 2 5 3 3 2
1 4 1 1 5 2 4 1 2 6
3 3 3 1 1 2 1 5 3 5
3 2 4 3 4 1 2 3 1 3
4 4 4 6 6 2 0 1 4 2
2 2 3 4 3 0 1 1 0 2
;
run;
* Print observations;
proc print data=poisson;
run;
* Tabulate data into frequencies;
proc freq data=poisson;
    tables y;
run;
quit;
```

SAS Output

Tabulate Poisson data 1
10:40 Friday, March 26, 2010

Obs	y
1	4
2	6
3	3
4	5
5	3

etc.

95	3
96	0
97	1
98	1
99	0
100	2

Tabulate Poisson data 3
10:40 Friday, March 26, 2010

The FREQ Procedure

y	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	6	6.00	6	6.00
1	15	15.00	21	21.00
2	17	17.00	38	38.00
3	29	29.00	67	67.00
4	20	20.00	87	87.00
5	6	6.00	93	93.00
6	5	5.00	98	98.00
7	1	1.00	99	99.00
8	1	1.00	100	100.00

We now want to compare these observed frequencies with those expected for the Poisson distribution. We first need to estimate the Poisson parameter λ from the observed data using $\bar{Y}$ (see Chapter 8 for a justification). We then calculate the Poisson probabilities for $\lambda = \bar{Y}$, obtaining $P[Y = y]$ for values of y that spans or better exceeds the range of y values in the data set. Because $P[Y = y]$ is the probability or proportion of observations that take the value y, the expected frequency with n observations is therefore equal to $n \times P[Y = y]$. We can then compare the observed frequencies with the expected ones generated using the Poisson distribution. These calculations can be automated using the SAS program listed below. The program first uses `proc univariate` to find n, $\bar{Y}$, and the sample variance s^2 for the observed frequencies. We let `proc univariate` know that the data are in the form of frequencies (the variable `obsfreq`), rather than individual observations, by adding the command `freq obsfreq`.

The program then passes these results to a `data` step where the Poisson probabilities and expected frequencies are calculated, which are then plotted across a range of y values using `proc gplot`. See SAS output and graph below. We first see that sample mean and variance are similar in magnitude ($\bar{Y} = 2.910$ vs. $s^2 = 2.628$), suggesting these data are close to Poisson (recall that $E[Y] = Var[Y] = \lambda$ for this distribution). In addition, the observed and expected frequencies are quite similar, again implying an adequate fit by the Poisson distribution. There are some small differences in the observed and expected frequencies, which is to be expected because the observed ones are random quantities.

SAS Program

```
* Poisson_fit.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Fitting the Poisson to frequency data';
data poisson;
    input y obsfreq;
    * Generate offset y values for plot;
    yexp = y - 0.1; yobs = y + 0.1;
    datalines;
0   6
1  15
2  17
3  29
4  20
```

```
5   6
6   5
7   1
8   1
9   0
10  0
;
run;
* Print data set;
proc print data=poisson;
run;
* Descriptive statistics, save ybar, n, and var to data file;
proc univariate data=poisson;
    var y;
    histogram y / vscale=count;
    freq obsfreq;
    output out=stats mean=ybar n=n var=var;
run;
* Print output data file;
proc print data=stats;
run;
* Calculate expected frequencies using ybar;
data poisfit;
    if _n_ = 1 then set stats;
    set poisson;
    poisprob = pdf('poisson',y,ybar);
    expfreq = n*poisprob;
run;
* Print observed and expected frequencies;
proc print data=poisfit;
run;
* Plot observed and expected frequencies;
proc gplot data=poisfit;
    plot expfreq*yexp=1 obsfreq*yobs=2 / overlay legend=legend1 vref=0 wvref=3
    vaxis=axis1 haxis=axis1;
    symbol1 i=needle v=circle c=red width=3 height=2;
    symbol2 i=needle v=square c=blue width=3 height=2;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
    legend1 label=(height=2) value=(height=2);
run;
quit;
```

SAS Output

Fitting the Poisson to frequency data 1
10:40 Friday, March 26, 2010

Obs	y	obsfreq	yexp	yobs
1	0	6	-0.1	0.1
2	1	15	0.9	1.1
3	2	17	1.9	2.1
4	3	29	2.9	3.1
5	4	20	3.9	4.1
6	5	6	4.9	5.1
7	6	5	5.9	6.1
8	7	1	6.9	7.1
9	8	1	7.9	8.1
10	9	0	8.9	9.1
11	10	0	9.9	10.1

Fitting the Poisson to frequency data 2
10:40 Friday, March 26, 2010

The UNIVARIATE Procedure
Variable: y

Freq: obsfreq

Moments

N	100	Sum Weights	100
Mean	2.91	Sum Observations	291
Std Deviation	1.62116681	Variance	2.62818182
Skewness	0.39509921	Kurtosis	0.31136421
Uncorrected SS	1107	Corrected SS	260.19
Coeff Variation	55.7101996	Std Error Mean	0.16211668

Basic Statistical Measures

Location		Variability	
Mean	2.910000	Std Deviation	1.62117
Median	3.000000	Variance	2.62818
Mode	3.000000	Range	8.00000

Interquartile Range	2.00000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	17.95003	Pr > |t|	<.0001
Sign	M	47	Pr >= |M|	<.0001
Signed Rank	S	2232.5	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	8.0
99%	7.5
95%	6.0
90%	5.0
75% Q3	4.0
50% Median	3.0
25% Q1	2.0
10%	1.0
5%	0.0
1%	0.0
0% Min	0.0

Fitting the Poisson to frequency data 3
10:40 Friday, March 26, 2010

The UNIVARIATE Procedure
Variable: y

Freq: obsfreq

Extreme Observations

---------Lowest--------			--------Highest--------		
Value	Freq	Obs	Value	Freq	Obs
0	6	1	4	20	5
1	15	2	5	6	6

2	17	3	6	5	7
3	29	4	7	1	8
4	20	5	8	1	9

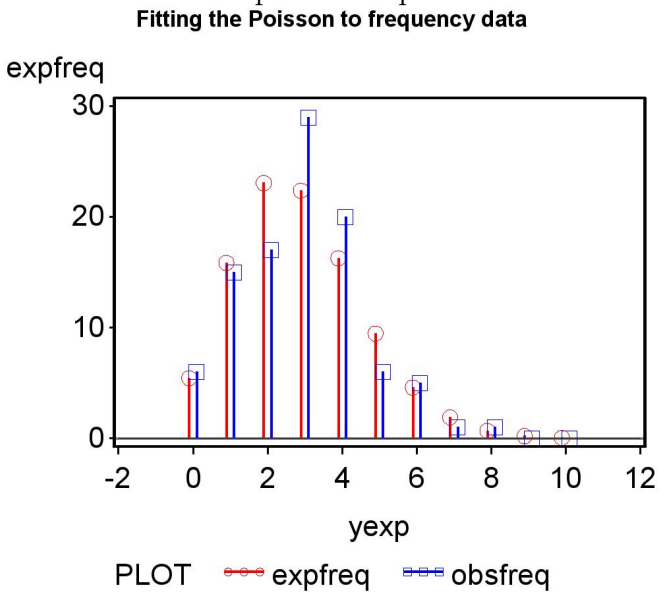
Fitting the Poisson to frequency data 4
10:40 Friday, March 26, 2010

Obs	n	ybar	var
1	100	2.91	2.62818

Fitting the Poisson to frequency data 5
10:40 Friday, March 26, 2010

Obs	n	ybar	var	y	obsfreq	yexp	yobs	poisprob	expfreq
1	100	2.91	2.62818	0	6	-0.1	0.1	0.05448	5.4476
2	100	2.91	2.62818	1	15	0.9	1.1	0.15852	15.8524
3	100	2.91	2.62818	2	17	1.9	2.1	0.23065	23.0653
4	100	2.91	2.62818	3	29	2.9	3.1	0.22373	22.3733
5	100	2.91	2.62818	4	20	3.9	4.1	0.16277	16.2766
6	100	2.91	2.62818	5	6	4.9	5.1	0.09473	9.4730
7	100	2.91	2.62818	6	5	5.9	6.1	0.04594	4.5944
8	100	2.91	2.62818	7	1	6.9	7.1	0.01910	1.9100
9	100	2.91	2.62818	8	1	7.9	8.1	0.00695	0.6947
10	100	2.91	2.62818	9	0	8.9	9.1	0.00225	0.2246
11	100	2.91	2.62818	10	0	9.9	10.1	0.00065	0.0654

Figure 5.8: Observed and expected frequencies - Poisson distribution



5.5.2 Corn borers - SAS demo

We now examine the spatial distribution of an insect pest, the European corn borer *Ostrinia nubilalis*, as reported by Bliss and Fisher (1953). The number of borers was recorded for 120 hills in which corn was planted. These data are already tabulated and can be directly inserted in the SAS program `poisson_fit.sas` (see below). For this example, we see that the Poisson distribution provides a relatively poor fit (see Fig. 5.9) - there are more zeroes ($y = 0$) and large values ($y \geq 7$) in the observed frequencies than predicted by the Poisson. We also note that the sample variance $s^2 = 7.770$ is considerably larger than the mean $\bar{Y} = 3.167$, while for the Poisson these two quantities should be equal. This finding also suggests that these data are not Poisson in distribution.

SAS Program

```
* Poisson_fit2.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Fitting the Poisson to frequency data';
data poisson;
        input y obsfreq;
        * Generate offset y values for plot;
        yexp = y - 0.1; yobs = y + 0.1;
        datalines;
0  24
1  16
2  16
3  18
4  15
5   9
6   6
7   5
8   3
9   4
10  3
11  0
12  1
;
run;
* Print data set;
proc print data=poisson;
run;
* Descriptive statistics, save ybar, n, and var to data file;
```

```
proc univariate data=poisson;
        var y;
        histogram y / vscale=count;
        freq obsfreq;
        output out=stats mean=ybar n=n var=var;
run;
* Print output data file;
proc print data=stats;
run;
* Calculate expected frequencies using ybar;
data poisfit;
        if _n_ = 1 then set stats;
        set poisson;
        poisprob = pdf('poisson',y,ybar);
        expfreq = n*poisprob;
run;
* Print observed and expected frequencies;
proc print data=poisfit;
run;
* Plot observed and expected frequencies;
proc gplot data=poisfit;
        plot expfreq*yexp=1 obsfreq*yobs=2 / overlay legend=legend1 vref=0 wvref=3
        vaxis=axis1 haxis=axis1;
        symbol1 i=needle v=circle c=red width=3 height=2;
        symbol2 i=needle v=square c=blue width=3 height=2;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
        legend1 label=(height=2) value=(height=2);
run;
quit;
```

SAS Output

Fitting the Poisson to frequency data 1
16:30 Monday, March 29, 2010

Obs	y	obsfreq	yexp	yobs
1	0	24	-0.1	0.1
2	1	16	0.9	1.1
3	2	16	1.9	2.1
4	3	18	2.9	3.1
5	4	15	3.9	4.1
6	5	9	4.9	5.1
7	6	6	5.9	6.1
8	7	5	6.9	7.1

9	8	3	7.9	8.1
10	9	4	8.9	9.1
11	10	3	9.9	10.1
12	11	0	10.9	11.1
13	12	1	11.9	12.1

Fitting the Poisson to frequency data 2
16:30 Monday, March 29, 2010

The UNIVARIATE Procedure
Variable: y

Freq: obsfreq

Moments

N	120	Sum Weights	120
Mean	3.16666667	Sum Observations	380
Std Deviation	2.78752724	Variance	7.77030812
Skewness	0.91183392	Kurtosis	0.32893349
Uncorrected SS	2128	Corrected SS	924.666667
Coeff Variation	88.0271761	Std Error Mean	0.25446526

Basic Statistical Measures

Location		Variability	
Mean	3.166667	Std Deviation	2.78753
Median	3.000000	Variance	7.77031
Mode	0.000000	Range	12.00000
		Interquartile Range	4.00000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	12.4444	Pr > |t|	<.0001
Sign	M	48	Pr >= |M|	<.0001
Signed Rank	S	2328	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	12
99%	10
95%	9
90%	7
75% Q3	5
50% Median	3
25% Q1	1
10%	0
5%	0
1%	0
0% Min	0

Fitting the Poisson to frequency data 3
16:30 Monday, March 29, 2010

The UNIVARIATE Procedure
Variable: y

Freq: obsfreq

Extreme Observations

---------Lowest--------			--------Highest--------		
Value	Freq	Obs	Value	Freq	Obs
0	24	1	7	5	8
1	16	2	8	3	9
2	16	3	9	4	10
3	18	4	10	3	11
4	15	5	12	1	13

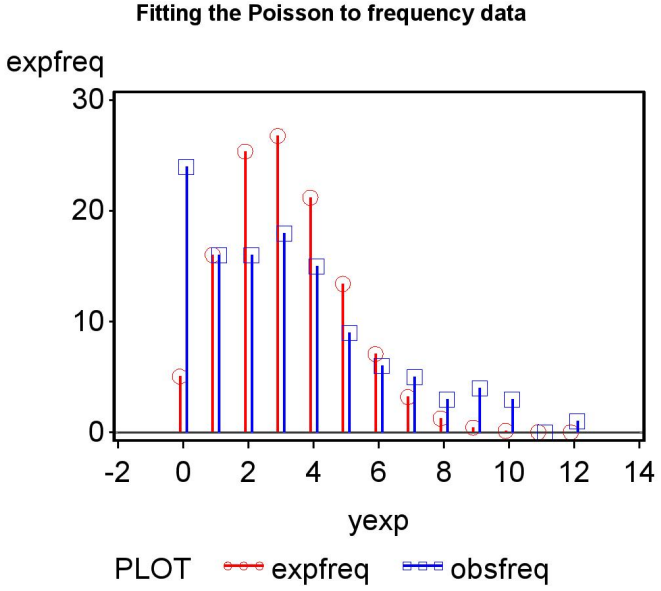
Fitting the Poisson to frequency data 4
16:30 Monday, March 29, 2010

Obs	n	ybar	var
1	120	3.16667	7.77031

Fitting the Poisson to frequency data 5
16:30 Monday, March 29, 2010

Obs	n	ybar	var	y	obsfreq	yexp	yobs	poisprob	expfreq
1	120	3.16667	7.77031	0	24	-0.1	0.1	0.04214	5.0573
2	120	3.16667	7.77031	1	16	0.9	1.1	0.13346	16.0147
3	120	3.16667	7.77031	2	16	1.9	2.1	0.21130	25.3565
4	120	3.16667	7.77031	3	18	2.9	3.1	0.22304	26.7652
5	120	3.16667	7.77031	4	15	3.9	4.1	0.17658	21.1892
6	120	3.16667	7.77031	5	9	4.9	5.1	0.11183	13.4198
7	120	3.16667	7.77031	6	6	5.9	6.1	0.05902	7.0827
8	120	3.16667	7.77031	7	5	6.9	7.1	0.02670	3.2041
9	120	3.16667	7.77031	8	3	7.9	8.1	0.01057	1.2683
10	120	3.16667	7.77031	9	4	8.9	9.1	0.00372	0.4462
11	120	3.16667	7.77031	10	3	9.9	10.1	0.00118	0.1413
12	120	3.16667	7.77031	11	0	10.9	11.1	0.00034	0.0407
13	120	3.16667	7.77031	12	1	11.9	12.1	0.00009	0.0107

Figure 5.9: Observed and expected frequencies - Poisson distribution



As an alternative to the Poisson, we can try fitting the negative binomial distribution using a similar SAS program. This distribution has two parameters, m and k, that must also be estimated before we can fit the distribution. The parameter m can be estimated using $\bar{Y}$ as with the Poisson, but k is best estimated using a technique called maximum likelihood (see Chapter 8). We will use a SAS procedure that can model count data using the negative binomial distribution, `proc genmod`, in order to estimate k (SAS Institute Inc. 2014). The output of `proc genmod` is manipulated in several `data` steps to combine these estimates with the observed frequency data, and then the negative binomial probabilities and expected frequencies calculated and plotted. See SAS program and output below.

We see that the expected frequencies for the negative binomial distribution provide a better match to the observed ones for this data set (Fig. 5.10). We also note that the variance predicted for the negative binomial distribution is close to the observed variance. From the negative binomial fit, we have $m = 3.167$ and $k = 1.760$, and so the estimated variance is $m + m^2/k = 3.167 + 3.167^2/1.760 = 7.459$, while the observed variance is $s^2 = 7.770$. This further implies the negative binomial provides a better fit to these data than the Poisson distribution.

SAS Program

```
* negbin_fit2.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Fitting the negative binomial to frequency data';
data negbin;
        input y obsfreq;
        * Generate offset y values for plot;
        yexp = y - 0.1; yobs = y + 0.1;
        datalines;
0  24
1  16
2  16
3  18
4  15
5   9
6   6
7   5
8   3
9   4
10  3
11  0
12  1
;
run;
* Print data set;
proc print data=negbin;
run;
* Descriptive statistics, save ybar, n, and var to data file;
proc univariate data=negbin;
        var y;
        histogram y / vscale=count;
        freq obsfreq;
        output out=stats mean=ybar n=n var=var;
run;
* Print output data file;
proc print data=stats;
run;
* Estimate m and k for the negative binomial distribution;
proc genmod data=negbin;
        model y = / dist=negbin;
        freq obsfreq;
        ods output ParameterEstimates=params;
run;
```

```
* Pick out value of m from genmod output;
data m;
        set params;
        if _n_ = 1;
        m = exp(Estimate);
        keep m;
run;
* Pick out value of k from genmod output;
data k;
        set params;
        if _n_ = 2;
        k = 1/Estimate;
        keep k;
run;
* Put m and k in one data file;
data params;
        merge m k;
run;
* Calculate expected frequencies using m and k;
data nbfit;
        if _n_ = 1 then set stats;
        if _n_ = 1 then set params;
        set negbin;
        nbprob = (gamma(k+y)/(gamma(y+1)*gamma(k)))*((m/(k+m))**y/(1+m/k)**k);
        expfreq = n*nbprob;
run;
* Print observed and expected frequencies;
proc print data=nbfit;
run;
* Plot observed and expected frequencies;
proc gplot data=nbfit;
        plot expfreq*yexp=1 obsfreq*yobs=2 / overlay legend=legend1 vref=0 wvref=3
        vaxis=axis1 haxis=axis1;
        symbol1 i=needle v=circle c=red width=3 height=2;
        symbol2 i=needle v=square c=blue width=3 height=2;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
        legend1 label=(height=2) value=(height=2);
run;
quit;
```

SAS Output

Fitting the negative binomial to frequency data 1
16:30 Monday, March 29, 2010

Obs	y	obsfreq	yexp	yobs
1	0	24	-0.1	0.1
2	1	16	0.9	1.1
3	2	16	1.9	2.1
4	3	18	2.9	3.1
5	4	15	3.9	4.1
6	5	9	4.9	5.1
7	6	6	5.9	6.1
8	7	5	6.9	7.1
9	8	3	7.9	8.1
10	9	4	8.9	9.1
11	10	3	9.9	10.1
12	11	0	10.9	11.1
13	12	1	11.9	12.1

Fitting the negative binomial to frequency data 2
16:30 Monday, March 29, 2010

The UNIVARIATE Procedure
Variable: y

Freq: obsfreq

Moments

N	120	Sum Weights	120
Mean	3.16666667	Sum Observations	380
Std Deviation	2.78752724	Variance	7.77030812
Skewness	0.91183392	Kurtosis	0.32893349
Uncorrected SS	2128	Corrected SS	924.666667
Coeff Variation	88.0271761	Std Error Mean	0.25446526

Basic Statistical Measures

Location		Variability	
Mean	3.166667	Std Deviation	2.78753

Median	3.000000	Variance	7.77031
Mode	0.000000	Range	12.00000
		Interquartile Range	4.00000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	12.4444	Pr > |t|	<.0001
Sign	M	48	Pr >= |M|	<.0001
Signed Rank	S	2328	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	12
99%	10
95%	9
90%	7
75% Q3	5
50% Median	3
25% Q1	1
10%	0
5%	0
1%	0
0% Min	0

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The UNIVARIATE Procedure
Variable: y

Freq: obsfreq

Extreme Observations

---------Lowest--------			--------Highest--------		
Value	Freq	Obs	Value	Freq	Obs

0	24	1	7	5	8
1	16	2	8	3	9
2	16	3	9	4	10
3	18	4	10	3	11
4	15	5	12	1	13

Fitting the negative binomial to frequency data 4
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Obs	n	ybar	var
1	120	3.16667	7.77031

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The GENMOD Procedure

Model Information

Data Set	WORK.NEGBIN
Distribution	Negative Binomial
Link Function	Log
Dependent Variable	y
Frequency Weight Variable	obsfreq

Number of Observations Read	13
Number of Observations Used	12
Sum of Frequencies Read	120
Sum of Frequencies Used	120
Missing Values	1

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	119	140.6185	1.1817
Scaled Deviance	119	140.6185	1.1817
Pearson Chi-Square	119	104.3325	0.8767
Scaled Pearson X2	119	104.3325	0.8767
Log Likelihood		92.3889	

Full Log Likelihood	-272.1343
AIC (smaller is better)	548.2685
AICC (smaller is better)	548.3711
BIC (smaller is better)	553.8435

Algorithm converged.

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept	1	1.1527	0.0858	0.9845	1.3209	180.40	<.0001
Dispersion	1	0.5680	0.1298	0.3136	0.8225		

NOTE: The negative binomial dispersion parameter was estimated by maximum likelihood.

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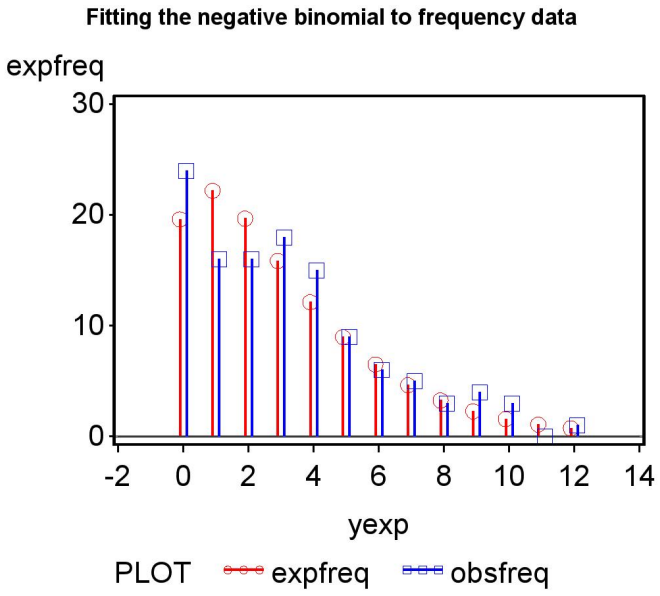
Obs	n	ybar	var	m	k	y	obsfreq	yexp	yobs	nbprob	expfreq
1	120	3.16667	7.77031	3.16667	1.76049	0	24	-0.1	0.1	0.16335	19.6023
2	120	3.16667	7.77031	3.16667	1.76049	1	16	0.9	1.1	0.18483	22.1793
3	120	3.16667	7.77031	3.16667	1.76049	2	16	1.9	2.1	0.16396	19.6748
4	120	3.16667	7.77031	3.16667	1.76049	3	18	2.9	3.1	0.13209	15.8503
5	120	3.16667	7.77031	3.16667	1.76049	4	15	3.9	4.1	0.10103	12.1237
6	120	3.16667	7.77031	3.16667	1.76049	5	9	4.9	5.1	0.07481	8.9770
7	120	3.16667	7.77031	3.16667	1.76049	6	6	5.9	6.1	0.05417	6.5008
8	120	3.16667	7.77031	3.16667	1.76049	7	5	6.9	7.1	0.03860	4.6319
9	120	3.16667	7.77031	3.16667	1.76049	8	3	7.9	8.1	0.02717	3.2599
10	120	3.16667	7.77031	3.16667	1.76049	9	4	8.9	9.1	0.01893	2.2722
11	120	3.16667	7.77031	3.16667	1.76049	10	3	9.9	10.1	0.01309	1.5714
12	120	3.16667	7.77031	3.16667	1.76049	11	0	10.9	11.1	0.00900	1.0797
13	120	3.16667	7.77031	3.16667	1.76049	12	1	11.9	12.1	0.00615	0.7379

Figure 5.10: Observed and expected frequencies - negative binomial distribution



5.6 Classifying spatial or temporal patterns

The spatial distribution of organisms, or the temporal occurrence of events like cases of disease, is often compared with the Poisson distribution. This distribution essentially assumes a random, independent distribution of organisms or events, and if the observed distribution differs from the Poisson then this could indicate some interesting biology. For example, if the observed frequencies have a distribution with more extreme values (low or high) than the Poisson, with $s^2 > \bar{Y}$, this implies organisms are unevenly distributed in space, or events in time. A pattern like this is often called an **overdispersed** distribution, or alternatively a clumped, aggregated, or contagious distribution (Pielou 1977, Begon et al. 2006). One method of quantifying the level of overdispersion is to fit the negative binomial distribution to the data and use the value of k as an index. Small values of k (say $k < 5$) imply an overdispersed distribution, while larger ones indicate a distribution close to Poisson. More rarely, an observed distribution may have fewer extreme values than the Poisson, with $s^2 < \bar{Y}$, implying the organisms are evenly distributed in space (or events in time). This is called an **underdispersed distribution**, also known as a regular, even, or repulsed distribution.

The figures below provide examples of spatial distributions that are overdispersed, Poisson, or underdispersed. Note the obvious clusters of organisms in the overdispersed example (Fig. 5.11). This might occur because the clusters are offspring from a single parent, the organisms are responding to resources that are clumped in space, or because the organisms are attracted to one another. The Poisson data also show a few clusters (Fig. 5.12), but these are chance occurrences. If we were to divide this graph into quadrats and count the number of organisms per quadrat, we would find the frequency distribution is close to Poisson. In contrast to the other examples, the organisms are spaced apart to some extent in the underdispersed example (Fig. 5.13). This could occur because they are territorial, compete for resources, or otherwise regulate their numbers in some fashion (Ridout & Besbeas 2004).

Figure 5.11: Overdispersed distribution of organisms in space

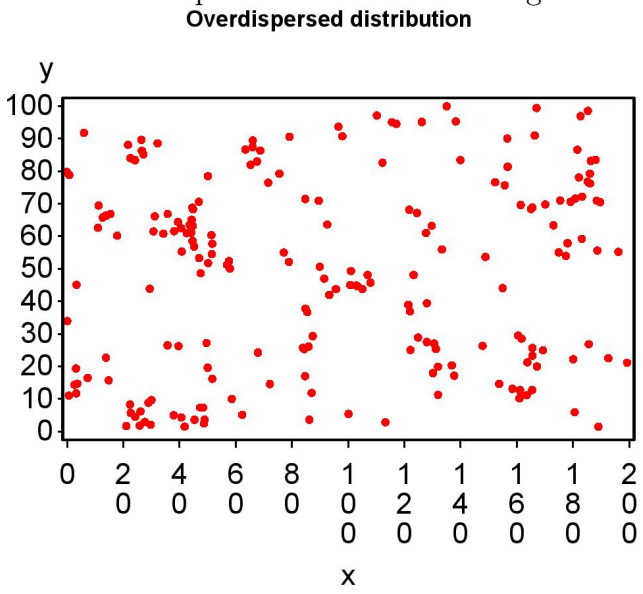


Figure 5.12: Poisson distribution of organisms in space

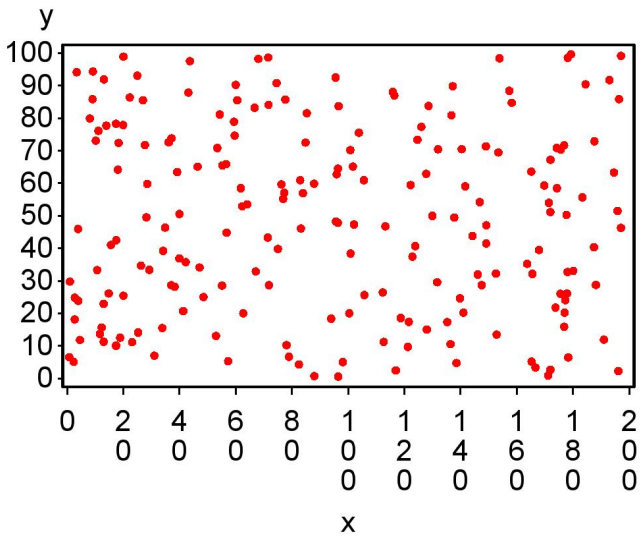
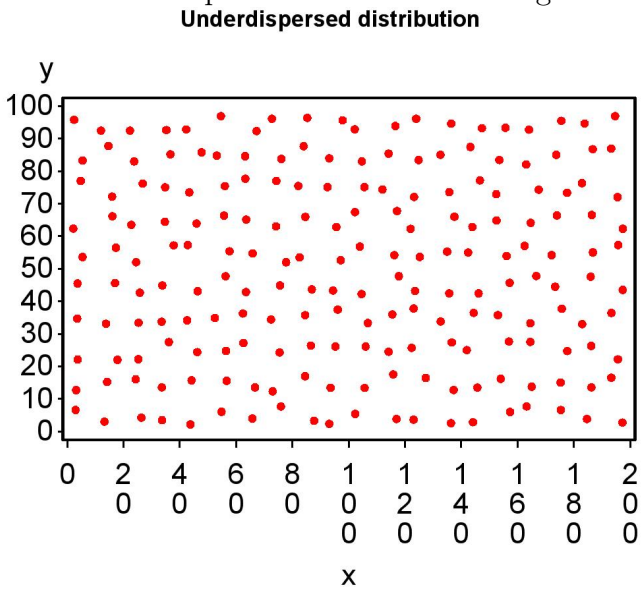


Figure 5.13: Underdispersed distribution of organisms in space



5.7 References

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5.8 Problems

1. Consider the dice cube example from Chapter 4, and define a random variable Y that is the number of spots showing on the dice cube. Find $E[Y]$ and $Var[Y]$ for this random variable. Show your work.

2. Suppose that a random variable Y has a discrete distribution with the following probabilities:

y	$P[Y = y]$
0	0.5000
1	0.2500
2	0.1250
3	0.0625
4	0.0625

   (a) What is the expected value of Y, or $E[Y]$?

   (b) What is the variance of Y, or $Var[Y]$?

3. An entomologist studies the spatial distribution of aphids in a field. They randomly select 100 locations within the field and count the number of aphids on the plants at each location. The following observed frequency distribution was obtained:

Aphids (y)	Frequency
0	19
1	22
2	16
3	10
4	11
5	11
6	6
7	2
8	1
9	1
10	1
11	0

(a) Use the SAS program `Poisson_fit.sas` to calculate $\bar{Y}$ and s^2, and generate a plot of the observed frequencies vs. those expected for the Poisson distribution. Attach your SAS program and output.

(b) Based on the above results, do the data have a Poisson distribution? Explain your answer using the pattern of observed and expected frequencies, and the values of $\bar{Y}$ and s^2. Is the pattern random (Poisson), overdispersed, or underdispersed?

(c) What are some possible biological explanations for this pattern?

4. A field is surveyed for golden mice (*Ochrotomys nuttalli*) using a grid of baited traps. A total of 100 traps were deployed and the number of mice counted in each trap. The following frequency distribution was obtained:

Mice (y)	Frequency
0	55
1	21
2	10
3	7
4	4
5	2
6	1
7	0
8	0

(a) Use the program `Poisson_fit.sas` to calculate to calculate $\bar{Y}$ and s^2, and generate a plot of the observed frequencies vs. those expected for the Poisson distribution. Attach your program and output.

(b) Based on the above results, do the data have a Poisson distribution? Explain your answer using the pattern of observed and expected frequencies, and the values of $\bar{Y}$ and s^2. Is the pattern random (Poisson), overdispersed, or underdispersed?

Chapter 6

Continuous Random Variables

We previously examined several different probability distributions for discrete random variables, in particular the binomial, Poisson, and negative binomial distributions. These distributions are suitable for modeling observations that are counts of some type, such as the number of plants in a quadrat or the number of females vs. males in a sample. Many variables in biology are continuous, however, such as the length and weight of organisms, quantities associated with populations such as birth, mortality, and growth rates, and chemical concentrations. We will now examine continuous random variables and their associated distributions that are used to model these quantities, in particular the **uniform and normal distributions**. The uniform distribution is often used to generate random sampling points in one- and two-dimensional areas. For example, we could use the uniform distribution to select a random point along a transect to sample, or a random x, y coordinate within a field to place a sampling quadrat. It also a useful starting point for understanding continuous distributions because of its simplicity. We then turn to the normal distribution, which forms the basis of many statistical procedures. Many biological variables have a distribution close to normal, or if initially non-normal can often be transformed to more closely resemble the normal distribution.

Discrete random variables have a function $f(y)$ that directly provides the probabilities for events that are integers, such as $Y = 0$, $Y = 3$, and so forth (see Chapter 5). However, events for continuous random variables are in the form of intervals. For example, we will be interested in finding the probability for events like $1 < Y < 3$ or $Y > 5$. Continuous random variables use a different kind of function, called a **probability density function**, to find

the probabilities for events. For an event like $1 < Y < 3$, probabilities are found by integrating the probability density function (finding the area under the function) over this interval. This process will be explained in more detail below. For many continuous random variables, such as the normal distribution, there exist tables of these integrals and probabilities for certain useful intervals. Note that events like $Y = 3$ have zero probability for continuous random variables, because this implies an interval of zero width and so the integral is zero. This makes some intuitive sense, because it is unlikely that a continuous quantity Y would take a value exactly equal to 3 to many decimal places.

6.1 Uniform distribution

Suppose that we have two constants, a and b, with $a < b$. A random variable Y has a uniform distribution if an observation is equally likely to occur anywhere between a and b, but never occurs outside this interval. The probability density for the uniform distribution is defined by the equation

$$f(y) = \frac{1}{b-a} \tag{6.1}$$

for $a \leq y \leq b$ (Mood et al. 1974). Outside of this interval, we have $f(y) = 0$. The quantities a and b are the parameters of the uniform distribution. The uniform distribution for $a = 0$, $b = 1$ is shown below (Fig. 6.1). The uniform distribution gets its name from the fact that its density is uniform over the interval a to b.

Note that the density simply describes a square with a length and width of one, implying an area equal to one. This is an important property of probability density functions in general – the area under $f(y)$ is always equal to one. Also shown is the uniform density for $a = 0$ and $b = 2$ (Fig. 6.2). It is lower but wider than the previous example, and also has an area of one.

Figure 6.1: Uniform probability density for $a = 0, b = 1$

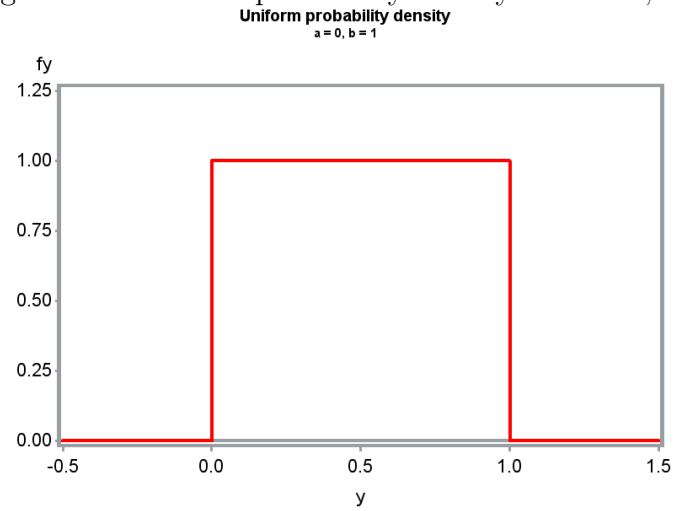
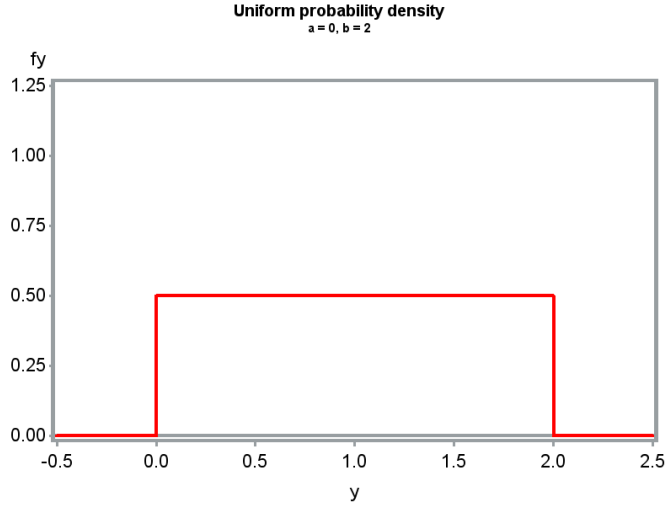


Figure 6.2: Uniform probability density for $a = 0, b = 2$



Probabilities for the uniform distribution are calculated by finding the area under the probability density function. This is relatively easy to do because of the simple form of the probability density. Suppose Y is a uniform random variable, and $a = 0$ and $b = 1$. What is the probability that an observed Y lies within the interval 0.5 to 0.75? We have

$$P[0.5 < Y < 0.75] = \int_{0.5}^{0.75} \frac{1}{b-a} dy \tag{6.2}$$

$$= \int_{0.5}^{0.75} \frac{1}{1-0} dy = y|_{0.5}^{0.75} \tag{6.3}$$

$$= 0.75 - 0.5 = 0.25. \tag{6.4}$$

We could also have found this probability without any calculus. It is just the area under $f(y)$ between 0.5 and 0.75, calculated as length $\times$ height $= (0.75 - 0.5) \times 1 = 0.25$.

Here are two more examples. Suppose that for $a = 0$ and $b = 2$, we want to find the probability that $0.2 < Y < 0.4$. The height of the density function in this case is $1/(b-a) = 1/(2-0) = 0.5$. We therefore have $P[0.2 < Y < 0.4] = (0.4 - 0.2) \times 0.5 = 0.1$. Now suppose we want the probability that $0 < Y < 2$. We have $P[0 < Y < 2] = (2-0) \times 0.5 = 1$. This also follows from the fact that $f(y)$ is a probability density function which has an area of one, and the interval $0 < Y < 2$ encompasses the entire range of $f(y)$.

The **cumulative distribution function** for a continuous random variable is defined as the quantity

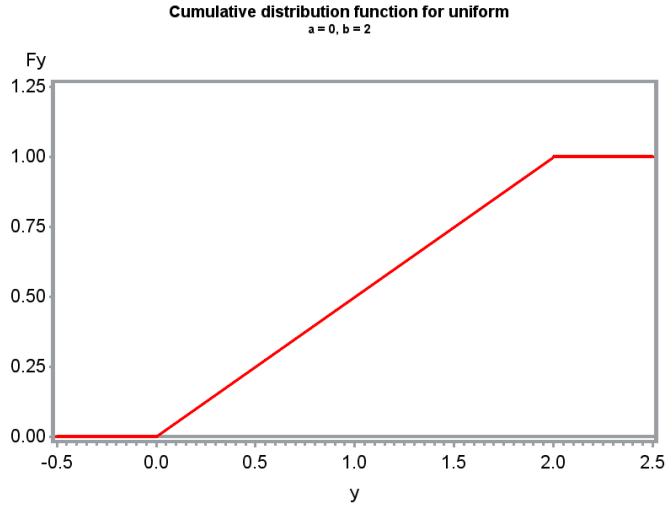
$$F(y) = P[Y < y] = \int_{-\infty}^{y} f(z)dz. \tag{6.5}$$

This function is just the probability to the left of y. The function $F(y)$ increases from 0 to 1 as y increases. If we carry out this integral for the uniform distribution, we get the function

$$F(y) = \frac{y-a}{b-a} \tag{6.6}$$

for $a \leq y \leq b$. In addition, $F(y) = 0$ for $y < a$, and $F(y) = 1$ for $y > b$. Figure 6.3 shows the cumulative distribution function for the uniform distribution corresponding to Fig. 6.2. Note that it increases linearly between

Figure 6.3: Cumulative distribution function for the uniform distribution, with $a = 0, b = 2$



a and b, as the probability to the left of y accumulates. The cumulative distribution function has many uses in statistics, especially for continuous random variables.

The uniform distribution has a number of common applications. It is possible to generate a stream of random numbers that have a uniform distribution using software, which can then be used to generate random observations for other distributions, including discrete distributions as well as the normal distribution. The uniform distribution can also be used to generate random sampling points along a transect for ecological studies, or random x, y coordinates for placing quadrats within an area (see below). It can also be used to generate random samples from a population, or randomly order treatments in an experiment.

6.1.1 Random sampling coordinates - SAS demo

A common application of the uniform distribution is to generate random sampling coordinates. SAS can generate random observations with a uniform distribution using the function `ranuni`. For this function, the parameter values of the uniform distribution are set at $a = 0$ and $b = 1$.

However, we will often want observations for other parameter values, es-

pecially other values of b. It can be shown that if Y has a uniform distribution with $a = 0$ and $b = 1$, then the variable $Y' = cY$ has a uniform distribution with $a = 0$ and $b = c$, where c is any positive number. This fact enables us to generate uniform random variables with any value of b.

For example, suppose we want to generate random sampling coordinates along a 100 m transect using the uniform distribution. If Y has a uniform distribution with $a = 0$ and $b = 1$, then $Y' = 100Y$ has a uniform distribution with $a = 0$ and $b = 100$. Values of Y generated in this fashion will give us sampling coordinates uniformly distributed between 0 and 100 m.

We will illustrate this process using a SAS program to generate random sampling coordinates for a 100 m transect and also a 200×100 m rectangular area. A call to `gplot` is used to plot the random coordinates. See SAS program and output below.

SAS Program

```
* randcoords.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Generate random sampling coordinates";
* Generate n random coordinates along a c m transect;
data transect;
        * Sample size n;
        n = 20;
        * Multiplying by c gives a uniform random variable with a=0, b=c;
        c = 100;
        do i = 1 to n;
                x = c*ranuni(0);
                output;
        end;
        drop i;
run;
* Print coordinates;
proc print data=transect;
run;
* Generate n random coordinates within a 200 x 100 m area;
data coords;
        * Sample size n;
        n = 200;
        * Multiplying by c_x gives a uniform random variable with a=0, b=c_x;
        c_x = 200;
        * Multiplying by c_y gives a uniform random variable with a=0, b=c_y;
        c_y = 100;
```

```
        do i = 1 to n;
                x = c_x*ranuni(0);
                y = c_y*ranuni(0);
                output;
        end;
        drop i;
run;
* Print first 25 coordinates;
proc print data=coords(obs=25);
run;
* Show coordinates as a scatterplot;
proc gplot data=coords;
        plot y*x / vaxis=axis1 haxis=axis2;
        symbol1 v=dot c=red;
        axis1 order=(0 to 100 by 10) label=(height=2) value=(height=2)
        width=3 major=(width=2) minor=none;
    axis2 order=(0 to 200 by 20) label=(height=2) value=(height=2)
        width=3 major=(width=2) minor=none;
run;
quit;
```

SAS Output

Generate random sampling coordinates 1
15:53 Monday, April 19, 2010

Obs	c	x
1	100	19.9499
2	100	76.3413
3	100	79.9041
4	100	15.7759
5	100	15.2421
6	100	71.3867
7	100	23.3531
8	100	73.9213
9	100	75.5294
10	100	55.6698
11	100	42.3700
12	100	67.0161
13	100	23.0314
14	100	17.1588
15	100	68.1973
16	100	20.1917
17	100	91.6066
18	100	50.2973

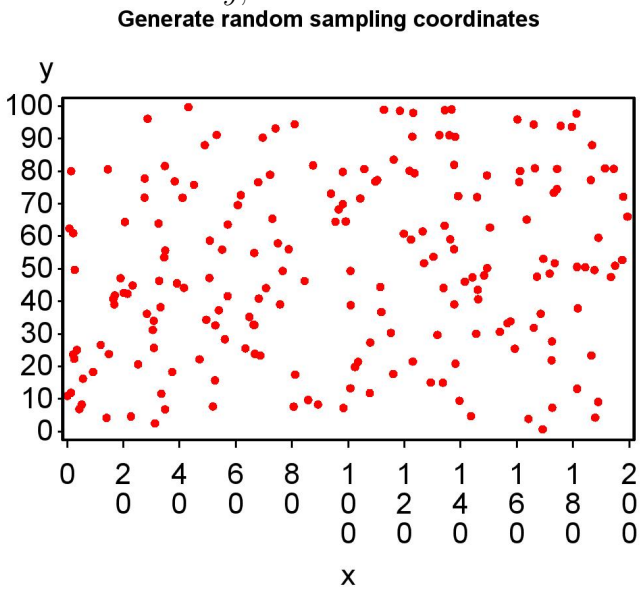
```
    19    100    84.9498
    20    100    36.2745
```

```
  Generate random sampling coordinates                    2
                                  15:53 Monday, April 19, 2010

 Obs    c_x    c_y       x          y

   1    200    100    154.862    21.3515
   2    200    100    160.414    70.8713
   3    200    100    118.344    57.3555
   4    200    100    154.958     4.8716
   5    200    100    173.834    80.7355
   6    200    100     40.852     1.9296
   7    200    100    116.088    94.5155
   8    200    100     13.003     5.9704
   9    200    100     58.785    96.1373
  10    200    100    190.694    18.8834
  11    200    100    180.953    29.0750
  12    200    100    100.127    42.8300
  13    200    100     75.700    47.8597
  14    200    100    127.454    59.8772
  15    200    100     27.703    35.4066
  16    200    100     16.360     7.5101
  17    200    100     43.722    18.8987
  18    200    100    177.311    55.2469
  19    200    100     41.933     2.2553
  20    200    100    101.261    39.6063
  21    200    100    146.369    48.9749
  22    200    100     44.071    96.6252
  23    200    100    146.298    88.8055
  24    200    100    158.129    43.9857
  25    200    100     58.123    66.6462
```

Figure 6.4: Random y, x coordinates for 200×100 m area



6.2 Normal distribution

The normal distribution plays an important role in statistics, with good reason. Biological variables often have a distribution that can be approximated by the normal or can be transformed to be normal. The normal distribution is thus a valid choice for modeling many variables encountered in practice. Many statistical quantities will also have a distribution approaching the normal for large sample sizes. For example, the distribution of the sample mean $\bar{Y}$ will approach the normal distribution as the sample size n increases, thanks to the central limit theorem (see Chapter 7). So, even if the underlying data are non-normal, statistics like $\bar{Y}$ will be normally-distributed for sufficiently large n.

The probability density for the normal distribution is defined by the function

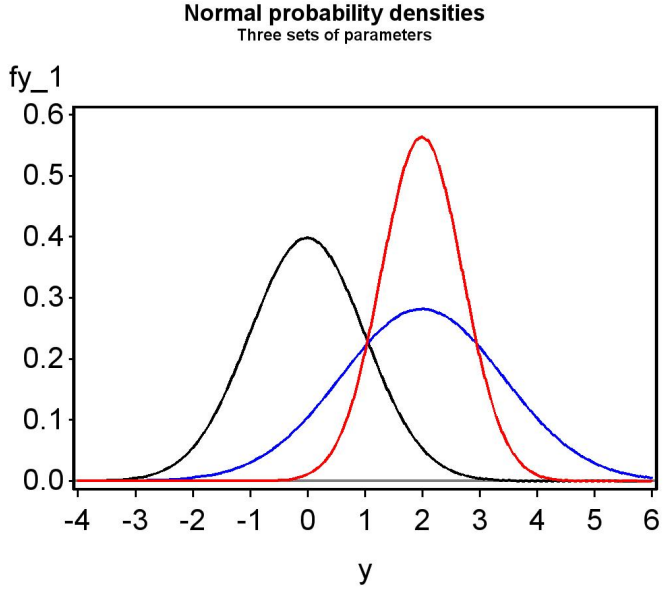
$$f(y) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(y-\mu)^2}{2\sigma^2}} \tag{6.7}$$

for $\infty < \mu < \infty$ and $\sigma^2 > 0$ (Mood et al. 1974). The normal distribution has

two parameters, μ and σ^2. The parameter μ is the mean of the distribution and basically controls its location, while σ^2 is its variance and determines its dispersion or spread. A random variable Y with a normal distribution is often written as $Y \sim N(\mu, \sigma^2)$, where the symbol '$\sim$' stands for 'is distributed as' while 'N' signifies the normal. A random variable with a **standard normal distribution** assumes that $\mu = 0$ and $\sigma^2 = 1$, or $Y \sim N(0, 1)$. The symbol Z is often used to denote a standard normal random variable.

Figure 6.5 shows the bell-shaped normal distribution for three different sets of μ and σ^2 values, and illustrates how these parameters affect its location and shape. As μ is increased the distribution shifts to the right, while an increase in σ^2 causes the distribution to spread out.

Figure 6.5: Three normal distributions



6.2.1 Normal distribution - SAS demo

The SAS program used to generate Fig. 6.5 is listed below. Three different sets of μ and σ^2 values are given in the `data` step of the program (feel free to experiment with other values). The different curves are specified in the `plot` statement for `proc gplot`. The `overlay` option is used to generate a single

graph with all three curves, each with different colors specified by the `symbol` statements.

SAS Program

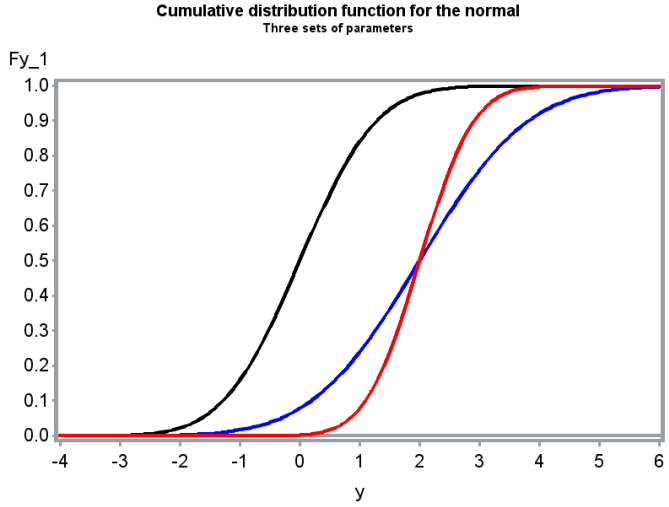
```
* normal_plot3.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Normal probability densities";
title2 "Three sets of parameters";
data normal_plot;
    * Three sets of normal parameters here;
    mu_1 = 0; sig2_1 = 1;
    mu_2 = 2; sig2_2 = 2;
    mu_3 = 2; sig2_3 = 0.5;
    * Minimum and maximum values of y;
    ymin = -4;
    ymax = 6;
    * Divisions between ymin and ymax (more = smoother graph);
    ydiv = 100;
    * Calculate step length;
    ylength = (ymax-ymin)/ydiv;
    * Find y and f(y) values for the plot;
    do i=0 to ydiv;
        y = ymin + i*ylength;
        * normal probability density function;
        fy_1 = (1/sqrt(2*3.14159*sig2_1))*exp(-((y-mu_1)**2)/(2*sig2_1));
        fy_2 = (1/sqrt(2*3.14159*sig2_2))*exp(-((y-mu_2)**2)/(2*sig2_2));
        fy_3 = (1/sqrt(2*3.14159*sig2_3))*exp(-((y-mu_3)**2)/(2*sig2_3));
        * Output y and fy1, fy2, fy3 to SAS data file;
        output;
    end;
run;
* Print data;
proc print data=normal_plot;
run;
* Plot probability density function;
proc gplot data=normal_plot;
    plot fy_1*y=1 fy_2*y=2 fy_3*y=3 / vref=0 wvref=3 vaxis=axis1 haxis=axis1 overlay;
    symbol1 i=join v=none c=black width=3;
    symbol2 i=join v=none c=blue width=3;
    symbol3 i=join v=none c=red width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

The cumulative distribution function for the normal distribution is defined as the quantity

$$F(y) = P[Y < y] = \int_{-\infty}^{y} f(z)dz = \int_{-\infty}^{y} \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(z-\mu)^2}{2\sigma^2}} dz. \tag{6.8}$$

The values of this integral have to be numerically calculated. Fig. 6.6 shows the cumulative distribution functions for the three normal distributions shown in Fig. 6.5. Note that the mean and variance for the different normal distributions affect the overall location and shape of $F(y)$.

Figure 6.6: Cumulative distribution function for three normal distributions



Like other continuous random variables, events for the normal distribution are in the form of intervals. We can calculate the probabilities for events by finding the area under the normal density function corresponding to the interval. This process is more difficult than for the uniform distribution because $f(y)$ has a more complex shape. However, there exist tables of the area under $f(y)$ for certain intervals that can be used for this purpose, as well as the SAS function `probnorm`. Table Z gives the probabilities for intervals of the form $Z < z$, where Z has a standard normal distribution and $z \geq 0$ (see Chapter 22). The first two digits of z are specified in the left-most column

of Table Z, while the third digit is the top row. The values within the table correspond to the probability that $Z < z$, or $P[Z < z]$, i.e., the cumulative distribution function for the standard normal.

6.2.2 Sample calculations - standard normal distribution

We illustrate how Table Z is used to calculate the probabilities for various events listed below. The general strategy is to sketch the interval on the standard normal bell curve, and deduce from this picture how to obtain the probability using Table Z.

1. Find the probability that $Z < 0.55$, or $P[Z < 0.55]$. From Table Z, we see that $P[Z < 0.55] = 0.7088$. See Fig. 6.7 for an illustration of this probability.

2. Find the probability that $0.40 < Z < 1.96$. In this case, the interval is not the same as shown in Table Z, and additional calculations are required. We first find the probabilities for the intervals $Z < 1.96$ and $Z < 0.4$ using Table Z. The probability for $0.40 < Z < 1.96$ should then be the difference between these two probabilities (see Fig. 6.8). We have $P[Z < 1.96] = 0.9750$ and $P[Z < 0.40] = 0.6554$ from Table Z, so $P[0.40 < Z < 1.96] = P[Z < 1.96] - P[Z < 0.40] = 0.9750 - 0.6554 = 0.3196$.

3. Find the probability that $Z > 0.55$. We will use the complement rule to obtain this probability (see Chapter 4). For any event A, we have $P[A^c] = 1 - P[A]$. If A is the event $Z < 0.55$, then A^C corresponds to $Z > 0.55$. Therefore, $P[Z > 0.55] = 1 - P[Z < 0.55] = 1 - 0.7088 = 0.2912$. See also Fig. 6.9.

4. Find the probability that $Z < -1.23$. This problem makes use of the symmetry of the standard normal distribution around zero, as well as the complement rule. By symmetry, we have $P[Z < -1.23] = P[Z > 1.23]$. The complement of $Z < 1.23$ is $Z > 1.23$, and so $P[Z > 1.23] = 1 - P[Z < 1.23] = 1 - 0.8907 = 0.1093$. See Fig. 6.10.

5. Find the probability that $-0.44 < Z < 2.15$. This problem can also be handled using symmetry and the complement rule. We first have

$P[Z < 2.15] = 0.9842$ using Table Z (Fig. 6.11). We then have $P[Z < -0.44] = P[Z > 0.44] = 1 - P[Z < 0.44] = 1 - 0.6700 = 0.3300$ by symmetry (Fig. 6.12). Therefore, $P[-0.44 < Z < 2.15] = P[Z < 2.15] - P[Z < -0.44] = 0.9842 - 0.3300 = 0.6542$.

6. Find a number z_0 such that $P[Z < z_0] = 0.95$. This problem is the inverse of the previous ones. Here, we want to find a value z_0 that gives a certain probability, rather than z_0 being a given quantity and determining the probability. To find z_0, we scan Table Z until we find a value that gives a probability close 0.95. We see that $z_0 = 1.64$ or 1.65 give approximately the right probability.

Figure 6.7: Sample calculation 1

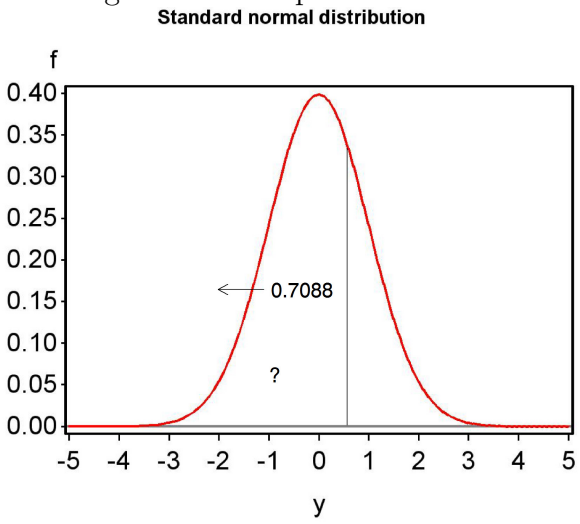


Figure 6.8: Sample calculation 2

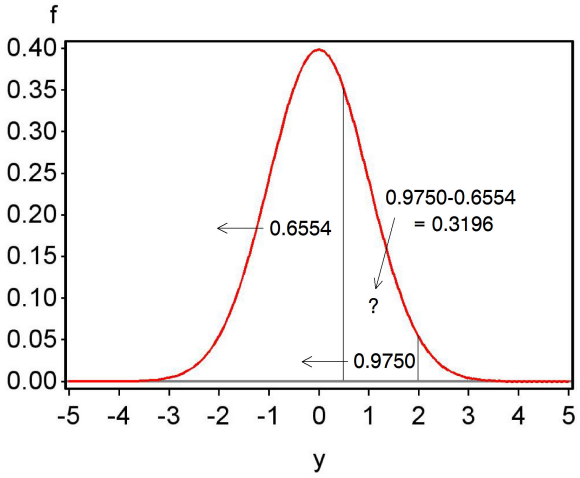


Figure 6.9: Sample calculation 3

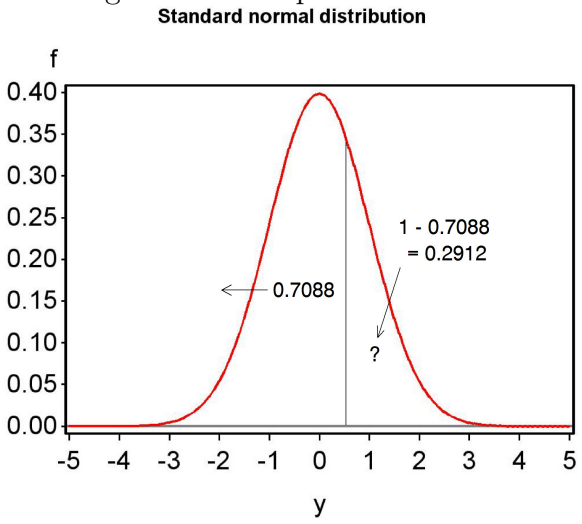


Figure 6.10: Sample calculation 4

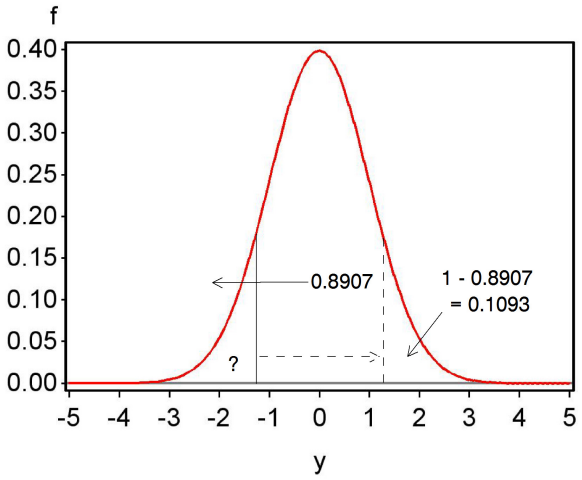


Figure 6.11: Sample calculation 5 - part 1

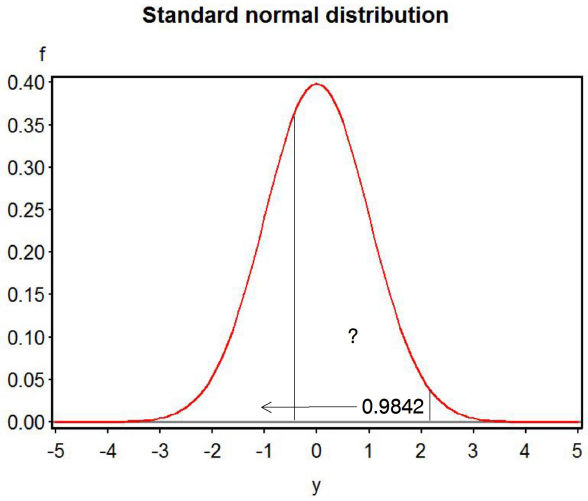
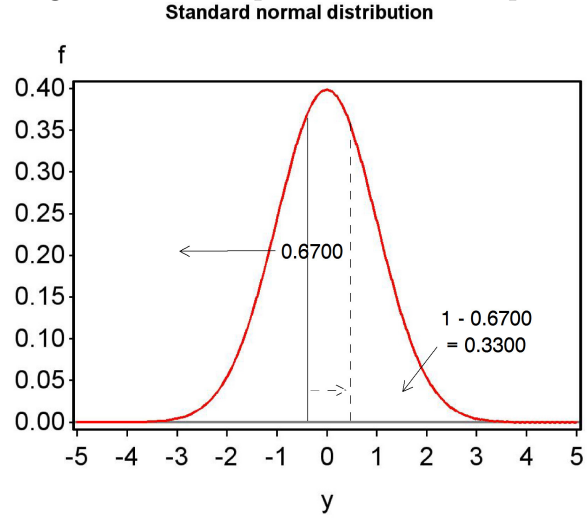


Figure 6.12: Sample calculation 5 - part 2



6.2.3 Sample calculations - other normal distributions

We now examine how probabilities can be calculated for normal distributions that are not standard normal. If $Y \sim N(\mu, \sigma^2)$, it can be shown that the quantity

$$Z = \frac{Y - \mu}{\sigma} \sim N(0, 1) \tag{6.9}$$

Thus, a random variable Y with a normal distribution having any μ or σ^2 can be transformed to a standard normal Z. The transformation works by first centering the random variable Y around zero by subtracting μ, and then dividing by σ so that it has a standard deviation and variance of one. Once Y is transformed to a standard normal Z, we can find probabilities for any event involving Y using Table Z. This process is illustrated below in several sample calculations.

1. Suppose that $Y \sim N(50, 16)$. Find the probability that $Y < 55$. First, we find $\sigma = \sqrt{\sigma^2} = \sqrt{16} = 4$. Using the above equation, we then have

$$\begin{aligned} P[Y < 55] &= P\left[Y - \mu < 55 - \mu\right] && (6.10)\\ &= P\left[\frac{Y - \mu}{\sigma} < \frac{55 - \mu}{\sigma}\right] && (6.11)\\ &= P\left[Z < \frac{55 - 50}{4}\right] && (6.12)\\ &= P[Z < 1.25]. && (6.13) \end{aligned}$$

   We then use Table Z to find that $P[Z < 1.25] = 0.8944$, and so $P[Y < 55] = 0.8944$.

2. Find the probability that $52 < Y < 56$, assuming $Y \sim N(50, 16)$. To find this probability, we first convert the problem to one involving Z. We have

$$\begin{aligned} P[52 < Y < 56] &= P\left[52 - \mu < Y - \mu < 56 - \mu\right] && (6.14)\\ &= P\left[\frac{52 - \mu}{\sigma} < \frac{Y - \mu}{\sigma} < \frac{56 - \mu}{\sigma}\right] && (6.15)\\ &= P\left[\frac{52 - 50}{4} < Z < \frac{56 - 50}{4}\right] && (6.16)\\ &= P[0.50 < Z < 1.50]. && (6.17) \end{aligned}$$

We next find the probabilities for the intervals $Z < 1.50$ and $Z < 0.50$ using Table Z, and then substract them to obtain $P[0.50 < Z < 1.50]$. We have $P[Z < 1.50] = 0.9332$ and $P[Z < 0.50] = 0.6915$, so $P[0.50 < Z < 1.50] = 0.9332 - 0.6915 = 0.2417$. Thus, $P[52 < Y < 56] = 0.2417$.

3. Find the probability that $Y > 54$. We have

$$P[Y > 54] = P\left[Y - \mu > 54 - \mu\right] \tag{6.18}$$
$$= P\left[\frac{Y - \mu}{\sigma} > \frac{54 - \mu}{\sigma}\right] \tag{6.19}$$
$$= P\left[Z > \frac{54 - 50}{4}\right] \tag{6.20}$$
$$= P[Z > 1.00]. \tag{6.21}$$

We next use the complement rule to obtain this probability. We have $P[Z > 1.00] = 1 - P[Z < 1.00] = 1 - 0.8413 = 0.1587$, so $P[Y > 54] = 0.1587$.

4. Find the probability that $Y < 46.5$. We have

$$P[Y < 46.5] = P\left[Y - \mu < 46.5 - \mu\right] \tag{6.22}$$
$$= P\left[\frac{Y - \mu}{\sigma} < \frac{46.5 - \mu}{\sigma}\right] \tag{6.23}$$
$$= P\left[Z < \frac{46.5 - 50}{4}\right] \tag{6.24}$$
$$= P[Z < -0.88]. \tag{6.25}$$

By symmetry, we have $P[Z < -0.88] = P[Z > 0.88]$. The complement of $Z < 0.88$ is $Z > 0.88$, and so $P[Z > 0.88] = 1 - P[Z < 0.88] = 1 - 0.8106 = 0.1093$. So, $P[Y < 46.5] = 0.1093$.

5. Find the probability that $46 < Z < 52$. We have

$$P[46 < Y < 52] = P\left[46 - \mu < Y - \mu < 52 - \mu\right] \tag{6.26}$$
$$= P\left[\frac{46 - \mu}{\sigma} < \frac{Y - \mu}{\sigma} < \frac{52 - \mu}{\sigma}\right] \tag{6.27}$$
$$= P\left[\frac{46 - 50}{4} < Z < \frac{52 - 50}{4}\right] \tag{6.28}$$
$$= P[-1.00 < Z < 0.50]. \tag{6.29}$$

We then use symmetry and the complement rule to find this probability involving Z. We first have $P[Z < 0.50] = 0.6915$ using Table Z. We then have $P[Z < -1.00] = P[Z > 1.00] = 1 - P[Z < 1.00] = 1 - 0.8413 = 0.1587$ by symmetry. Therefore, $P[-1.00 < Z < 0.50] = P[Z < 0.50] - P[Z < -1.00] = 0.6915 - 0.1587 = 0.5328$, and so $P[46 < Y < 52] = 0.5328$.

6. Find a number y_0 such that $P[Y < y_0] = 0.70$. This problem can also be handled by converting it to one involving Z. We have

$$P[Y < y_0] = P[Y - \mu < y_0 - \mu] \tag{6.30}$$
$$= P\left[\frac{Y - \mu}{\sigma} < \frac{y_0 - \mu}{\sigma}\right] \tag{6.31}$$
$$= P\left[Z < \frac{y_0 - 50}{4}\right] \tag{6.32}$$
$$= P[Z < z_0] \tag{6.33}$$

where $z_0 = \frac{y_0 - 50}{4}$. We then search for a value of z_0 such that $P[Z < z_0] = 0.70$, and obtain $z_0 = 0.52$ from Table Z. We then solve for y_0 as follows:

$$z_0 = \frac{y_0 - 50}{4} \tag{6.34}$$
$$0.52 = \frac{y_0 - 50}{4} \tag{6.35}$$
$$4(0.52) = y_0 - 50 \tag{6.36}$$
$$2.08 = y_0 - 50 \tag{6.37}$$
$$2.08 + 50 = y_0 \tag{6.38}$$
$$52.08 = y_0. \tag{6.39}$$

So, $y_0 = 52.08$ is the answer. In general, one would have $z_0 = \frac{y_0 - \mu}{\sigma}$, so $y_0 = \sigma z_0 + \mu$ for any σ and μ.

6.3 Expected values and variance for continuous distributions

We saw earlier how a theoretical mean, variance, and standard deviation could be calculated for a discrete random variable, using the concept of expectation and its probability distribution. The same concepts can be extended to continuous random variables and probability densities.

Let Y be a continuous random variable with some probability density. The expected value of Y, or its theoretical mean, is defined by the equation

$$E[Y] = \int_{-\infty}^{\infty} yf(y)dy \tag{6.40}$$

where $f(y)$ is the probability density of Y, and the integral is carried out over the interval $-\infty$ to ∞ (Mood et al. 1974). This equation is analogous to the definition of expected value for a discrete random variable, except that we use integration rather than summation to make the calculation.

Similar to discrete random variables, we can also define the theoretical variance of a continuous random variable using expectation. The variance of a continuous random variable Y is defined as

$$Var[Y] = E[(Y - E[Y])^2] = \int_{-\infty}^{\infty} (y - E[Y])^2 f(y)dy. \tag{6.41}$$

We can directly calculate these quantities for the uniform distribution. Recall from calculus that $\int udu = u^2/2$. We therefore have

$$E[Y] = \int_{-\infty}^{\infty} yf(y)dy = \int_a^b \frac{y}{b-a}dy \tag{6.42}$$

$$= \frac{1}{b-a}\frac{y^2}{2}|_a^b = \frac{1}{b-a}\frac{b^2-a^2}{2} \tag{6.43}$$

$$= \frac{(b-a)(b+a)}{2(b-a)} = \frac{b+a}{2} \tag{6.44}$$

Thus, the expected value (or theoretical mean) of a uniform random variable is located at the center of the interval, midway between a and b. It can also be shown using the above formula that the variance of the uniform distribution is

$$Var[Y] = \frac{(b-a)^2}{12} \tag{6.45}$$

The theoretical standard deviation is just the square root of this quantity.

What are these quantities for the normal distribution? Recall that the normal distribution is specified by the two parameters μ and σ^2. If $Y \sim N(\mu, \sigma^2)$, it can be shown (by evaluating the above integrals using the normal density) that

$$E[Y] = \mu \tag{6.46}$$

and

$$Var[Y] = \sigma^2. \tag{6.47}$$

Thus, the parameters μ and σ^2 for this distribution are the theoretical mean and variance $E[Y]$ and $Var[Y]$.

6.4 Continuous random variables and samples

Suppose we have a set of observations and want to determine if they can be modeled using the normal distribution. We now develop a graphical method of comparing these observed data with the pattern expected for the normal distribution, called a **normal quantile plot**. These plots exist for other continuous distributions as well, and are generally called quantile-quantile plots. The idea is to plot the quantiles for the observed data vs. the quantiles for the normal distribution, with the quantiles for the normal on the x-axis and the data quantiles on the y-axis. If the data are normally distributed, then this plot will resemble a straight diagonal line. This occurs because we are essentially plotting the quantiles for one normal distribution (the data) vs. the quantiles for the normal distribution itself (Wilk & Gnanadesikan 1968). This is like plotting the function $y = ax$, which is the equation of a line with slope a. See Chapter 3 for a review of quantiles such as the median, the 25% and 75% quartiles, and so forth.

Normal quantile plots are constructed as follows. Suppose we have five data points that take the values 2.1, 1.4, 3.9, 7.7, and 8.9. We first rank or order the data points from smallest to largest:

$$1.4, 2.1, 3.9, 7.7, 8.9. \tag{6.48}$$

We then determine a probability p corresponding to each data point using the formula $p = (r_i - 3/8)/(n + 1/4)$, where r_i is the rank order of the ith

data point and n is the sample size:

$$0.1190, 0.3095, 0.5, 0.6905, 0.8810. \tag{6.49}$$

The idea here is to associate a particular probability p with each data point, depending on its rank order. Note that the median of these data (the value 3.9) corresponds to $p = 0.5$. The values 3/8 and 1/4 in the formula are there to prevent p from taking the value 0 or 1 for the largest and smallest ranks. These are the values used by SAS for this purpose (SAS Institute Inc. 2014), although other ones have been suggested (Harter 1984, Makkonen 2008).

We then determine the quantiles of the standard normal distribution that correspond to the values of p for these data, using Table Z. For example, suppose we want to find a value z_0 such that $P[Z < z_0] = 0.5$, the median of the standard normal distribution. We see from Table Z that $z_0 = 0$ give the correct probability. For $p = 0.6905$, we find that $z_0 = 0.50$ gives close to the correct probability. We can similarly find the values of z_0 for the other values of p, to obtain:

$$-1.18, -0.50, 0, 0.50, 1.18. \tag{6.50}$$

The last step is to plot the rank ordered data vs. the normal quantiles, with the ordered data on the y-axis and corresponding normal quantiles on the x-axis. If the data are normally distributed, there will be a linear relationship between the observed data and the normal quantiles, and the normal quantile plot will be a straight line. If the data are non-normal, however, all manner of curved relationships are possible.

6.4.1 Elytra lengths - SAS demo

We previously examined a data set involving the elytra lengths of male and female *T. dubius* beetles and calculated various descriptive statistics using `proc univariate` (see Chapter 3). We now examine whether these data are normally-distributed using normal quantile plots. A normal quantile plot is requested by adding the command `qqplot` with the `normal` option to the program (see below). A histogram and fitted normal curve can also be generated using the `histogram` command with the `normal` option. Separate analyses are requested for male and female beetles using a `by` statement, because the two sexes differ in size and could also have potentially different distributions. We observe that the normal quantile plots for female beetles is close to linear, suggesting a normal distribution, while the males show some curvature.

SAS Program

```
* normal_quantile_plot.sas;
options pageno=1 linesize=80;
title 'Fitting the normal to elytra data';
data elytra;
    input sex $ length;
    datalines;
M   4.9
F   5.2
M   4.9
F   4.2
F   5.7

etc.

M   5.1
F   4.4
M   4.8
M   4.6
F   3.7
;
run;
* Descriptive statistics, histograms, and normal quantile plots;
proc univariate plots data=elytra;
    * Separate analyses for each sex;
    class sex;
    var length;
    histogram length/ vscale=count normal(w=3) wbarline=3 waxis=3 height=4;
    qqplot length / normal waxis=3 height=4;
    symbol1 h=3;
run;
quit;
```

SAS Output

Fitting the normal to elytra data 1
11:03 Thursday, May 13, 2010

The UNIVARIATE Procedure
Variable: length
sex = F

Moments

N	60	Sum Weights	60
Mean	4.94	Sum Observations	296.4
Std Deviation	0.48544929	Variance	0.23566102
Skewness	-0.521146	Kurtosis	0.16125847
Uncorrected SS	1478.12	Corrected SS	13.904
Coeff Variation	9.82690878	Std Error Mean	0.06267123

Basic Statistical Measures

Location		Variability	
Mean	4.940000	Std Deviation	0.48545
Median	5.000000	Variance	0.23566
Mode	5.200000	Range	2.20000
		Interquartile Range	0.70000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	78.82404	Pr > |t|	<.0001
Sign	M	30	Pr >= |M|	<.0001
Signed Rank	S	915	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	5.9
99%	5.9
95%	5.7

90%	5.5
75% Q3	5.3
50% Median	5.0
25% Q1	4.6
10%	4.3
5%	4.0
1%	3.7
0% Min	3.7

Fitting the normal to elytra data 4
11:03 Thursday, May 13, 2010

The UNIVARIATE Procedure
Variable: length
sex = M

Moments

N	70	Sum Weights	70
Mean	4.71285714	Sum Observations	329.9
Std Deviation	0.44977335	Variance	0.20229607
Skewness	-0.896502	Kurtosis	1.00307174
Uncorrected SS	1568.73	Corrected SS	13.9584286
Coeff Variation	9.5435388	Std Error Mean	0.0537582

Basic Statistical Measures

Location		Variability	
Mean	4.712857	Std Deviation	0.44977
Median	4.800000	Variance	0.20230
Mode	5.000000	Range	2.40000
		Interquartile Range	0.50000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	87.66769	Pr > |t|	<.0001
Sign	M	35	Pr >= |M|	<.0001
Signed Rank	S	1242.5	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	5.80
99%	5.80
95%	5.20
90%	5.15
75% Q3	5.00
50% Median	4.80
25% Q1	4.50
10%	4.00
5%	3.80
1%	3.40
0% Min	3.40

Fitting the normal to elytra data 7
11:03 Thursday, May 13, 2010

The UNIVARIATE Procedure
sex = F
Fitted Normal Distribution for length

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	4.94
Std Dev	Sigma	0.485449

Goodness-of-Fit Tests for Normal Distribution

Test	----Statistic-----		------p Value------	
Kolmogorov-Smirnov	D	0.10387776	Pr > D	0.105
Cramer-von Mises	W-Sq	0.07705508	Pr > W-Sq	0.228
Anderson-Darling	A-Sq	0.50377430	Pr > A-Sq	0.206

Quantiles for Normal Distribution

------Quantile------

Percent	Observed	Estimated
1.0	3.70000	3.81068
5.0	4.00000	4.14151
10.0	4.30000	4.31787
25.0	4.60000	4.61257
50.0	5.00000	4.94000
75.0	5.30000	5.26743
90.0	5.50000	5.56213
95.0	5.70000	5.73849
99.0	5.90000	6.06932

Fitting the normal to elytra data 8
11:03 Thursday, May 13, 2010

The UNIVARIATE Procedure
sex = M
Fitted Normal Distribution for length

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	4.712857
Std Dev	Sigma	0.449773

Goodness-of-Fit Tests for Normal Distribution

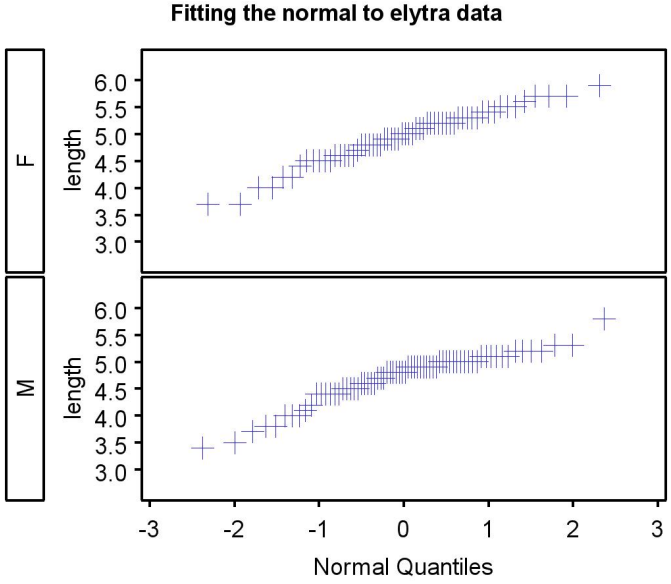
Test		----Statistic-----		------p Value------
Kolmogorov-Smirnov	D	0.16252783	Pr > D	<0.010
Cramer-von Mises	W-Sq	0.34087445	Pr > W-Sq	<0.005
Anderson-Darling	A-Sq	1.99478432	Pr > A-Sq	<0.005

Quantiles for Normal Distribution

	------Quantile------	
Percent	Observed	Estimated
1.0	3.40000	3.66653
5.0	3.80000	3.97305
10.0	4.00000	4.13645

```
25.0    4.50000     4.40949
50.0    4.80000     4.71286
75.0    5.00000     5.01622
90.0    5.15000     5.28926
95.0    5.20000     5.45267
99.0    5.80000     5.75919
```

Figure 6.13: Normal quantile plot for beetle elytra - females and males



6.4.2 Development time - SAS demo

We now examine a data set involving the development time of *T. dubius* beetles in various stages, in particular the time from the larval to prepupal stage, and then from the prepupal to adult stage (Reeve et al. 2003). See program below for details of this analysis. We see that the normal quantile plots for both stages are quite nonlinear, suggesting a distribution different from normal. This is a reflection of the skewed distributions of development time we saw earlier for these data (Chapter 3). Skewed and nonnormal distributions are a common feature of insect development data (Wagner et al. 1984).

SAS Program

```
* normal_quantile_plot_2.sas;
options pageno=1 linesize=80;
title 'Fitting the normal to development data';
data devel_time;
    input time_pp time_adult;
    datalines;
34  65
31  48
29   .
30  55
32  62

etc.

29   .
29 108
31 103
33   .
29  92
;
run;
* Descriptive statistics, histograms, and normal quantile plots;
proc univariate plots data=devel_time;
    var time_pp time_adult;
    histogram time_pp time_adult / vscale=count normal(w=3) wbarline=3 waxis=3 height=4;
    qqplot time_pp time_adult / normal waxis=3 height=4;
    symbol1 h=3;
run;
quit;
```

SAS Output

Fitting the normal to development data 1
08:08 Thursday, April 29, 2010

The UNIVARIATE Procedure
Variable: time_pp

Moments

N	96	Sum Weights	96
Mean	31.3541667	Sum Observations	3010
Std Deviation	3.32764866	Variance	11.0732456
Skewness	0.75038358	Kurtosis	0.04666776
Uncorrected SS	95428	Corrected SS	1051.95833
Coeff Variation	10.6130987	Std Error Mean	0.33962672

Basic Statistical Measures

Location		Variability	
Mean	31.35417	Std Deviation	3.32765
Median	31.00000	Variance	11.07325
Mode	30.00000	Range	14.00000
		Interquartile Range	5.00000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	92.31949	Pr > |t|	<.0001
Sign	M	48	Pr >= |M|	<.0001
Signed Rank	S	2328	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	41
99%	41
95%	39
90%	36

75% Q3	34
50% Median	31
25% Q1	29
10%	27
5%	27
1%	27
0% Min	27

Fitting the normal to development data 4
08:08 Thursday, April 29, 2010

The UNIVARIATE Procedure
Fitted Normal Distribution for time_pp

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	31.35417
Std Dev	Sigma	3.327649

Goodness-of-Fit Tests for Normal Distribution

Test	----Statistic-----		------p Value------	
Kolmogorov-Smirnov	D	0.13138957	Pr > D	<0.010
Cramer-von Mises	W-Sq	0.26720735	Pr > W-Sq	<0.005
Anderson-Darling	A-Sq	1.73548398	Pr > A-Sq	<0.005

Quantiles for Normal Distribution

	------Quantile------	
Percent	Observed	Estimated
1.0	27.0000	23.6129
5.0	27.0000	25.8807
10.0	27.0000	27.0896
25.0	29.0000	29.1097
50.0	31.0000	31.3542
75.0	34.0000	33.5986
90.0	36.0000	35.6187

95.0	39.0000	36.8277
99.0	41.0000	39.0954

Fitting the normal to development data 5
08:08 Thursday, April 29, 2010

The UNIVARIATE Procedure
Variable: time_adult

Moments

N	68	Sum Weights	68
Mean	75.3529412	Sum Observations	5124
Std Deviation	26.3465791	Variance	694.14223
Skewness	0.51461555	Kurtosis	-0.6244048
Uncorrected SS	432616	Corrected SS	46507.5294
Coeff Variation	34.9642346	Std Error Mean	3.19499201

Basic Statistical Measures

Location		Variability	
Mean	75.35294	Std Deviation	26.34658
Median	68.00000	Variance	694.14223
Mode	42.00000	Range	105.00000
		Interquartile Range	46.50000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	23.5847	Pr > |t|	<.0001
Sign	M	34	Pr >= |M|	<.0001
Signed Rank	S	1173	Pr >= |S|	<.0001

Quantiles (Definition 5)

Quantile	Estimate
100% Max	147.0
99%	147.0

95%	116.0
90%	110.0
75% Q3	99.0
50% Median	68.0
25% Q1	52.5
10%	43.0
5%	42.0
1%	42.0
0% Min	42.0

Fitting the normal to development data 8
08:08 Thursday, April 29, 2010

The UNIVARIATE Procedure
Fitted Normal Distribution for time_adult

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	75.35294
Std Dev	Sigma	26.34658

Goodness-of-Fit Tests for Normal Distribution

Test	----Statistic-----		------p Value------	
Kolmogorov-Smirnov	D	0.12461617	Pr > D	<0.010
Cramer-von Mises	W-Sq	0.22866485	Pr > W-Sq	<0.005
Anderson-Darling	A-Sq	1.43281773	Pr > A-Sq	<0.005

Quantiles for Normal Distribution

	-------Quantile------	
Percent	Observed	Estimated
1.0	42.0000	14.0616
5.0	42.0000	32.0167
10.0	43.0000	41.5884
25.0	52.5000	57.5824
50.0	68.0000	75.3529

```
75.0     99.0000     93.1234
90.0    110.0000    109.1174
95.0    116.0000    118.6892
99.0    147.0000    136.6442
```

Figure 6.14: Development time- larval to prepupal stage

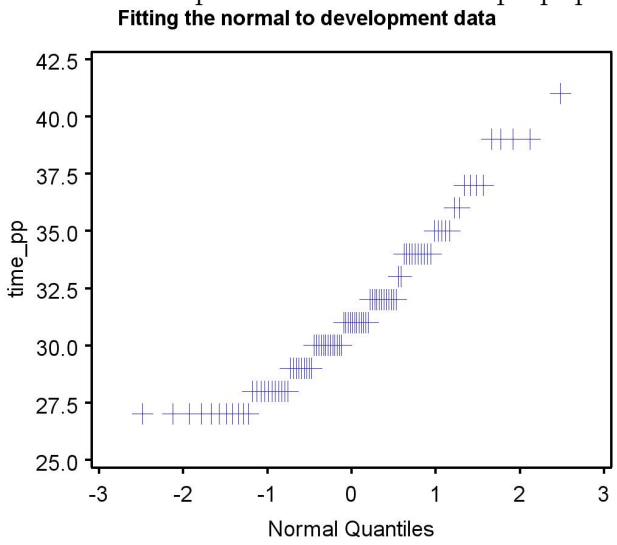
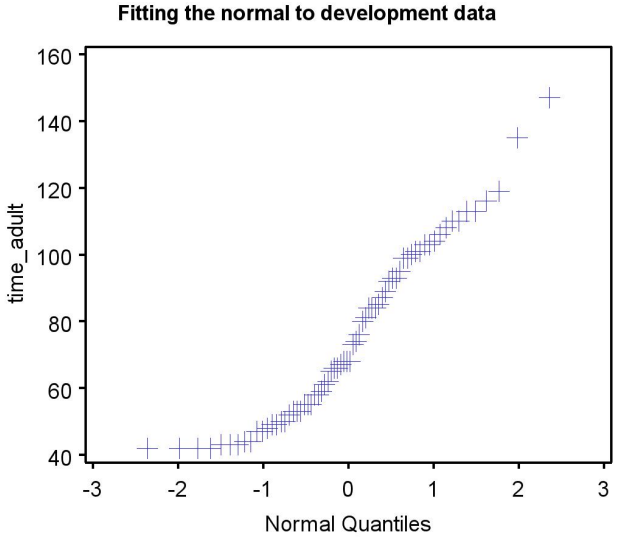


Figure 6.15: Development time - prepupal to adult stage



6.5 References

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6.6 Problems

1. A random variable Y has a uniform probability density with $a = 0$ and $b = 2$.

   (a) What is the expected value of Y, or $E[Y]$? What is the variance of Y, or $Var[Y]$?

   (b) What are the 25%, 50%, and 80% quantiles or percentiles of Y?

   (c) Find the probability that $Y < 0.05$.

   (d) Find a symmetric interval centered around $y = 1$ that has a probability of 0.95.

2. Suppose that Y has a normal distribution with $\mu = 1$ and $\sigma^2 = 3$, or $Y \sim N(1, 3)$. Find the following quantities using Table Z.

   (a) The probability that $Y > 2$.

   (b) The probability that $1 < Y < 3$.

   (c) The probability that $Y < 0.5$.

   (d) The probability that Y is not inside the interval given in b.

   (e) A value of y_0 such that the probability that $Y < y_0$ is 0.9.

3. Suppose that Y has a normal distribution with $\mu = 2$ and $\sigma^2 = 4$, or $Y \sim N(2, 4)$. Find the following quantities using Table Z:

   (a) The probability that $Y < 2.5$.

   (b) The probability that $0.5 < Y < 2.5$.

   (c) The probability that $Y < 1$.

   (d) The probability that Y is not inside the interval given in b.

   (e) A value of y_0 such that the probability that $Y < y_0$ is 0.4.

Chapter 7

Expected Value, Variance, and Samples

7.1 Expected value and variance

Previously, we determined the expected value and variance for a random variable Y, which we can think of as a single observation from a distribution. We will now extend these concepts to a linear function of Y and also the sum of n random variables. We will use these results to derive the expected value and variance of the sample mean $\bar{Y}$ and variance s^2, and so describe their basic statistical properties. The idea of an unbiased estimator is also expressed in terms of expected values, and we will show that $\bar{Y}$ and s^2 are unbiased estimators of the theoretical mean and variance of Y, i.e., $E[Y]$ and $Var[Y]$. This is true regardless of the distribution of Y.

We begin by reviewing the definition of expected value and variance. Recall that if Y has a discrete distribution, the expected value (theoretical mean) of Y, or $E[Y]$, is given by the equation

$$E[Y] = \sum_y yP[Y = y] = \sum_y yf(y). \tag{7.1}$$

Here $f(y)$ is the probability distribution of Y, with the summation is taken over all possible values of y. If Y has a continuous distribution, the expected value is defined as the integral

$$E[Y] = \int_{-\infty}^{\infty} yf(y)dy, \tag{7.2}$$

where $f(y)$ is the probability density of Y. For both discrete and continuous random variables, the expected value is essentially a weighted average of all possible values of Y, with the weights being probabilities or densities.

We also defined the theoretical variance of a random variable using expectation. The variance of a random variable Y, denoted by $Var[Y]$, is defined as

$$Var[Y] = E[(Y - E[Y])^2] = \sum_y (y - E[Y])^2 P[Y = y] \tag{7.3}$$

$$= \sum_y (y - E[Y])^2 f(y). \tag{7.4}$$

The variance is a measure of the dispersion of the distribution of Y. The variance of a continuous random variable Y is similarly defined as

$$Var[Y] = E[(Y - E[Y])^2] = \int_{-\infty}^{\infty} (y - \mu)^2 f(y) dy. \tag{7.5}$$

Table 7.1 summarizes the expected value and variance for the different distributions we have examined so far. These quantities are a function of the parameters in the distribution. Note that for the binomial, Poisson, negative binomial and uniform distributions, there is some relationship between $E[Y]$ and $Var[Y]$, because the formulas share the same parameters. For example, in the Poisson distribution the theoretical mean and variance are both equal to λ. This is not the case for the normal distribution, where the mean and variance are two separate parameters.

Table 7.1: Expected value and variance for five common probability distributions

Distribution	Parameters	$E[Y]$	$Var[Y]$
Binomial	l, p	lp	$lp(1-p)$
Poisson	λ	λ	λ
Negative binomial	m, k	m	$m + m^2/k$
Uniform	a, b	$\frac{a+b}{2}$	$\frac{(b-a)^2}{12}$
Normal	μ, σ^2	μ	σ^2

The significance of this result is that many statistical procedures assume the mean and variance are unrelated, because they are based on the normal

distribution. If we wish to apply these procedures to other distributions, we will need to transform the observations to reduce the relationship between the mean and variance. This type of transformation is known as a **variance-stabilizing transformation** (see Chapter 15).

7.2 Linear functions and sums - expected value and variance

Before we turn to samples, we first need to determine the expected value of a linear function of Y. Let Y be a random variable with any distribution, and define a new variable $Y' = aY + b$, where a and b are constants. This is called a linear function of Y because there is a straight-line relationship between Y' and Y. What is the expected value of Y', or $E[Y']$? It can be shown that

$$E[Y'] = E[aY + b] = aE[Y] + b. \tag{7.6}$$

Thus, multiplying a random variable by a constant and then adding another constant just shifts the theoretical mean in the same way (Mood et al. 1974). This result holds for random variables with either a discrete or continuous distribution.

Now suppose we have n random variables of any type, $Y_1, Y_2, \ldots, Y_n$, which may or may not be independent. The random variables could also have unequal means and variances, and even different distributions. What is the expected value of the sum of these variables? One can show that

$$E[Y_1 + Y_2 + \ldots + Y_n] = E[Y_1] + E[Y_2] + \ldots + E[Y_n] = \sum E[Y_i]. \tag{7.7}$$

So, **the expected value of a sum is equal to the sum of the expected values** (Mood et al. 1974).

We will now examine how the theoretical variance is affected by a linear function. Let Y be a variable with any distribution with an associated variance of $Var[Y]$. Define a new random variable $Y' = aY + b$, where a and b are constants. What is the variance of Y', or $Var[Y']$? It can be shown that

$$Var[Y'] = Var[aY + b] = a^2 Var[Y]. \tag{7.8}$$

This implies that a linear function of a random variable increases its variance by a factor of a^2, with b playing no role in the variance. This makes intuitive

sense, because multiplying a random variable by a constant (a) should affect its breadth or dispersion, while adding a constant (b) only shifts its location and not its dispersion.

Now suppose we have n random variables of any type, $Y_1, Y_2, \ldots, Y_n$. The random variables can have unequal means and variances, but we will assume they are independent. What is the variance of the sum of these observations? It can be shown that

$$Var[Y_1 + Y_2 + \ldots + Y_n] = Var[Y_1] + Var[Y_2] + \ldots + Var[Y_n] = \sum Var[Y_i]. \tag{7.9}$$

Thus, **the variance of a sum is equal to the sum of the variances** (Mood et al. 1974). As you add more and more random variables together, the variance of the sum also increases. This result only holds when the random variables are independent of each other – if they were dependent a much more complicated formula would be required. This is one advantage of working with a random sample in which the observations are independent, because it simplifies parameter estimation and other statistical procedures (see Chapter 8).

7.3 Sample mean - expected value and variance

We will now use the preceding results to find the expected value and variance of the sample mean. Suppose we have a set of observations $Y_1, Y_2, \ldots, Y_n$ drawn from some statistical population, say the body lengths of n randomly selected individuals. The random variables Y_i are independent, and because they are drawn from the same population, they also have the same expected value $E[Y_i]$ and variance $Var[Y_i]$.

The sample mean is defined using the familiar formula:

$$\bar{Y} = \frac{\sum_{i=1}^{n} Y_i}{n}. \tag{7.10}$$

What is the expected value of the sample mean or $\bar{Y}$? Using our results for sums of variables and linear transformations, we have

$$E[\bar{Y}] = E\left[\frac{\sum Y_i}{n}\right] = \frac{E[\sum Y_i]}{n} = \frac{\sum E[Y_i]}{n} = \frac{nE[Y_i]}{n} = E[Y_i]. \tag{7.11}$$

The expected value of the mean is thus equal to the expected value of the individual variables (Mood et al. 1974).

The fact that $E[\bar{Y}] = E[Y_i]$ means that $\bar{Y}$ is an **unbiased estimator** of the theoretical mean of the distribution of Y_i. In less technical terms, it implies that on average $\bar{Y}$ will be equal to the underlying mean of the random variable Y_i. This is often a desirable property in an estimator, although there are useful biased estimators as well.

We also need to calculate the theoretical variance of the sample mean, written as $Var[\bar{Y}]$. Using the properties of the expected value and variance, we have

$$Var[\bar{Y}] = Var\left[\frac{\sum Y_i}{n}\right] = \frac{Var[\sum Y_i]}{n^2} = \frac{\sum Var[Y_i]}{n^2} = \frac{nVar[Y_i]}{n^2} = \frac{Var[Y_i]}{n}. \tag{7.12}$$

Thus, the variance of the sample mean is the variance of Y_i divided by n (Mood et al. 1974).

What does this result imply? **As you collect larger and larger samples, the variance of the sample mean $\bar{Y}$ becomes smaller.** In other words, $\bar{Y}$ becomes less variable when it includes more data. This result underlies many of the desirable effects of larger sample sizes in statistics, including better estimates of parameters (Chapter 8), smaller confidence intervals (Chapter 9), and statistical tests with more power (Chapter 10).

The standard deviation of the sample mean $\bar{Y}$ is defined to be the square root of the above quantity:

$$\sqrt{Var[\bar{Y}]} = \sqrt{\frac{Var[Y_i]}{n}} = \frac{\sqrt{Var[Y_i]}}{\sqrt{n}}. \tag{7.13}$$

This formula makes it clear that the standard deviation of the mean is a function of the standard deviation of the individual observations and the sample size used in the mean. The common name for this quantity is the **standard error**. In general, a standard error is the standard deviation of a particular statistic, in this case the sample mean $\bar{Y}$.

7.4 Sample variance - expected value

Recall that the sample variance is defined using the formula

$$s^2 = \frac{\sum_{i=1}^{n}(Y_i - \bar{Y})^2}{n-1}. \tag{7.14}$$

It can be shown that $E[s^2] = Var[Y_i]$, implying that the sample variance is an unbiased estimator of the underlying variance of Y_i.

It is important to note that all our results for the sample mean $\bar{Y}$ and variance s^2 hold true for any distribution, not just the normal distribution. The basic requirement is that the observations Y_1, $Y_2, \ldots$, Y_n are randomly drawn from some statistical population, implying they are independent and have the same expected value $E[Y_i]$ and variance $Var[Y_i]$.

7.5 Sample calculations and simulation - SAS demo

As an example of these rules of expectation and variance, suppose that Y has a normal distribution with mean $\mu = 1$ and variance $\sigma^2 = 1$, namely $Y \sim N(1, 1)$. Suppose we want to find the expected value and variance of $Y' = 2Y + 1$. Note that Y' is a linear function of Y with $a = 2$ and $b = 1$. Using the formulas for the expected value and variance of a linear function, we have $E[Y'] = aE[Y] + b = 2E[Y] + 1 = 2(1) + 1 = 3$, and also $Var[Y'] = a^2 Var[Y] = 2^2 Var[Y] = 4(1) = 4$.

Now suppose we have three variables Y_1, Y_2, and Y_3 with the same distribution as above, and assumed to be independent. What is the expected value and variance of the sum of these two variables, $Y_1 + Y_2 + Y_3$? Using the formulas for sums of random variables, we have $E[Y_1 + Y_2 + Y_3] = E[Y_1] + E[Y_2] + E[Y_3] = 1 + 1 + 1 = 3$, and $Var[Y_1 + Y_2 + Y_3] = Var[Y_1] + Var[Y_2] + Var[Y_3] = 1 + 1 + 1 = 3$.

We can also calculate the expected value and variance of the sample mean $\bar{Y}$ for Y_1, Y_2, and Y_3. Using the preceding results, we have $E[\bar{Y}] = E[Y_i] = 1$, and $Var[\bar{Y}] = Var[Y_i]/n = 1/3$.

We can verify that these theoretical rules for the expected value and variance have some basis in reality by conducting an experiment. Recall that the expected value for a random variable can also be thought of as the sample mean $\bar{Y}$ for an infinite number of observations of that random variable. Similarly, its theoretical variance is the sample variance s^2 of an infinite number of observations. It is easy to generate a very large number of observations using SAS, and then compare the result predicted by these theoretical rules with the sample mean and variance of the observations. The SAS program listed below first generates 100,000 observations having the

Table 7.2: Expected value and variance

	Theory		Simulation	
Variable	$E[\cdot]$	$Var[\cdot]$	$\bar{Y}$	s^2
Y	1	1	1.002	0.999
Y'	3	4	3.003	3.997
$Y_1 + Y_2 + Y_3$	3	3	3.009	3.018
$\bar{Y}$	1	1/3	1.003	0.335
s^2	1	-	1.001	-

specified distribution $[Y, Y_i \sim N(1,1)]$ in a `data` step. Formulas are then used to calculate Y', $Y_1 + Y_2 + Y_3$, $\bar{Y}$, and s^2. The SAS procedure `proc univariate` is then used to calculate the sample mean and variance of these quantities. See SAS output below.

If the theory involving expected values and variances is correct, it should predict the behavior of the mean and variance in this large sample. A comparison between the results predicted using our expected value formulas and the observed simulation results is given in Table 7.2. The theoretical predictions and sample mean and variance are in close agreement.

Notice also from the SAS output that the distributions of Y', $Y_1 + Y_2 + Y_3$, and $\bar{Y}$ appear to be normally distributed (see Fig. 7.2 - 7.4). In fact, linear functions and sums of normal random variables are always normally distributed, as is the sample mean. This may not be the case for variables with other distributions. We also see that the variance of $\bar{Y}$ is lower than Y (1/3 vs. 1), an important property of this statistic (see Fig. 7.2 vs. 7.4).

SAS Program

```
* Linear.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Demonstration of expected value and variance rules';
data linear;
        * Loop to generate 100000 random observations;
        do i = 1 to 100000;
                a = 2;
                b = 1;
                * Generate y, y1, y2, y3 with N(1,2) distribution;
                mu = 1; sig2 = 1;
                y = sqrt(sig2)*rannor(0) + mu;
                y1 = sqrt(sig2)*rannor(0) + mu;
                y2 = sqrt(sig2)*rannor(0) + mu;
                y3 = sqrt(sig2)*rannor(0) + mu;
                * Calculate a linear function of y, then sum, mean, and s2;
                yprime = a*y + b;
                ysum = y1 + y2 + y3;
                ybar = ysum/3;
                s2 = ((y1-ybar)**2+(y2-ybar)**2+(y3-ybar)**2)/(3-1);
                output;
        end;
run;
* Print simulated data, first 25 observations;
proc print data=linear(obs=25);
run;
* Descriptive statistics, histograms, and normal quantile plots;
proc univariate data=linear;
        var y yprime ysum ybar s2;
        histogram y yprime ysum ybar s2 / vscale=count normal(w=3)
        haxis=axis1 vaxis=axis2 wbarline=3 waxis=3 height=4;
        qqplot y yprime ysum ybar s2 / normal waxis=3 height=4;
        symbol1 h=3;
        axis1 order=(-6 to 12 by 0.2);
        axis2 order=(0 to 10000 by 2000);
run;
quit;
```

SAS Output

Demonstration of expected value and variance rules 1
11:24 Tuesday, August 30, 2011

Obs	i	a	b	mu	sig2	y	y1	y2	y3
1	1	2	1	1	1	2.40343	1.16387	1.40458	-0.42999
2	2	2	1	1	1	0.88987	1.40552	0.96997	-0.03135
3	3	2	1	1	1	3.33301	0.32146	-0.29182	-1.54156
4	4	2	1	1	1	1.07186	2.12205	1.59675	0.77104
5	5	2	1	1	1	2.00620	1.49543	1.58517	0.17379
6	6	2	1	1	1	0.19762	2.14458	0.60593	4.30395
7	7	2	1	1	1	-0.81464	1.75308	1.30842	0.60105
8	8	2	1	1	1	2.80298	1.06916	0.78637	0.65529
9	9	2	1	1	1	-0.56801	1.22604	0.15375	0.29170
10	10	2	1	1	1	-0.36265	1.34079	-0.36673	-2.41139
11	11	2	1	1	1	0.18388	-1.54080	1.11047	1.30994
12	12	2	1	1	1	1.62454	1.05907	0.65800	2.33563
13	13	2	1	1	1	1.83046	1.43715	-0.37539	0.83023
14	14	2	1	1	1	0.63400	0.38407	2.14804	1.30881
15	15	2	1	1	1	-0.87082	1.83641	0.60312	-0.56471
16	16	2	1	1	1	1.30249	2.13018	1.30823	0.54144
17	17	2	1	1	1	1.11287	-0.95954	-0.10480	-0.40775
18	18	2	1	1	1	1.58593	0.50497	1.22156	-0.10476
19	19	2	1	1	1	0.94855	1.95200	-0.19290	0.73783
20	20	2	1	1	1	2.76269	-1.04592	0.28742	2.47228
21	21	2	1	1	1	1.35196	1.55843	-0.65659	0.11237
22	22	2	1	1	1	0.90882	1.64321	1.11038	0.58658
23	23	2	1	1	1	-0.11425	1.50310	0.71810	-0.02761

Obs	yprime	ysum	ybar	s2
1	5.80686	2.13846	0.71282	0.99400
2	2.77973	2.34414	0.78138	0.54282
3	7.66603	-1.51192	-0.50397	0.90147
4	3.14372	4.48984	1.49661	0.46383
5	5.01240	3.25439	1.08480	0.62446
6	1.39523	7.05446	2.35149	3.45095
7	-0.62927	3.66255	1.22085	0.33754
8	6.60595	2.51082	0.83694	0.04474
9	-0.13603	1.67149	0.55716	0.34031
10	0.27470	-1.43732	-0.47911	3.52919
11	1.36775	0.87961	0.29320	2.53262
12	4.24908	4.05270	1.35090	0.76748

13	4.66092	1.89199	0.63066	0.85120
14	2.26799	3.84092	1.28031	0.77850
15	-0.74163	1.87482	0.62494	1.44171
16	3.60497	3.97985	1.32662	0.63128
17	3.22575	-1.47209	-0.49070	0.18780
18	4.17186	1.62176	0.54059	0.44073
19	2.89710	2.49693	0.83231	1.15685
20	6.52538	1.71377	0.57126	3.15485
21	3.70392	1.01421	0.33807	1.26478
22	2.81764	3.34017	1.11339	0.27912
23	0.77151	2.19358	0.73119	0.58590

Obs	i	a	b	mu	sig2	y	y1	y2	y3
24	24	2	1	1	1	2.08307	0.88622	1.07412	-0.47401
25	25	2	1	1	1	0.78354	3.10312	0.25982	1.98184

Obs	yprime	ysum	ybar	s2
24	5.16614	1.48633	0.49544	0.71371
25	2.56709	5.34477	1.78159	2.05116

The UNIVARIATE Procedure
Variable: y

Moments

N	100000	Sum Weights	100000
Mean	1.00156793	Sum Observations	100156.793
Std Deviation	0.99964147	Variance	0.99928307
Skewness	0.00181563	Kurtosis	-0.0101015
Uncorrected SS	200241.14	Corrected SS	99927.3079
Coeff Variation	99.8076557	Std Error Mean	0.00316114

Demonstration of expected value and variance rules 6
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The UNIVARIATE Procedure
Variable: yprime

Moments

N	100000	Sum Weights	100000
Mean	3.00313586	Sum Observations	300313.586
Std Deviation	1.99928294	Variance	3.99713229
Skewness	0.00181563	Kurtosis	-0.0101015
Uncorrected SS	1301591.73	Corrected SS	399709.232
Coeff Variation	66.5731767	Std Error Mean	0.00632229

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The UNIVARIATE Procedure
Variable: ysum

Moments

N	100000	Sum Weights	100000
Mean	3.00918311	Sum Observations	300918.311
Std Deviation	1.73737373	Variance	3.01846747
Skewness	0.01349896	Kurtosis	0.01654247
Uncorrected SS	1207362.03	Corrected SS	301843.729
Coeff Variation	57.7357264	Std Error Mean	0.00549406

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The UNIVARIATE Procedure
Variable: ybar

Moments

N	100000	Sum Weights	100000
Mean	1.00306104	Sum Observations	100306.104
Std Deviation	0.57912458	Variance	0.33538527
Skewness	0.01349896	Kurtosis	0.01654247
Uncorrected SS	134151.337	Corrected SS	33538.1921
Coeff Variation	57.7357264	Std Error Mean	0.00183135

```
Demonstration of expected value and variance rules            15
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                    The UNIVARIATE Procedure
                         Variable:  s2

                              Moments

N                         100000    Sum Weights             100000
Mean                   1.00110176    Sum Observations    100110.176
Std Deviation          1.00051177    Variance            1.00102381
Skewness               2.05787409    Kurtosis            6.75960569
Uncorrected SS         200321.854    Corrected SS         100101.38
Coeff Variation        99.9410659    Std Error Mean       0.0031639
```

Figure 7.1: Frequency distribution for $Y \sim N(1, 1)$

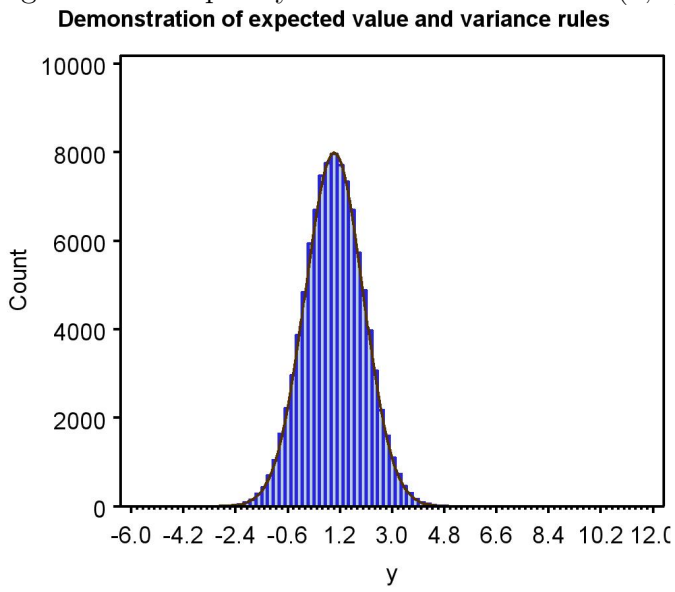


Figure 7.2: Frequency distribution for $Y' = 2Y + 1$

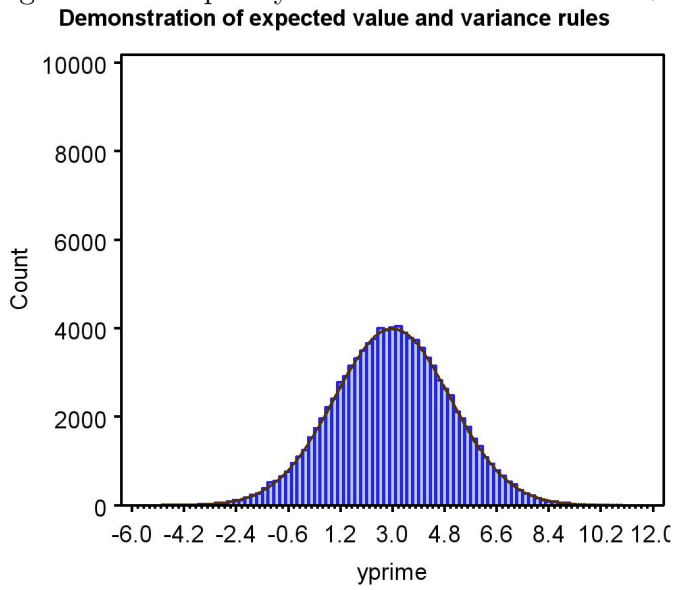


Figure 7.3: Frequency distribution for $Y_1 + Y_2 + Y_3$

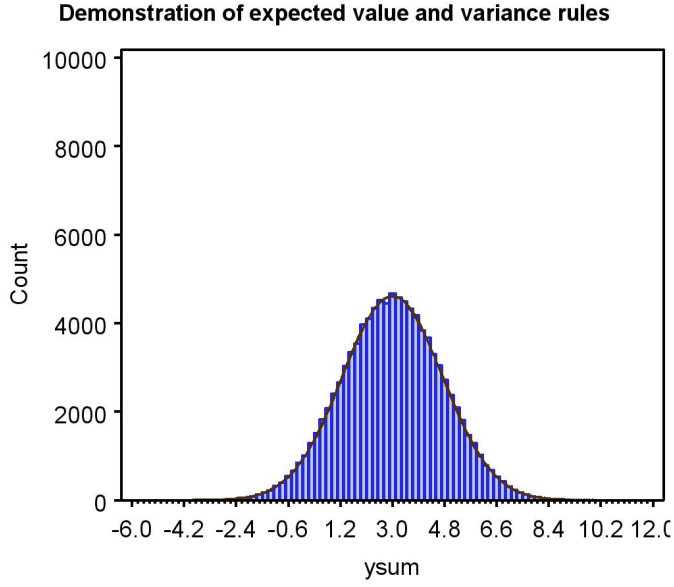
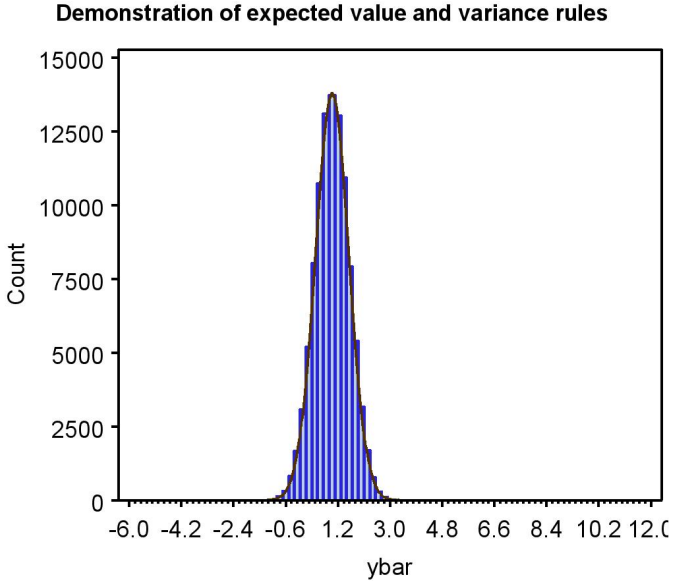


Figure 7.4: Frequency distribution for $\bar{Y}$



7.6 Central limit theorem

Suppose we randomly draw a sample $Y_1, Y_2, \ldots, Y_n$ of size n from some statistical population. In this situation, the observations are independent and have a common expected value $E[Y_i]$ and variance $Var[Y_i]$. **They may have any probability distribution, known or unknown.**

The **central limit theorem** states that the distribution of the sample mean of these random variables, namely $\bar{Y}$, approaches a normal distribution with mean $E[Y_i]$ and variance $Var[Y_i]$ as the sample size n becomes large (Mood et al. 1974). In particular, we have $\bar{Y} \sim N(E[Y_i], Var[Y_i]/n)$ for large n. The central limit theorem also holds for sums of random variables, and in this case we have $\sum Y_i \sim N(nE[Y_i], nVar[Y_i])$ for large n. **These results are true for any probability distribution - $\bar{Y}$ and $\sum Y_i$ will have a normal distribution for large sample sizes.** Note also that the variance of $\bar{Y}$ decreases as the sample size n increases. We would also expect this from our earlier results concerning the variance of $\bar{Y}$.

7.6.1 Central limit theorem - SAS demo

The operation of the central limit theorem can be demonstrated in a simple experiment using a SAS program (see below). The program models Y as a Poisson random variable with $\lambda = 1$, implying $E[Y_i] = 1$ and $Var[Y_i] = 1$. Sample means are then generated for different sample sizes, ranging from $n = 1$ to $n = 100$, in a SAS `data` step. A total of 100,000 sample means are generated for each value of n in the simulation. The program then used `proc univariate` to calculate summary statistics for these data, as well as histograms and normal quantile plots (not shown). See SAS output below.

Examining the histograms, we see that as n increases the distribution of $\bar{Y}$ approaches the normal distribution. A sample size of $n = 50$ appears sufficient to produce a distribution almost indistinguishable from normal. What is especially interesting here is that fact that the Poisson is a discrete random variable, yet the distribution of $\bar{Y}$ approaches the normal distribution, a continuous random variable.

We also observe that the variance of $\bar{Y}$ decreases as the sample size n increases, as predicted by the central limit theorem and our earlier results on the variance of $\bar{Y}$. See Table 7.3.

Table 7.3: Mean and variance of $\bar{Y}$

n	Mean of $\bar{Y}$	Variance of $\bar{Y}$	$E[Y_i]$	$Var[Y_i]/n$
1	0.997	0.998	1.000	1.000
5	1.001	0.201	1.000	0.200
10	1.000	0.100	1.000	0.100
50	1.000	0.020	1.000	0.020

SAS Program

```
* central_limit_theorem.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Demonstration of central limit theorem in action';
data cntrlmt;
        * Loop to generate 100000 random observations;
        do i = 1 to 100000;
                * A single Poisson observations with lambda = 1;
                y1 = ranpoi(0,1);
                * Mean of 5 Poisson observations;
                y5 = 0;
                do j = 1 to 5;
                        y5 = y5 + ranpoi(0,1);
                end;
                y5 = y5/5;
                * Mean of 10 Poisson observations;
                y10 = 0;
                do j = 1 to 10;
                        y10 = y10 + ranpoi(0,1);
                end;
                y10 = y10/10;
                * Mean of 50 Poisson observations;
                y50 = 0;
                do j = 1 to 50;
                        y50 = y50 + ranpoi(0,1);
                end;
                y50 = y50/50;
                * Mean of 100 Poisson observations;
                output;
        end;
        drop i j;
run;
* Print simulated data (first 25 observations);
```

```
proc print data=cntrlmt(obs=25);
run;
* Descriptive statistics, histograms, and normal quantile plots;
proc univariate data=cntrlmt;
        var y1 y5 y10 y50;
        histogram y1 y5 y10 y50 / vscale=count normal(w=3) wbarline=3 waxis=3 height=4;
        qqplot y1 y5 y10 y50 / normal waxis=3 height=4;
        symbol1 h=3;
run;
quit;
```

SAS Output

Demonstration of central limit theorem in action 1
09:25 Thursday, May 6, 2010

Obs	y1	y5	y10	y50
1	3	1.4	0.7	1.08
2	0	1.0	1.3	1.22
3	0	0.4	0.6	0.98
4	1	1.6	0.9	0.96
5	1	0.4	1.0	1.18
6	1	1.4	1.1	0.76
7	0	1.0	0.8	0.88
8	1	1.0	1.1	0.96
9	2	1.8	1.0	1.10
10	4	1.0	1.0	1.00
11	0	0.4	1.3	0.94
12	1	0.2	1.1	0.84
13	0	1.8	0.9	0.80
14	1	1.2	0.8	1.10
15	0	0.8	0.8	0.86
16	2	0.6	0.7	1.00
17	0	1.4	0.8	1.00
18	1	2.2	0.6	0.98
19	0	1.6	0.4	1.26
20	2	0.6	0.9	0.80
21	1	0.8	0.7	0.86
22	0	0.8	1.3	0.90
23	4	1.0	0.9	0.84
24	2	1.2	0.7	1.04
25	0	0.8	0.8	0.82

Demonstration of central limit theorem in action 2
09:25 Thursday, May 6, 2010

The UNIVARIATE Procedure
Variable: y1

Moments

N	100000	Sum Weights	100000
Mean	0.99708	Sum Observations	99708
Std Deviation	0.99900023	Variance	0.99800145
Skewness	0.99072058	Kurtosis	0.937192
Uncorrected SS	199216	Corrected SS	99799.1474
Coeff Variation	100.192585	Std Error Mean	0.00315912

Demonstration of central limit theorem in action 5
09:25 Thursday, May 6, 2010

The UNIVARIATE Procedure
Variable: y5

Moments

N	100000	Sum Weights	100000
Mean	1.00095	Sum Observations	100095
Std Deviation	0.44812041	Variance	0.20081191
Skewness	0.45210017	Kurtosis	0.19930735
Uncorrected SS	120271.08	Corrected SS	20080.9897
Coeff Variation	44.7695104	Std Error Mean	0.00141708

Demonstration of central limit theorem in action 8
09:25 Thursday, May 6, 2010

The UNIVARIATE Procedure
Variable: y10

Moments

N	100000	Sum Weights	100000
Mean	1.000173	Sum Observations	100017.3
Std Deviation	0.31593491	Variance	0.09981487
Skewness	0.31057365	Kurtosis	0.11464285
Uncorrected SS	110015.99	Corrected SS	9981.38701

Coeff Variation	31.5880264	Std Error Mean	0.00099907

Demonstration of central limit theorem in action 11
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The UNIVARIATE Procedure
Variable: y50

Moments

N	100000	Sum Weights	100000
Mean	1.0000688	Sum Observations	100006.88
Std Deviation	0.14104417	Variance	0.01989346
Skewness	0.12418096	Kurtosis	0.00838865
Uncorrected SS	102003.086	Corrected SS	1989.32593
Coeff Variation	14.1034468	Std Error Mean	0.00044602

Figure 7.5: Frequency distribution for Y

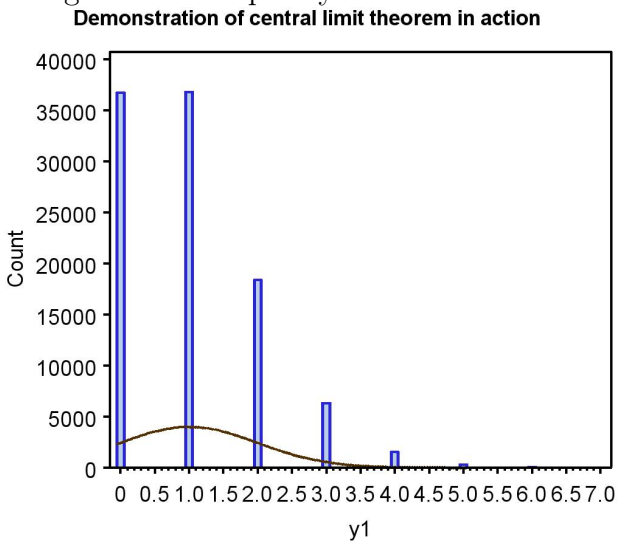


Figure 7.6: Frequency distribution for $\bar{Y}$ with $n = 5$

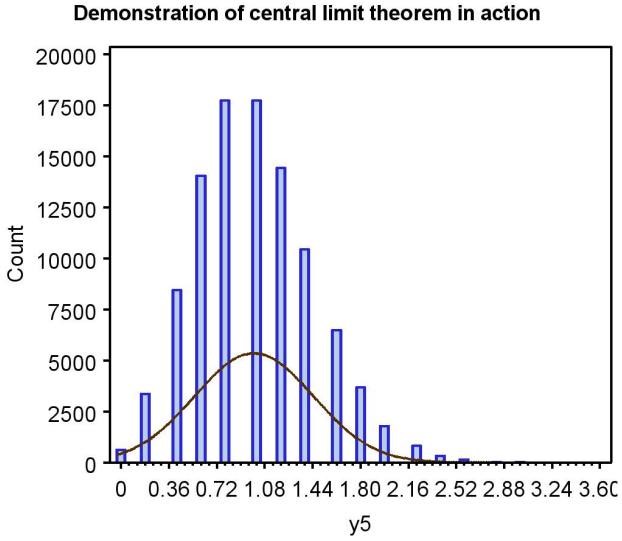


Figure 7.7: Frequency distribution for $\bar{Y}$ with $n = 10$

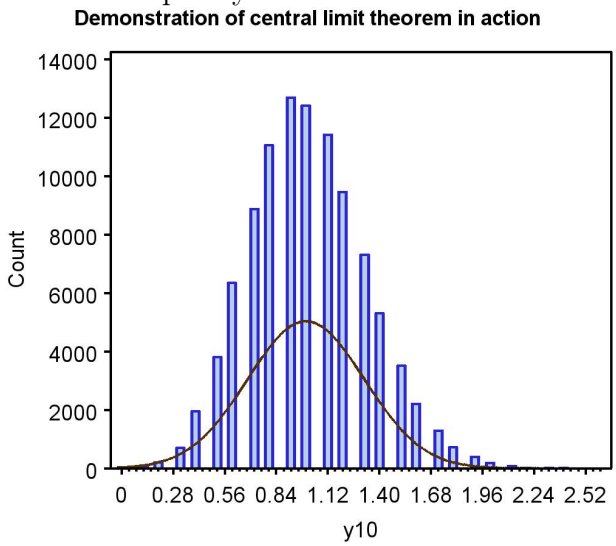
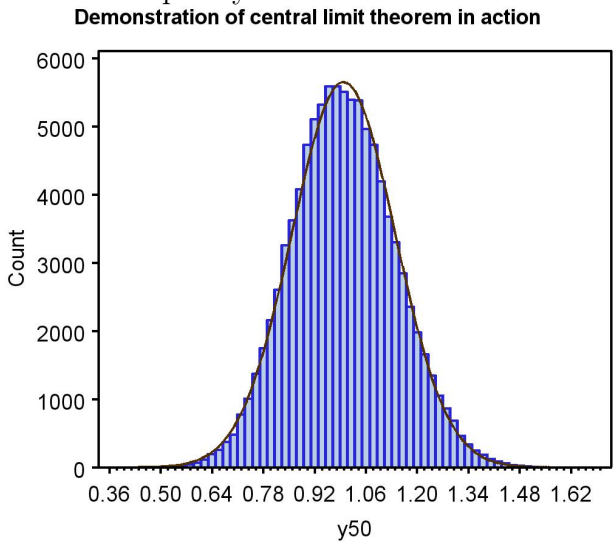


Figure 7.8: Frequency distribution for $\bar{Y}$ with $n = 50$



7.7 Applications of the central limit theorem

The central limit theorem provides a potential explanation why so many biological variables like the length of an organism and other continuous variables are apparently normal in distribution. These variables are often under the control of multiple genes and environmental factors that can behave like sums and means of random variables, and so their combined effect should generate a normal distribution of outcomes by the central limit theorem (Hartl & Clark 1989).

The theorem also applies to measurements of ecological variables like population density. To estimate population density, we often average the results of several quadrats (or whatever sampling units) to yield a single number for a given location. By the central limit theorem, these average densities will have a normal distribution for sufficiently large n.

Most of the statistical methods we will study are based on the assumption that the observations in a study or experiment have a normal distribution. This would seem a risky assumption, since many natural processes yield random variables that are not strictly normal, some examples being count data that are better modeled using the binomial and Poisson distributions. However, the tests themselves are often based on means that are assumed to have a normal distribution. The central limit theorem guarantees these means are normal provided sample sizes are sufficiently large. Thus, statistical tests based on normality should be valid for non-normal data given large enough sample sizes (see Stewart-Oaten 1995 for further discussion).

The central limit may not be sufficient to guarantee normality for smaller sample sizes, and so other approaches may be needed. One possibility would be a transformation of the observations to make their distribution closer to normal (Chapter 15). If that fails, there are nonparametric statistical procedures (Chapter 16) that are valid for any distribution, as well as ones that allow the use of other probability distributions.

7.8 References

Hartl, D. L. & Clark, A. G. *Principles of Population Genetics, Second Edition*. Sinauer Associates, Inc., Sunderland, MA.

Mood, A. M., Graybill, F. A. & Boes, D. C. (1974) *Introduction to the Theory of Statistics*. McGraw-Hill, Inc., New York, NY.

Stewart-Oaten, A. (1995) Rules and judgments in statistics: three examples. *Ecology* 76: 2001-2009.

7.9 Problems

1. Let Y_1, Y_2, and Y_3 be three independent random variables with $E[Y_i] = 2$ and $Var[Y_i] = 1$. Using the rules for expected value and variance, calculate the expected value and variance of the following quantities:

   (a) $3Y_1 + 1$.

   (b) $Y_1 + Y_2 + Y_3$.

   (c) $(Y_1 + Y_2 + Y_3)/3$.

2. Suppose that Y_1, Y_2, and Y_3 are three independent random variables, with $E[Y_i] = 3$ and $Var[Y_i] = 2$. Using the rules for expected value and variance, calculate the expected value and variance of the following quantities:

   (a) $0.5Y_2 + 2$.

   (b) $(Y_1 + Y_2 + Y_3)/3$.

   (c) $2(Y_1 + Y_2) + 3$.

3. The exponential distribution is often used to model the time until an event happens, such as the radioactive decay of an atom or mortality processes in population models. The probability density for the exponential distribution is defined as

$$f(y) = \frac{e^{-y/\lambda}}{\lambda} \tag{7.15}$$

   for $y \geq 0$. The distribution has one parameter, λ, which is the mean decay time ($E[Y] = \lambda$). A single random observation with an exponential distribution can be generated in SAS using the expression `ranexp(0)*lambda`. Modify the program `central_limit.sas` so that is generates exponential observations instead of Poisson ones, using $\lambda = 2$. Discuss how the distribution of $\bar{Y}$ changes as the sample size increases.

Chapter 8

Sampling and Estimation

We discuss in this chapter two topics that are critical to most statistical analyses. The first is **random sampling**, which is a method for obtaining observations from a statistical population that has many advantages. After obtaining a random sample, the next step of the analysis is the selection of a probability distribution to model the observations, such as the Poisson or normal distributions. One then seeks to **estimate the parameters** of these distributions (λ, μ, σ^2, etc.) using the information contained in the random sample, the second topic of this chapter. We will examine one common method of parameter estimation called maximum likelihood.

8.1 Random samples

A basic assumption of many statistical procedures is that the observations are a **random sample** from a statistical population (see Chapter 3). A sample from a statistical population is a random sample if (1) each element of the population has an equal probability of being sampled, and (2) the observations in the sample are independent (Thompson 2002). This definition has a number of implications. It implies that a random sample will resemble the statistical population from which it is drawn, especially as the sample size n increases, because each element of the population has an equal chance of being in the sample. Random sampling also implies there is no connection or relationship between the observations in the sample, because they are independent of one another.

What are some ways of obtaining a random sample? Suppose we are

interested in the distribution of body length for insects of a given species, say in a particular forest. This defines the statistical population of interest. One way to obtain a random sample would be to number all the insects, and then write the numbers on pieces of paper and place them in a hat. After mixing the pieces, one would draw n numbers from the hat (without peeking) and collect only those insects corresponding to these numbers. Although impractical, because of difficulties in locating and numbering individual insects, this method would in fact yield a random sample of the insect population. Each member of the insect population would have an equal probability of being selected from the hat, and the observations would also be independent. This method of sampling is more useful for statistical populations were the number of elements or members is relatively small and can be individually identified, as in surveys of human populations (Thompson 2002).

A more feasible way of sampling insects would be to place traps in the forest and in this way sample the population. If we want to successfully approximate a random sample with our trapping scheme, however, some knowledge of the biology of the organism is essential. For example, suppose that insect size varies in space because of differences in food plants or microclimate. A single trap deployed at only one location could therefore yield insects different in length than those in the overall population. A better sampling scheme would deploy multiple traps at several locations within the forest. The location of the traps could be randomly chosen to avoid conscious or unconscious biases by the trapper, such as deploying the traps close to a road for convenience. There is also the problem that insects susceptible to trapping could differ in length from the general population. This implies that the population actually sampled could differ from the target statistical population, and a careful analyst would consider this possibility. Thus, the biology of the organism plays an integral role in designing an appropriate sampling scheme.

8.2 Parameter estimation

Suppose we have obtained a random sample from some statistical population, say the lengths of insects trapped in a forest, or the counts of the insects in each trap. The first step faced by the analyst is to chose a probability distribution to model the data in the sample. For insect lengths, a normal distribution could be a plausible model, while counts of the insects per trap

might have a Poisson distribution. Once a distribution has been selected, the next task is to estimate the parameters of the distribution using the sample data. The dominant method of parameter estimation in modern statistics is **maximum likelihood**. This method has a number of desirable statistical properties although it can also be computationally intensive.

Maximum likelihood obtains estimates of the parameters using a mathematical function (see Chapter 2) known as the likelihood function. The likelihood function gives the probability or density of the observed data as a function of the parameters in the probability distribution. For example, the likelihood function for Poisson data would be a function of the Poisson parameter λ. We then seek the maximum value of the likelihood function (hence the name maximum likelihood) across the potential range of parameter values. The parameter values that maximize the likelihood are the maximum likelihood estimates. In other words, **the maximum likelihood estimates are the parameter values that give the largest probability (or probability density) for the observed data.**

8.2.1 Maximum likelihood for Poisson data

We will first illustrate estimation using maximum likelihood with a random sample drawn from a statistical population where the observations are Poisson. For simplicity, let $n = 3$ and suppose the observed values are $Y_1 = 8$, $Y_2 = 5$, and $Y_3 = 6$. We begin by calculating the probability of observing this sample, which in fact is its likelihood function. Because we have a random sample, the Y_i values are independent of each other, and so this probability is the product of the probability for each Y_i. We have

$$L(\lambda) = P[Y_1 = 8] \times P[Y_2 = 5] \times P[Y_3 = 6] \tag{8.1}$$

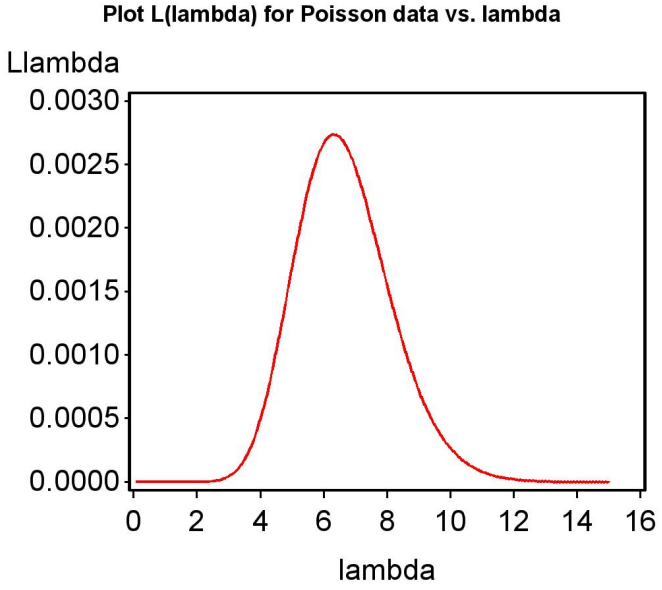
$$= \frac{e^{-\lambda}\lambda^8}{8!} \times \frac{e^{-\lambda}\lambda^5}{5!} \times \frac{e^{-\lambda}\lambda^6}{6!} \tag{8.2}$$

The notation $L(\lambda)$ is used for likelihood functions and indicates the likelihood is a function of the parameter λ of the Poisson distribution. The method of maximum likelihood estimates λ by finding the value of λ that maximizes this function (Mood *et al.* 1974). Note that the location of the maximum will vary with the data in the sample.

We can find the maximum likelihood estimate graphically by plotting $L(\lambda)$ as function of λ (Fig. 8.1). For these particular data values, the maximum occurs at $\lambda = 6.3$, and so the maximum likelihood estimate (often

abbreviated MLE) of λ is this value. This is also the value of $\bar{Y}$ for these data, which suggests that $\bar{Y}$ might be the maximum likelihood estimator of λ in general. This can also be shown mathematically using derivatives. Let y_1,

Figure 8.1: Plot of $L(\lambda)$ vs. λ



y_2, and y_3 be the observed values of Y_1, Y_2, and Y_3. The likelihood function can then be written as

$$L(\lambda) = \frac{e^{-\lambda}\lambda^{y_1}}{y_1!} \times \frac{e^{-\lambda}\lambda^{y_2}}{y_2!} \times \frac{e^{-\lambda}\lambda^{y_3}}{y_3!} = \frac{e^{-3\lambda}\lambda^{y_1+y_2+y_3}}{y_1!y_2!y_3!} \tag{8.3}$$

We want to find the maximum of $L(\lambda)$ (Eq. 8.3), which should occur when the derivative of this function with respect to λ equals zero. This follows because the derivative is the slope of a function, and at the maximum the slope is equal to zero. Differentiating $L(\lambda)$ with respect to λ and simplifying, we obtain

$$\frac{dL(\lambda)}{d\lambda} = \frac{e^{-3\lambda}}{y_1!y_2!y_3!}\left[(y_1+y_2+y_3)\lambda^{y_1+y_2+y_3-1} - 3\lambda^{y_1+y_2+y_3}\right]. \tag{8.4}$$

This derivative can only equal zero if the term in square brackets is zero:

$$\left[(y_1+y_2+y_3)\lambda^{y_1+y_2+y_3-1} - 3\lambda^{y_1+y_2+y_3}\right] = 0 \tag{8.5}$$

or

$$(y_1 + y_2 + y_3)\lambda^{y_1+y_2+y_3-1} = 3\lambda^{y_1+y_2+y_3}. \tag{8.6}$$

Canceling the quantity $\lambda^{y_1+y_2+y_3}$ from both sides of this equation, we find that

$$(y_1 + y_2 + y_3)\lambda^{-1} = 3, \tag{8.7}$$

or

$$\hat{\lambda} = \frac{y_1 + y_2 + y_3}{3}. \tag{8.8}$$

Note that this is the sample mean $\bar{Y}$ for $n = 3$, and it is can be shown that $\bar{Y}$ is the maximum likelihood estimator of λ for any n. Statisticians often write the estimator of a parameter like λ using the notation $\hat{\lambda}$, pronounced 'λ-hat.' An **estimator** can be thought of as the formula or recipe for obtaining an estimate of a parameter, with the **estimate** itself obtained by plugging actual data values into the estimator.

8.2.2 Poisson likelihood function - SAS demo

We can use a SAS program to further illustrate the behavior of the likelihood function for Poisson data (see program listing below). In particular, we will show how $L(\lambda)$ changes as the observed data and the sample size n changes. The program first generates n random Poisson observations for a specified Poisson parameter value of $\lambda = 6$ (`mu_parameter = 6`). It then plots $L(\lambda)$ across a range of λ values. In this scenario we actually know the underlying value of λ and can see how well maximum likelihood estimates its value. See SAS program below.

The program makes extensive use of loops in the data step, to generate the Poisson data and also values of the likelihood function for different values of λ. One new feature of this program is the use of a SAS macro variable(SAS Institute Inc. 2014). In this case, a macro variable labeled `n` is defined and assigned a value of 3 using the command

```
%let n = 3;
```

We can then refer to this value throughout the program using the notation `&n`. Otherwise, if we wanted to change the sample size n in the program we would have to type in a new value everywhere sample size is used in the calculations.

SAS program

```
* likepois_random.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Plot L(lambda) for Poisson data vs. lambda";
data likepois;
    * Generate n random Poisson observations with parameter lambda;
    %let n = 3;
    lambda_parameter = 6;
    array ydata (&n) y1-y&n;
    do i=1 to &n;
        ydata(i) = ranpoi(0,lambda_parameter);
    end;
    * Find likelihood as function of lambda;
    do lambda=0.1 to 15 by 0.1;
        Llambda = 1;
        do i=1 to &n;
            Llambda = Llambda*pdf('poisson',ydata(i),lambda);
        end;
        output;
    end;
run;
* Print data;
proc print data=likepois;
run;
* Plot likelihood as a function of lambda;
proc gplot data=likepois;
    plot Llambda*lambda=1 / vaxis=axis1 haxis=axis1;
    symbol1 i=join v=none c=red width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

Examining the SAS output and graphs from the first two runs of the program (Fig. 8.2, 8.3), we see that the likelihood function is different. This is because the observed data are different for each run. The peak in the likelihood function always occurs at the value of $\bar{Y}$ for each data set, and this is the maximum likelihood estimate of λ.

The last run shows the effect of increasing the sample size in the program, from $n = 3$ to $n = 10$. Note that the peak of the likelihood function lies quite close to the specified value $\lambda = 6$ (Fig. 8.4). This illustrates an important property of maximum likelihood estimators - they converge on the true value

as $n \to \infty$. This property is known as consistency in mathematical statistics.

SAS output

Plot L(lambda) for Poisson data vs. lambda 1
11:12 Tuesday, January 26, 2010

Obs	lambda_ parameter	y1	y2	y3	i	lambda	Llambda
1	6	6	5	2	4	0.1	4.2871E-19
2	6	6	5	2	4	0.2	2.6018E-15
3	6	6	5	2	4	0.3	3.7512E-13
4	6	6	5	2	4	0.4	1.1697E-11
5	6	6	5	2	4	0.5	1.5762E-10
6	6	6	5	2	4	0.6	.000000001
7	6	6	5	2	4	0.7	.000000007
8	6	6	5	2	4	0.8	.000000029
9	6	6	5	2	4	0.9	.000000099
10	6	6	5	2	4	1.0	.000000288
11	6	6	5	2	4	1.1	.000000737
12	6	6	5	2	4	1.2	.000001692
13	6	6	5	2	4	1.3	.000003548
14	6	6	5	2	4	1.4	.000006888
15	6	6	5	2	4	1.5	.000012512
16	6	6	5	2	4	1.6	.000021449
17	6	6	5	2	4	1.7	.000034945
18	6	6	5	2	4	1.8	.000054426
19	6	6	5	2	4	1.9	.000081428
20	6	6	5	2	4	2.0	.000117511
21	6	6	5	2	4	2.1	.000164154
22	6	6	5	2	4	2.2	.000222642
23	6	6	5	2	4	2.3	.000293959
24	6	6	5	2	4	2.4	.000378689
25	6	6	5	2	4	2.5	.000476944

etc.

Figure 8.2: Plot of $L(\lambda)$ vs. λ for $n = 3$, first run

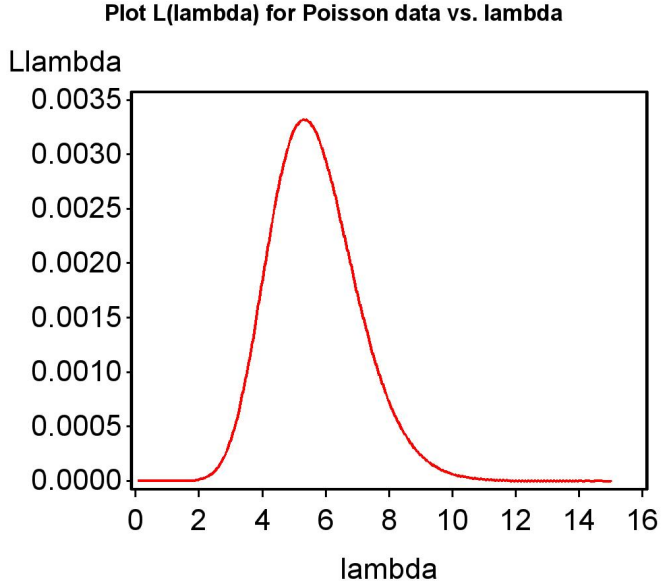


Figure 8.3: Plot of $L(\lambda)$ vs. λ for $n = 3$, second run

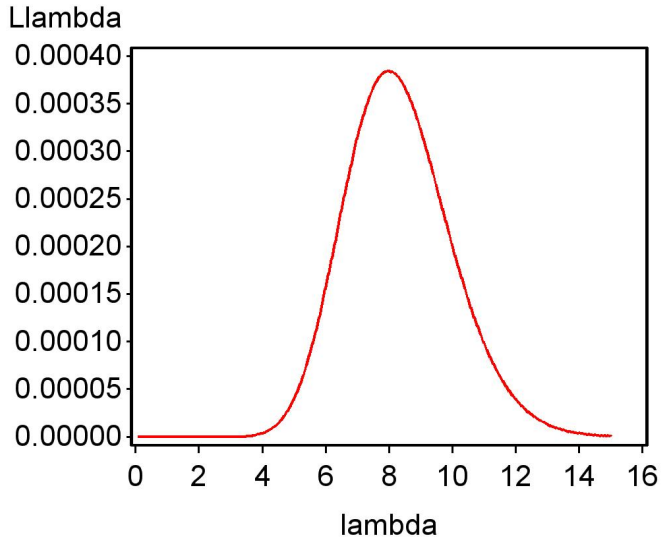
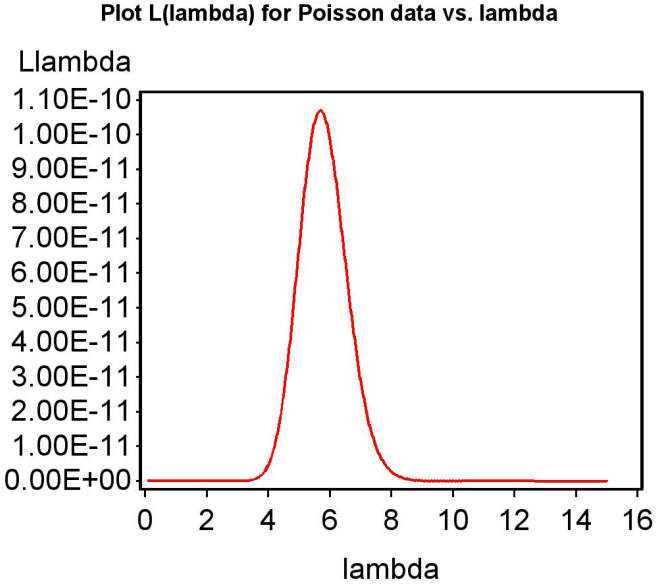


Figure 8.4: Plot of $L(\lambda)$ vs. λ for $n = 10$



8.2.3 Maximum likelihood for normal data

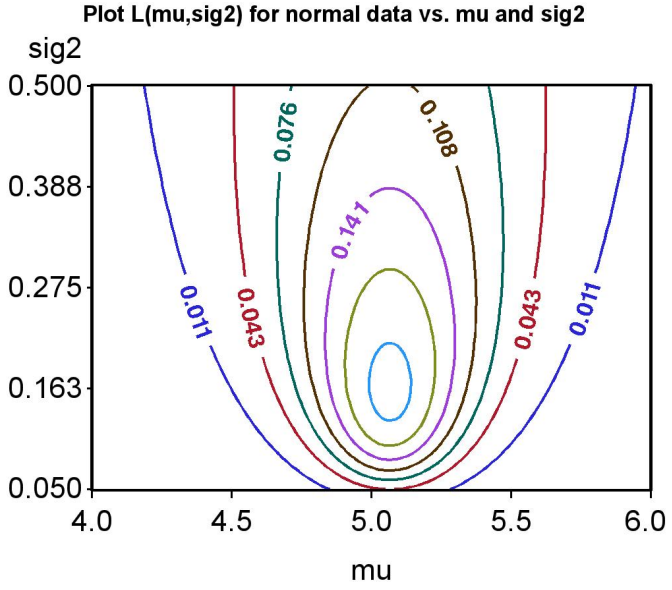
Now suppose we draw a random sample from a population with a normal distribution, such as body lengths, etc. For simplicity, let $n = 3$ again and the observed values be $Y_1 = 4.5$, $Y_2 = 5.4$, and $Y_3 = 5.3$. The likelihood function in this case is the probability density values for the observed data:

$$L(\mu, \sigma^2) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(4.5-\mu)^2}{\sigma^2}} \times \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(5.4-\mu)^2}{\sigma^2}} \times \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(5.3-\mu)^2}{\sigma^2}}. \tag{8.9}$$

Note that the terms in the likelihood for normal data are probability densities, instead of probabilities as with Poisson data.

We can find the maximum likelihood estimate graphically by plotting $L(\mu, \sigma^2)$ as function of μ and σ^2. The likelihood function in this case describes a dome-shaped surface (Fig. 8.5). With these particular data, the maximum occurs at about $\mu = 5.07$ and $\sigma^2 = 0.16$, and so these are the maximum likelihood estimates of μ and σ^2.

Figure 8.5: Plot of $L(\mu, \sigma^2)$ vs. μ and σ^2



Using a bit of calculus, it can be shown that the maximum likelihood estimators of these parameters are, for any sample size n:

$$\hat{\mu} = \bar{Y} \tag{8.10}$$

and

$$\hat{\sigma}^2 = \frac{\Sigma_{i=1}^{n}(Y_i - \bar{Y})^2}{n}. \tag{8.11}$$

Note that does not quite equal the sample variance s^2, which uses $n - 1$ (rather than n) in the denominator:

$$s^2 = \frac{\Sigma_{i=1}^{n}(Y_i - \bar{Y})^2}{n-1}. \tag{8.12}$$

Recall that s^2 is an unbiased estimator of σ^2, and so $\hat{\sigma}^2$ derived using maximum likelihood is actually a biased estimator of σ^2. It would consistently generate values that underestimate σ^2 because n is greater than $n - 1$. For cases like this one where bias is known, most analysts would use a bias-corrected version of the maximum likelihood estimator (i.e., $n - 1$ rather than n in the denominator).

8.2.4 Normal likelihood function - SAS demo

We will use another SAS program to illustrate the behavior of the likelihood function for normal data. The program first generates n random normal observations for a specified, known value of $\mu = 5$ and $\sigma^2 = 0.25$. It then plots the likelihood function across a range of possible μ and σ^2 values. See SAS program below.

Examining the SAS output and graphs from the first two runs of the program, we see that the likelihood function changes with the observed data. The peak always occurs at $\hat{\mu}$ and $\hat{\sigma}^2$ for each data set. The last run shows the effect of increasing the sample size from $n = 3$ to $n = 10$. Note that the peak of the likelihood function lies quite close to the specified values of $\mu = 5$ and $\sigma^2 = 0.25$. This again illustrates the consistency of maximum likelihood estimates.

SAS program

```
* likenorm_random.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Plot L(mu,sig2) for normal data vs. mu and sig2";
data likenorm;
    * Generate n random normal observations with parameters mu and sig2;
    %let n = 3;
    mu_parameter = 5; sig2_parameter = 0.25; sig_parameter = sqrt(sig2_parameter);
    array ydata (&n) y1-y&n;
    do i=1 to &n;
        ydata(i) = mu_parameter + sig_parameter*rannor(0);
    end;
    * Find likelihood as a function of mu and sig2;
    do mu=4 to 6 by 0.01;
        do sig2=0.05 to 0.5 by 0.01;
            sig = sqrt(sig2);
            Lmusig2 = 1;
            do i=1 to &n;
                Lmusig2 = Lmusig2*pdf('normal',ydata(i),mu,sig);
            end;
            output;
        end;
    end;
run;
* Print data, first 25 observations;
proc print data=likenorm(obs=25);
run;
* Plot likelihood as a function of mu and sig2;
* Contour plot version;
proc gcontour data=likenorm;
    plot sig2*mu=Lmusig2 / autolabel nolegend vaxis=axis1 haxis=axis1;
    symbol1 height=1.5 font=swissb width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

SAS output

Plot L(mu,sig2) for normal data vs. mu and sig2 1
14:55 Wednesday, June 2, 2010

Obs	mu_parameter	sig2_parameter	sig_parameter	y1	y2	y3	i	mu	sig2	sig	Lmu_sig2
1	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.05	0.22361	1.8951E-21
2	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.06	0.24495	5.4739E-18
3	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.07	0.26458	1.5654E-15
4	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.08	0.28284	1.0597E-13
5	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.09	0.30000	2.7536E-12
6	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.10	0.31623	3.6678E-11
7	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.11	0.33166	3.0097E-10
8	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.12	0.34641	.000000002
9	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.13	0.36056	.000000007
10	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.14	0.37417	.000000026
11	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.15	0.38730	.000000076
12	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.16	0.40000	.000000193
13	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.17	0.41231	.000000437
14	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.18	0.42426	.000000900
15	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.19	0.43589	.000001709
16	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.20	0.44721	.000003034
17	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.21	0.45826	.000005081
18	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.22	0.46904	.000008092
19	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.23	0.47958	.000012340
20	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.24	0.48990	.000018118
21	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.25	0.50000	.000025733
22	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.26	0.50990	.000035493
23	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.27	0.51962	.000047701
24	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.28	0.52915	.000062644
25	5	0.25	0.5	5.86023	4.75626	4.95540	4	4	0.29	0.53852	.000080587

Figure 8.6: Plot of $L(\mu, \sigma^2)$ vs. μ and σ^2 for $n = 3$, first run

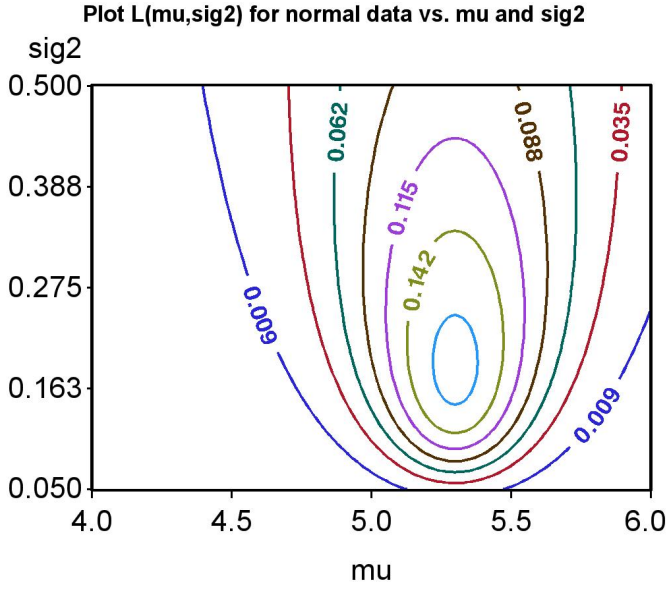


Figure 8.7: Plot of $L(\mu, \sigma^2)$ vs. μ and σ^2 for $n = 3$, second run

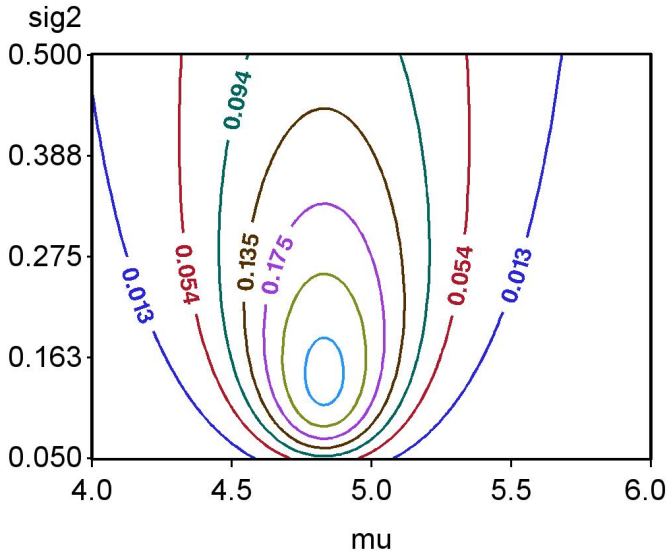
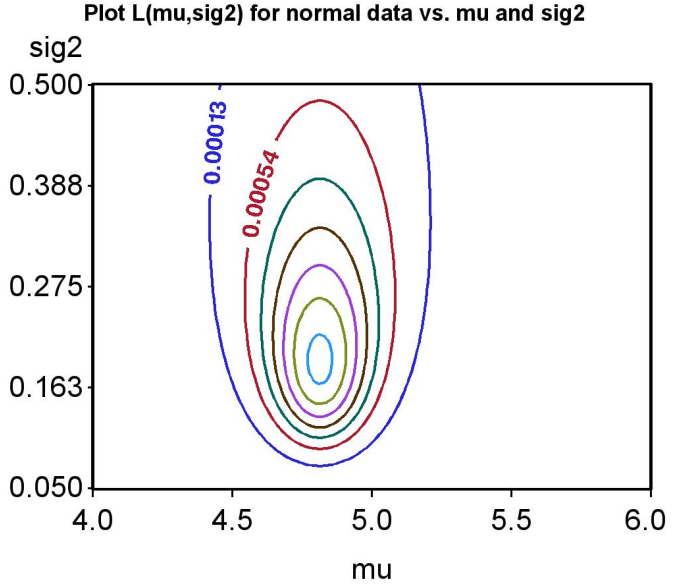


Figure 8.8: Plot of $L(\mu, \sigma^2)$ vs. μ and σ^2 for $n = 10$



8.3 Optimality of maximum likelihood estimates

Why should we use maximum likelihood estimates? There are other methods of parameter estimation, but maximum likelihood estimates are optimal in a number of ways (Mood *et al.* 1974). We have already seen that they are **consistent**, approaching the true parameter values as sample size increases. Increasing the sample size also reduces the variance of these estimators. We can observe this behavior for $\hat{\mu} = \bar{Y}$, the estimator of μ for the normal distribution. Recall that the variance of $\bar{Y}$ is σ^2/n, which decreases for large n. Maximum likelihood estimates are also **asymptotically unbiased**, meaning their expected value approaches the true value of the parameter as the sample size n increases. We can see this in operation for $\hat{\sigma}^2$ (Eq. 8.11), the maximum likelihood estimator of σ^2, vs. s^2 (Eq. 8.12), an unbiased estimator of σ^2. Note that the difference between n vs. $n-1$ in the denominator becomes very small as n increases. Finally, maximum likelihood estimates are **asymptotically normal**, meaning their distribution approaches the normal distribution for large n.

There are other uses for the likelihood function besides parameter estimation. We will later see how the likelihood function can be used to develop statistical tests called likelihood ratio tests. Many of the statistical tests we will study are actually likelihood ratio tests. Likelihood methods provide an essential tool for developing new statistical procedures, provided that we can specify a probability distribution for the data.

8.4 References

Mood, A. M., Graybill, F. A. & Boes, D. C. (1974) *Introduction to the Theory of Statistics*. McGraw-Hill, Inc., New York, NY.

Thompson, S. K. (2002) *Sampling*. John Wiley & Sons, Inc., New York, NY.

SAS Institute Inc. (2014) *SAS 9.4 Macro Language: Reference, Fourth Edition*. SAS Institute Inc., Cary, NC.

8.5 Problems

1. The exponential distribution is a continuous distribution that is used to model the time until a particular event occurs. For example, the time when a radioactive particle decays is often modeled using an exponential distribution. If a variable Y has a exponential distribution, then its probability density is given by the formula

$$f(y) = \frac{e^{-y/\lambda}}{\lambda} \tag{8.13}$$

for $y \geq 0$. The distribution has one parameter, λ, which is the mean decay time ($E[Y] = \lambda$).

   (a) Use SAS and the program `fplot.sas` to plot the exponential probability density with $\lambda = 2$, for $0 \leq y \leq 5$. Attach your SAS program and output.

   (b) Suppose you have a sample of four observations y_1, y_2, y_3 and y_4 from the exponential distribution. What would be the likelihood function for these observations?

   (c) Plot the likelihood function for $y_1 = 1$, $y_2 = 2$, $y_3 = 2$ and $y_4 = 3$ over a range of λ values. Show that the maximum occurs at $\hat{\lambda} = \bar{Y}$, the maximum likelihood estimator of λ. Attach your SAS program and output.

2. The geometric distribution is a discrete distribution that is used to model the time until a particular event occurs. Consider tossing a coin – the number of tosses before a head appears would have a geometric distribution. If a variable Y has a geometric distribution, then the probability that Y takes a particular value y is given by the formula

$$P[Y = y] = f(y) = p(1-p)^y \tag{8.14}$$

where p is the probability of observing the event on a particular trial, and $y = 0, 1, 2, \ldots, \infty$. The distribution has only one parameter, p.

   (a) Use SAS and the program `fplot.sas` to plot this probability distribution for $p = 0.5$, for $y = 0, 1, \ldots, 10$. Attach your SAS program and output.

(b) Suppose you have a sample of three observations y_1, y_2, and y_3 from the geometric distribution. What would be the likelihood function for these observations?

(c) Plot the likelihood function for $y_1 = 1$, $y_2 = 2$, and $y_3 = 3$ over a range of p values. Show that the maximum occurs at $\hat{p} = 1/(\bar{Y} + 1)$, the maximum likelihood estimator of p. Attach your SAS program and output.

Chapter 9

Confidence Intervals

In the preceding chapter, we examined the maximum likelihood method for estimating the parameters of a statistical population, using a random sample from that population. For example, if we have a sample from a population with a normal distribution, we can estimate the parameter μ of this population using the sample mean $\bar{Y}$. What we will now examine is a common method for characterizing the precision of these estimates, known as **confidence intervals**. Given an estimate $\bar{Y}$ of μ, say, we will learn how to calculate an interval that will contain the true population μ with a certain probability. A narrow interval indicates the parameter μ is reliably estimated, while a broad one indicates substantial uncertainty as to its value.

9.1 Preliminaries to confidence intervals

We now discuss some material that is essential for the construction of confidence intervals and later in hypothesis testing. We first review some results from Chapter 8 on parameter estimation for the normal distribution, then derive some new results. We then examine some distributions associated with sampling from the normal distributions, not surprisingly called **sampling distributions**.

9.1.1 Parameters and estimates

Confidence intervals are based on estimates of population parameters, such as μ and σ^2 for populations with a normal distribution. Our previous results

on parameter estimation suggest that $\bar{Y}$ and s^2 are reasonable estimators of μ and σ^2. The sample standard deviation $s = \sqrt{s^2}$ is typically used to estimate the population standard deviation σ.

We also want to estimate the variance and standard deviation of the sample mean $\bar{Y}$. Recall that for a random sample Y_1, Y_2, ... Y_n with any distribution,

$$Var[\bar{Y}] = \frac{Var[Y_i]}{n} \tag{9.1}$$

where $Var[Y_i]$ is the variance of Y_i (Chapter 7). For a random sample where the observations are normal, this translates to

$$Var[\bar{Y}] = \frac{\sigma^2}{n} \tag{9.2}$$

because $Var[Y_i] = \sigma^2$ for the normal. If we use s^2 to estimate σ^2, we can therefore estimate $Var[\bar{Y}]$ using s^2/n and $\sigma/\sqrt{n}$ using $s/\sqrt{n}$.

The table below summarizes the different parameters, their estimators, and common terminology for these quantities:

Table 9.1: Parameters and their estimators

Parameter	Estimator	Terminology
μ	$\bar{Y}$	Sample mean
σ^2	s^2	Sample variance
σ	s	Sample standard deviation
$\frac{\sigma^2}{n}$	$\frac{s^2}{n}$	Sample variance of the mean
$\frac{\sigma}{\sqrt{n}}$	$\frac{s}{\sqrt{n}}$	Standard error of the mean

The term **standard error** always refers to the standard deviation of a statistic, such as $\bar{Y}$. The term standard deviation used without qualification usually means the standard deviation s of items in a random sample from a population.

9.1.2 Sampling distributions

In this section, we will first examine the probability distribution of the estimator $\bar{Y}$. We then examine the distributions of some quantities involving $\bar{Y}$ and the sample variance s^2, known as sampling distributions. These sampling distributions will be used to construct confidence intervals and also play an important role in hypothesis testing (Chapter 10).

Distribution of $\bar{Y}$

Suppose we have a random sample $Y_1, Y_2, ..., Y_n$ from a statistical population with a normal distribution, in particular that $Y_i \sim N(\mu, \sigma^2)$ and are independent of each other. It can be shown that

$$\bar{Y} \sim N\left(\mu, \frac{\sigma^2}{n}\right). \tag{9.3}$$

Thus, the sample mean of normal observations also has a normal distribution with the same mean μ, but with variance equal to σ^2/n, not σ^2 (Mood *et al.* 1974).

Note that the distribution of $\bar{Y}$ will be approximately normal for any distribution provided n is large, thanks to the central limit theorem. Thus, for large sample sizes we have $\bar{Y} \sim N(E[Y], Var[Y]/n)$ for any probability distribution. This result has important statistical implications. **Confidence intervals and hypothesis testing procedures often assume that $\bar{Y}$ is normally distributed, and this will be approximately true if n is sufficiently large.** These statistical procedures are therefore robust to departures from normality in the data for large n.

We also learned earlier that if $Y \sim N(\mu, \sigma^2)$, then the transformed variable $(Y - \mu)/\sigma$ has a standard normal distribution, or $(Y - \mu)/\sigma = Z \sim N(0, 1)$. Combining these two results, we find that

$$\frac{\bar{Y} - \mu}{\sqrt{\sigma^2/n}} = \frac{\bar{Y} - \mu}{\sigma/\sqrt{n}} \sim N(0, 1) \tag{9.4}$$

Thus, the quantity $\frac{\bar{Y}-\mu}{\sigma/\sqrt{n}}$ has a standard normal distribution. We will use this sampling distribution to obtain a confidence interval for μ, for the case where σ^2 is known from other information.

We will also need to find certain intervals with a specified probability using the standard normal distribution, in order to construct confidence intervals. In general, we will need to find a positive value c such that

$$P[-c_\alpha < Z < c_\alpha] = 1 - \alpha \tag{9.5}$$

for this purpose, where typically $\alpha = 0.05$ or 0.01. The values of c_α that satisfy this probability are often called **critical values**, a term that also applies to other probability distributions. We use the notation c_α because

this quantity depends on the value of α. To find c_α, we first express this probability in terms of Table Z. We have

$$\begin{aligned} P[-c_\alpha < Z < c_\alpha] &= P[Z < c_\alpha] - P[Z < -c_\alpha] && (9.6)\\ &= P[Z < c_\alpha] - (1 - P[Z < c_\alpha]) && (9.7)\\ &= 2P[Z < c_\alpha] - 1. && (9.8) \end{aligned}$$

If we set $2P[Z < c_\alpha] - 1 = 1 - \alpha$ and rearrange, we get

$$P[Z < c_\alpha] = (2 - \alpha)/2 = 1 - \alpha/2. \tag{9.9}$$

Therefore, we examine Table Z for a value of c_α such that $P[Z < c_\alpha] = 1 - \alpha/2$. For $\alpha = 0.05$, we would look for $c_{0.05}$ such that $P[Z < c_{0.05}] = 1 - 0.05/2 = 0.975$ and find that $c_{0.05} = 1.96$ is answer. Similarly, for $\alpha = 0.01$ we seek $c_{0.01}$ such that $P[Z < c_{0.01}] = 1 - 0.01/2 = 0.995$. There is no value in Table Z that gives quite this probability, although we can see 2.57 and 2.58 are close. The exact answer is $c_{0.01} = 2.576$.

t distribution

Another important sampling distribution is the t distribution. This distribution has a single parameter, called the degrees of freedom, that governs the shape of the distribution. It can be shown that the quantity

$$\frac{\bar{Y} - \mu}{s/\sqrt{n}} \sim t_{n-1} \tag{9.10}$$

(Mood *et al.* 1974). Here the symbol 't_{n-1}' stands for the t distribution with $n-1$ degrees of freedom, where n is the sample size in $\bar{Y}$. Degrees of freedom is often abbreviated as '*df*'.

The t distribution resembles the standard normal distribution in being bell-shaped, except that it has more probability in the tails and less in the center of the distribution (Fig. 9.1). Roughly speaking, the t distribution has heavier tails than the normal because $\bar{Y}$ and s are both random quantities in Eq. 9.10, making their ratio more variable than for Eq. 9.4 where only $\bar{Y}$ is random. However, as $n \to \infty$ the t distribution does approach the standard normal distribution. We will use this sampling distribution to obtain a confidence interval for μ, when σ^2 is estimated using the sample variance s^2.

What is the origin of the term degrees of freedom? Recall that the sample standard deviation s is obtained from the sample variance, calculated using the formula

$$s^2 = \frac{\Sigma_{i=1}^{n}(Y_i - \bar{Y})^2}{n-1}. \tag{9.11}$$

Notice that the sample variance s^2 is composed of terms of the form $Y_i - \bar{Y}$. Although there are n of these terms, they also sum to zero ($\Sigma_i^n(Y_i - \bar{Y}) = 0$). This implies that if $n-1$ terms are known, we can always determine the remaining term because of this relationship, implying there are really only $n-1$ free, independent terms in s^2 (Mood et al. 1974). Hence the name degrees of freedom.

Figure 9.1: Plot of the t distribution for different degrees of freedom

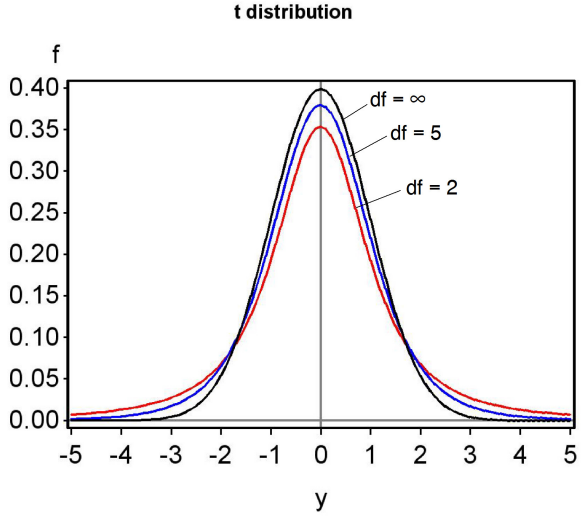


Table T gives the quantiles of the t distribution for different values of the degrees of freedom and the cumulative probability p. We will also need to find intervals of the form

$$P[-c_{\alpha,df} < T < c_{\alpha,df}] = 1 - \alpha, \tag{9.12}$$

where $c_{\alpha,df}$ is a positive number, T has a t distribution, for $\alpha = 0.05$ or 0.01. We use the notation $c_{\alpha,df}$ because this quantity will depend on both α and the degrees of freedom. We proceed as before by expressing this probability

in terms of Table T. We have

$$P[-c_{\alpha,df} < T < c_{\alpha,df}] = P[T < c_{\alpha,df}] - P[T < -c_{\alpha,df}] \tag{9.13}$$
$$= P[T < c_{\alpha,df}] - (1 - P[T < c_{\alpha,df}]) \tag{9.14}$$
$$= 2P[T < c_{\alpha,df}] - 1. \tag{9.15}$$

If we set $2P[T < c_{\alpha,df}] - 1 = 1 - \alpha$ and rearrange, we get

$$2(1 - P[T < c_{\alpha,df}]) = \alpha. \tag{9.16}$$

Because $P[T < c_{\alpha,df}]$ is essentially p for this table, we simply look across the row corresponding to $2(1-p)$ at the top and find the column corresponding to α. For $\alpha = 0.05$, we see that for $df = 10$ the answer is $c_{0.05,10} = 2.228$. For $\alpha = 0.01$ and $df = 10$, the answer is $c_{0.01,10} = 3.169$.

χ^2 distribution

One other common sampling distribution is the χ^2 (chi-square) distribution, which also has a parameter called the degrees of freedom. It can be shown that the quantity

$$\frac{(n-1)s^2}{\sigma^2} \sim \chi^2_{n-1} \tag{9.17}$$

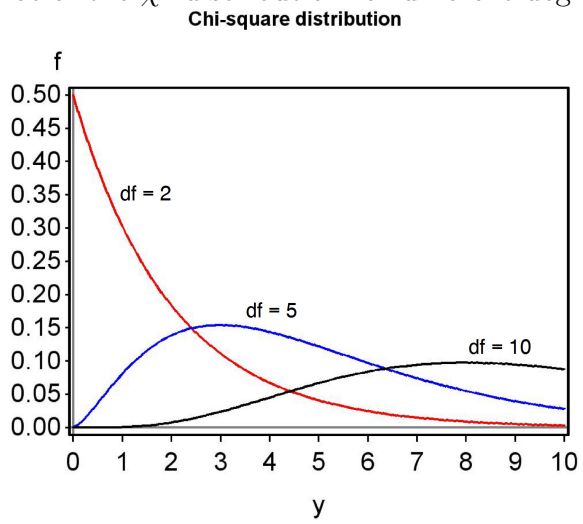
(Mood *et al.* 1974). Here the symbol 'χ^2_{n-1}' stands for a χ^2 distribution with $n-1$ degrees of freedom. The degrees of freedom parameter controls the shape of the χ^2 distribution (Fig. 9.2). The χ^2 distribution is only defined for positive values, because s^2 is always positive, and its distribution shifts to the right (large values become more likely) as n and the degrees of freedom increases. We will use this sampling distribution to obtain a confidence interval for σ^2 and σ.

Table C gives the quantiles of the χ^2 distribution for different values of the degrees of freedom and the cumulative probability p. We will need to find the probabilities for certain intervals, but this is more complicated with the χ^2 distribution because it is asymmetrical, unlike the normal or t distributions. In this case, we want to find two positive numbers $c_{\alpha/2,df}$ and $c_{1-\alpha/2,df}$ such that

$$P[c_{\alpha/2,df} < X < c_{1-\alpha/2,df}] = 1 - \alpha, \tag{9.18}$$

where X has a χ^2 distribution and $\alpha = 0.05$ or $\alpha = 0.01$. The subscripts $\alpha/2$ and $1-\alpha/2$ for c essentially correspond to values of p in Table C. This gives

Figure 9.2: Plot of the χ^2 distribution for different degrees of freedom



the correct probability because

$$P[c_{\alpha/2,df} < X < c_{1-\alpha/2,df}] = P[X < c_{1-\alpha/2,df}] - P[X < c_{\alpha/2,df}] \quad (9.19)$$

$$= 1 - \alpha/2 - \alpha/2 = 1 - \alpha. \quad (9.20)$$

To see how these values are obtained from Table C, suppose that $\alpha = 0.05$ and $df = 10$. To find $c_{\alpha/2,df} = c_{0.05/2,10} = c_{0.025,10}$, we look in the column for $p = 0.025$ and row for $df = 10$, and obtain $c_{0.025,10} = 3.247$. To find $c_{1-\alpha/2,df} = c_{1-0.05/2,10} = c_{0.975,10}$, we look in the column for $p = 0.975$ and row for $df = 10$, and obtain $c_{0.975,10} = 20.483$.

Now suppose that $\alpha = 0.01$. Using the same technique, we find that $c_{\alpha/2,df} = c_{0.01/2,10} = c_{0.005,10} = 2.156$, and $c_{1-\alpha/2,df} = c_{1-0.01/2,10} = c_{0.995,10} = 25.188$.

9.2 Confidence intervals

We now have the information needed to calculate confidence intervals. We will begin with a simple but unrealistic case, finding a confidence interval for μ when σ^2 is known through other means. This case is unrealistic because σ^2 is almost always estimated from the data, but the calculations are simple and

illustrate a general method for finding confidence intervals. We then turn to finding a confidence intervals for μ, and then σ^2, where all parameters are estimated from the data.

9.2.1 Confidence intervals for μ when σ^2 is known

We will use the fact that the quantity $\frac{\bar{Y}-\mu}{\sigma/\sqrt{n}}$ has a standard normal distribution to find a confidence interval for μ. Suppose that α is given and we have found c_α such that

$$P\left[-c_\alpha < Z < c_\alpha\right] = 1-\alpha. \tag{9.21}$$

(see previous section). Substituting $\frac{\bar{Y}-\mu}{\sigma/\sqrt{n}}$ for Z we obtain

$$P\left[-c_\alpha < \frac{\bar{Y}-\mu}{\sigma/\sqrt{n}} < c_\alpha\right] = 1-\alpha. \tag{9.22}$$

Multiplying both sides by $\sigma/\sqrt{n}$ gives you

$$P\left[-c_\alpha\frac{\sigma}{\sqrt{n}} < \bar{Y}-\mu < c_\alpha\frac{\sigma}{\sqrt{n}}\right] = 1-\alpha. \tag{9.23}$$

Multiplying all parts inside the brackets by -1 reverses the signs and inequalities to give

$$P\left[c_\alpha\frac{\sigma}{\sqrt{n}} > \mu-\bar{Y} > -c_\alpha\frac{\sigma}{\sqrt{n}}\right] = 1-\alpha. \tag{9.24}$$

We now add to $\bar{Y}$ to all parts inside the brackets to give

$$P\left[\bar{Y}+c_\alpha\frac{\sigma}{\sqrt{n}} > \mu > \bar{Y}-c_\alpha\frac{\sigma}{\sqrt{n}}\right] = 1-\alpha, \tag{9.25}$$

or equivalently

$$P\left[\bar{Y}-c_\alpha\frac{\sigma}{\sqrt{n}} < \mu < \bar{Y}+c_\alpha\frac{\sigma}{\sqrt{n}}\right] = 1-\alpha. \tag{9.26}$$

We call the terms $\bar{Y}-c_\alpha\frac{\sigma}{\sqrt{n}}$ and $\bar{Y}+c_\alpha\frac{\sigma}{\sqrt{n}}$ the lower and upper $100(1-\alpha)\%$ confidence limits for μ (Mood et al. 1974). Confidence intervals are often reported in the form $(\bar{Y}-c_\alpha\frac{\sigma}{\sqrt{n}}, \bar{Y}+c_\alpha\frac{\sigma}{\sqrt{n}})$. Note that the center of the

confidence interval is at $\bar{Y}$, our estimate of μ. This interval would be expected to include the true value of μ with a probability of $1 - \alpha$, because this was the probability set in Eq. 9.21.

It is common practice to set $\alpha = 0.05$, which corresponds to a $100(1 - 0.05)\% = 95\%$ confidence interval. For this case, we would have $c_\alpha = c_{0.05} = 1.96$ (see previous section). Therefore, the 95% confidence interval would be

$$(\bar{Y} - 1.96\frac{\sigma}{\sqrt{n}}, \bar{Y} + 1.96\frac{\sigma}{\sqrt{n}}). \tag{9.27}$$

We would expect this interval to include the true μ with a probability of 0.95, or 95% of the time. However, it follows that the interval will miss μ with a probability of 0.05, or 5% of the time. **This is an important feature of confidence intervals - they will often but not always enclose the true parameter value for the population, with the probability set by α.**

If we wanted to be more certain of including μ, we could choose a smaller α, say $\alpha = 0.01$, which corresponds to a $100(1 - 0.01)\% = 99\%$ confidence interval. Here we have $c_\alpha = c_{0.01} = 2.576$, and so the 99% confidence interval would be

$$(\bar{Y} - 2.576\frac{\sigma}{\sqrt{n}}, \bar{Y} + 2.576\frac{\sigma}{\sqrt{n}}). \tag{9.28}$$

A 99% confidence interval will necessarily be broader than a 95% one, because it is constructed to have a higher probability of including μ.

Confidence intervals - sample calculation

Suppose we have a sample of $n = 10$ elytra from female *T. dubius* beetles, with the values listed below:

```
5.0 5.1 5.2 5.9 4.8 5.5 4.8 5.1 5.0 5.1
```

For this sample, we calculate that $\bar{Y} = 5.150$. Suppose we have *a priori* knowledge that $\sigma = 0.3$, although that would be rare in practice. We will calculate a 95% and 99% confidence interval for μ.

The formula for a 95% confidence interval is

$$(\bar{Y} - 1.96\frac{\sigma}{\sqrt{n}}, \bar{Y} + 1.96\frac{\sigma}{\sqrt{n}}). \tag{9.29}$$

Substituting $n = 10, \bar{Y} = 5.150$, and $\sigma = 0.3$ in the above formula, we obtain

$$(5.150 - 1.96\frac{0.3}{\sqrt{10}}, 5.150 + 1.96\frac{0.3}{\sqrt{10}}), \tag{9.30}$$

or

$$(5.150 - 0.186, 5.150 + 0.186), \tag{9.31}$$

or

$$(4.964, 5.336). \tag{9.32}$$

So, the 95% confidence interval for μ is $(4.964, 5.336)$.

For a 99% confidence interval, we use the formula

$$(\bar{Y} - 2.576\frac{\sigma}{\sqrt{n}}, \bar{Y} + 2.576\frac{\sigma}{\sqrt{n}}). \tag{9.33}$$

Substituting as before, we obtain

$$(5.150 - 2.576\frac{0.3}{\sqrt{10}}, 5.150 + 2.576\frac{0.3}{\sqrt{10}}), \tag{9.34}$$

or

$$(5.150 - 0.244, 5.150 + 0.244), \tag{9.35}$$

or

$$(4.906, 5.394). \tag{9.36}$$

The 99% confidence interval is therefore $(4.906, 5.394)$. Note that the 99% confidence interval is broader than the 95% one, because its lower limit is lower and upper limit higher.

9.2.2 Confidence intervals for μ when σ^2 is estimated

Confidence intervals for μ can also be derived when σ^2 is estimated using the sample variance s^2, as will usually be the case in practice. We will make use of the fact that

$$\frac{\bar{Y} - \mu}{s/\sqrt{n}} \sim t_{n-1}. \tag{9.37}$$

Using Table T, we can find a value of $c_{\alpha,n-1}$ for $n-1$ degrees of freedom such that the following equation is true:

$$P\left[-c_{\alpha,n-1} < \frac{\bar{Y} - \mu}{s/\sqrt{n}} < c_{\alpha,n-1}\right] = 1 - \alpha. \tag{9.38}$$

Rearranging this equation using the same procedures as before, we obtain

$$P\left[\bar{Y} - c_{\alpha,n-1}\frac{s}{\sqrt{n}} < \mu < \bar{Y} + c_{\alpha,n-1}\frac{s}{\sqrt{n}}\right] = 1 - \alpha. \tag{9.39}$$

The terms $\bar{Y} - c_{\alpha,n-1}\frac{s}{\sqrt{n}}$ and $\bar{Y} + c_{\alpha,n-1}\frac{s}{\sqrt{n}}$ are the lower and upper $100(1-\alpha)\%$ confidence limits for μ (Mood et al. 1974). The interval would be reported in the form $(\bar{Y} - c_{\alpha,n-1}\frac{s}{\sqrt{n}}, \bar{Y} + c_{\alpha,n-1}\frac{s}{\sqrt{n}})$. The center of the confidence interval is located at $\bar{Y}$, the estimate of μ.

For example, if we let $\alpha = 0.05$ this corresponds to a 95% confidence interval of the form

$$(\bar{Y} - c_{0.05,n-1}\frac{s}{\sqrt{n}}, \bar{Y} + c_{0.05,n-1}\frac{s}{\sqrt{n}}). \tag{9.40}$$

The value of $c_{0.05,n-1}$ would need to be determined from Table T, using the column for $2(1-p) = \alpha = 0.05$ and the row for $n-1$ degrees freedom.

For $\alpha = 0.01$, we obtain a 99% confidence interval of the form

$$(\bar{Y} - c_{0.01,n-1}\frac{s}{\sqrt{n}}, \bar{Y} + c_{0.01,n-1}\frac{s}{\sqrt{n}}). \tag{9.41}$$

In this case, we would use the column for $2(1-p) = \alpha = 0.01$ to find the value of $c_{0.01,n-1}$, using $n-1$ degrees freedom.

Confidence interval for μ - sample calculation

We return to the elytra data set, for which we previously calculated that $\bar{Y} = 5.150$, $s^2 = 0.109$, and $s = 0.331$ for $n = 10$. We will calculate 95% and 99% confidence intervals for μ.

The formula for a 95% confidence interval is

$$(\bar{Y} - c_{0.05,n-1}\frac{s}{\sqrt{n}}, \bar{Y} + c_{0.05,n-1}\frac{s}{\sqrt{n}}). \tag{9.42}$$

For $n = 10$, we have $df = n - 1 = 10 - 1 = 9$. For a 95% confidence interval, we therefore look up $c_{0.05,n-1} = c_{0.05,9}$ using the column for $2(1-p) = 0.05$ in Table T, choosing the value for 9 degrees of freedom. We obtain $c_{0.05,9} = 2.262$. Substituting $n = 10, \bar{Y} = 5.150, s = 0.331$, and $c_{0.05,9} = 2.262$ in the above formula, we obtain

$$(5.150 - 2.262\frac{0.331}{\sqrt{10}}, 5.150 + 2.262\frac{0.331}{\sqrt{10}}), \tag{9.43}$$

or

$$(5.150 - 0.237, 5.150 + 0.237), \tag{9.44}$$

or

$$(4.913, 5.387). \tag{9.45}$$

So, the 95% confidence interval for μ is $(4.913, 5.387)$. For a 99% confidence interval, we find $c_{0.01,n-1} = c_{0.01,9}$ for $2(1-p) = 0.01$ and 9 degrees of freedom in Table T, obtaining $c_{0.01,9} = 3.250$. Substituting this value in the above formula, we obtain

$$(5.150 - 3.250\frac{0.331}{\sqrt{10}}, 5.150 + 3.250\frac{0.331}{\sqrt{10}}), \tag{9.46}$$

or

$$(5.150 - 0.340, 5.150 + 0.349), \tag{9.47}$$

or

$$(4.810, 5.490). \tag{9.48}$$

The 99% confidence interval is therefore $(4.810, 5.490)$, and as expected is broader than the 95% one.

9.2.3 Confidence intervals for σ^2 and σ

Confidence intervals for σ^2 and σ can also be derived, using the fact that

$$\frac{(n-1)s^2}{\sigma^2} \sim \chi^2_{n-1} \tag{9.49}$$

Using Table C for the χ^2 distribution, we can find values $c_{\alpha/2,n-1}$ and $c_{1-\alpha/2,n-1}$ for $n-1$ degrees of freedom such that the following equation is true:

$$P\left[c_{\alpha/2,n-1} < \frac{(n-1)s^2}{\sigma^2} < c_{1-\alpha/2,n-1}\right] = 1 - \alpha. \tag{9.50}$$

We now rearrange this equation to obtain a confidential interval for σ^2. If we take the inverse of all the inside terms, we obtain

$$P\left[\frac{1}{c_{\alpha/2,n-1}} > \frac{\sigma^2}{(n-1)s^2} > \frac{1}{c_{1-\alpha/2,n-1}}\right] = 1 - \alpha. \tag{9.51}$$

Note that taking the inverse changes the direction of the inequality signs. Multiplying each term by $(n-1)s^2$ we obtain

$$P\left[\frac{(n-1)s^2}{c_{\alpha/2,n-1}} > \sigma^2 > \frac{(n-1)s^2}{c_{1-\alpha/2,n-1}}\right] = 1-\alpha, \tag{9.52}$$

or equivalently

$$P\left[\frac{(n-1)s^2}{c_{1-\alpha/2,n-1}} < \sigma^2 < \frac{(n-1)s^2}{c_{\alpha/2,n-1}}\right] = 1-\alpha. \tag{9.53}$$

The terms $\frac{(n-1)s^2}{c_{1-\alpha/2,n-1}}$ and $\frac{(n-1)s^2}{c_{\alpha/2,n-1}}$ are the lower and upper $100(1-\alpha)\%$ confidence limits for σ^2, and the interval $(\frac{(n-1)s^2}{c_{1-\alpha/2,n-1}}, \frac{(n-1)s^2}{c_{\alpha/2,n-1}})$ is a $100(1-\alpha)\%$ confidence interval for σ^2 (Mood et al. 1974). The confidence interval for σ^2 is not symmetrical around the value s^2, our estimate of σ^2.

For a 95% confidence interval with $\alpha = 0.05$, the confidence interval formula is

$$\left(\frac{(n-1)s^2}{c_{0.975,n-1}}, \frac{(n-1)s^2}{c_{0.025,n-1}}\right) \tag{9.54}$$

To find $c_{0.025,n-1}$, we look across the top row of Table C and find the column corresponding to $p = 0.025$, then look for the row corresponding to $n-1$ degrees of fredom. To find $c_{0.975,n-1}$, we use the column corresponding to $p = 0.975$, again looking for the row with $n-1$ degrees of freedom.

For a 99% confidence interval with $\alpha = 0.01$, the confidence interval formula is

$$\left(\frac{(n-1)s^2}{c_{0.995,n-1}}, \frac{(n-1)s^2}{c_{0.005,n-1}}\right) \tag{9.55}$$

To find $c_{0.005,n-1}$, we use the column corresponding to $p = 0.005$, while the column for $c_{0.995,n-1}$ corresponds to $p = 0.995$. We again use the entries corresponding to $n-1$ degrees of freedom.

We can also obtain a confidence interval for $\sigma = \sqrt{\sigma^2}$ by taking the square root of the above confidence limits. In particular, a confidence interval for σ would be $(\sqrt{\frac{(n-1)s^2}{c_{1-\alpha/2,n-1}}}, \sqrt{\frac{(n-1)s^2}{c_{\alpha/2,n-1}}})$.

Confidence interval for σ^2 and σ - sample calculation

Recall the elytra data set, for which $\bar{Y} = 5.150$ and $s^2 = 0.109$ for $n = 10$. Calculate a 95% and 99% confidence interval for σ^2 and then σ.

The formula for a 95% confidence interval is

$$\left(\frac{(n-1)s^2}{c_{0.975,n-1}}, \frac{(n-1)s^2}{c_{0.025,n-1}}\right) \tag{9.56}$$

For $n = 10$, we have $df = n - 1 = 10 - 1 = 9$.

For a 95% confidence interval, with $\alpha = 0.05$, we find from Table C that $c_{0.025,n-1} = c_{0.025,9} = 2.700$, and $c_{0.975,n-1} = c_{0.975,9} = 19.023$. Substituting $n = 10, \bar{Y} = 5.132, s^2 = 0.110, c_{0.025,9} = 2.700$ and $c_{0.975,9} = 19.023$ in the above formula, we obtain

$$\left(\frac{(10-1)0.109}{19.023}, \frac{(10-1)0.109}{2.700}\right) \tag{9.57}$$

or

$$(0.052, 0.363). \tag{9.58}$$

So, the 95% confidence interval for σ^2 is $(0.052, 0.363)$. To obtain a 95% confidence interval for σ we simply take the square root of these values, or $(\sqrt{0.052}, \sqrt{0.363}$, to obtain $(0.228, 0.603)$.

For a 99% confidence interval, the formula is

$$\left(\frac{(n-1)s^2}{c_{0.995,n-1}}, \frac{(n-1)s^2}{c_{0.005,n-1}}\right) \tag{9.59}$$

We use Table C to find $c_{0.005,n-1} = c_{0.005,9} = 1.735$, and $c_{0.995,n-1} = c_{0.995,9} = 23.589$. Substituting these values in the above formula, we obtain

$$\left(\frac{(10-1)0.109}{23.589}, \frac{(10-1)0.109}{1.735}\right) \tag{9.60}$$

or

$$(0.042, 0.565). \tag{9.61}$$

The 99% confidence interval of σ^2 is therefore $(0.042, 0.565)$. To obtain a 99% confidence interval for σ, we take the square root and obtain $(0.205, 0.752)$. Note that the 99% intervals are wider than the corresponding 95% ones.

9.2.4 Confidence intervals - SAS demo

These same calculations can be readily accomplished using `proc univariate` in SAS (SAS Institute Inc. 2014). We obtain 95% confidence intervals

by including the option `cibasic` in the `proc univariate` line of the program. 99% confidence intervals may be obtained by specifying `alpha=0.01` in the `proc univariate` line. See SAS program below and attached output.

We obtain results similar to our earlier calculations. SAS finds that the 95% confidence interval for μ is $(4.913, 5.387)$, while one for σ^2 is $(0.052, 0.365)$ and σ is $(0.228, 0.604)$. The 99% confidence intervals can be found further in the output.

9.2.5 Confidence interval size

Confidence intervals are a method of characterizing the precision of parameter estimates, with narrower intervals generally indicating a population parameter like μ is better estimated. How then can an investigator reduce the size of these confidence intervals? The simplest way is to increase the sample size n on which the estimate is based. This reduces the size of confidence intervals for μ because it reduces the magnitude of the quantity $c_{\alpha,n-1}s/\sqrt{n}$, which determines the width of the interval (see Eq. 9.26). Most of this effect is through the $\sqrt{n}$ term here, but $c_{\alpha,n-1}$ also becomes smaller for larger n. Increasing the sample size n also reduces the size of the confidence intervals for σ^2 and σ, although the mechanism is more complex in this case.

SAS Program

```
* Confidence_intervals.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Confidence intervals for elytra data';
data elytra;
    input length;
    datalines;
5.0
5.1
5.2
5.9
4.8
5.5
4.8
5.1
5.0
5.1
;
run;
* Print data set;
proc print data=elytra;
run;
* Generate 95% confidence intervals and plots;
title2 "95% confidence intervals";
proc univariate cibasic plots data=elytra;
    var length;
    histogram length / vscale=count normal(w=3) wbarline=3 waxis=3 height=4;
    qqplot length / normal waxis=3 height=4;
    symbol1 h=3;
run;
* Generate 99% confidence intervals;
title2 "99% confidence intervals";
proc univariate cibasic alpha = 0.01 data=elytra;
    var length;
run;
quit;
```

SAS Output

Confidence intervals for elytra data 1
10:50 Thursday, June 3, 2010

Obs	length
1	5.0
2	5.1
3	5.2
4	5.9
5	4.8
6	5.5
7	4.8
8	5.1
9	5.0
10	5.1

Confidence intervals for elytra data 2
95% confidence intervals
10:50 Thursday, June 3, 2010

The UNIVARIATE Procedure
Variable: length

Moments

N	10	Sum Weights	10
Mean	5.15	Sum Observations	51.5
Std Deviation	0.33082389	Variance	0.10944444
Skewness	1.42698649	Kurtosis	2.26518149
Uncorrected SS	266.21	Corrected SS	0.985
Coeff Variation	6.4237648	Std Error Mean	0.1046157

Basic Statistical Measures

Location		Variability	
Mean	5.150000	Std Deviation	0.33082
Median	5.100000	Variance	0.10944
Mode	5.100000	Range	1.10000
		Interquartile Range	0.20000

Basic Confidence Limits Assuming Normality

Parameter	Estimate	95% Confidence Limits	
Mean	5.15000	4.91334	5.38666
Std Deviation	0.33082	0.22755	0.60396
Variance	0.10944	0.05178	0.36476

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	49.22779	Pr > |t|	<.0001
Sign	M	5	Pr >= |M|	0.0020
Signed Rank	S	27.5	Pr >= |S|	0.0020

Confidence intervals for elytra data 5
99% confidence intervals
10:50 Thursday, June 3, 2010

The UNIVARIATE Procedure
Variable: length

Moments

N	10	Sum Weights	10
Mean	5.15	Sum Observations	51.5
Std Deviation	0.33082389	Variance	0.10944444
Skewness	1.42698649	Kurtosis	2.26518149
Uncorrected SS	266.21	Corrected SS	0.985
Coeff Variation	6.4237648	Std Error Mean	0.1046157

Basic Statistical Measures

Location		Variability	
Mean	5.150000	Std Deviation	0.33082
Median	5.100000	Variance	0.10944
Mode	5.100000	Range	1.10000
		Interquartile Range	0.20000

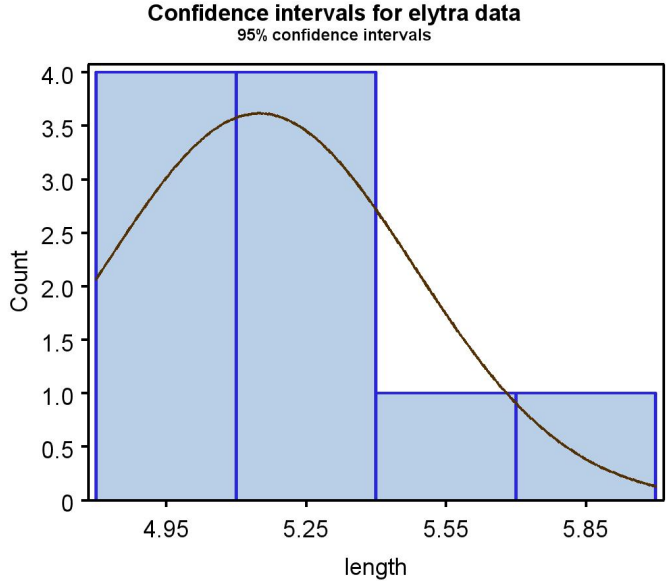
Basic Confidence Limits Assuming Normality

Parameter	Estimate	99% Confidence Limits	
Mean	5.15000	4.81002	5.48998
Std Deviation	0.33082	0.20434	0.75349
Variance	0.10944	0.04176	0.56775

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	49.22779	Pr > |t|	<.0001
Sign	M	5	Pr >= |M|	0.0020
Signed Rank	S	27.5	Pr >= |S|	0.0020

Figure 9.3: Histogram for elytra data



9.3 References

Mood, A. M., Graybill, F. A. & Boes, D. C. (1974) *Introduction to the Theory of Statistics*. McGraw-Hill, Inc., New York, NY.

SAS Institute Inc. (2014) *Base SAS 9.4 Procedures Guide: Statistical Procedures, Third Edition.* SAS Institute Inc., Cary, NC, USA

9.4 Problems

1. Ten adult female *Daphnia ambigua* (Lei and Armitage 1980) were cultured under laboratory conditions, and their longevity (days) determined. The following data were obtained.

```
28 4 22 21 17 21 22 26 15 19
```

   (a) Find $\bar{Y}$, s^2, and s for these data, then calculate a 95% confidence interval for μ, σ^2 and then σ. Show all your calculations.

   (b) Find a 99% confidence interval for μ, σ^2 and then σ. Show your calculations.

   (c) Use SAS to find the same confidence intervals as in parts a and b. List the confidence intervals and test results below. Attach your SAS program(s) and output.

2. A study was conducted to measure the population growth rate of a laboratory culture of nematodes. A hundred nematodes were each added to 8 petri dishes of a new growth media, and the number of offspring counted one generation later. The number of offspring divided by the initial number of organisms (100) provides an estimate of λ, the finite growth rate of the population. It is customary to log-transform the values of λ in such studies, yielding $r = \ln(\lambda)$. The following values of r were obtained:

```
2.1 0.8 1.8 1.9 0.8 1.7 0.5 1.6
```

   (a) Find $\bar{Y}$, s^2, and s for these data, then calculate a 95% confidence interval for μ, σ^2 and then σ. Show all your calculations.

   (b) Find a 99% confidence interval for μ, σ^2 and then σ. Show your calculations.

   (c) Use SAS to find the same confidence intervals as in parts a and b. List the confidence intervals and test results below. Attach your SAS program(s) and output.

Chapter 10

Hypothesis Testing

We previously examined how the parameters for a probability distribution can be estimated using a random sample and maximum likelihood (Chapter 8), as then showed how confidence intervals provide a measure of the reliability of these estimates (Chapter 9). In hypothesis testing, the subject of this chapter, we examine the consistency of observed data sets with a null hypothesis, commonly a statement about the parameter values within a statistical model. We conduct a statistical test of this null hypothesis, with the result being a decision to accept or reject the null hypothesis based on the magnitude of a quantity called a P value. Small values of P indicate a test result inconsistent with the null hypothesis, suggesting it might be false and some alternative hypothesis more valid. In the following, we discuss the different components and steps of hypothesis testing.

10.1 The null and alternative hypotheses

As an example of hypothesis testing, suppose that we rear n tilapia on a commercial diet, and want to compare their body size with ones reared using a natural diet. Fish reared on natural food are already known to have a weight of 500 g at a certain age, and weight is normally distributed. We could test whether the fish reared on the commercial diet have the same mean weight as ones reared on natural food (500 g) using the **null hypothesis** that $\mu = 500$ g, where μ is the mean parameter for the normal distribution. This can be written as $H_0 : \mu = 500$ g, where H_0 stands for null hypothesis. Null hypotheses of this type can be written more generally as $H_0 : \mu = \mu_0$, where

μ_0 is the hypothesized mean of the distribution. For the tilapia problem, we would have $\mu_0 = 500$ g.

An **alternative hypothesis** for this example is that the mean weight of tilapia on commercial diet is different from 500 g. This can be written as $H_1 : \mu \neq 500$ g, where H_1 stands for the alternative hypothesis. Alternative hypotheses of this type are written generally as $H_1 : \mu \neq \mu_0$. We may also be interested in particular values of the alternative mean, such as $H_1 : \mu = 490$ g or $H_1 : \mu = 530$ g, or more generally $H_1 : \mu = \mu_1$.

10.2 Test statistics

A test statistic is a quantity that measures the consistency of the observed data with the null hypothesis. Test statistics are usually chosen so that large values occur when the data are inconsistent with H_0. What would be a suitable test statistic for the tilapia problem, using $H_0 : \mu = \mu_0$ as the null hypothesis? Suppose we rear n fish on the commercial diet, and then calculate the sample mean $\bar{Y}$ of their weights. The statistic $\bar{Y}$ is an estimator of the true mean μ for this statistical population, which may or may not be equal to the μ_0 under the null hypothesis. A value of $\bar{Y}$ substantially greater than μ_0, or smaller than μ_0, would be inconsistent with H_0. This suggests using the quantity $\bar{Y} - \mu_0$ as the test statistic for the problem. What about the other parameter for the normal distribution, σ^2 or σ? For simplicity, we will assume that it is a known quantity, although this is rare in practice. We will then employ the test statistic

$$Z_s = \frac{\bar{Y} - \mu_0}{\sigma/\sqrt{n}} \tag{10.1}$$

to test $H_0 : \mu = \mu_0$ (Bickel & Doksum 1977). We use this statistic because it has a standard normal distribution under H_0 ($Z_s \sim N(0, 1)$, see Chapter 9) which makes it straightforward to employ the test. Note that Z_s becomes large (positive or negative) if the sample mean $\bar{Y}$ differs greatly from μ_0. Tests based on the standard normal distribution are called Z tests.

10.3 Acceptance and rejection regions – Type I error

Given a suitable test statistic, how large must it be before we decide the data are inconsistent with H_0? This is determined by finding an interval that defines an **acceptance region** for the test, and its complement, called the **rejection** or **critical region** (Bickel & Doksum 1977). We then accept H_0 if the test statistic falls within the acceptance region, and reject H_0 if it falls outside or lies on its boundary. The boundaries of the acceptance and rejection regions are determined by setting the probability of a Type I error. **A Type I error is defined as the test rejecting H_0 when H_0 is true. The probability of committing a Type I error is called the Type I error rate, usually denoted with the symbol α.** It is common practice to set $\alpha = 0.05$, meaning there is a 1 in 20 chance that the test will reject H_0 even when it is true. It follows that the probability of the test accepting H_0 if it is true is $1 - \alpha$. For $\alpha = 0.05$, we have $1 - \alpha = 1 - 0.05 = 0.95$.

The acceptance region is determined as follows. Suppose that $H_0 : \mu = \mu_0$ is true. Because the test statistic $Z_s \sim N(0, 1)$ under H_0, the following is a true statement:

$$P[-c_\alpha < Z_s < c_\alpha] = P[-c_\alpha < \frac{\bar{Y} - \mu_0}{\sigma/\sqrt{n}} < c_\alpha] = 1 - \alpha. \tag{10.2}$$

The quantity c_α would be chosen using Table Z to satisfy this equation (for details see Chapter 9). The interval $(-c_\alpha, c_\alpha)$ is the acceptance region of a test with a Type I error rate of α. Under H_0, the test statistic Z_s would lie within this interval with probability $1 - \alpha$ and outside this region with probability α, which is the required Type I error rate. The rejection region would be the complement of the acceptance region, i.e., all values on the boundary or outside of $(-c_\alpha, c_\alpha)$.

For example, with $\alpha = 0.05$ we find that $c_{0.05} = 1.96$, and so we would accept H_0 if Z_s lies within $(-1.96, 1.96)$ and reject H_0 if it lies outside this interval or exactly on the boundary (see Fig. 10.1). The acceptance region for this test can also be expressed using absolute values - we would accept H_0 if $|Z_s| < 1.96$ and reject it if $|Z_s| \geq 1.96$.

The acceptance region becomes larger (and the rejection region smaller) for smaller α values. For $\alpha = 0.01$, we find that $c_{0.01} = 2.576$ and so the acceptance region is $(-2.576, 2.576)$ (Fig. 10.2). Using absolute values, we

would accept H_0 if $|Z_s| < 2.576$ and reject it otherwise. Using a smaller value of α indicates we are more concerned about making a Type I error. For $\alpha = 0.01$ there is only a 1 in 100 chance we would reject H_0 if H_0 were true, but this also reduces the power of the test (see below) to detect whether H_0 is false.

The acceptance and rejection regions we just developed are for a **two-tailed test**, which tests the null hypothesis $H_0 : \mu = \mu_0$ with $H_1 : \mu \neq \mu_0$ the alternative hypothesis. This test statistic will reject H_0 for either large and small values of the test statistic Z_s, which occurs when $\bar{Y}$ is greater than μ_0 or less than μ_0. We will later examine the behavior of **one-tailed tests**, where the null is $H_0 : \mu = \mu_0$ while the alternative is of the form $H_1 : \mu > \mu_0$, or $H_1 : \mu < \mu_0$. Note that the two alternative hypotheses here specify that μ is either greater or less than μ_0.

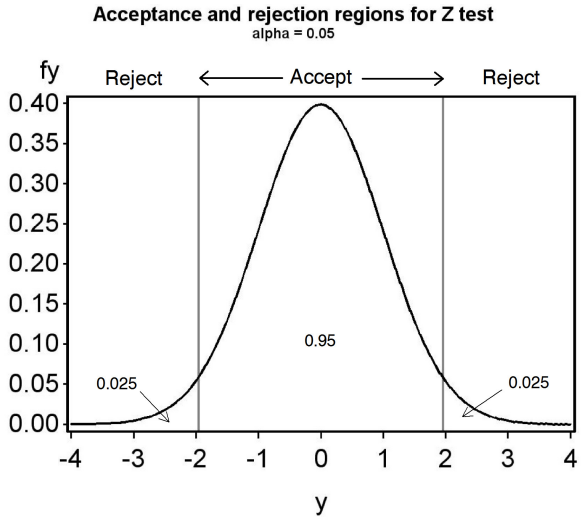


Figure 10.1: Acceptance and rejection regions for a one-sample Z test, $\alpha = 0.05$. Also shown is the distribution of Z_s under H_0.

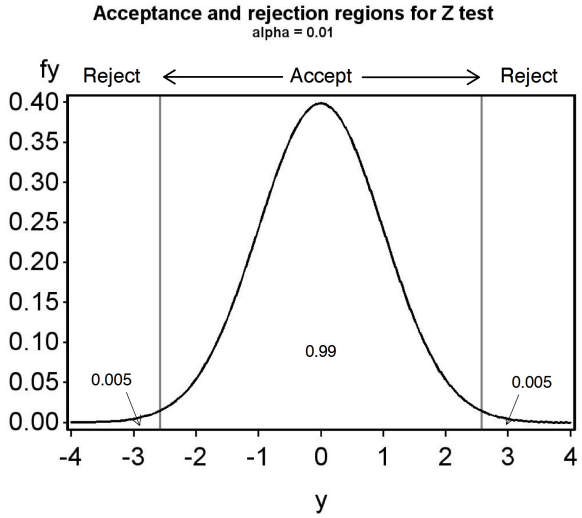


Figure 10.2: Acceptance and rejection regions for a one-sample Z test, $\alpha = 0.01$. Also shown is the distribution of Z_s under H_0.

10.3.1 One-sample Z test - sample calculation

We will now do an example of this test, known as a one-sample Z test. Recall the tilapia diet example, where it is known that fish reared on natural food have a mean weight of 500 g. We rear $n = 10$ fish on a commercial diet, and want to compare the weight of fish on the commercial diet with ones reared on natural food. In particular, we want to test $H_0 : \mu = 500$ g. We find that $\bar{Y} = 495$ g for the fish reared on the commercial diet, and already know that $\sigma^2 = 49$ g^2, so $\sigma = 7$ g. Because $\bar{Y} = 495$ g is less than 500 g, it already appears that the commercial diet produces smaller fish than natural food, but a statistical test is still needed to provide convincing evidence against H_0. For the test statistic, we have

$$Z_s = \frac{\bar{Y} - \mu_0}{\sigma/\sqrt{n}} = \frac{495 - 500}{7/\sqrt{10}} = \frac{-5}{2.214} = -2.258 \tag{10.3}$$

For a Type I error rate of $\alpha = 0.05$, the acceptance region for Z_s is $(-1.96, 1.96)$. $Z = -2.258$ lies outside this interval, so we would reject H_0 at the $\alpha = 0.05$ level. For $\alpha = 0.01$ the acceptance region is $(-2.576, 2.576)$. Because Z_s lies within this interval, we would accept H_0 at this α level. Thus, the decision to accept or reject H_0 depends on both the test statistic value and the value of α.

10.4 P values

As noted above, the value of α can affect whether we accept or reject H_0. Rather than force a particular α on the analyst, the test results can also be presented in the form of a P value. **A P value is defined as the smallest value of α for which one can just reject** H_0 (Bickel & Doksum 1977). It is calculated by finding an α such that the test statistic Z_s is equal to c_α.

Recall from Chapter 9 that c_α is defined so that the following equation is true:

$$P[Z < c_\alpha] = 1 - \alpha/2. \tag{10.4}$$

To find the P value for the tilapia example, we substitute the test statistic value Z_s for c_α in the above equation, ignoring the fact that Z_s is negative. We have

$$P[Z < Z_s] = P[Z < 2.258] = 1 - \alpha/2. \tag{10.5}$$

From Table Z, we see that $P[Z < 2.258] \approx 0.9881$. We then solve the equation

$$0.9881 = 1 - \alpha/2 \tag{10.6}$$

for α to obtain the P value. We have $\alpha = 2(1 - 0.9881) = 0.0238$. This is the P value for the test, reported as $P = 0.0238$. Given the P value, the analyst or other interested parties can decide for themselves whether to reject or accept H_0.

A P value can also be thought of as the probability of obtaining a test statistic equal to or more extreme than the observed one, under the null hypothesis. We can see this from a graph of the acceptance and rejection regions for the tilapia example, where $Z_s = -2.258$ and $P = 0.0238$ (Fig. 10.3). The probabilities outside the acceptance region correspond to $P[Z_s \leq -2.258]$ and $P[Z_s \geq 2.258]$, which are the probabilities of observing values of Z_s equal to or more extreme than the observed value of $Z_s = -2.258$. The two definitions of a P value are equivalent.

A P value is also a measure of the consistency of the observed data with the null hypothesis. If the P value is large, say $P > 0.05$, then the observed data generated a test statistic value that is fairly likely under the null hypothesis. On the other hand, if P is small then the observed data has generated a test statistic that is unlikely under the null hypothesis. This suggests the observed data are inconsistent with the null hypothesis, and the null may be false.

There are specific phrases generally used to describe the significance of a statistical test result. If a test yields $P \leq 0.05$, it is described as being **significant**, while if $P \leq 0.01$ it is **highly significantly**. If $P > 0.05$ the test is described as **nonsignificant**. The tilapia example with $P = 0.0272$ would be described as significant because $0.0272 < 0.05$, but not highly significant.

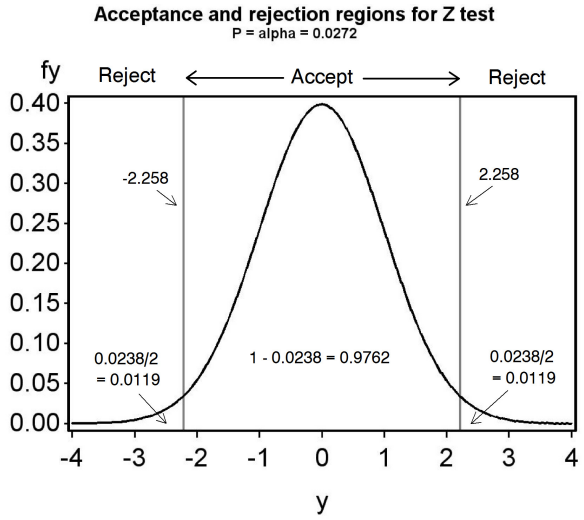


Figure 10.3: Acceptance-rejection region for a one-sample Z test, exact $P = 0.0238$

10.5 Type II error and power

Suppose now that H_0 is actually false and some alternative hypothesis H_1 is true. **A Type II error is defined as failing to reject H_0 when H_0 is false**. The probability of committing a Type II error is called the Type II error rate, usually denoted by the symbol β. It follows that the probability of the test rejecting H_0 if it is false is $1 - \beta$, and this quantity is called the **power** of the test (Bickel & Doksum 1977). High power values indicate the test is capable of detecting departures from the null hypothesis.

The power and Type II error rate of a statistical test depends on the sample size n of the test, the standard deviation of the observations σ, the Type I error rate α, and the particular alternative hypothesis chosen. An analyst interested in determining the power of a test will fix some of these values, often α and σ, and then examine how changes in n and the alternative hypothesis affect power. This procedure is called a **power analysis**. A power value of 0.8 is believed to be adequate in most situations (Cohen 1988). This implies that a statistical test will reject H_0 when it is false 80% of the time.

It is relatively easy to calculate the power for a one-sample Z test, using the distribution of Z_s under H_1. Suppose that we choose $\alpha = 0.05$, so that the acceptance region is the interval $(-1.96, 1.96)$, and that the alternative hypothesis is $H_1 : \mu = \mu_1$ for some μ_1. Under $H_0 : \mu = \mu_0$ the test statistic has a standard normal distribution, implying $Z_s \sim N(0, 1)$, but what is its distribution under H_1? Using the expected value and variance rules in Chapter 7, one can show that

$$E[Z_s] = \frac{\mu_1 - \mu_0}{\sigma/\sqrt{n}} = \phi \tag{10.7}$$

and also that $Var[Z_s] = 1$. So, Z_s has the same variance under both H_1 and H_0, but the mean under H_1 is equal to ϕ, not zero as under H_0. It follows that under H_1 the test statistic $Z_s \sim N(\phi, 1)$. The probability of rejecting H_0 when H_1 is true, the power of the test, is the probability that Z_s lies outside the interval $(-1.96, 1.96)$, or

$$\text{power} = P[Z_s \leq -1.96] + P[Z_s \geq 1.96]. \tag{10.8}$$

The Type II error rate β can be calculated as $1-$power, or directly by finding

$$\beta = P[-1.96 < Z_s < 1.96] \tag{10.9}$$

when H_1 is true.

Fig. 10.4 shows the power and Type II error for the tilapia example with $H_0 : \mu = 500$ vs. a particular alternative hypothesis, $H_1 : \mu = 495$. We assume $\sigma = 7$ as before, with $n = 10$ and $\alpha = 0.05$. For this alternative hypothesis, we have

$$\phi = \frac{\mu_1 - \mu_0}{\sigma/\sqrt{10}} = \frac{(495 - 500)}{7/\sqrt{10}} = -2.26. \tag{10.10}$$

Thus, under H_1 we have $Z_s \sim N(-2.26, 1)$, and this distribution is shown as well as the distribution of Z_s under H_0 and the acceptance and rejection regions for the test. The power is the area Z_s under H_1 outside the acceptance region, while β is the area in the region.

What happens to the power as we vary μ_1? Suppose now that $H_1 : \mu_1 = 490$ is the alternative hypothesis. As we can see from Fig. 10.5, in this case the power is substantially higher and β is lower. Fig. 10.6 shows how power changes as we vary μ_1 across a range of values. Power is quite high (nearly 1) for μ_1 far from μ_0, but approaches a minimum value of α for μ_1 near μ_0. The minimum power is α, not zero, because the test will reject H_0 even if it is true ($\mu_1 = \mu_0$) at this rate.

Power is also affected by sample size. If we use $H_1 : \mu = 495$ and increase the sample size from $n = 10$ to $n = 20$, this also increases the power (Fig. 10.7). However, an increase in the standard deviation from $\sigma = 7$ to $\sigma = 10$ lowers the power (Fig. 10.8).

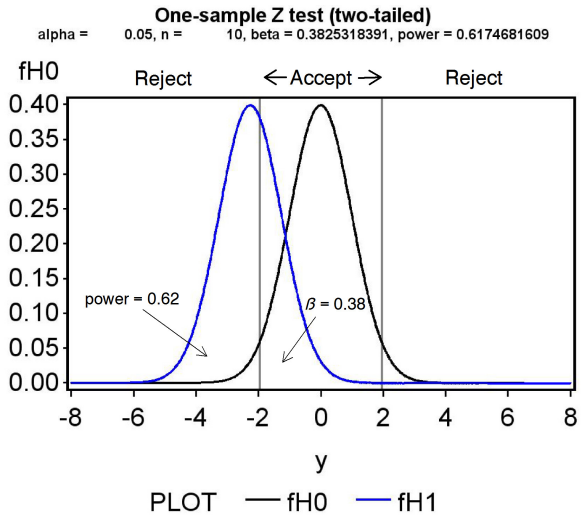


Figure 10.4: Distribution of Z_s under $H_1 : \mu = 495$, with $\sigma = 7, n = 10$ ($\phi = -2.26$). Almost all of the power occurs to the left of the acceptance region, but there is also a small amount to the right. Also shown is the distribution of Z_s under H_0.

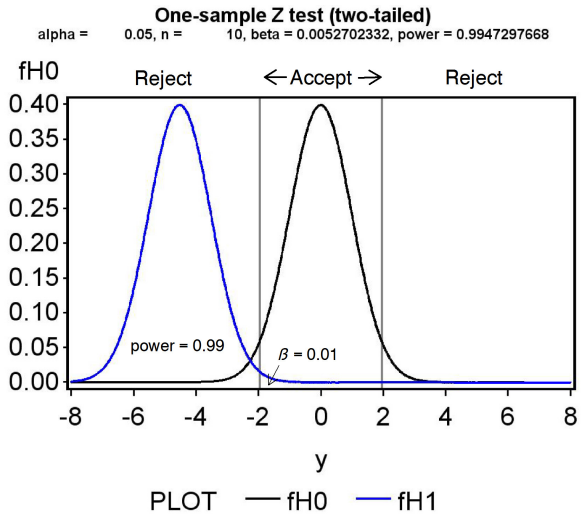


Figure 10.5: Distribution of Z_s under $H_1 : \mu = 490$, with $\sigma = 7, n = 10$ ($\phi = -4.52$).

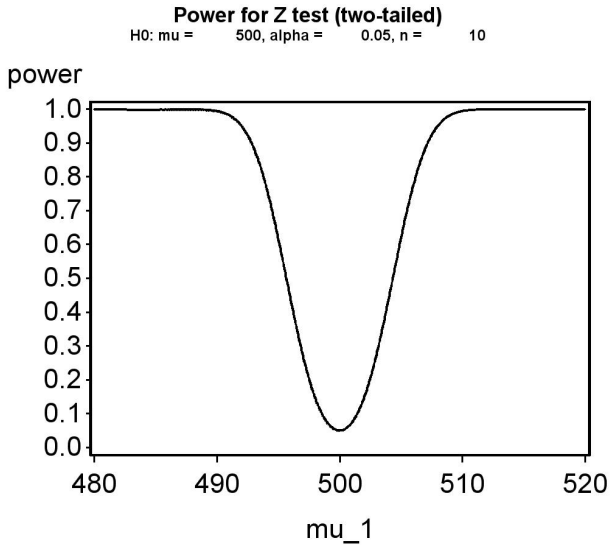


Figure 10.6: Power across a range of μ_1 values, for $H_0 : \mu = 500$, $\sigma = 7$, and $n = 10$

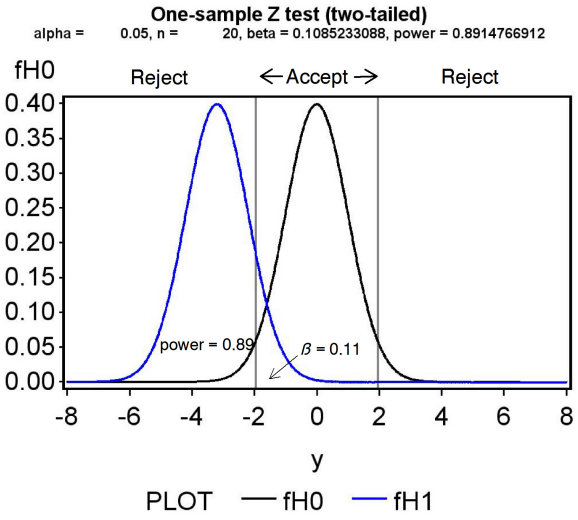


Figure 10.7: Distribution of Z_s under $H_1 : \mu = 495$, with $\sigma = 7, n = 20$ ($\phi = -3.19$).

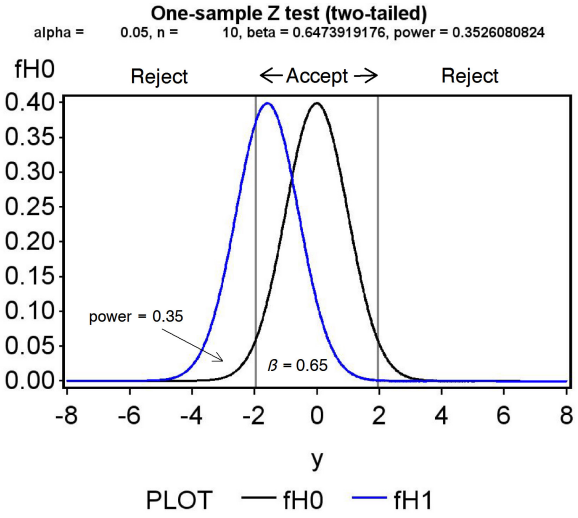


Figure 10.8: Distribution of Z_s under $H_1 : \mu = 495$, with $\sigma = 10, n = 10$ ($\phi = -1.58$).

Table 10.1: Effects on power and the Type II error rate β of changes in various parameters. The arrows indicate if a particular quantity increases or decreases.

Parameter	Direction	ϕ	power	β
$\lvert\mu_1 - \mu_0\rvert$	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$
n	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$
σ	$\uparrow$	$\downarrow$	$\downarrow$	$\uparrow$
α	$\uparrow$	no change	$\uparrow$	$\downarrow$

All of these effects on power can be understood through their influence on ϕ. Any change in a parameter value that makes ϕ larger increases power and reduces β, because it shifts the distribution of Z_s under H_1 away from the acceptance and into the rejection region. Thus, large differences between μ_1 and μ_0, large n, and small σ will all increase power because they increase ϕ. Conversely, similar values of μ_1 and μ_0, small n, and large σ would all reduce power. Table 10.1 summarizes how the different parameter values influence ϕ, power, and the Type II error rate β. Also shown is the effect of the Type I error rate α on power. If an investigator can accept a larger value of α, so that Type I errors are more common, this reduces the acceptance and increases the rejection region size, and thus increases power.

Note that a sufficiently large value of n can generate a large value of ϕ, even when μ_1 and μ_0 are close or σ is large. Thus, large sample sizes can yield adequate power even when the data are noisy, or the two means are similar in value. This basically arises from the inverse relationship between the variance of $\bar{Y}$ and n, i.e., $Var[\bar{Y}] = \sigma^2/n$, which is incorporated in the test statistic Z_s (see Eqn. 10.1).

10.6 Summary table

A common way of summarizing the different outcomes in hypothesis testing is the table below. The null hypothesis H_0 can be either true or false. If H_0 is true, then the test may accept H_0 and make a correct decision, or reject it and make a Type I error, with a Type I error rate of α. If H_0 is false, then the test may accept H_0 and make a Type II error with an error rate of β, or reject it and make a correct decision.

Table 10.2: Table summarizing the different outcomes in hypothesis testing, with the corresponding Type I (α) and Type II (β) error rates.

	Accept H_0	Reject H_0
H_0 true	Correct 1-α	Type I error α
H_0 false	Type II error β	Correct 1-β = power

10.7 One-sample t test

In the preceding, we used the test statistic Z_s to test $H_0 : \mu = \mu_0$ vs. $H_1 : \mu \neq \mu_0$, for the case where σ^2 or σ was known. Although this simplifies the statistics, in most cases we will need to estimate σ^2 and σ from the data using the sample variance s^2 and standard deviation s. We then use the test statistic

$$T_s = \frac{\bar{Y} - \mu_0}{s/\sqrt{n}} \tag{10.11}$$

to conduct the test (Bickel & Doksum 1977). T_s has a t distribution with $n-1$ degrees of freedom under H_0 (see Chapter 9). The following is therefore a true statement:

$$P[-c_{\alpha,n-1} < T_s < c_{\alpha,n-1}] = P[-c_{\alpha,n-1} < \frac{\bar{Y} - \mu_0}{s/\sqrt{n}} < c_{\alpha,n-1}] = 1 - \alpha. \tag{10.12}$$

The quantity $c_{\alpha,n-1}$ would be chosen using Table T, using the entry for $2(1-p)$ corresponding to α and the appropriate degrees of freedom (see Chapter 9). The interval $(-c_{\alpha,n-1}, c_{\alpha,n-1})$ is the acceptance region of a test with a Type I error rate of α, while the rejection region is its complement.

For example, with $\alpha = 0.05$ and $n = 10$, we have $c_{0.05,9} = 2.262$. We would therefore accept H_0 if T_s lies within $(-2.262, 2.262)$, and reject it if T_s lies outside this interval (see Fig. 10.9). Using absolute values, we would accept H_0 if $|T_s| < 2.262$ and reject it otherwise. For $\alpha = 0.01$ and $n = 10$, we have $c_{0.01,9} = 3.250$, and would accept H_0 if T_s lies within $(-3.250, 3.250)$ and reject it otherwise.

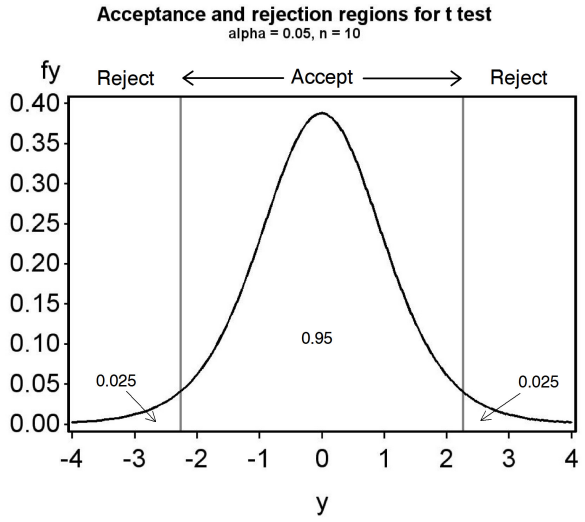


Figure 10.9: Acceptance and rejection regions for a one-sample t test, $\alpha = 0.05, n = 10$. The distribution shown is for the t distribution with $n - 1 = 9$ degrees of freedom.

10.7.1 One-sample t test - sample calculation

Recall the tilapia example, and suppose that $\bar{Y} = 493$ g and $s^2 = 48.2$ g^2, so that $s = 6.94$ g, with $n = 10$. We wish to test $H_0 : \mu = 500$ g vs. $H_1 : \mu \neq 500$ g. For the test statistic, we have

$$T_s = \frac{\bar{Y} - \mu_0}{s/\sqrt{n}} = \frac{493 - 500}{6.94/\sqrt{10}} = \frac{-7}{2.19} = -3.196 \tag{10.13}$$

For $\alpha = 0.05$, the acceptance region for T_s is $(-2.262, 2.262)$ with $n - 1 = 10 - 1 = 9$ degrees of freedom (Fig. 10.9). $T_s = -3.196$ lies outside this interval, so we would reject H_0 at the $\alpha = 0.05$ level. For $\alpha = 0.01$ the acceptance region is $(-3.250, 3.250)$. Because T_s lies within this interval, we would accept H_0 at this α level. We can also determine a P value for this test using Table T. The P value is found by scanning along the row in the table corresponding to 9 degrees of freedom, looking for two values that bracket T_s while ignoring its sign. We see that the values 2.821 and 3.250 bracket $T_s = -3.196$. Looking at the values for $2(1 - p)$, which correspond to α, this implies that $0.010 < P < 0.020$. This is the best accuracy that can be

accomplished using Table T, and to obtain an exact P value would require the use of SAS.

10.7.2 Hypothesis testing - SAS demo

We will use a small subset of our larger data set on elytra length (of the predatory beetle *Thanasimus dubius*) to illustrate hypothesis testing using SAS. The data are from a rearing study of insects reared on an artificial diet, and we want to compare their size to wild individuals. The subset data are for eight female *T. dubius* and are listed below:

```
5.2 4.2 5.7 5.4 4.0 4.5 5.2 4.2
```

Suppose that wild predators have an elytral length of 5.2 mm. This suggests testing $H_0 : \mu = 5.2$ mm vs. $H_1 : \mu \neq 5.2$ mm. We can conduct a one-sample t test for this null hypothesis using `proc univariate`, by adding the option `mu0=5.2` as an option. See SAS program and output listed below. The test statistic T_s and its P value are listed on one line in SAS output:

```
Student's t    t  -1.74574    Pr > |t|    0.1244
```

We see that $T_s = -1.75$ for this test. What is its P value? The notation `Pr > |t|` in the printout is shorthand for the $P[T_s < -1.75]+P[T_s > 1.75]$, the P value for this two-tailed test. We thus have $P = 0.1244$, a non-significant test result because $P > 0.05$. The degrees of freedom for the test are not reported by SAS, but are equal to $n - 1 = 8 - 1 = 7$. A sentence reporting this test result in a scientific journal would be something like 'A one-sample t test comparing the elytra length of individuals reared on artificial diet vs. wild individuals was non-significant ($t_7 = -1.75, P = 0.1244$).' Note that the degrees of freedom are reported as a subscript on the test statistic.

SAS Program

```
* one-sample_t_test.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'One-sample t-test for elytra data';
data elytra;
    input sex $ length;
    datalines;
F   5.2
F   4.2
F   5.7
F   5.4
F   4.0
F   4.5
F   5.2
F   4.2
;
run;
* Generate t test and plots;
proc univariate mu0=5.2 data=elytra;
    var length;
    histogram length / vscale=count normal(w=3) wbarline=3 waxis=3 height=4;
    qqplot length / normal waxis=3 height=4;
    symbol1 h=3;
run;
quit;
```

SAS Output

```
                    One-sample t-test for elytra data                    1
                                  13:34 Wednesday, June 23, 2010

                        The UNIVARIATE Procedure
                          Variable:  length

                                Moments

        N                          8    Sum Weights                8
        Mean                     4.8    Sum Observations          38.4
        Std Deviation      0.64807407    Variance                0.42
        Skewness           0.07137842    Kurtosis           -1.9577259
        Uncorrected SS         187.26    Corrected SS              2.94
        Coeff Variation    13.5015431    Std Error Mean     0.22912878
```

Basic Statistical Measures

Location		Variability	
Mean	4.800000	Std Deviation	0.64807
Median	4.850000	Variance	0.42000
Mode	4.200000	Range	1.70000
		Interquartile Range	1.10000

Note: The mode displayed is the smallest of 2 modes with a count of 2.

Tests for Location: Mu0=5.2

Test	-Statistic-		-----p Value------	
Student's t	t	-1.74574	Pr > |t|	0.1244
Sign	M	-1	Pr >= |M|	0.6875
Signed Rank	S	-7.5	Pr >= |S|	0.1563

Quantiles (Definition 5)

Quantile	Estimate
100% Max	5.70
99%	5.70
95%	5.70
90%	5.70
75% Q3	5.30
50% Median	4.85
25% Q1	4.20
10%	4.00
5%	4.00
1%	4.00
0% Min	4.00

10.7.3 Power analysis for one-sample t tests - SAS demo

A power analysis can be used to determine an adequate sample size n for a one-sample t test, as well as many other statistical tests. To conduct a power analysis, you need to specify a null and alternative hypothesis, a Type I error rate α, and have some estimate of the standard deviation σ of the population in question. The analysis then calculates the power for a range of n values. **The idea is to choose a value of n that gives power close to 0.8, often regarded as an adequate level of power (Cohen 1988).** The power analysis for a one-sample t test involves the same quantity

$$\phi = \frac{\mu_1 - \mu_0}{\sigma/\sqrt{n}} \tag{10.14}$$

as for the one-sample Z test, and its power is influenced by the same factors (see Table 10.1). The power calculation involves the **non-central t distribution** with a non-centrality parameter of ϕ. One subtle difference is that acceptance and rejection regions for the t test depends on n through the degrees of freedom, unlike the Z test. Larger values of n lead to smaller values of $c_{\alpha,n-1}$, shrinking the acceptance region and affecting the power calculation in this way.

Returning to the elytra example, suppose we want to test if the length of predators reared on an artificial diet differs from wild individuals, which have a length of 5.2 mm. This implies $H_0 : \mu = 5.2$ mm. For biological reasons, we are interested in detecting an decrease or increase in length of approximately 10% on the artificial diet, about 0.5 mm. This suggests an alternative hypothesis of the form $H_1 : \mu = 5.2 - 0.5 = 4.7$ mm (or $H_1 : \mu = 5.2 + 0.5 = 5.7$ mm). How many predators need to be reared on artificial diet to give a power of at least 0.8? Assume we already have an estimate of σ from another study, say $s = 0.6$ mm, and let $\alpha = 0.05$.

We can use `proc power` to find the sample size n that gives this power (SAS Institute Inc. 2014). See program and output below. We first specify a one-sample t test using the `onesamplemeans` option, followed by values for μ under H_0 (`nullmean = 5.2`), σ (`stddev = 0.6`), and μ under H_1 (`mean = 4.7`). The default value of α is 0.05. We then specify a range of sample sizes (n) for which we want the power to be calculated, using the option `ntotal = 2 to 20 by 1`. This finds the power for $n = 2, 3, \ldots, 20$. The `power = .` option tells SAS solve for power (there are other possibilities, like finding n for a given power value). The option `plot x=n` generates a low quality plot of power vs. n. We

can generate a better plot by sending the results of `proc power` to `gplot`, using an `ods output` option. We see that a sample size of $n = 14$ gives power > 0.8 for this scenario. While power increases rapidly for small sample sizes, there are diminishing returns once the power exceeds about 0.8. In other words, obtaining higher power values requires many more observations.

SAS Program

```
* One-sample_t_test_power2.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Power analysis for one-sample t test';
proc power;
    ods output Plotcontent=plotdata;
    onesamplemeans
        nullmean = 5.2
        stddev = 0.6
        mean = 4.7
        ntotal = 2 to 20 by 1
        power = . ;
    plot x=n;
run;
* Plot power vs. sample size in a nicer graph;
proc gplot data=plotall;
    plot power*ntotal=1 / vaxis=axis1 haxis=axis1 overlay;
    symbol1 i=join v=dot c=red width=3 height=2;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
quit;
```

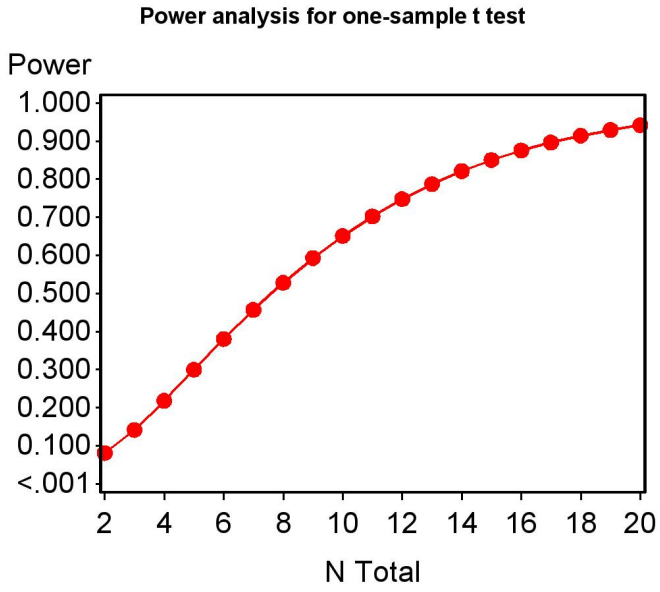


Figure 10.10: Power vs. n for a one-sample t test, for $H_0 : \mu = 5.2$ vs. $H_1 : \mu = 4.7$, with $\sigma = 0.6$ and $\alpha = 0.05$.

SAS Output

Power analysis for one-sample t test 1
13:34 Wednesday, June 23, 2010

The POWER Procedure
One-sample t Test for Mean

Fixed Scenario Elements

Distribution	Normal
Method	Exact
Null Mean	5.2
Mean	4.7
Standard Deviation	0.6
Number of Sides	2
Alpha	0.05

Computed Power

Index	N Total	Power
1	2	0.081
2	3	0.142
3	4	0.218
4	5	0.300
5	6	0.381
6	7	0.457
7	8	0.528
8	9	0.593
9	10	0.651
10	11	0.703
11	12	0.748
12	13	0.788
13	14	0.822
14	15	0.851
15	16	0.876
16	17	0.897
17	18	0.915
18	19	0.930
19	20	0.942

10.8 One-tailed t test

The tests we have examined so far are known as two-tailed tests. They are called this because the test statistic Z_s or T_s can detect departures from $H_0 : \mu = \mu_0$ in both directions, for $H_1 : \mu > \mu_0$ and $H_1 : \mu < \mu_0$, although the alternative for these tests is usually written more compactly as $H_1 : \mu \neq \mu_0$. We will now examine one-tailed tests, which have the same null hypothesis but the alternative is one direction or the other.

Suppose we are interested in testing $H_0 : \mu = \mu_0$ vs. $H_1 : \mu > \mu_0$. We can use the same test statistic as before, namely

$$T_s = \frac{\bar{Y} - \mu_0}{s/\sqrt{n}}. \tag{10.15}$$

If H_1 is true, we would expect to see $\bar{Y}$ values larger than μ_0, and so T_s would be positive. We would reject H_0 if T_s was sufficiently positive, with the acceptance and rejection regions determined as before by controlling the Type I error rate. Therefore, if the Type I error rate is α we want to determine a constant $c'_{\alpha,n-1}$ such that the following statement is true:

$$P[T_s < c'_{\alpha,n-1}] = 1 - \alpha \tag{10.16}$$

The quantity $c'_{\alpha,n-1}$ would be chosen using Table T, using the entry for p corresponding to $1 - \alpha$. We would accept H_0 if $T_s < c'_{\alpha,n-1}$ and reject it if $T_s \geq c'_{\alpha,n-1}$.

For example, with $\alpha = 0.05$ so that $p = 0.95$, and $n = 10$ (degrees of freedom $= n - 1 = 10 - 1 = 9$), we have $c'_{0.05,9} = 1.833$. We would therefore accept H_0 if $T_s < 1.833$ and reject it if $T_s \geq 1.833$ (see Fig. 10.11). For $\alpha = 0.01$ and $n = 10$, we have $c'_{0.01,9} = 2.822$, and would accept H_0 if $T_s < 2.822$ and reject it otherwise.

If we now wish to test $H_0 : \mu = \mu_0$ vs. $H_1 : \mu < \mu_0$, we would use the same test statistic as above. However, if H_1 is true we would expect $\bar{Y}$ to be smaller than μ_0, and so T_s would be negative. To determine the acceptance and rejection regions we would find $c'_{\alpha,n-1}$ in the same way as above, except we would use its negative. We would accept H_0 if $T_s > -c'_{\alpha,n-1}$ and reject it if $T_s \leq -c'_{\alpha,n-1}$. For example, if $\alpha = 0.05$ and $n = 10$, we would accept H_0 if $T_s > -1.833$ and reject it if $T_s \leq -1.833$ (Fig. 10.12). For $\alpha = 0.01$, we would accept H_0 if $T_s > -2.822$ and reject it otherwise.

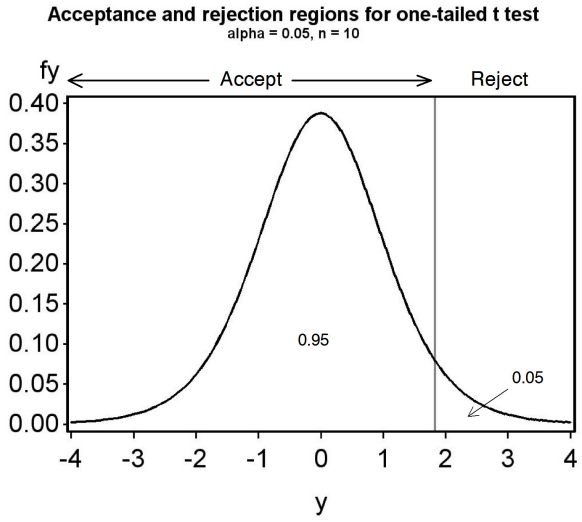


Figure 10.11: Acceptance and rejection regions for one-tailed t test, $H_0 : \mu = \mu_0$ vs. $H_1 : \mu > \mu_0$, for $\alpha = 0.05$ and $n = 10$.

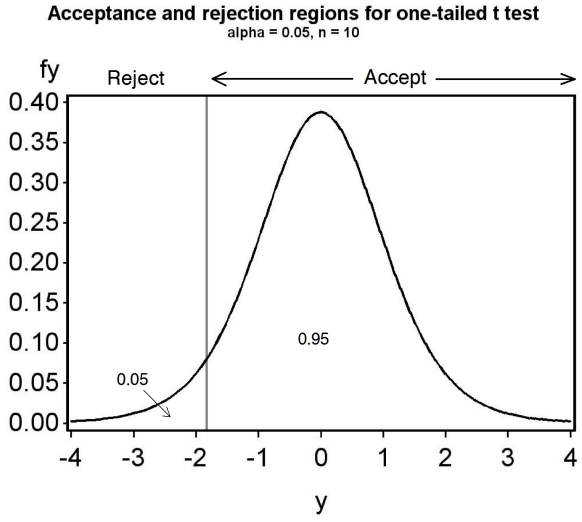


Figure 10.12: Acceptance and rejection regions for a one-tailed t test, $H_0 : \mu = \mu_0$ vs. $H_1 : \mu < \mu_0$, for $\alpha = 0.05$ and $n = 10$.

10.8.1 One-tailed t test - sample calculation

Recall the tilapia example, with $\bar{Y} = 493$ g, $s^2 = 48.2$ g^2, $s = 6.94$ g, and $n = 10$. Suppose we are only interested in detecting diets that produce fish of lower weight than natural food, implying we wish to test $H_0 : \mu = 500$ g vs. $H_1 : \mu < 500$ g. The test statistic value is again

$$T_s = \frac{\bar{Y} - \mu_0}{s/\sqrt{n}} = \frac{493 - 500}{6.94/\sqrt{10}} = \frac{-7}{2.19} = -3.196 \tag{10.17}$$

For $\alpha = 0.05$ and $n - 1 = 10 - 1 = 9$ degrees of freedom, we have $-c'_{0.05,9} = -1.833$. Because $T_s = -3.916 < -1.833$, we would reject H_0 at the $\alpha = 0.05$ level. For $\alpha = 0.01$, we have $-c'_{0.01,9} = -2.821$, and again $T_s = -3.196 < -2.821$. Thus, we can also reject H_0 at the $\alpha = 0.01$ level. We could continue this process with successively smaller values of α by scanning the row corresponding to 9 degrees of freedom in Table T, but cannot reject H_0 for smaller ones. Therefore, we have $P < 0.01$ for this test.

Suppose we had wanted to test $H_0 : \mu = 500$ g vs. $H_1 : \mu > 500$ g using the same data and test statistic value, namely $T_s = -3.196$. The scenario here could be that we want a commercial diet that actually increases the weight of tilapia over natural food, and are not interested in ones that yield lower weights. In this case, for $\alpha = 0.05$ we would not reject H_0, because $T_s = -3.196 < 1.833$. The test is non-significant, with $P > 0.05$.

10.8.2 One-tailed t test - SAS demo

Recall the elytra length example, where we tested $H_0 : \mu = 5.2$ mm vs. $H_1 : \mu \neq 5.2$ mm using SAS. While there is no option for one-tailed tests in `proc univariate`, we can reinterpret the output and so derive a P value for a one-tailed test.

Suppose we want to test $H_0 : \mu = 5.2$ mm vs. $H_1 : \mu < 5.2$ mm. This implies we want to test whether predators reared on artificial diet are smaller than those reared on natural food, which have a length of 5.2 mm. This would be reasonable if we want to detect diets that are deficient in some manner. If H_1 were true we would expect to see a negative value of T_s, because $\bar{Y}$ would likely be smaller than μ_0. This is what occurred in the SAS output, because $\bar{Y} = 4.8 < 5.2$ mm and $T_s = -1.75$:

```
Student's t    t  -1.74574    Pr > |t|    0.1244
```

The one-tailed P value in this case is simply half the two-tailed P value, or P(one-tailed) = P(two-tailed)/2= 0.1244/2 = 0.0622. This is because the two-tailed test gives the P value for both tails (see Fig. 10.9), but for this one-tailed test we only need the probability for the left tail of the t distribution (Fig. 10.12).

Now suppose we want to test $H_0 : \mu = 5.2$ mm vs. $H_1 : \mu > 5.2$ mm. This implies we want to test whether predators reared on artificial diet are larger than those reared on natural food. If H_1 were true we would expect to see a positive value of T_s, because $\bar{Y}$ would likely be greater than μ_0. This is not what occurred in the SAS output, because $\bar{Y} = 4.8 < 5.2$ mm and $T_s = -1.75$. The P value should therefore be large in this case, and in fact the one-tailed P value is $1 - P$(two-tailed) $= 1 - 0.1244/2 = 0.9378$. This is the probability for the right tail of the t distribution, which is large because T_s is negative.

We can distill the above procedures to a simple rule that will convert the SAS two-tailed P value to the appropriate one-tailed one. Assume $H_0 : \mu = \mu_0$ is the null hypothesis. **If the test statistic favors the alternative hypothesis, then the one-tailed P value is P(two-tailed)/2, otherwise it is $1 - P$(two-tailed)/2.** For example, if we have $H_1 : \mu > \mu_0$ and $T_s > 0$, the test statistic favors H_1 and the P value is P(two-tailed)/2. This procedure also works for tests calculated by hand. You first find the P value for the two-tailed test, then convert it to a one-tailed P value using the same rule.

10.8.3 One-tailed tests - a warning

As discussed above, the P value for a one-tailed test may sometimes be half the two-tailed P value. This makes it tempting to employ a one-tailed test after a two-tailed test yields a nonsignificant result. However, the proper procedure is to determine whether a one-tailed alternative hypothesis and test is appropriate for the situation **before** conducting the test. For example, artificial diets for insects are unlikely to yield larger insects than natural diets, and so it seems reasonable to use an alternative hypothesis of the form $H_1 : \mu < \mu_0$, where μ_0 is the size of insects reared on natural foods. This choice of an alternative hypothesis can be justified based on prior knowledge of the system.

10.9 Confidence intervals and hypothesis testing

Confidence intervals are typically used as measures of the accuracy or reliability of parameter estimates, but can also be used for hypothesis testing. Why might you do this? There are cases where the statistical software only provides confidence intervals for a parameter, but a test can still be developed using these intervals. Also, a publication may only provide confidence intervals for a parameter, but the reader can still conduct a test if required using these intervals. Some statisticians argue that this makes confidence intervals more useful than hypothesis testing, because they also provide information on the magnitude of a population parameter, and how reliably it is estimated (see Yaccoz 1991).

We will now demonstrate how a confidence interval for μ is equivalent to a one-sample t test. Recall that a $100(1-\alpha)\%$ confidence interval for μ has the form

$$\left(\bar{Y} - c_{\alpha,n-1}\frac{s}{\sqrt{n}}, \bar{Y} + c_{\alpha,n-1}\frac{s}{\sqrt{n}}\right) \tag{10.18}$$

(see Chapter 9). Suppose that we want to test $H_0 : \mu = \mu_0$. If we accept H_0 when this confidence interval includes μ_0, and reject it if the interval does not include μ_0, this is an α level test of H_0, equivalent to running a one-sample t test.

To see this connection, note that we would accept H_0 if μ_0 was inside this interval, or

$$\bar{Y} - c_{\alpha,n-1}\frac{s}{\sqrt{n}} < \mu_0 < \bar{Y} + c_{\alpha,n-1}\frac{s}{\sqrt{n}}. \tag{10.19}$$

Rearranging these inequalities, we see it is equivalent to saying

$$-c_{\alpha,n-1} < \frac{\bar{Y} - \mu_0}{s/\sqrt{n}} < c_{\alpha,n-1}, \tag{10.20}$$

or

$$-c_{\alpha,n-1} < T_s < c_{\alpha,n-1}, \tag{10.21}$$

where T_s is the test statistic for a one-sample t test. We would reject H_0 if T_s falls outside this interval. Note that this acceptance region is exactly the same as for the t test with Type I error rate of α, which is of the form $(-c_{\alpha,n-1}, c_{\alpha,n-1})$. Thus, the test based on a $100(1-\alpha)\%$ confidence interval

is equivalent to an α level test. In particular, a 95% confidence interval is equivalent to an $\alpha = 0.05$ test.

Conversely, it is often possible to reverse this process and obtain a confidence interval from a statistical test. The procedure is called 'inverting the test' (Bickel & Doksum 1977).

10.10 Likelihood ratio tests

We saw earlier how statisticians use the concept of maximum likelihood to estimate population parameters (Chapter 8). The maximum likelihood method begins by constructing a likelihood function based on the distribution of the data (Poisson, normal, etc.) and the observed data. We then maximize the likelihood as a function of the parameters of the distribution (μ, σ^2, etc). The values of the parameters that maximize the likelihood are the maximum likelihood estimates of the parameters. The likelihood function is not a fixed quantity but instead varies with the observed data, so that different data sets yield different estimates of the population parameters. Maximum likelihood estimators have desirable statistical properties and in many cases yield estimators that seem reasonable (like using $\bar{Y}$ to estimate μ).

Likelihood methods can also be used to develop statistical tests called **likelihood ratio tests**. These tests also have desirable statistical properties and in many cases are identical to classical statistical tests. Likelihood methods thus provide a theoretical framework for many statistical problems, including parameter estimation, confidence intervals, and hypothesis testing. The main drawback of these methods is that one must be willing to specify the distribution of the data, be it Poisson, binomial, normal, or more exotic distributions.

10.10.1 Example of a likelihood ratio test

We will now develop a likelihood ratio test that leads to the familiar one-sample t test (Mood et al. 1974) . We suppose that the data are normally distributed and we wish to test $H_0 : \mu = \mu_0$ vs. $H_1 : \mu \neq \mu_0$. A random sample with n observations has been obtained.

We can think of H_0 and H_1 as two different statistical models for the data. Under H_0, the data are assumed to be normally distributed with $\mu = \mu_0$, but

can have any value of σ^2 because this parameter is left unspecified. Under H_1, the data are permitted to have any value of μ and σ^2.

The first step in constructing a likelihood ratio test is to find the maximum likelihood estimates of the parameters for each of these two statistical models. We have already dealt with this problem for the model specified by H_1 – this is just maximum likelihood estimation of μ and σ^2 for the normal distribution. The same methods can be used to estimate σ^2 under H_0, but we will not go into the details.

This process can be illustrated by plotting the likelihood function as a function of μ and σ^2. To make things more concrete, we show the likelihood function for a data set with three data points ($Y_1 = 4.5$, $Y_2 = 5.3$, and $Y_3 = 5.4$). Also shown is a possible null hypothesis for these data, such as $H_0 : \mu = 4.7$. See figure below.

The maximum likelihood estimates of μ and σ^2 under H_1 are the values of μ and σ^2 found at the peak of the likelihood function. However, the maximum likelihood estimate of σ^2 under H_0 occurs at a different location. Because μ is fixed at 4.7 under H_0, σ^2 is only free to vary along the vertical line shown in the figure. The maximum likelihood estimate of σ^2 under H_0 is the value of σ^2 that maximizes the likelihood along this line.

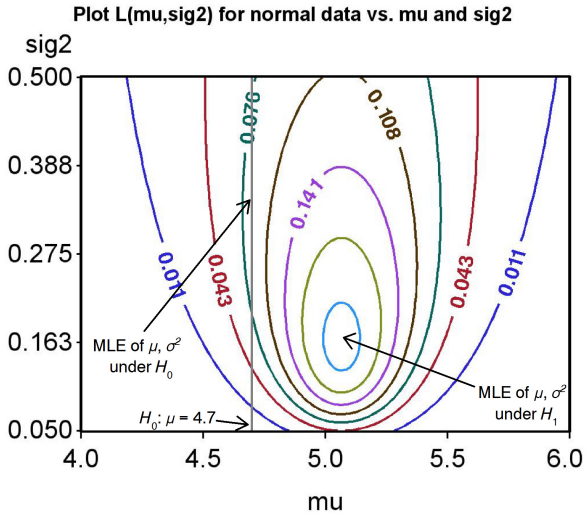


Figure 10.13: Likelihood ratio test for $H_0 : \mu = 4.7$

We are now ready to construct the likelihood ratio test statistic. Let L_{H_0}

be the maximum height of the likelihood surface under H_0, which occurs at the maximum likelihood estimate of σ^2 under H_0. Similarly, let let L_{H_1} be the maximum height under H_1, which occurs at the estimates of μ and σ^2 under H_1. The test statistic λ is just the ratio of these two quantities:

$$\lambda = \frac{L_{H_0}}{L_{H_1}}. \tag{10.22}$$

How does this statistic behave? If H_0 is true, the peak of the likelihood function will be near the vertical line, and the height of the likelihood function will be similar at the two locations. This implies a value of $\lambda \approx 1$ because $L_{H_0} \approx L_{H_1}$. If H_0 is false and H_1 true, however, we would expect to see $L_{H_0} < L_{H_1}$ and so $\lambda < 1$. We would therefore reject H_0 for sufficiently small values of λ.

More formally, we reject H_0 if $\lambda < c$ and accept H_0 otherwise. The value of c is determined using the Type I error rate α and the distribution of λ under H_0.

An alternate form of the test uses $-2\ln(\lambda)$ rather than λ itself, and rejects H_0 for values of $-2\ln(\lambda) > d$, where d is a constant that controls the Type I error rate. This form of the test rejects for large values of the test statistic, similar to other tests we have developed. Note that

$$-2\ln(\lambda) = 2\ln(L_{H_1}) - 2\ln(L_{H_0}) \tag{10.23}$$

by the properties of logarithms, and is a positive quantity. SAS provides values of the likelihood function in this format for some statistical procedures, and these can be used to construct likelihood ratio tests.

How is the likelihood ratio test related to a t test? It can be shown mathematically that the value of the test statistic

$$T_s = \frac{\bar{Y} - \mu_0}{s/\sqrt{n}} \tag{10.24}$$

is directly proportional to $-2\ln(\lambda)$, the likelihood ratio test statistic (Mood et al. 1974). The figure below plots the value of $-2\ln(\lambda)$ vs. T_s for a scenario matching our example data set. We observe there is a one-to-one correspondence between the two test statistics. When such a correspondence occurs between two test statistics, the tests are considered to be statistically equivalent. We will later see that many statistical tests are in fact likelihood ratio tests. These include tests in analysis of variance, regression, and methods for categorical data such as χ^2 tests.

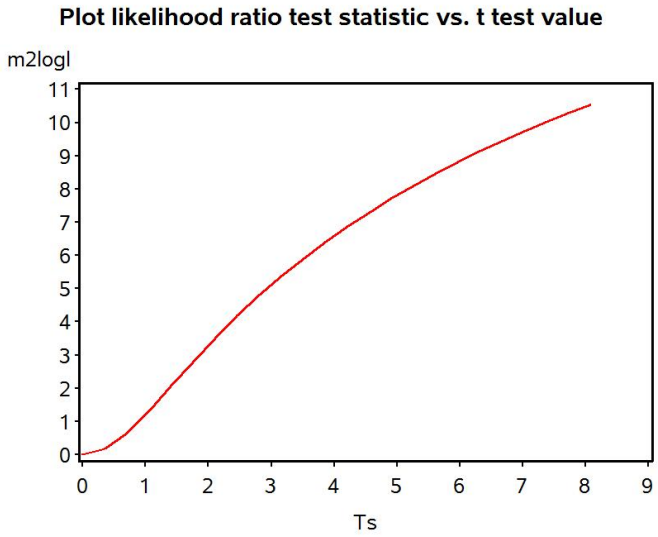


Figure 10.14: Likelihood ratio vs. t test statistics.

10.11 References

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10.12 Problems

1. A company that rears beneficial insects produces lacewings (Chrysopidae: Neuroptera) whose mean length is 10 mm. A new method of rearing is being tested and the company wants to determine if the new method changes lacewing length. A sample of 10 insects is collected for the new method, yielding the following lengths:

```
10.3  14.1  11.5  9.9  12.6  9.7  11.0  9.5  12.4  13.5
```

   (a) Test whether the lacewings produced using the new method have the same length as before ($H_0 : \mu = 10$ vs. $H_1 : \mu \neq 10$), using a two-tailed test and Table T. Provide a P value and discuss the significance of the test. Show your calculations.

   (b) Suppose the company is only interested in rearing methods that yield larger lacewing lengths, because bigger is better with beneficial insects. Test $H_0 : \mu = 10$ vs. $H_1 : \mu > 10$. Provide a P value and discuss the significance of the test.

   (c) Use SAS and `proc univariate` to carry out the same two tests. What are the exact P values for these tests? Attach your SAS program and printout.

2. A study is done to measure the concentration of a particular chemical (ppm) in drinking water, with samples taken at eight locations. The samples were analyzed and the following results obtained:

```
23 20 24 20 23 24 21 22
```

   (a) Test whether the concentration of the chemical is significantly different from 20 ppm, the level set by the EPA, using a two-tailed test and Table T. Provide a P value and discuss the significance of the test. Show your calculations.

   (b) The EPA actually requires that the concentration of the chemical be equal to or below 20 ppm. Test whether the chemical concentration exceeds this level using a one-tailed test and Table T. In particular, test $H_0 : \mu = 20$ vs. $H_1 : \mu > 20$. Provide a P value and discuss the significance of the test.

(c) Use SAS and `proc univariate` to carry out the same two tests. What are the exact P values for these tests? Attach your SAS program and printout.

Chapter 11

Analysis of Variance (One-Way)

We now develop a statistical procedure for comparing the means of two or more groups, known as analysis of variance or ANOVA. These groups might be the result of an experiment in which organisms are exposed to different treatments. Alternately, the groups might be different species or different age classes of the same species, populations in different locations, or different genetic families. The test works by comparing the variance among the group means to the variance of the observations within each group – if the variance among group means is large (implying differences in their means) relative to the variance within groups, the test is significant. This chapter will examine tests for one-way ANOVA, in which a single factor like a treatment affects the observations. More complex designs are possible in which several factors may influence the observations and may also interact (see Chapter 14 and 19).

What do the data look like for a one-way ANOVA design? Suppose we are interested in trapping bark beetles (Coleoptera: Curculionidae: Scolytinae) using different chemical baits, which could involve the beetle's sex pheromones or odors of the trees they colonize. Suppose that three different baits (A, B, and C), with $a = 3$ denoting the number of treatments. The baits are deployed on traps in the forest, with $n = 5$ replicate traps for each bait type. A typical experimental design would establish 15 traps in the forest, and then randomly assign a bait to each trap. After a period of time, the traps would be checked and the number of insects caught in each trap recorded (Table 11.1). Because the data are counts, it would not be normally

distributed but more likely have a Poisson or negative binomial distribution (see Chapter 5). However, it is often possible to **transform** count data to have a distribution closer to the normal by taking the square root or log of the counts (see Chapter 15). The third column in Table 11.1 shows the count data after applying a log transformation. The notation Y_{ij} is often used to refer to the observations in ANOVA designs. The i subscript refers to the group or treatment, while j is the observation within the treatment. For example, Y_{13} refers to the third observation in the first treatment, which is 2.41.

Another one-way ANOVA design for bark beetles might simply look at variability in their density across sites. Suppose there is a large collection of study sites, and we randomly select five sites for trapping. Five traps are deployed at each of the five sites and the number of beetles caught per trap is recorded. Data for a study of this type are listed below, also with a log transformation (Table 11.2). There appears to be substantial variability in beetle abundance across sites, with Site 4 having very high beetle catches, while Site 5 has low ones.

The data sets presented in this section represent **balanced designs**, because there are the same number of replicates for each treatment or group. An **unbalanced design** would have an unequal number of replicates, possibly very unequal. We will present tests and theory for balanced designs in this chapter, because this greatly simplifies the formulas and equations. However, these results can be readily extended to unbalanced designs, and unbalanced designs require no changes in the SAS programs presented.

Table 11.1: Example 1 - Bark beetles captured in a trapping experiment comparing the attraction to different baits. There were three baits (A, B, and C) and five replicate traps per bait treatment. Also shown are the log-transformed counts (Y_{ij}) and subscript values (i, j), and some preliminary one-way ANOVA calculations.

Treatment	Count	$Y_{ij} = \log_{10}(\text{Count})$	i	j	$\bar{Y}_{i\cdot}$	$(Y_{ij} - \bar{Y}_{i\cdot})^2$	$\sum(Y_{ij} - \bar{Y}_{i\cdot})^2$
A	373	2.57	1	1		0.0441	
A	126	2.10	1	2		0.0676	
A	255	2.41	1	3	2.3600	0.0025	0.2110
A	138	2.14	1	4		0.0484	
A	379	2.58	1	5		0.0484	
B	25	1.40	2	1		0.0999	
B	64	1.81	2	2		0.0088	
B	62	1.79	2	3	1.7160	0.0055	0.1325
B	71	1.85	2	4		0.0180	
B	54	1.73	2	5		0.0002	
C	449	2.65	3	1		0.1832	
C	249	2.40	3	2		0.0317	
C	69	1.84	3	3	2.2220	0.1459	0.4581
C	199	2.30	3	4		0.0061	
C	84	1.92	3	5		0.0912	

Table 11.2: Example 2 - Bark beetles captured in a trapping study comparing their abundance at five randomly chosen study sites. There were five replicate traps per site. Also shown are the log-transformed counts (Y_{ij}) and subscript values (i, j), and some preliminary one-way ANOVA calculations.

Site	Count	$Y_{ij} = \log_{10}(\text{Count})$	i	j	$\bar{Y}_{i\cdot}$	$(Y_{ij} - \bar{Y}_{i\cdot})^2$	$\sum(Y_{ij} - \bar{Y}_{i\cdot})^2$
1	137	2.14	1	1		0.0164	
1	101	2.00	1	2		0.0001	
1	113	2.05	1	3	2.0120	0.0014	0.1598
1	48	1.68	1	4		0.1102	
1	155	2.19	1	5		0.0317	
2	156	2.19	2	1		0.0784	
2	165	2.22	2	2		0.0625	
2	652	2.81	2	3	2.4700	0.1156	0.4730
2	179	2.25	2	4		0.0484	
2	757	2.88	2	5		0.1681	
3	278	2.44	3	1		0.0376	
3	197	2.29	3	2		0.0019	
3	95	1.98	3	3	2.2460	0.0708	0.3419
3	395	2.60	3	4		0.1253	
3	83	1.92	3	5		0.1063	
4	2540	3.40	4	1		0.4956	
4	613	2.79	4	2		0.0088	
4	200	2.30	4	3	2.6960	0.1568	0.7600
4	251	2.40	4	4		0.0876	
4	390	2.59	4	5		0.0112	
5	18	1.26	5	1		0.0044	
5	16	1.20	5	2		0.0000	
5	11	1.04	5	3	1.1940	0.0237	0.0459
5	21	1.32	5	4		0.0159	
5	14	1.15	5	5		0.0019	

11.1 ANOVA models

We now examine the statistical models that are used in one-way ANOVA. There are two models for one-way ANOVA, known as fixed or random effects models, but sometimes called Model I and II. This classification is based on how the groups in the design are defined or generated. We begin by defining fixed and random effects, then present the statistical models and hypotheses for each type.

11.1.1 Fixed and random effects

For groups generated by different treatments in an experiment, or purposely chosen groups of organisms such as different species, sexes, or ages, the groups are classified as **fixed effects**. They are called fixed effects because these groups are the only ones of interest to the investigator, and the only ones on which a statistical inference can be made (Littell et al. 1996, McCulloch and Searle 2001). They are also incorporated in statistical models as fixed parameters. Groups that are generated by a process of random sampling are classified as a **random effects** (Littell et al. 1996, McCulloch and Searle 2001). For example, suppose we want to examine the fish populations in a large number of lakes, and are interested in how body length varies across lakes. If we randomly sample the lakes to be examined, from a large collection of lakes, then lake would be classified as a random effect. In many genetic experiments, families are chosen at random from a larger collection of families, making family a random effect. Random effects are incorporated in statistical models as random variables, typically with a normal distribution.

These definitions suggest a simple test for fixed vs. random effects – if the groups are a random sample from a large collection you have a random effect, otherwise a fixed effect. Although it is usually possible to declare an effect as either fixed or random, in practice it is sometimes difficult to decide. For example, suppose that a particular organism occurs at only a small number of locations. If we randomly select a subset of these locations to sample, seemingly implying a random effect, the overall number of locations is still finite. In this scenario, location may be better classified as a fixed effect.

11.1.2 Fixed effects model

Suppose that we want to model the observations in the bark beetle trapping experiment, Example 1, where different baits are used. Recall that the symbol Y_{ij} stand for the jth observation in the ith treatment group, where $i = 1, 2, 3$ and $j = 1, 2, 3, 4, 5$. For example, $Y_{11} = 2.57$ and $Y_{12} = 2.10$, while $Y_{32} = 2.40$ (see Table 11.1). One commonly used model for such a design is

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \tag{11.1}$$

where μ is a parameter setting the grand mean (the overall mean) of the observations, α_i is the deviation from the grand mean caused by the ith treatment (McCulloch and Searle 2001), and $\epsilon_{ij} \sim N(0, \sigma^2)$. It is usually assumed that $\sum \alpha_i = 0$, i.e., the treatment effect terms sum to zero. The ϵ_{ij} term represents random departures from the mean value for the ith treatment, due to natural variability among the observations. The ϵ_{ij} values are also assumed to be independent (Chapter 4). In practice, these parameters would be unknown but could be estimated from the data. The same model can be used to describe the observations for experiments with any number of treatments (any a value) as well as replicates per treatments (any n), as well as experiments where the number of observations is unequal among treatments.

It follows that for the ith treatment, $E[Y_{ij}] = \mu + \alpha_i$ and $Var[Y_{ij}] = \sigma^2$, using the rules for expected values and variances. Thus, for the ith treatment we have $Y_{ij} \sim N(\mu + \alpha_i, \sigma^2)$. We can illustrate how the different parameters work in this model with a diagram that plots the distribution for each group. Suppose that we want to model an experiment similar to the bark beetle trapping one, with $a = 3$ treatments. Suppose that $\mu = 2.1$, $\alpha_1 = 0.25$, $\alpha_2 = -0.40$, and $\alpha_3 = 0.15$, with $\sigma^2 = 0.1$. Fig. 11.1 shows the distribution of the observations in each treatment group. Note that the means for treatment 1 and 3 are shifted upward from the grand mean by their positive values of α_i, while the mean for treatment 2 is shifted downward by its negative value. The distribution for each treatment has the same variance, namely $\sigma^2 = 0.1$.

The usual objective in ANOVA is to test whether the means of the different groups are significantly different, implying there is treatment or group effect. In terms of the fixed effects model, this amounts to testing whether the α_i values are significantly different from zero, because it is these parameters that produce shifts in the group means from the grand mean. More formally, we are interested in testing the null hypothesis H_0 : all $\alpha_i = 0$.

Under H_0, all the groups have the same mean μ because the α_i terms are zero (Fig. 11.2). The alternative hypothesis would be H_1 : some $\alpha_i \neq 0$, i.e., there is some treatment effect on some (perhaps all) groups (Fig. 11.1). We will discuss how this null hypothesis is actually tested later in the chapter.

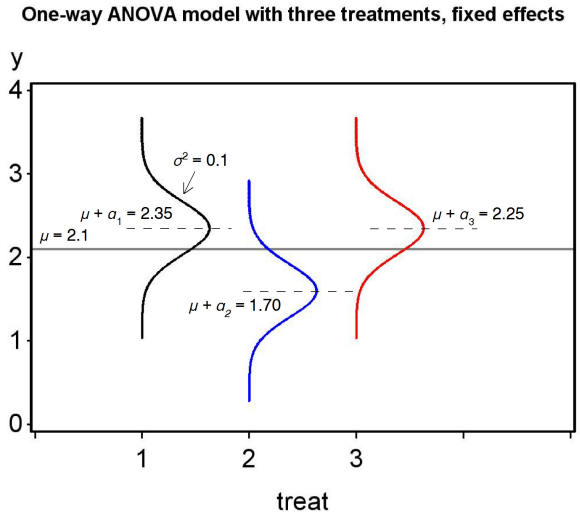

Figure 11.1: Fixed effects model for one-way ANOVA, under H_1 : some $\alpha_i \neq 0$.

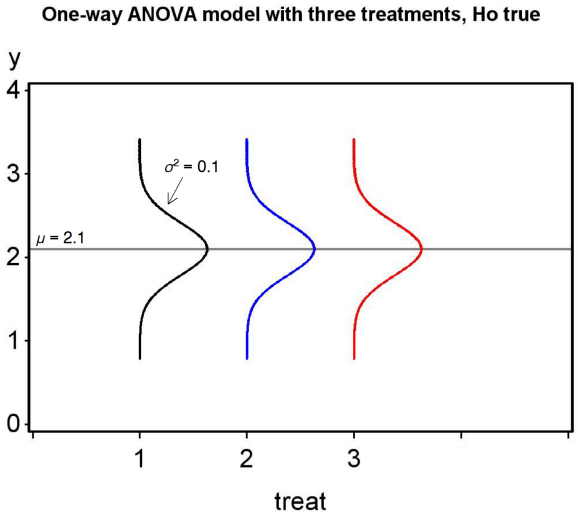

Figure 11.2: Fixed effects model for one-way ANOVA, under H_0 : all $\alpha_i = 0$.

11.1.3 Random effects model

Suppose that we now want to model the variability in bark beetle abundance across different sites, such as in the Example 2 study. Let Y_{ij} stand for the jth observation at the ith sampled site, with $i = 1, 2, 3, 4, 5$ and $j = 1, 2, 3, 4, 5$. We have $Y_{11} = 4.92$, $Y_{12} = 4.62$, and so forth (see Table 11.2). A common statistical model for this design is

$$Y_{ij} = \mu + A_i + \epsilon_{ij} \tag{11.2}$$

where μ is again a parameter setting the grand mean (the overall mean) of the observations, with A_i a random deviation from the grand mean due to the ith site (McCulloch and Searle 2001), and $\epsilon_{ij} \sim N(0, \sigma^2)$. It is assumed that A_i is normally distributed with mean zero and variance σ_A^2, or $A_i \sim N(0, \sigma_A^2)$. Note that in the random effects model the group effect is indeed a random variable, one whose variance is unknown but can be estimated from the data. The variances σ_A^2 and σ^2 are collectively called the **variance components** of the model.

For the ith group sampled, it can be shown that $E[Y_{ij}] = \mu + A_i$ and $Var[Y_{ij}] = \sigma^2$, using the rules for expected values and variances. Thus, for the ith treatment we have $Y_{ij} \sim N(\mu + A_i, \sigma^2)$. Because the A_i values are themselves random quantities, however, the expected value is itself a random quantity and would differ for each group sampled. We again illustrate how the model works using a diagram showing the distribution for each group. Suppose that we want to model a study similar to the second bark beetle one (Table 11.2), with $a = 5$ sites randomly selected from a larger collection of sites. Suppose that $\mu = 2.1$ and $\sigma^2 = 0.1$. The first time we did this study, we might see a pattern like Fig. 11.3. If we redid the study and randomly selected another five sites, we would get a different pattern (Fig. 11.4). This illustrates that this model is not static like the fixed effects one, but instead would vary with the sites actually sampled. In the random effects model, we are usually interested in testing whether the variance of A_i is zero vs. greater than zero, or $H_0 : \sigma_A^2 = 0$ vs. $H_1 : \sigma_A^2 > 0$. Under $H_0 : \sigma_A^2 = 0$, all the A_i values must be zero (to give $\sigma_A^2 = 0$), and so all the groups have the same mean μ. A plot of the model under H_0 would therefore be similar to Fig. 11.2. This null hypothesis is tested in the same way as the one for the fixed effects model (see below).

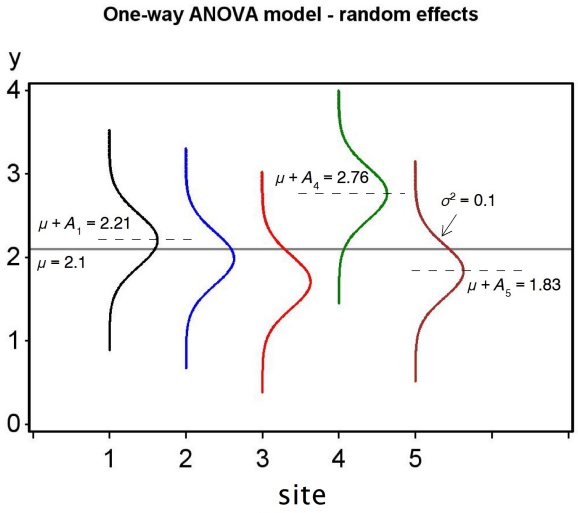

Figure 11.3: Random effects model for one-way ANOVA, for the first time sites are sampled.

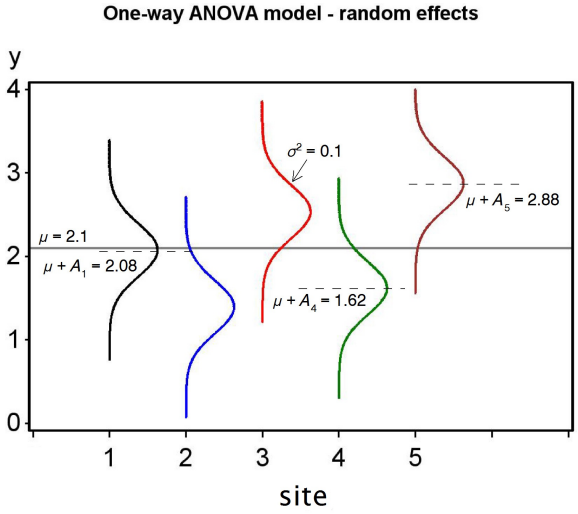

Figure 11.4: Random effects model for one-way ANOVA, for the second time sites are sampled.

11.2 Hypothesis testing for ANOVA

We now develop a statistical test for the null hypotheses in both fixed and random effects models, either H_0 : all $\alpha_i = 0$ or $H_0 : \sigma_A^2 = 0$. We will first present the test and explain how it works in terms of different estimates of the variance, then later show it is another example of a likelihood ratio test.

11.2.1 Sums of squares and mean squares

Suppose the data are described by a fixed effects model, for which the hypotheses are H_0 : all $\alpha_i = 0$ vs. H_1 : some $\alpha_i \neq 0$. It is clear that if H_1 is true, then the observations for the different groups will be shifted from the grand mean, as shown in Fig. 11.1, and in particular $Y_{ij} \sim N(\mu + \alpha_i, \sigma^2)$ for each group. For a random effects model, we have $H_0 : \sigma_A^2 = 0$ vs. $H_1 : \sigma_A^2 > 0$. If H_1 is true, we would also expected the observations for the different groups to be shifted away from the grand mean (Fig. 11.3), and in particular $Y_{ij} \sim N(\mu + A_i, \sigma^2)$. How can we estimate this shift in actual data? How large must this shift be to be judged statistically significant?

We begin by calculating the means for each group using the data. These are labeled as $\bar{Y}_{i\cdot}$ and are called group means. The '$\cdot$' subscript implies the mean was calculated using all the observations in that group ($j = 1, 2, \ldots, n$). We then calculate the mean of the group means, called the grand mean and labeled as $\bar{\bar{Y}}$. If the ith group is shifted from the grand mean, we can measure this shift using the quantity $\bar{Y}_{i\cdot} - \bar{\bar{Y}}$. In fact, this quantity estimates α_i for the ith group, and so is a direct measure of any group effect (see section on maximum likelihood estimation below). If these quantities are small then this suggests H_0 might be true, whereas if they are large this provides evidence for H_1. We can obtain a single measure of these shifts by squaring and summing them across all groups, to obtain a quantity called the sum of squares among groups or SS_{among}, because it measures variation in the observations among groups:

$$SS_{among} = n \sum_{i=1}^{a} (\bar{Y}_{i\cdot} - \bar{\bar{Y}})^2. \tag{11.3}$$

Note the sample size n in this expression, which we will justify below. To make this quantity more concrete, we will calculate SS_{among} for Example 1, the bark beetle trapping experiment. We first calculate the sample mean for

each group for the log-transformed data, as shown in Table 11.1. Then, the grand mean is estimated using the mean of these means, or

$$\bar{\bar{Y}} = \frac{\sum_{i=1}^{a} \bar{Y}_{i\cdot}}{a} = \frac{2.3600 + 1.7160 + 2.2220}{3} = \frac{6.2980}{3} = 2.0993. \tag{11.4}$$

We then have

$$SS_{among} = n \sum_{j=1}^{a} (\bar{Y}_{i\cdot} - \bar{\bar{Y}})^2 \tag{11.5}$$

$$= 5 \left[(2.3600 - 2.0993)^2 + (1.7160 - 2.0993)^2 + (2.2220 - 2.0993)^2 \right] \tag{11.6}$$

$$= 5 \left[0.0680 + 0.1469 + 0.0151 \right] \tag{11.7}$$

$$= 1.1500 \tag{11.8}$$

SS_{among} has $a-1$ degrees of freedom, where a is the number of groups. There are $a-1$ degrees of freedom because there are a terms of the form $\bar{Y}_{i\cdot} - \bar{\bar{Y}}$ in the sum of squares, but these sum to zero so there are really only $a-1$ independent terms (similar to the $n-1$ degrees of freedom for the sample variance s^2). The next step is to convert SS_{among} to a sample variance, dividing it by $a-1$. This quantity is called the mean square among groups:

$$MS_{among} = \frac{SS_{among}}{a-1} = \frac{n \sum_{j=1}^{a} (\bar{Y}_{i\cdot} - \bar{\bar{Y}})^2}{a-1}. \tag{11.9}$$

For the bark beetle experiment, we find that

$$MS_{among} = \frac{SS_{among}}{a-1} = \frac{1.1500}{3-1} = 0.5750. \tag{11.10}$$

So, what variance does MS_{among} estimate? If H_0 is true and there are no group effects, we would expect $\bar{Y}_{i\cdot}$ to have a variance of σ^2/n, because it is a sample mean composed of n observations in the ith group (which have a variance of σ^2). MS_{among} estimates this variance multiplied by n, because of the n term in numerator, and so actually estimates $n\sigma^2/n = \sigma^2$. On the other hand, if H_1 is true then there are group effects, and we would expect the group means to be shifted away from the grand mean. This should increase the size of MS_{among}. **Thus, MS_{among} estimates σ^2 if H_0 is true but becomes larger if H_1 is true.**

We next develop an estimate of the variance σ^2 that is free of any effects, fixed or random. This variance estimate is based on a quantity called the sum of squares within groups or SS_{within}, because it measures variation of the observations within each group. It is defined by the formula

$$SS_{within} = \sum_{i=1}^{a}\sum_{j=1}^{n}(Y_{ij} - \bar{Y}_{i\cdot})^2 \tag{11.11}$$

$$= \sum_{j=1}^{n}(Y_{1j} - \bar{Y}_{1\cdot})^2 + \ldots + \sum_{j=1}^{n}(Y_{aj} - \bar{Y}_{a\cdot})^2. \tag{11.12}$$

It has $a(n-1)$ degrees of freedom, because there are a sum of squares terms each with $n-1$ degrees of freedom. We can obtain an estimate of σ^2 by dividing this sum of squares by its degrees of freedom, to obtain the mean square within groups:

$$MS_{within} = \frac{SS_{within}}{a(n-1)} = \frac{\sum_{i=1}^{a}\sum_{j=1}^{n}(Y_{ij} - \bar{Y}_{i\cdot})^2}{a(n-1)}. \tag{11.13}$$

This quantity estimates σ^2 because it simply averages estimates of σ^2 for each group. With some rearrangement, we can write MS_{within} as

$$MS_{within} = \frac{\sum_{i=1}^{a}\sum_{j=1}^{n}(Y_{ij} - \bar{Y}_{i\cdot})^2}{a(n-1)} \tag{11.14}$$

$$= \frac{\sum(Y_{1j} - \bar{Y}_{1\cdot})^2 + \ldots + \sum(Y_{aj} - \bar{Y}_{a\cdot})^2}{a(n-1)} \tag{11.15}$$

$$= \frac{\frac{\sum(Y_{1j} - \bar{Y}_{1\cdot})^2}{n-1} + \ldots + \frac{\sum(Y_{aj} - \bar{Y}_{a\cdot})^2}{n-1}}{a} \tag{11.16}$$

$$= \frac{s_1^2 + \ldots + s_a^2}{a}. \tag{11.17}$$

Each term in the numerator of this expression is the sample variance s^2 for each group, which is then averaged across all groups to yield an overall or **pooled** estimate of σ^2. The word 'pooled' in statistics often indicates a combined estimate of a variance. It can also be shown that $E[MS_{within}] = \sigma^2$, regardless of any group effects.

We now calculate MS_{within} for the bark beetle experiment. We first need to calculate the quantity $(Y_{ij} - \bar{Y}_{i\cdot})^2$ for the observations in each group and

then sum these for each group (see Table 11.1). Summing these quantities in turn across all groups, we obtain

$$SS_{within} = 0.2110 + 0.1325 + 0.4581 = 0.8016. \tag{11.18}$$

(11.19)

We then have

$$MS_{within} = \frac{SS_{within}}{a(n-1)} = \frac{0.8016}{3(5-1)} = 0.0668. \tag{11.20}$$

(11.21)

There is one more sum of squares that can be calculated in one-way ANOVA, the total sum of squares. It is defined as

$$SS_{total} = \sum_{i=1}^{a}\sum_{j=1}^{n}(Y_{ij} - \bar{\bar{Y}})^2. \tag{11.22}$$

It measures the variability of the observations around the grand mean of the data ($\bar{\bar{Y}}$) and has $an - 1$ degrees of freedom. Applying this formula to the Example 1 data set, we obtain $SS_{total} = 1.9516$ after much calculation.

An interesting feature of the sum of squares is that they add to the total sum of squares, as do the degrees of freedom. In particular, we have

$$SS_{among} + SS_{within} = SS_{total} \tag{11.23}$$

and

$$(a-1) + a(n-1) = an - 1. \tag{11.24}$$

Thus, the sum of squares and degrees of freedom can be neatly partitioned into components corresponding to among group and within group variation. We will illustrate this relationship further in the section below on ANOVA tables.

11.2.2 F statistic and distribution

We next describe the statistic used to test H_0 : all $\alpha_i = 0$ for the fixed effect model, and $H_0 : \sigma_A^2 = 0$ for the random effects one. It is simply the ratio of MS_{among} and MS_{within}, or

$$F_s = \frac{MS_{among}}{MS_{within}}. \tag{11.25}$$

If H_0 is true for either model, both MS_{among} and MS_{within} estimate σ^2 and we would expect their ratio, F_s, to be small and on the order of one. However, if H_0 is false and H_1 is true, we would expect MS_{among} to become larger and F_s to increase. We would therefore reject H_0 for large values of F_s.

To complete our testing procedure and find P values, we need to know the distribution of F_s under H_0. It turns out this statistic has an F distribution under H_0, whose shape and location is governed by two parameters, the degrees of freedom for MS_{among} and MS_{within}. These are called the numerator and denominator degrees of freedom, which we abbreviate as df_1 and df_2. In particular, for one-way ANOVA we have $df_1 = a - 1$ and $df_2 = a(n - 1)$. Figure 11.5 shows the F distribution for three different sets of parameter values. Note that distribution can have a maximum at $y = 0$ for small values of df_1, while larger values of df_2 decrease the probability in the right tail of the distribution.

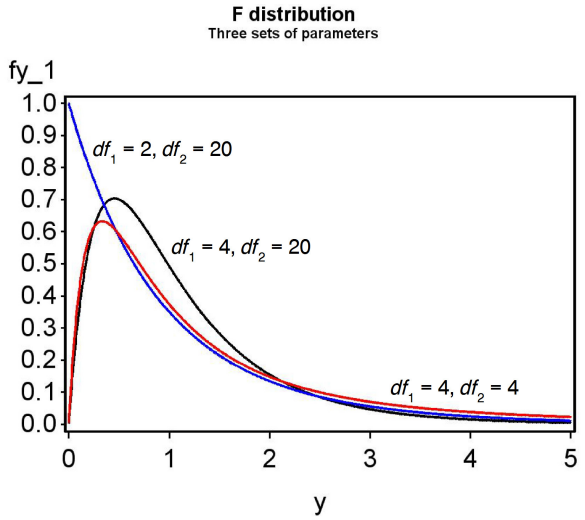

Figure 11.5: The F distribution for three different sets of parameter values

Table F gives the quantiles of the F distribution for different values of the degrees of freedom and the cumulative probability p. Statistical tests that make use of the F distribution are typically called F tests.

Calculating the test statistic F_s for the bark beetle experiment, we have

$$F_s = \frac{MS_{among}}{MS_{within}} = \frac{0.5750}{0.0668} = 8.6078, \tag{11.26}$$

$$\tag{11.27}$$

with $df_1 = a - 1 = 3 - 1 = 2$ and $df_2 = a(n-1) = 3(5-1) = 12$.

As with previous tests, we seek acceptance and rejection regions for a particular value of α, the Type I error rate. In particular, we seek a quantity c_{α,df_1,df_2} such that

$$P[0 < F_s < c_{\alpha,df_1,df_2}] = 1 - \alpha. \tag{11.28}$$

The region is of this form because the test is designed to reject H_0 for large values of F_s, and accept it for small ones. To find c_{α,df_1,df_2}, we look in Table F for the column corresponding to $1 - p = \alpha$, for the appropriate degrees of freedom. The acceptance region would therefore be $(0, c_{\alpha,df_1,df_2})$, and we would reject H_0 if F_s lies outside this region.

For $\alpha = 0.05$, $df_1 = 2$, and $df_2 = 12$, we see from Table F that $c_{0.05,2,12} = 3.885$. Our acceptance region is therefore $(0, 3.885)$, and we reject H_0 at the $\alpha = 0.05$ level if $F_s \geq 3.885$ (Fig. 11.6). We see this is the case because $F_s = 8.6078 > 3.885$. We can continue this process for increasingly smaller α and eventually find that for $\alpha = 0.005$ we can still reject H_0, but not for $\alpha = 0.001$. We therefore have $P < 0.005$ for this test, because $\alpha = 0.005$ is the smallest value of α for which we can reject H_0 (see Chapter 10). An F test in ANOVA would often be reported as follows: 'There was a highly significant difference among the different baits in the number of bark beetles trapped ($F_{2,12} = 8.6078, P < 0.005$).' Note that the degrees of freedom are given as subscripts.

11.2.3 ANOVA tables

We can organize the different sum of squares and mean squares into an ANOVA table. It lists the different sources of variation in the data (among, within, and total), their degrees of freedom, sums of squares and mean squares, and then the F statistic and its P value. Table 11.3 shows the general layout of such a table for one-way ANOVA designs, while Table 11.4 gives the results for the Example 1 analysis. Note the additive relationship for the degrees of freedom and sum of squares.

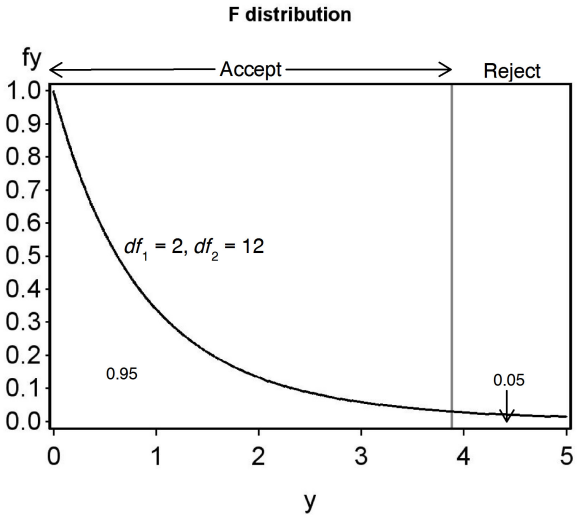

Figure 11.6: Acceptance and rejection regions for $\alpha = 0.05$

Table 11.3: General ANOVA table for one-way designs with a groups and n observations per group, showing formulas for different mean squares and the F test.

Source	*df*	Sum of squares	Mean square	F_s
Among	$a-1$	$SS_{among} = n\sum_{i=1}^{a}(\bar{Y}_{i\cdot} - \bar{Y})^2$	$MS_{among} = SS_{among}/(a-1)$	MS_{among}/MS_{within}
Within	$a(n-1)$	$SS_{within} = \sum_{i=1}^{a}\sum_{j=1}^{n}(Y_{ij} - \bar{Y}_{i\cdot})^2$	$MS_{within} = SS_{within}/a(n-1)$	
Total	$an-1$	$SS_{total} = \sum_{i=1}^{a}\sum_{j=1}^{n}(Y_{ij} - \bar{Y})^2$		

Table 11.4: ANOVA table for the Example 1 data set, including a P value for the test.

Source	*df*	Sum of squares	Mean square	F_s	P
Among	2	1.1500	0.5750	8.6078	< 0.005
Within	12	0.8016	0.0668		
Total	14	1.9516			

11.2.4 One-way ANOVA for Example 1 - SAS demo

The same calculations for the bark beetle experiment can be carried out in SAS using `proc glm` (SAS Institute Inc. 2014a). This procedure is primarily intended for fixed effects ANOVA models, with `proc mixed` the best choice for random effects models. However, the F test would be the same in either procedure.

The SAS program for one-way ANOVA is a bit more complicated than previous programs, so we will examine it a section at a time. The first step is to read in the observations using a `data` step, with one variable denoting the treatment (`treat`) and a second the number of beetles captured (`count`). As discussed earlier, it is common to log-transform count data, and so we generate a variable `y` that is the $\log 10$ (*log* base 10) of `count`. The `data` step is followed by a `print` statement to print the data set. See section below.

```
* bark_beetle_experiment.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "One-way ANOVA for bark beetle trapping experiment";
data bark_beetle;
        input treat $ count;
        * Apply transformations here;
        y = log10(count);
        datalines;
A   373
A   126
A   255

etc.

C   199
C    84
;
run;
* Print data set;
proc print data=bark_beetle;
run;
```

We next plot the data using the SAS procedure `gplot` (SAS Institute Inc. 2014b). The basic idea is to plot, for each treatment group, the individual data points along with their mean ($\bar{Y}$) $\pm$ one standard error ($s/\sqrt{n}$). The `plot` statement tells `gplot` to plot the variable `y` on the y-axis and `treat` on the x-axis of the plot. The appearance of the points is controlled by the

`symbol1` statement, which among other things specifies that the points be plotted along with their means ± one standard error, with the means joined by a line, using the option `i=std1mjt`. Other options in the `symbol` statement control the type and size of the points, and line width. The `vaxis=axis1` and `haxis=axis1` options control the visual appearance of the x- and y-axes. See below.

```
* Plot means, standard errors, and observations;
proc gplot data=bark_beetle;
        plot y*treat=1 / vaxis=axis1 haxis=axis1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
```

The next section of the program conducts the one-way ANOVA and F test using `proc glm`. The `class` statement tells SAS that the variable `treat` is the one that defines different groups in the ANOVA (see listing below). The `model` statement basically tells SAS the form of the ANOVA model. Recall that the model for fixed effects one-way ANOVA is given by the equation

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}. \tag{11.29}$$

If we equate Y_{ij} with `y`, and α_i with `treat`, we see there are similarities between the fixed effects model and the SAS `model` statement. In fact, SAS assumes you want a grand mean μ unless otherwise specified, as well as the error term ϵ_{ij}. As we examine more complex ANOVA models in later chapters, we will see there is nearly a one-to-one correspondence between these models and the corresponding SAS `model` statement.

```
* One-way ANOVA with all fixed effects;
proc glm data=bark_beetle;
        class treat;
        model y = treat;
        * Calculate means for each group;
        means treat;
        output out=resids p=pred r=resid;
run;
```

The `means` statement causes `glm` to calculate means for each `treat` group. The other statements generate graphs that are used to examine some of the assumptions of ANOVA – we will defer their discussion to later chapters.

The complete SAS program and output are listed below. The output shows the same F test in three different locations within the `proc glm` output.

The first is in a format resembling an ANOVA table, and then two other times corresponding to Type I and III sums of squares. These are different ways of calculating the sums of squares and tests, with Type III sums of squares more generally useful for ANOVA designs. For one-way ANOVA the results are the same, and we see that there was a highly significant difference among groups ($F_{2,12} = 8.60, P = 0.0048$). Inspection of the graph and means suggests that treatment A caught the most beetles, followed by C and then B.

SAS Program

```
* bark_beetle_experiment.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "One-way ANOVA for bark beetle trapping experiment";
data bark_beetle;
        input treat $ count;
        * Apply transformations here;
        y = log10(count);
        datalines;
A   373
A   126
A   255
A   138
A   379
B    25
B    64
B    62
B    71
B    54
C   449
C   249
C    69
C   199
C    84
;
run;
* Print data set;
proc print data=bark_beetle;
run;
* Plot means, standard errors, and observations;
proc gplot data=bark_beetle;
        plot y*treat=1 / vaxis=axis1 haxis=axis1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
```

```
* One-way ANOVA with all fixed effects;
proc glm data=bark_beetle;
        class treat;
        model y = treat;
        * Calculate means for each group;
        means treat;
        output out=resids p=pred r=resid;
run;
goptions reset=all;
title "Diagnostic plots to check ANOVA assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
        plot resid*pred=1 / vaxis=axis1 haxis=axis1;
        symbol1 v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
        qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

One-way ANOVA for bark beetle trapping experiment 1
09:32 Tuesday, August 31, 2010

Obs	treat	count	y
1	A	373	2.57171
2	A	126	2.10037
3	A	255	2.40654
4	A	138	2.13988
5	A	379	2.57864
6	B	25	1.39794
7	B	64	1.80618
8	B	62	1.79239
9	B	71	1.85126
10	B	54	1.73239
11	C	449	2.65225
12	C	249	2.39620
13	C	69	1.83885
14	C	199	2.29885
15	C	84	1.92428

One-way ANOVA for bark beetle trapping experiment 2
09:32 Tuesday, August 31, 2010

The GLM Procedure

Class Level Information

Class	Levels	Values
treat	3	A B C

Number of Observations Read	15
Number of Observations Used	15

One-way ANOVA for bark beetle trapping experiment 3
09:32 Tuesday, August 31, 2010

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.14818176	0.57409088	8.60	0.0048
Error	12	0.80114853	0.06676238		
Corrected Total	14	1.94933029			

R-Square	Coeff Var	Root MSE	y Mean
0.589013	12.30880	0.258384	2.099182

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	2	1.14818176	0.57409088	8.60	0.0048

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	2	1.14818176	0.57409088	8.60	0.0048

One-way ANOVA for bark beetle trapping experiment 4
09:32 Tuesday, August 31, 2010

The GLM Procedure

Level of treat	N	y Mean	y Std Dev
A	5	2.35942757	0.22948244
B	5	1.71603276	0.18282085
C	5	2.22208543	0.33793710

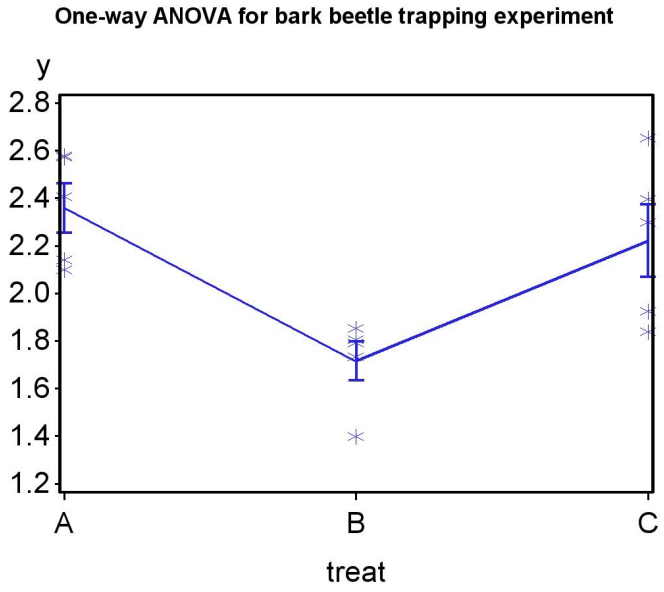

Figure 11.7: Means and standard errors for three treatments

11.2.5 One-way ANOVA for Example 2 - sample calculation

We will conduct an F test for our second data set, involving a study of bark beetles trapped at five different sites ($a = 5$) selected at random from a collection of sites, with five traps per site ($n = 5$). This implies a random effects model, and we are therefore interested in testing $H_0 : \sigma_A^2 = 0$ vs. $H_1 : \sigma_A^2 > 0$. Some preliminary calculations for the F test are shown in Table 11.2. We first find the mean $\bar{Y}_{i\cdot}$ for each site, then calculate the grand mean as the average of the site means:

$$\bar{\bar{Y}} = \frac{\sum_{i=1}^{a} \bar{Y}_{i\cdot}}{a} \tag{11.30}$$

$$= \frac{2.0120 + 2.4700 + 2.2460 + 2.6960 + 1.1940}{5} \tag{11.31}$$

$$= \frac{10.6180}{5} = 2.1236. \tag{11.32}$$

We then have

$$SS_{among} = n\sum_{j=1}^{a}(\bar{Y}_{i\cdot} - \bar{\bar{Y}})^2 \tag{11.33}$$

$$= 5\left[(2.0120 - 2.1236)^2 + \ldots + (1.1940 - 2.1236)^2\right] \tag{11.34}$$

$$= 5\left[0.0125 + 0.1200 + 0.0150 + 0.3276 + 0.8642\right] \tag{11.35}$$

$$= 6.6965 \tag{11.36}$$

We next calculate MS_{among}:

$$MS_{among} = \frac{SS_{among}}{a-1} = \frac{6.6965}{5-1} = 1.6741. \tag{11.37}$$

$$\tag{11.38}$$

Now we find SS_{within}, first calculating $(Y_{ij} - \bar{Y}_{i\cdot})^2$ for the observations in each group and then summing these for each group (see Table 11.2). Summing these quantities in turn across all groups, we obtain

$$SS_{within} = 0.1598 + 0.4730 + 0.3419 + 0.7600 + 0.0459 = 1.7806. \tag{11.39}$$

$$\tag{11.40}$$

We then have

$$MS_{within} = \frac{SS_{within}}{a(n-1)} = \frac{1.7806}{5(5-1)} = 0.0890. \tag{11.41}$$

$$\tag{11.42}$$

Calculating the test statistic F_s, we obtain

$$F_s = \frac{MS_{among}}{MS_{within}} = \frac{1.6741}{0.0890} = 18.8101, \tag{11.43}$$

$$\tag{11.44}$$

with $df_1 = a - 1 = 4 - 1 = 4$ and $df_2 = a(n-1) = 5(5-1) = 20$. From Table F, we find $P < 0.001$. The variance among sites is highly significant ($F_{4,12} = 18.8101, P < 0.001$.

11.2.6 One-way ANOVA for Example 2 - SAS demo

We can carry out the F test as well as estimate the variance components (σ_A^2 and σ^2) for the random effects model using SAS. The first section of the program involving the `data` step and `gplot` graph is similar to the fixed effects program. The next section of the program fits the random effects model to the data and conducts the F test, using `proc mixed` (see listing below). As before, the `class` statement tells SAS that the variable `site` is the one that defines different groups in the ANOVA. Now recall that the model for random effects one-way ANOVA is given by the equation

$$Y_{ij} = \mu + A_i + \epsilon_{ij}. \tag{11.45}$$

Note that A_i corresponds to `site` in the bark beetle study. In `proc mixed`, fixed effects in the model are placed in a `model` statement, while any random effects are listed in a `random` statement (SAS Institute Inc. 2014a). Because our random effects model only has one random effect, `site`, this is listed in the `random` statement. There are no fixed effects in this model, so the `model` statement lists nothing after the equals sign. The option `ddfm=kr` specifies a general method of calculating the degrees of freedom that works well under many circumstances, including more complicated models.

```
* One-way ANOVA with random effects - F test;
proc mixed method=type3 data=bark_beetle;
        class site;
        model y = / ddfm=kr;
        random site;
run;
* One-way ANOVA with random effects - variance components;
proc mixed cl data=bark_beetle;
        class site;
        model y = / ddfm=kr outp=resids;
        random site;
run;
```

Why is `proc mixed` invoked twice in this program? The first one generates the F statistic for testing $H_0 : \sigma_A^2 = 0$ vs. $H_1 : \sigma_A^2 > 0$, using the option `method=type3`. This is not the default in `proc mixed`, which appears more designed to estimate the variance components in random effects (Littell et al. 1996). If we drop this option, as in the second `proc mixed` statement, we

get only these estimates and no F test. Confidence intervals for the variance components are requested using the `cl` option. The variance components estimated in the second `proc mixed` using a version of maximum likelihood, the preferred method of estimating these quantities.

The complete SAS program and output are listed below. The variance among sites is highly significant ($F_{4,12} = 18.77, P < 0.0001$. The second call to `proc mixed` provide estimates and confidence intervals for the two variance components and confidence intervals. We have $\hat{\sigma}^2_A = 0.3174$ for which the 95% confidence interval is $(0.1093, 3.1458)$, and $\hat{\sigma}^2 = 0.0893$ with confidence interval $(0.0523, 0.1863)$. From these results, we see that the variance among sites is considerably greater than the variance within sites ($0.3174 > 0.0893$).

SAS Program

```
* bark_beetle_random.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "One-way ANOVA for bark beetle sampling study";
data bark_beetle;
        input site $ count;
        * Apply transformations here;
        y = log10(count);
        datalines;
1   137
1   101
1   113
1    48
1   155
2   156
2   165
2   652
2   179
2   757
3   278
3   197
3    95
3   395
3    83
4  2540
4   613
4   200
4   251
4   390
5    18
```

```
5    16
5    11
5    21
5    14
;
run;
* Print data set;
proc print data=bark_beetle;
run;
* Plot means, standard errors, and observations;
proc gplot data=bark_beetle;
        plot y*site=1 / vaxis=axis1 haxis=axis1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* One-way ANOVA with random effects - F test;
proc mixed method=type3 data=bark_beetle;
        class site;
        model y = / ddfm=kr;
        random site;
run;
* One-way ANOVA with random effects - variance components;
proc mixed cl data=bark_beetle;
        class site;
        model y = / ddfm=kr outp=resids;
        random site;
run;
goptions reset=all;
title "Diagnostic plots to check ANOVA assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
        plot resid*pred=1 / vaxis=axis1 haxis=axis1;
        symbol1 v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
        qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

One-way ANOVA for bark beetle sampling study 1
14:44 Tuesday, September 20, 2011

Obs	site	count	y
1	1	137	2.13672
2	1	101	2.00432
3	1	113	2.05308
4	1	48	1.68124
5	1	155	2.19033
6	2	156	2.19312
7	2	165	2.21748
8	2	652	2.81425
9	2	179	2.25285
10	2	757	2.87910
11	3	278	2.44404
12	3	197	2.29447
13	3	95	1.97772
14	3	395	2.59660
15	3	83	1.91908
16	4	2540	3.40483
17	4	613	2.78746
18	4	200	2.30103
19	4	251	2.39967
20	4	390	2.59106
21	5	18	1.25527
22	5	16	1.20412
23	5	11	1.04139
24	5	21	1.32222
25	5	14	1.14613

One-way ANOVA for bark beetle sampling study 2
14:44 Tuesday, September 20, 2011

The Mixed Procedure

Model Information

Data Set	WORK.BARK_BEETLE
Dependent Variable	y
Covariance Structure	Variance Components
Estimation Method	Type 3

Residual Variance Method	Factor
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
site	5	1 2 3 4 5

Dimensions

Covariance Parameters	2
Columns in X	1
Columns in Z	5
Subjects	1
Max Obs Per Subject	25

Number of Observations

Number of Observations Read	25
Number of Observations Used	25
Number of Observations Not Used	0

Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square
site	4	6.706318	1.676580	Var(Residual) + 5 Var(site)
Residual	20	1.786777	0.089339	Var(Residual)

Type 3 Analysis of Variance

Source	Error Term	Error DF	F Value	Pr > F
site	MS(Residual)	20	18.77	<.0001
Residual	.	.	.	.

One-way ANOVA for bark beetle sampling study 3
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The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
site	0.3174
Residual	0.08934

Fit Statistics

-2 Res Log Likelihood	25.1
AIC (smaller is better)	29.1
AICC (smaller is better)	29.7
BIC (smaller is better)	28.3

One-way ANOVA for bark beetle sampling study 4
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The Mixed Procedure

Model Information

Data Set	WORK.BARK_BEETLE
Dependent Variable	y
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
site	5	1 2 3 4 5

Dimensions

Covariance Parameters	2
Columns in X	1
Columns in Z	5
Subjects	1
Max Obs Per Subject	25

Number of Observations

Number of Observations Read	25
Number of Observations Used	25
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	46.39671929	
1	1	25.08857565	0.00000000

Convergence criteria met.

One-way ANOVA for bark beetle sampling study 5
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The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate	Alpha	Lower	Upper
site	0.3174	0.05	0.1093	3.1458
Residual	0.08934	0.05	0.05229	0.1863

Fit Statistics

-2 Res Log Likelihood	25.1
AIC (smaller is better)	29.1

```
AICC (smaller is better)        29.7
BIC (smaller is better)         28.3
```

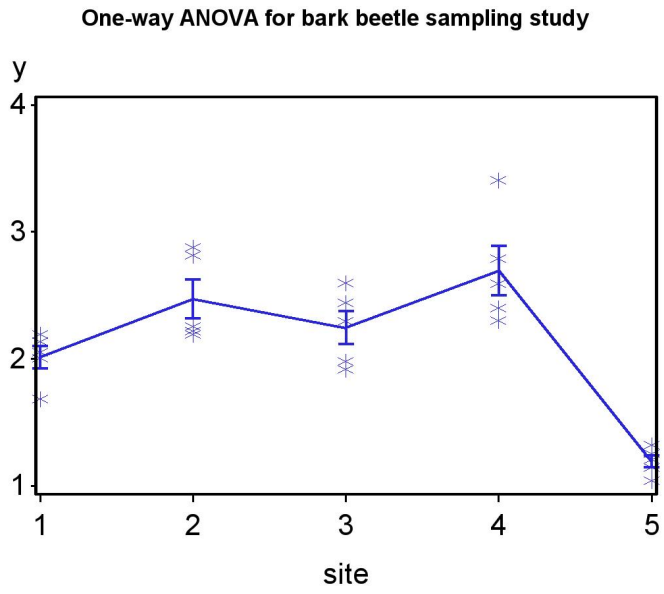

Figure 11.8: Means and standard errors for five study sites

11.3 Maximum likelihood estimates

This section sketches how the parameters in one-way ANOVA can be estimated using maximum likelihood. Recall that the likelihood for a random sample of three observations ($Y_1 = 4.5, Y_2 = 5.4, Y_2 = 5.3$) from a normal distribution (see Chapter 8) was of the form

$$L(\mu, \sigma^2) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(4.5-\mu)^2}{\sigma^2}} \times \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(5.4-\mu)^2}{\sigma^2}} \times \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(5.3-\mu)^2}{\sigma^2}}. \tag{11.46}$$

We found maximum likelihood estimates of the normal distribution parameters by maximizing this quantity with respect to μ and σ^2.

Suppose now we have a data set that can be modeled using the fixed effects one-way ANOVA model, in particular

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}. \tag{11.47}$$

This model has a number of parameters to estimate, such as μ, α_i for $i = 1, 2, \ldots, a$, and σ^2. What would the likelihood function look like for these data? Consider the first group for the bark beetle experiment (Example 1), for which we have $Y_{11} = 2.576$, $Y_{12} = 2.10$, $Y_{13} = 2.41$, $Y_{14} = 2.14$, and $Y_{15} = 2.58$. For the first group the model assumes that $Y_{1j} \sim N(\mu + \alpha_1, \sigma^2)$, and so the likelihood would be

$$L_1 = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(2.57-(\mu+\alpha_1))^2}{\sigma^2}} \times \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(2.10-(\mu+\alpha_1))^2}{\sigma^2}} \times \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(2.41-(\mu+\alpha_1))^2}{\sigma^2}} \tag{11.48}$$

$$\times \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(2.14-(\mu+\alpha_1))^2}{\sigma^2}} \times \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(2.58-(\mu+\alpha_1))^2}{\sigma^2}}. \tag{11.49}$$

The likelihood L_2 for the second group would be similar, except that $Y_{2j} \sim N(\mu + \alpha_2, \sigma^2)$, and L_3 similarly defined. The overall likelihood would then be defined as

$$L(\mu, \alpha_1, \alpha_2, \alpha_3, \sigma^2) = L_1 \times L_2 \times L_3. \tag{11.50}$$

Finding the maximum likelihood estimates involves maximizing this quantity with respect to the parameters $\mu, \alpha_1, \alpha_2, \alpha_3$, and σ^2. The likelihood for

designs with any number of treatment groups and replicates would be similar. Using a bit of calculus to find the maximum, it can be shown that the maximum likelihood estimates of these parameters, in general, are

$$\hat{\mu} = \bar{\bar{Y}}, \tag{11.51}$$

$$\hat{\alpha}_i = \bar{Y}_{i\cdot} - \bar{\bar{Y}}, \tag{11.52}$$

and

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n}(Y_{ij} - \bar{Y}_{i\cdot})^2}{a(n-1)} = MS_{within}. \tag{11.53}$$

(McCulloch & Searle 2001). These estimators seem quite reasonable. They use the grand mean of the data, $\bar{\bar{Y}}$, to estimate the grand mean μ of the model, and the difference between the ith group mean and the grand mean, $\bar{Y}_{i\cdot} - \bar{\bar{Y}}$, to estimate the deviation from the group mean α_i. Note that $\hat{\sigma}^2$ is equal to MS_{within}, which we have already encountered in our ANOVA calculations.

Suppose now we have a data set suited to the random effects model, in particular

$$Y_{ij} = \mu + A_i + \epsilon_{ij}. \tag{11.54}$$

This model has three parameters to be estimated: μ, σ_A^2, and σ^2. The likelihood for this model is more complex because of the random effect A_i, but one can show that the maximum likelihood estimators of these parameters are

$$\hat{\mu} = \bar{\bar{Y}}, \tag{11.55}$$

$$\hat{\sigma}_A^2 = \frac{MS_{among} - MS_{within}}{n}, \tag{11.56}$$

and

$$\hat{\sigma}^2 = MS_{within}. \tag{11.57}$$

An intuitive explanation of the formula for $\hat{\sigma}_A^2$ is that MS_{among} incorporates variance from both A_i and ϵ_{ij}, while MS_{within} only has ϵ_{ij}. Subtracting MS_{within} from MS_{among} leaves only the variance due to A_i, so that the numerator of this expression estimates $n\sigma_A^2$. We then divide by n to obtain an estimate of σ_A^2.

Suppose that for an unusual data set we obtain $MS_{among} < MS_{within}$, implying a negative estimate of $\hat{\sigma}_A^2 = 0$ according to the above equation. An inherent feature of maximum likelihood is that is restricts variance components to plausible values (McCulloch & Searle 2001), so in this case it would

simply say that $\hat{\sigma}_A^2 = 0$, the smallest possible nonnegative value. This would be reflected in the SAS output for `proc mixed`, which would report that the variance component in question was zero. The estimates presented here are actually obtained using a variant of maximum likelihood called restricted maximum likelihood or REML. This method is the default in SAS, and has some theoretical advantages over straight maximum likelihood (McCulloch and Searle 2001).

11.4 F test as a likelihood ratio test

The F test in one-way ANOVA can be derived as a likelihood ratio test, similar to the development of the t test in Chapter 10. We first find the maximum likelihood estimates of various parameters under H_1 vs. H_0, where the parameters under consideration are the ANOVA model parameters. Recall that the observations in the fixed effects model are described as

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \tag{11.58}$$

where μ is the grand mean, α_i is the effect of the ith treatment, and $\epsilon_{ij} \sim N(0, \sigma^2)$. This is the statistical model under the alternative hypothesis, where $\alpha_i \neq 0$ for some i. Under H_0 : all $\alpha_i = 0$, the model reduces to just

$$Y_{ij} = \mu + \epsilon_{ij}. \tag{11.59}$$

We would need to find the maximum likelihood estimates under both H_1 (see previous section) and H_0, as well as L_{H_0} and L_{H_1}, the maximum height of the likelihood function under H_0 and H_1. We would then use the likelihood ratio test statistic

$$\lambda = \frac{L_{H_0}}{L_{H_1}}. \tag{11.60}$$

It can be shown that there is a one-to-one correspondence between $-2\ln(\lambda)$ and F_s in one-way ANOVA, and so the F test is actually a likelihood ratio test (McCulloch & Searle 2001). A similar argument can be made to justify the F test for the random effects model. Like all likelihood ratio tests, large values of the test statistic $-2\ln(\lambda)$ or F_s indicate a lower value of the likelihood under H_0 relative to H_1, and thus a poorer fit of the H_0 model.

11.5 One-way ANOVA and two-sample t tests

There is an alternative to one-way ANOVA when there are only two groups to be compared, the two-sample t test. Let μ_1 be the mean of the first group and μ_2 the second one, and suppose that the two groups have the same variance σ^2 and sample size n. We are interested in testing $H_0 : \mu_1 = \mu_2$ vs. $H_1 : \mu_1 \neq \mu_2$, to determine if there are differences in the means of the two groups. Under H_0, the test statistic

$$T_s = \frac{(\bar{Y}_{1\cdot} - \bar{Y}_{2\cdot})}{\sqrt{\frac{s_1^2+s_2^2}{2}}} \sim t_{2(n-1)}. \tag{11.61}$$

Here $\bar{Y}_{1\cdot}$ and $\bar{Y}_2$ are the sample means for each group, and s_1^2 and s_2^2 the sample variances. For a Type I error rate of α, the acceptance region of the test would be the interval $(-c_{\alpha,2(n-1)}, c_{\alpha,2(n-1)})$, where $c_{\alpha,2(n-1)}$ is determined using Table T (see Chapter 10). We would reject H_0 if it falls on the edge or outside this interval. There are also versions of this test statistic for unequal sample sizes.

Although a two-sample t test is often used for comparing two groups, in the form above it is equivalent to the F test in one-way ANOVA. To see this, note that $T_s^2 = F_s$ for one-way ANOVA with two groups. It can also be shown that the acceptance and rejection regions are the same for the two tests. Unlike ANOVA, though, a two-sample t test can also be used for one-tailed alternative hypotheses, such as $H_1 : \mu_1 > \mu_2$ or $H_1 : \mu_1 < \mu_2$. The procedure is similar to one-sample t tests for one-tailed alternatives (see Chapter 10).

11.5.1 Two-sample t test for Example 1 - SAS demo

We can illustrate this test by comparing treatment A and B in the Example 1 study, deleting the data for the third treatment. See SAS program and output below. The `data` and `proc gplot` portions of the program are similar to our previous one-way ANOVA code. The two-sample t test is carried using `proc ttest` (SAS Institute Inc. 2014a), with the `class` statement indicating the variable that codes for different groups (`treat`), while the `var` statement designates the dependent variable (`y`). We see there is a highly significant difference between treatment A and B ($t_8 = 4.90, P = 0.0012$), with treatment A catching more beetles than B (Fig. 11.9).

SAS Program

```
* bark_beetle_experiment_ttest.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Two-sample t test for bark beetle trapping experiment";
data bark_beetle;
        input treat $ count;
        * Apply transformations here;
        y = log10(count);
        datalines;
A   373
A   126
A   255
A   138
A   379
B    25
B    64
B    62
B    71
B    54
;
run;
* Print data set;
proc print data=bark_beetle;
run;
* Plot means, standard errors, and observations;
proc gplot data=bark_beetle;
        plot y*treat=1 / vaxis=axis1 haxis=axis1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Two-sample t test;
proc ttest data=bark_beetle;
        class treat;
        var y;
run;
quit;
```

SAS Output

Two-sample t test for bark beetle trapping experiment 1
16:16 Thursday, May 22, 2014

Obs	treat	count	y
1	A	373	2.57171
2	A	126	2.10037
3	A	255	2.40654
4	A	138	2.13988
5	A	379	2.57864
6	B	25	1.39794
7	B	64	1.80618
8	B	62	1.79239
9	B	71	1.85126
10	B	54	1.73239

s

Two-sample t test for bark beetle trapping experiment 2
16:16 Thursday, May 22, 2014

The TTEST Procedure

Variable: y

treat	N	Mean	Std Dev	Std Err	Minimum	Maximum
A	5	2.3594	0.2295	0.1026	2.1004	2.5786
B	5	1.7160	0.1828	0.0818	1.3979	1.8513
Diff (1-2)		0.6434	0.2075	0.1312		

treat	Method	Mean	95% CL Mean		Std Dev
A		2.3594	2.0745	2.6444	0.2295
B		1.7160	1.4890	1.9430	0.1828
Diff (1-2)	Pooled	0.6434	0.3408	0.9460	0.2075
Diff (1-2)	Satterthwaite	0.6434	0.3382	0.9486	

treat	Method	95% CL Std Dev	
A		0.1375	0.6594
B		0.1095	0.5253
Diff (1-2)	Pooled	0.1401	0.3975
Diff (1-2)	Satterthwaite		

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	8	4.90	0.0012
Satterthwaite	Unequal	7.6194	4.90	0.0014

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	4	4	1.58	0.6704

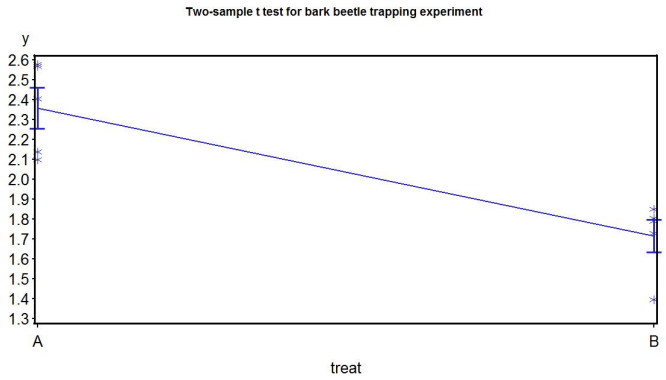

Figure 11.9: Means and standard errors for treatment A and B

11.6 References

McCulloch, C. E. & Searle, S. R. (2001) *Generalized, Linear, and Mixed Models*. John Wiley & Sons, Inc., New York, NY.

Littell, R. C., Milliken, G. A., Stroup, W. W. & Wolfinger, R. D. (1996) *The SAS System for Mixed Models*. SAS Institute Inc., Cary, NC.

SAS Institute Inc. (2014a) *SAS/STAT 13.2 Users Guide.* SAS Institute Inc., Cary, NC.

SAS Institute Inc. (2014b) *SAS/GRAPH 9.4: Reference, Third Edition.* SAS Institute Inc., Cary, NC.

Winer, B. J., Brown, D. R. & Michels, K. M. (1991) *Statistical Principles in Experimental Design*, 3rd edition. McGraw-Hill, Inc., Boston, MA.

11.7 Problems

1. A doctor conducts an experiment in which men are placed on four different diets, consisting of a standard weight loss regimen (a control treatment) and three new diets (Diets 1, 2, 3). The weight losses (lbs) after six months are given in the following table.

Control	Diet 1	Diet 2	Diet 3
19.5	20.0	20.8	25.9
20.5	16.4	17.4	25.9
16.6	11.9	16.7	25.8
19.3	22.1	16.8	22.5

   (a) Test whether there is a significant difference among the four treatments using one-way ANOVA, using manual calculations. Report the P value and discuss the significance of the test, and then interpret the results of the experiment. Show all your calculations.

   (b) Repeat the analysis using SAS and `proc glm`. Attach your program and output.

2. An experiment was conducted on the fecundity of a predatory insect reared on an artificial diet using four different concentrations of the preservative sorbic acid: (1) no sorbic acid, (2) 0.1% sorbic acid, (3) 0.2% sorbic acid, and (4) 0.5% sorbic acid. Twenty insects were reared at each concentration and the fecundity of the resulting adults measured. See table below.

Treatment	Observations
No sorbic acid	87, 124, 105, 87, 100, 89, 95, 79, 102, 112
	92, 87, 115, 96, 111, 90, 86, 92, 109, 76
0.1% sorbic acid	105, 94, 97, 94, 83, 97, 107, 99, 104, 83
	101, 71, 100, 75, 87, 106, 88, 99, 90, 74
0.2% sorbic acid	73, 94, 81, 83, 100, 98, 76, 91, 68, 82
	92, 105, 76, 82, 95, 96, 101, 89, 92, 67
0.5% sorbic acide	83, 54, 86, 76, 74, 81, 79, 72, 80, 78
	70, 83, 83, 85, 90, 70, 85, 94, 82, 75

   Test whether there is a difference among the four treatments using one-way ANOVA and SAS. Interpret the results of this analysis, providing a P value and discussing the significance of the test. Using a graph,

explain what happens to fecundity as the concentration of sorbic acid changes.

Chapter 12

Power Analysis for One-Way ANOVA

Recall that the power of a statistical test is the probability of rejecting H_0 when H_0 is false, and some alternative hypothesis H_1 is true. We saw earlier (Chapter 10) that power for one-sample Z and t tests is a function of the quantity

$$\phi = \frac{(\mu_1 - \mu_0)}{\sigma/\sqrt{n}}, \tag{12.1}$$

where μ_1 and μ_0 are the means under H_1 and H_0, σ is the standard deviation of the observations, and n is the sample size. Anything that increases ϕ increases the power of the test, including greater differences between μ_1 and μ_0, decreasing σ, or increasing the sample size n. Larger values of the Type I error rate α also increase the power of the test, because they make it more likely the test will reject H_0 under any circumstances. Although one-way ANOVA is a more complicated design, we will see that exactly the same factors influence the power of its associated F test.

A power analysis for a one-way ANOVA design is usually conducted before running the experiment or study. This is known as a **prospective power analysis**. We then use the information from this analysis to refine our experimental design, most often the sample sizes needed for each treatment group to yield adequate power. Conversely, a **retrospective power analysis** is one conducted after an experiment or study, using the results from the study in the power calculation. This is a controversial procedure that some statisticians find questionable (Steidl et al. 1997).

Cohen (1988) recommends using a default power value of 0.8 when designing an experiment, if there is no other basis for setting the power. One reason is that achieving higher power values usually requires disproportionately larger sample sizes. He also recommends a power value of 0.8 on the basis of the ratio of Type II (β) to Type I error (α). He suggests that an optimal ratio of β/α is about four, implying that Type I errors are four times more serious than Type II errors. If you use $\alpha = 0.05$ as the Type I error rate, and choose power = 0.8, then $\beta = 1 - \text{power} = 0.2$, and so $\beta/\alpha = 4$.

12.1 Power analysis for one-way ANOVA

Suppose we want to design an experiment involving several treatments that has adequate power. Assuming we know the treatments we will apply, the first step in a power analysis is to specify the actual values of the treatment means under H_1, the alternative hypothesis. If the experiment has five treatments, we might speculate that the treatment means take the following values under H_1:

$$H_1 : \mu_1 = 20, \mu_2 = 22, \mu_3 = 22, \mu_4 = 25, \mu_5 = 18. \tag{12.2}$$

For example, these values could be the final weights of fish reared on five different diets. This is the form of H_1 needed by `proc power` (SAS Institute Inc. 2014). We can also express H_1 in terms of the usual model for this design, the fixed effects model of the form

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}. \tag{12.3}$$

In terms of the parameters of this model, H_1 is equivalent to saying

$$H_1 : \alpha_1 = -1.4, \alpha_2 = 0.6, \alpha_3 = 0.6, \alpha_4 = 3.6, \alpha_5 = -3.4, \tag{12.4}$$

where $\alpha_i = \mu_i - \mu$, and μ is the grand mean ($\mu = \sum \mu_i/5 = 107/5 = 21.4$) (Winer et al. 1991; Montgomery 1997).

The null hypothesis in terms of group means would have the form

$$H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu, \tag{12.5}$$

where μ is the grand mean. This is equivalent to the usual null hypothesis for one-way ANOVA, which is $H_0 : \alpha_i = 0$ for all i.

We also need to specify a standard deviation σ for the power analysis. We could potentially estimate σ from similar studies in the literature or through a pilot study. If the paper provides an ANOVA table, we can estimate σ using $\sqrt{MS_{within}} = \sqrt{MS_{error}}$. SAS actually calculates this quantity and labels it `Root MSE` – see previous printouts for `proc glm`. In other situations, you may not know σ precisely but can specify a plausible range of values. Continuing our example, we suppose that previous experiments suggest $\sigma = 3$.

To calculate the power for this example, we also need to specify a sample size n for the treatments. Usually we are interested in determining the power for a range of n values, so we can determine the minimal sample size to needed to reject H_0 with adequate power. Most power analyses assume an equal sample size for each treatment, because this usually yields a higher power than unbalanced designs. We also need to specify the Type I error rate for the overall ANOVA, and $\alpha = 0.05$ is customary.

The power is then calculated using the distribution of the statistic F_s under H_1, called the non-central F distribution (the distribution under H_0 is the F distribution). The non-central F distribution has three parameters, the usual two degrees of freedom plus an additional parameter λ, defined by the formula

$$\lambda = \frac{n \sum_{i=1}^{a} \alpha_i^2}{\sigma^2}, \tag{12.6}$$

where $\alpha_i = \mu_i - \mu$, and $\mu = \sum \mu_i / a$ (Winer et al. 1991, Montgomery 1997). Note that λ is a function of the α_i values, σ, and the sample size n. The non-central F distribution is equal to the F distribution when $\lambda = 0$, which can only happen if there are no treatment effects and $\alpha_i = 0$ for all i. As the value of λ increases, however, the noncentral F distribution will shift to the right, away from the position held by the F distribution. Note the similarity of this quantity with ϕ, which determines the power for one-sample Z and t tests.

Figure 12.1 shows the F and noncentral F distributions for the power analysis example described above, with $a = 5$, the α_i values as specified, and $\sigma = 3$. We also assume for the moment that $n = 5$, and set $\alpha = 0.05$. For this design, we have $df_1 = a - 1 = 5 - 1 = 4$, $df_2 = a(n-1) = 5(5-1) = 20$. For $\alpha = 0.05$, we would reject H_0 if F_s, the test statistic for one-way ANOVA (Chapter 11), exceeded 2.866 (see Table F). We also need to calculate a value of λ for the noncentral F distribution. We have

$$\lambda = \frac{5\left[(-1.4)^2 + 0.6^2 + 0.6^2 + 3.6^2 + (-3.4)^2\right]}{3^2} = 15.111. \tag{12.7}$$

We see that the noncentral F lies to the right of the F distribution, because λ is fairly large in this example. What is the power of the test? It is the area of the noncentral F distribution lying to the right of 2.866, because this is the probability that F_s will exceed 2.866 under H_1, i.e., the probability of rejecting H_0 if it is false and H_1 is true.

What would happen to the power for other values of n or σ, or for that matter smaller or larger differences among groups under H_1 (implying smaller or larger α_i values)? Any change that increases the value of λ will increase the power of the test, because it reduces the amount of overlap between the two distributions. Examining λ, we see that larger n, larger differences among groups, and smaller σ values would all increase λ and so increase the power of the test. Larger α (Type I error rate) values also increase the power of the test, because they reduce the acceptance and increase the rejection region size. Sample size n also has an effect on power through the acceptance region – larger n reduces its upper boundary through its effect on $df_2 = a(n-1)$. Fig. 12.2 shows the F and noncentral F distributions for the power example, now using $n = 8$. Note how the overlap between the two distributions is reduced for larger n, increasing the power of the test. See Table 12.1 for a summary of how these factors affect power and β.

Table 12.1: Effects on power and the Type II error rate β of changes in various parameters. The arrows indicate if a particular quantity increases or decreases.

Parameter	Direction	λ	power	β
α_i values	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$
n	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$
σ	$\uparrow$	$\downarrow$	$\downarrow$	$\uparrow$
α	$\uparrow$	no change	$\uparrow$	$\downarrow$

The effect of n on λ implies that a sufficient large sample size can generate adequate power, even when the α_i values are small or σ is large. Thus, large sample sizes should make it possible to detect small treatment effects, and can also compensate for noisy data.

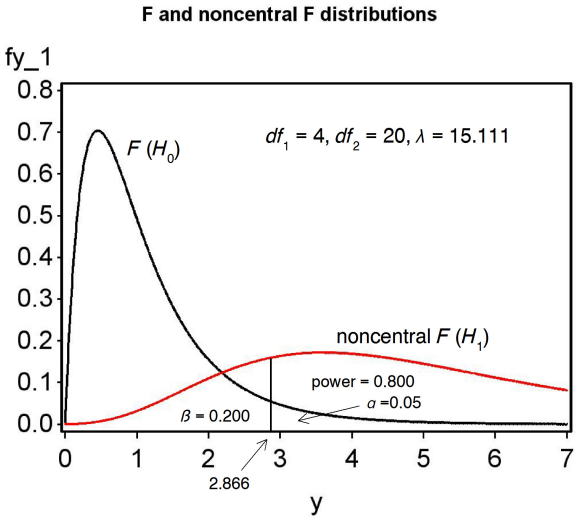

Figure 12.1: The F and noncentral F distributions for the power example, using $n = 5$.

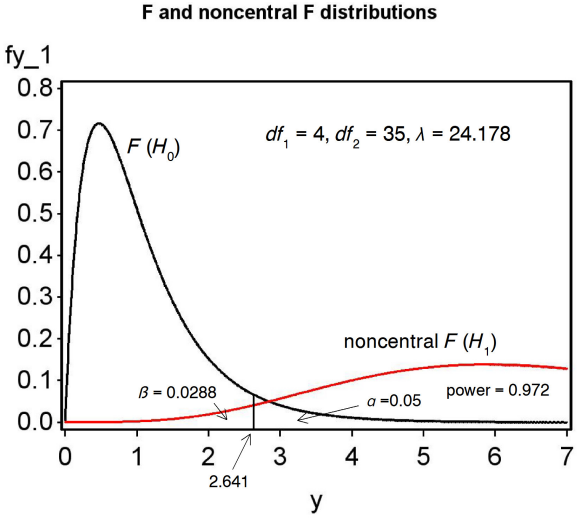

Figure 12.2: The F and noncentral F distributions for the power example, for $n = 8$.

12.2 Power analysis - SAS Demo

SAS makes power analysis relatively easy and provides specific methods for one-way ANOVA and many other designs. Consider our previous example involving five different treatments. We are interested in determining the power of a one-way ANOVA, when the following alternative hypothesis is true:

$$H_1 : \mu_1 = 20, \mu_2 = 22, \mu_3 = 22, \mu_4 = 25, \mu_5 = 18. \tag{12.8}$$

We need another piece of information for the power analysis, the value of σ. From preliminary studies or a previously published paper, we estimate that $\sigma = 3$. We also specify the Type I error rate, setting $\alpha = 0.05$.

This is everything required to carry out a power analysis using `proc power` (SAS Institute Inc. 2014). We first specify that we want a power analysis for one-way ANOVA using the option `onewayanova`. The means for each treatment group are specified using the `groupmeans` option, with the means listed in parentheses. See program listing below.

The values of σ and α are similarly specified using the `stddev` and `alpha` options. We are interested in determining the power for a range of n values, the sample size per group. This is specified using the `npergroup` option. You can either give a list of n values or use the syntax `x to y by z` to specify a sequence of values.

The `power` option is specified as a missing value (a period), because we want SAS to solve for power as a function of sample size per group. The `plot` command generates a low quality plot of power vs. sample size with no options to improve its appearance. We can generate a better-looking graph by sending the plot data to an output data file using the `ods output` command, then plotting it using `gplot` with the usual options to thicken the lines and increase the text size.

We see that power increases rapidly with sample size per group (n), from both the graph and SAS output. A power value of 0.8 is achieved for $n = 5$ in this example.

SAS Program

```
* oneway_power.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Power Analysis for One-Way Anova';
proc power;
        ods output Plotcontent=plotdata;
        onewayanova
                groupmeans = (20 22 22 25 18)
                stddev = 3
                alpha = 0.05
                npergroup = 2 to 20 by 1
                power = . ;
        plot x=n;
run;
* Plot power vs. sample size in a nicer graph;
proc gplot data=plotdata;
        plot power*npergroup=1 / vaxis=axis1 haxis=axis1 legend=legend1;
        symbol1 i=join v=dot c=black width=3 height=2;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
        legend1 label=(height=2) value=(height=2);
run;
quit;
```

SAS Output

Power Analysis for One-Way Anova 1
09:19 Tuesday, August 24, 2010

The POWER Procedure
Overall F Test for One-Way ANOVA

Fixed Scenario Elements

Method	Exact
Alpha	0.05
Group Means	20 22 22 25 18
Standard Deviation	3

Computed Power

Index	N Per Group	Power
1	2	0.222
2	3	0.456
3	4	0.657
4	5	0.800
5	6	0.891
6	7	0.944
7	8	0.972
8	9	0.987
9	10	0.994
10	11	0.997
11	12	0.999
12	13	>.999
13	14	>.999
14	15	>.999
15	16	>.999
16	17	>.999
17	18	>.999
18	19	>.999
19	20	>.999

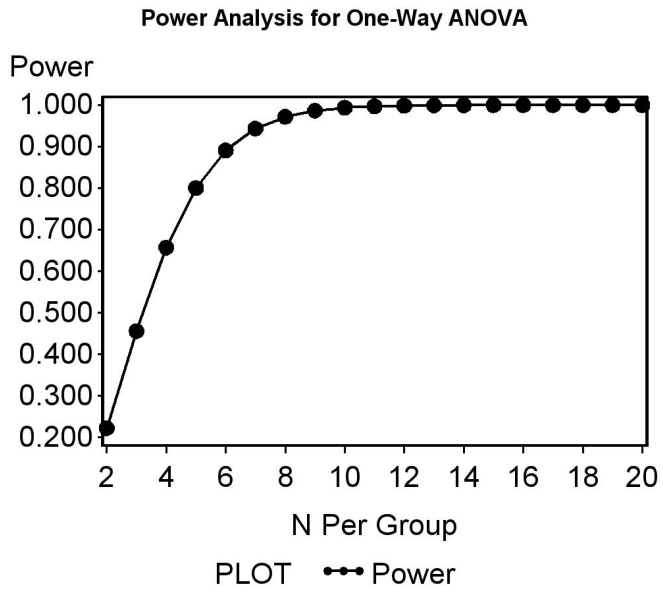

Figure 12.3: Power vs. n for the power example.

12.3 Power analysis continued - SAS demo

It is often worthwhile to compare power curves for different values of σ and α, to see how these influence power. We can obtain this from `proc power` by specifying several different values of these parameters. We will examine the results for $\alpha = 0.05$ vs. 0.01 and $\sigma = 3$ vs. 6. These are requested by listing both values under the `alpha` and `stddev` statements. A better quality graph is again generated by exporting the plot information and plotting it using `gplot`. See SAS program and output below.

We see that low α (the Type I error rate) reduces the power of the test across all sample sizes, because low α makes it harder to reject H_0 under any circumstance. Larger values of σ also decrease the power at all sample sizes. The larger the value of σ, the more variable the data, and the harder it is for the statistical test to distinguish between the null and alternative hypotheses. Adequate power is only obtained for a larger sample size.

SAS Program

```
* oneway_power2.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Power Analysis for One-Way Anova';
proc power;
        ods output Plotcontent=plotdata;
        onewayanova
                groupmeans = (20 22 22 25 18)
                stddev = 3 6
                alpha = 0.05 0.01
                npergroup = 2 to 20 by 1
                power = . ;
        plot x=n;
run;
* Plot power vs. sample size in a nicer graph;
proc gplot data=plotdata;
        plot power*npergroup=plotcurve / vaxis=axis1 haxis=axis1 legend=legend1;
        symbol1 i=join v=circle c=black width=3 height=2;
        symbol2 i=join v=plus c=black width=3 height=2;
        symbol3 i=join v=circle c=black l=2 width=3 height=2;
        symbol4 i=join v=plus c=black l=2 width=3 height=2;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
        legend1 label=(height=2) value=(height=2);
run;
quit;
```

SAS Output

Power Analysis for One-Way Anova 1
12:09 Wednesday, August 25, 2010

The POWER Procedure
Overall F Test for One-Way ANOVA

Fixed Scenario Elements

Method	Exact
Group Means	20 22 22 25 18

Computed Power

Index	Alpha	Std Dev	N Per Group	Power
1	0.05	3	2	0.222
2	0.05	3	3	0.456
3	0.05	3	4	0.657
4	0.05	3	5	0.800
5	0.05	3	6	0.891
6	0.05	3	7	0.944
7	0.05	3	8	0.972
8	0.05	3	9	0.987
9	0.05	3	10	0.994
10	0.05	3	11	0.997
11	0.05	3	12	0.999
12	0.05	3	13	>.999
13	0.05	3	14	>.999
14	0.05	3	15	>.999
15	0.05	3	16	>.999
16	0.05	3	17	>.999
17	0.05	3	18	>.999
18	0.05	3	19	>.999
19	0.05	3	20	>.999
20	0.05	6	2	0.088
21	0.05	6	3	0.136
22	0.05	6	4	0.189
23	0.05	6	5	0.245
24	0.05	6	6	0.303
25	0.05	6	7	0.361

26	0.05	6	8	0.418
27	0.05	6	9	0.474
28	0.05	6	10	0.527
29	0.05	6	11	0.577
30	0.05	6	12	0.624
31	0.05	6	13	0.668
32	0.05	6	14	0.708
33	0.05	6	15	0.744
34	0.05	6	16	0.777
35	0.05	6	17	0.806
36	0.05	6	18	0.833
37	0.05	6	19	0.856
38	0.05	6	20	0.876

Power Analysis for One-Way Anova 2
12:09 Wednesday, August 25, 2010

The POWER Procedure
Overall F Test for One-Way ANOVA

Computed Power

Index	Alpha	Std Dev	N Per Group	Power
39	0.01	3	2	0.059
40	0.01	3	3	0.185
41	0.01	3	4	0.359
42	0.01	3	5	0.538
43	0.01	3	6	0.691
44	0.01	3	7	0.806
45	0.01	3	8	0.885
46	0.01	3	9	0.935
47	0.01	3	10	0.965
48	0.01	3	11	0.981
49	0.01	3	12	0.991
50	0.01	3	13	0.995
51	0.01	3	14	0.998
52	0.01	3	15	0.999
53	0.01	3	16	>.999
54	0.01	3	17	>.999
55	0.01	3	18	>.999
56	0.01	3	19	>.999
57	0.01	3	20	>.999

58	0.01	6	2	0.019
59	0.01	6	3	0.036
60	0.01	6	4	0.057
61	0.01	6	5	0.084
62	0.01	6	6	0.116
63	0.01	6	7	0.152
64	0.01	6	8	0.191
65	0.01	6	9	0.233
66	0.01	6	10	0.277
67	0.01	6	11	0.323
68	0.01	6	12	0.369
69	0.01	6	13	0.415
70	0.01	6	14	0.460
71	0.01	6	15	0.505
72	0.01	6	16	0.548
73	0.01	6	17	0.589
74	0.01	6	18	0.628
75	0.01	6	19	0.664
76	0.01	6	20	0.699

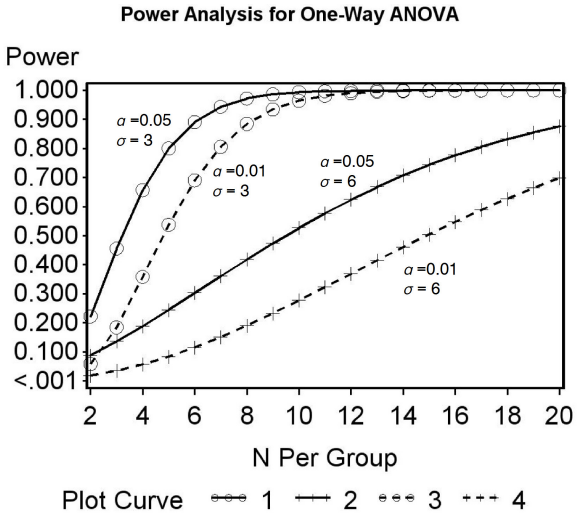

Figure 12.4: Power vs. n for the power example, for $\alpha = 0.05$ vs. 0.01, and $\sigma = 3$ vs. 6.

12.4 Power analysis continued - SAS demo

The SAS procedure `power` can be used to directly find the sample size n for a power of 0.8. One way is to simply read the value of n from a power vs. sample size graph, choosing the smallest n that gives power greater than or equal to 0.8. Returning to the first output we generated using `power` with $\alpha = 0.05$ and $\sigma = 3$, we see that for $n = 5$ the power exactly equals 0.8, so this is our sample size.

Alternately, you can set a power value of 0.8 and have `proc power` find the sample size. We first set the power option equal to 0.8 in the program, then change the `npergroup` option to a missing value, which tells `power` to solve for it. See program below and attached SAS output. SAS indicates that a sample size of $n = 5$ would give power = 0.8. This is the same result as obtained earlier by inspecting the power curve. For this particular example, there was a value of n that gave exactly the required power. More often, `power` will provide an n that guarantees power ≥ 0.8, not exactly 0.8.

SAS Program

```
* oneway_power3.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Power Analysis for One-Way ANOVA';
proc power;
        onewayanova
                groupmeans = (20 22 22 25 18)
                stddev = 3
                alpha = 0.05
                npergroup = .
                power = 0.8;
run;
quit;
```

SAS Output

Power Analysis for One-Way ANOVA 1
12:09 Wednesday, August 25, 2010

The POWER Procedure
Overall F Test for One-Way ANOVA

Fixed Scenario Elements

Method	Exact
Alpha	0.05
Group Means	20 22 22 25 18
Standard Deviation	3
Nominal Power	0.8

Computed N Per Group

Actual Power	N Per Group
0.800	5

12.5 References

Cohen, J. (1988) *Statistical Power Analysis for the Behavioral Sciences, Second Edition.* Lawrence Erlbaum Associates, Inc., Hillsdale, NJ.

Montgomery, D. C. (1997) *Design and Analysis of Experiments.* John Wiley & Sons, New York, NY.

SAS Institute Inc. (2014) *SAS/STAT 13.2 Users Guide.* SAS Institute Inc., Cary, NC.

Steidl, R. J., Hayes, J. P. & Schauber, E. (1997) Statistical power analysis in wildlife research. *Journal of Wildlife Management* 61: 270-279.

Winer, B. J., Brown, D. R. & Michels, K. M. (1991) *Statistical Principles in Experimental Design.* McGraw-Hill, Inc., Boston, MA.

12.6 Problems

1. Suppose you want to compare the effect of four different diets on the weight of prawns reared in aquaculture ponds. There is a standard diet (S) and three other diets (A, B and C) that will be fed to prawns in replicate ponds. Relative to diet S, you would like to see a 20% increase in weight on diet A, a 20% increase on diet B, and a 30% increase on diet C. If the mean weight on diet S is 100 g, this translates into the following alternative hypothesis:

$$H_1 : \mu_S = 100, \mu_A = 120, \mu_B = 120, \mu_C = 130. \tag{12.9}$$

   From previous studies the researchers estimate that $\sigma = 22$. Assume a Type I error rate of $\alpha = 0.05$.

   (a) Use SAS and `proc power` to determine the sample size per treatment (number of ponds) necessary to give power ≥ 0.8. Attach your SAS program and output.

   (b) Repeat the same analysis for $\alpha = 0.01$. How does this change in the Type I error rate affect the sample size? Why?

2. Suppose you want to compare the effect of five different diets on the weight of fish reared in aquaculture. There is a control diet (C) and four other diets (D1, D2, D3, and D4). Relative to diet S, you would like to see a 10% increase in weight on diet D1, a 15% increase on diet D2, and 20% increases on diets D3 and D4. If the weight on the control diet C is 100 g, this translates into the following alternative hypothesis:

$$H_1 : \mu_C = 100, \mu_{D1} = 110, \mu_{D2} = 115, \mu_{D3} = 120, \mu_{D4} = 120. \tag{12.10}$$

   Previous studies suggest that $\sigma = 10$. Assume a Type I error rate of $\alpha = 0.05$.

   (a) Use SAS to determine the sample size per treatment necessary to give power ≥ 0.8. Attach your program and output.

   (b) Repeat the same analysis for the following alternative hypothesis:

$$H_1 : \mu_C = 100, \mu_{D1} = 105, \mu_{D2} = 108, \mu_{D3} = 110, \mu_{D4} = 110. \tag{12.11}$$

   How does this change affect the sample size? Why?

Chapter 13

Multiple Comparisons

One-way ANOVA, as well as more complex variants, provides a test of an overall null hypothesis of the form $H_0 : \alpha_i = 0$ for all i vs. $H_1 :$ some $\alpha_i \neq 0$. If we obtain a small P value for this test, it provides evidence against H_0 and in favor of H_1. However, this overall test provides little information on whether particular groups are different. We now turn to statistical methods designed to compare pairs of groups for one-way ANOVA designs. These procedures allow comparisons to be made among all possible pairs of groups, or sometimes one group vs. all others, and are collectively called **multiple comparisons**. Although multiple comparisons are often conducted in association with ANOVA, they are in fact stand-alone procedures (Hsu 1996). There is no need to conduct an ANOVA before using these procedures, although SAS will generate an overall F test regardless. Moreover, significant differences between groups in multiple comparisons may not coincide with a significant overall F test, or vice versa.

13.1 Models for multiple comparisons

The statistical model for multiple comparisons is basically the one-way ANOVA model expressed in a different form. The one-way ANOVA model is

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}, \tag{13.1}$$

where μ is the grand mean, α_i is the deviation from the grand mean caused by the ith group, and $\epsilon_{ij} \sim N(0, \sigma^2)$. For multiple comparison procedures it

is common to define $\mu_i = \mu + \alpha_i$, and so the one-way model becomes

$$Y_{ij} = \mu_i + \epsilon_{ij}. \tag{13.2}$$

We can think of μ_i as the mean of the ith group, where there are a total groups.

Now consider two groups i and j in a study which have means μ_i and μ_j, where $i \neq j$. We will be interested in estimating the difference in the means of these two groups, $\mu_i - \mu_j$, and finding a confidence interval to accompany this estimate for all possible pairs of groups. We will also be interested in testing whether the means of the two groups are equal, namely $H_0 : \mu_i = \mu_j$ or equivalently $H_0 : \mu_i - \mu_j = 0$, again for all possible pairs of groups. For a study with a groups, this amounts to $a(a-1)/2$ pairs of groups. For example, if there are $a = 3$ groups there are $3(3-1)/2 = 3$ possible pairwise comparisons (groups 1-2, 2-3, and 1-3). There are multiple comparison methods that provide estimates, confidence intervals, and tests, while others provide only tests but have more statistical power. The basic purpose of these procedures is to statistically test which pairs of treatments are different, and provide some idea of the magnitude of the difference. We will examine three procedures in this category, known as **all possible pairwise comparisons**. The procedures are called Fisher's least significant difference, the Tukey procedure, and the Ryan-Einot-Gabriel-Welsch (REGW) procedure (Hsu 1996).

For experiments that have a clearly identifiable control group, it may be appropriate to compare each group with only the control. For example, suppose the control is a standard drug treatment for a disease. We may only be interested in treatments that give a significantly better (or maybe worse) result compared to the control, and are not interested in other comparisons among the treatments. For a study with a groups including the control, this amounts to $a - 1$ pairs of groups with the control. For example, if there are $a = 3$ groups with the first group ($i = 1$) the control, there are $3 - 1 = 2$ possible comparisons (groups 1-2 and 1-3). We will examine Dunnett's procedure in this category, known as **multiple comparisons with a control** (Hsu 1996).

13.2 Error rates in multiple comparisons

There are two error rates commonly used to describe multiple comparison procedures. One is the **per comparison** error rate, which is the Type I

error rate for a single test comparing a single pair of groups. This rate is like that used in other statistical tests we have encountered, where only a single test is considered. The second is the **experimentwise error rate**, or **EER**. **The EER is defined as the probability of one or more Type I errors (rejecting H_0 when it is true) in a set of comparisons.**

Why do we need two error rates? Multiple comparison procedures such as the ones mentioned above can involve a substantial number of statistical tests, one test for each pair of groups. For example, with $a = 5$ groups there would be $5(5-1)/2 = 10$ possible pairwise comparisons, while for $a = 10$ groups we would have $10(10-1)/2 = 45$ comparisons! Given this many comparisons and tests, it is quite possible that some pairs would yield a significant test result even if the null hypothesis were true, i.e., we would reject $H_0 : \mu_i = \mu_j$ for one or more pairs of groups, even though there is no difference between the groups. For example, suppose that the per comparison error rate is set at the typical $\alpha = 0.05$ value, which amounts to a 1 in 20 chance of rejecting H_0 when it is true. Given $a = 10$ and 45 total tests, we would expect to see a few significant test results just by chance. This difficulty has been called the **multiplicity problem** (Westfall et al. 1999).

To see the magnitude of the multiplicity problem, we can plot the EER for the least significant difference procedure, which controls the per comparison error rate but not the EER. Fig. 13.1 shows a plot of the EER vs. the number of groups or treatments (a). The least significant difference procedure is a t test that compares the means for each pair of groups, with each test conducted at the same α level, in this case $\alpha = 0.05$. We see that the EER, and the number of pairwise comparisons, increases rapidly with the number of groups. Thus, it becomes more likely that any significant differences reported among groups are in fact Type I errors. In contrast, methods designed to control the EER, such as the Tukey procedure, would maintain an EER of 0.05 regardless of the number of groups. These tests manage the EER by essentially reducing the per comparison error rate for each test. **The penalty of controlling the EER is a loss of power to detect differences among groups where they do exist.**

Multiple comparison procedures have been the subject of considerable controversy in the ecological and statistical literature. Several tests you may encounter in the literature, such as least significant difference, Fisher's protected least significant difference, Duncan's multiple range test, and the Student-Newman-Keuls test, were very popular because they gave significant results more often than competing methods. Unfortunately, these particular

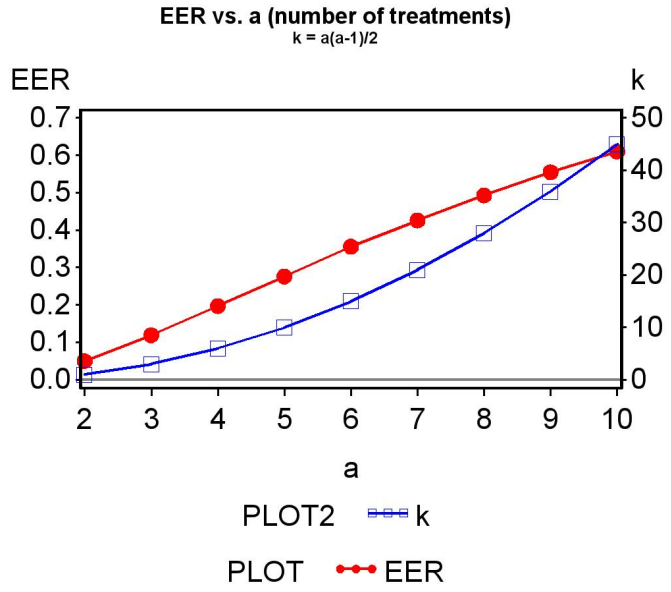

Figure 13.1: Plot of the experimentwise error rate vs. a, the number of treatments or groups, using $\alpha = 0.05$ for each comparison. Also shown is the number of pairwise comparisons ($k = a(a-1)/2$) vs. a.

tests do not control the experimentwise error rate (Day & Quinn 1989, Hsu 1996).

Another error rate that is becoming popular is the **false discovery rate** or **FDR** (Benjamini & Hochberg 1995).. **This is defined as the proportion of Type I errors in a set of comparisons.** Procedures that use the FDR have more power than those controlling the EER, but with more Type I errors. We will examine the rationale for FDR procedures later in the chapter.

13.3 All pairwise comparisons

This section examines three different methods for all pairwise comparisons among groups, the least significant difference, Tukey, and REGW methods. The least significant difference method does not control the EER, but is simple in form and a useful starting point. It provides estimates and confidence

intervals for $\mu_i - \mu_j$, the difference between the group means for any pair of groups, as well as a statistical test for $H_0 : \mu_i - \mu_j$. The Tukey procedure is similar to the least significant difference except that it controls the EER. We also examine the REGW method, an example of a **multiple range test**. Multiple range procedures only provide tests, not confidence intervals, but are more powerful procedures.

13.3.1 Least significant difference

We first develop confidence intervals and construct statistical tests for the least significant difference procedure, using methods similar to those in Chapter 9 and 10. For multiple comparisons, we are interested in estimating $\mu_i - \mu_j$ and finding a confidence interval for this quantity. It seems reasonable to use $\bar{Y}_i - \bar{Y}_j$ to estimate $\mu_i - \mu_j$, but what is the variance of this estimate? Using the rules for calculating the variance of a sum of random variables (Chapter 7), we have

$$Var[\bar{Y}_i - \bar{Y}_j] = Var[\bar{Y}_i] + (-1)^2 Var[\bar{Y}_j] = \sigma^2/n + \sigma^2/n = 2\sigma^2/n. \quad (13.3)$$

ANOVA provides an estimate of σ^2, namely MS_{within}, and so we can estimate the variance of $\bar{Y}_i - \bar{Y}_j$ using the quantity $2MS_{within}/n$, which has $a(n-1)$ degrees of freedom. Using these results, it can be shown that the quantity

$$\frac{(\bar{Y}_i - \bar{Y}_j) - (\mu_i - \mu_j)}{\sqrt{\frac{2MS_{within}}{n}}} \sim t_{a(n-1)}. \quad (13.4)$$

We use this quantity to first derive a confidence interval for $\mu_i - \mu_j$. Using Table T, we can find a value of $c_{\alpha,a(n-1)}$ for $a(n-1)$ degrees of freedom such that the following equation is true:

$$P\left[-c_{\alpha,a(n-1)} < \frac{(\bar{Y}_i - \bar{Y}_j) - (\mu_i - \mu_j)}{\sqrt{\frac{2MS_{within}}{n}}} < c_{\alpha,a(n-1)}\right] = 1 - \alpha. \quad (13.5)$$

Rearranging this equation, we obtain

$$P\left[\bar{Y}_i - \bar{Y}_j - c_{\alpha,a(n-1)}\sqrt{\frac{2MS_{within}}{n}} < \mu_i - \mu_j < \bar{Y}_i - \bar{Y}_j + c_{\alpha,a(n-1)}\sqrt{\frac{2MS_{within}}{n}}\right] = 1 - \alpha. \quad (13.6)$$

The confidence interval would therefore be the interval

$$\left(\bar{Y}_i - \bar{Y}_j - c_{\alpha,a(n-1)}\sqrt{\frac{2MS_{within}}{n}}, \bar{Y}_i - \bar{Y}_j + c_{\alpha,a(n-1)}\sqrt{\frac{2MS_{within}}{n}}\right). \quad (13.7)$$

The center of the confidence interval is located at $\bar{Y}_i - \bar{Y}_j$, the estimate of $\mu_i - \mu_j$. We will later illustrate how this interval is calculated in a SAS demo of the least significant difference procedure.

Now suppose we want to test $H_0 : \mu_i = \mu_j$ or equivalently $H_0 : \mu_i - \mu_j = 0$. Under H_0, the test statistic

$$T_s = \frac{(\bar{Y}_i - \bar{Y}_j) - 0}{\sqrt{\frac{2MS_{within}}{n}}} = \frac{(\bar{Y}_i - \bar{Y}_j)}{\sqrt{\frac{2MS_{within}}{n}}} \sim t_{a(n-1)}. \quad (13.8)$$

Using a Type I error rate of α, the acceptance region of the test would be the interval $(-c_{\alpha,a(n-1)}, c_{\alpha,a(n-1)})$, where $c_{\alpha,a(n-1)}$ is determined using Table T (see Chapter 10). We would reject H_0 if it falls on the edge or outside this interval.

We can rearrange the test given above into a different form, one that is commonly used for multiple comparisons. Recall that one would accept H_0 if T_s falls inside the acceptance region $(-c_{\alpha,a(n-1)}, c_{\alpha,a(n-1)})$, which implies

$$-c_{\alpha,a(n-1)} < \frac{(\bar{Y}_i - \bar{Y}_j)}{\sqrt{\frac{2MS_{within}}{n}}} < c_{\alpha,a(n-1)}. \quad (13.9)$$

We can rearrange this into the form

$$-c_{\alpha,a(n-1)}\sqrt{\frac{2MS_{within}}{n}} < \bar{Y}_i - \bar{Y}_j < c_{\alpha,a(n-1)}\sqrt{\frac{2MS_{within}}{n}}, \quad (13.10)$$

or

$$-LSD < \bar{Y}_i - \bar{Y}_j < LSD, \quad (13.11)$$

where

$$LSD = c_{\alpha,a(n-1)}\sqrt{\frac{2MS_{within}}{n}}. \quad (13.12)$$

The quantity LSD is called the least significant difference. We would accept H_0 if $\bar{Y}_i - \bar{Y}_j$ falls inside the interval $(-LSD, LSD)$, or equivalently if $|\bar{Y}_i - \bar{Y}_j| < LSD$. Conversely, we would reject H_0 if $|\bar{Y}_i - \bar{Y}_j| \geq LSD$. This

same rule applies to any pair of groups, because LSD would take the same value. Any pair of means that equals or exceeds this value is declared to be significantly different.

The confidence intervals we derived for $\mu_i - \mu_j$ can also be expressed in this format. In particular, the confidence interval would have the form

$$\left(\bar{Y}_i - \bar{Y}_j - LSD, \bar{Y}_i - \bar{Y}_j + LSD\right). \tag{13.13}$$

13.3.2 Least significant difference - SAS demo

Kneitel & Lessin (2010) studied the effect of eutrophication on vernal pools in California. They were interested in the effect of eutrophication (nutrient addition) on algae cover during the period the pools were filled with water, as well as vascular plant cover later in the season. Experimental pools were subjected to five different treatments: low, medium, high, and very high nutrient addition levels, and a control to which no nutrients were added. We will use a simplified data set from this study to illustrate the least significant difference procedure in SAS. We first examine the data involving algae cover. Algae cover was expressed as a percentage of the pool covered, and for data of this type it is common to transform the data. The data were first converted to a proportion by dividing the percentage by 100, then the arcsine-square root transformation applied (see Chapter 15). See the `data` step in the SAS program below.

The program is similar to our previous one-way ANOVA programs, with the addition of a `means` statement within `proc glm`:

```
means treat / t cldiff lines;
```

This statement requests a mean for each level of `treat`, the treatment variable (SAS Institute Inc. 2014). The `t` option requests the least significant difference procedure, because it is essentially a t test. The option `cldiff` requests 95% confidence intervals for $\mu_i - \mu_j$ for all pairs of groups, while `lines` generates a diagram that indicates which pairs of groups are significantly different at the $\alpha = 0.05$ level. See the full program listing and SAS output below.

According to the one-way ANOVA results, there was a highly significant difference among the nutrient treatments ($F_{4,20} = 4.76, P < 0.0073$). Confidence intervals for $\mu_i - \mu_j$ and $\mu_j - \mu_i$ are given for every pair of groups. For example, SAS gives a confidence interval for $\mu_{\text{medium}} - \mu_{\text{control}}$ as well as $\mu_{\text{control}} - \mu_{\text{medium}}$. Also shown in the output is the diagram generated by the

`lines` command. **Treatments with different letters are significantly different, while if they have the same letter they are not significantly different.** According to the letters, the very high, high, and medium treatments are significantly different from the low and control treatments, while there were no significant differences within these two groups. This lettering scheme can also be used to indicate significant differences among treatments within a graph (Fig. 13.2).

SAS Program

```
* Kneitel_2010_algae_lsd2.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Multiple comparisons for algae cover';
title2 'Data from Kneitel and Lessin (2010)';
data kneitel;
        input treat $ richness total algae;
        * Apply transformations here;
        y = arsin(sqrt(algae/100));
        datalines;
Control   8   78    1
Control   5   84    7
Control  10  115   45
Control   7  200  100
Control   6   72   20
Low       8   73   15
Low       7  124   70
Low       8  116   50
Low       8   92    5
Low       7  138   60
Medium    7  124   85
Medium    8  116   80
Medium    8  145   60
Medium    6  154  100
Medium    7  129   90
High      6  134   95
High      7  138   95
High      8  103   70
High      8  119   75
High          6  132   80
VeryHigh  6  148   95
VeryHigh  5  134   95
VeryHigh  5  119  100
VeryHigh  5  117   90
VeryHigh  5  129   80
```

```
;
run;
* Print data set;
proc print data=kneitel;
run;
* Plot means, standard errors, and observations;
proc gplot data=kneitel;
        plot y*treat=1 / vaxis=axis1 haxis=axis1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* One-way anova with comparisons;
proc glm data=kneitel;
        class treat;
        model y = treat;
        output out=resids p=pred r=resid;
        * LSD or Students t - only controls the per comparison error rate;
        means treat / t cldiff lines;
run;
goptions reset=all;
title "Diagnostic plots to check anova assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
        plot resid*pred=1 / vaxis=axis1 haxis=axis1;
        symbol1 v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
        qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

Multiple comparisons for algae cover 1
Data from Kneitel and Lessin (2010)
15:51 Tuesday, July 3, 2012

Obs	treat	richness	total	algae	y
1	Control	8	78	1	0.10017
2	Control	5	84	7	0.26776
3	Control	10	115	45	0.73531
4	Control	7	200	100	1.57080
5	Control	6	72	20	0.46365
6	Low	8	73	15	0.39770
7	Low	7	124	70	0.99116
8	Low	8	116	50	0.78540
9	Low	8	92	5	0.22551
10	Low	7	138	60	0.88608
11	Medium	7	124	85	1.17310
12	Medium	8	116	80	1.10715
13	Medium	8	145	60	0.88608
14	Medium	6	154	100	1.57080
15	Medium	7	129	90	1.24905
16	High	6	134	95	1.34528
17	High	7	138	95	1.34528
18	High	8	103	70	0.99116
19	High	8	119	75	1.04720
20	High	6	132	80	1.10715
21	VeryHigh	6	148	95	1.34528
22	VeryHigh	5	134	95	1.34528
23	VeryHigh	5	119	100	1.57080
24	VeryHigh	5	117	90	1.24905
25	VeryHigh	5	129	80	1.10715

Multiple comparisons for algae cover 2
Data from Kneitel and Lessin (2010)
15:51 Tuesday, July 3, 2012

The GLM Procedure

Class Level Information

Class	Levels	Values

```
          treat             5    Control High Low Medium VeryHigh


                    Number of Observations Read          25
                    Number of Observations Used          25


                      Multiple comparisons for algae cover                    3
                      Data from Kneitel and Lessin (2010)
                                                15:51 Tuesday, July 3, 2012

                              The GLM Procedure

Dependent Variable: y

                                        Sum of
 Source                       DF        Squares    Mean Square   F Value   Pr > F

 Model                         4     2.13816313     0.53454078      4.76   0.0073

 Error                        20     2.24444069     0.11222203

 Corrected Total              24     4.38260382


              R-Square     Coeff Var      Root MSE        y Mean

              0.487875      33.68371      0.334996      0.994533


 Source                       DF      Type I SS    Mean Square   F Value   Pr > F

 treat                         4     2.13816313     0.53454078      4.76   0.0073


 Source                       DF    Type III SS    Mean Square   F Value   Pr > F

 treat                         4     2.13816313     0.53454078      4.76   0.0073


                      Multiple comparisons for algae cover                    4
                      Data from Kneitel and Lessin (2010)
                                                15:51 Tuesday, July 3, 2012

                              The GLM Procedure
```

t Tests (LSD) for y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.112222
Critical Value of t	2.08596
Least Significant Difference	0.442

Comparisons significant at the 0.05 level are indicated by ***.

treat Comparison	Difference Between Means	95% Confidence Limits		
VeryHigh - Medium	0.1263	-0.3157	0.5682	
VeryHigh - High	0.1563	-0.2857	0.5983	
VeryHigh - Low	0.6663	0.2244	1.1083	***
VeryHigh - Control	0.6960	0.2540	1.1379	***
Medium - VeryHigh	-0.1263	-0.5682	0.3157	
Medium - High	0.0300	-0.4119	0.4720	
Medium - Low	0.5401	0.0981	0.9820	***
Medium - Control	0.5697	0.1277	1.0116	***
High - VeryHigh	-0.1563	-0.5983	0.2857	
High - Medium	-0.0300	-0.4720	0.4119	
High - Low	0.5100	0.0681	0.9520	***
High - Control	0.5397	0.0977	0.9816	***
Low - VeryHigh	-0.6663	-1.1083	-0.2244	***
Low - Medium	-0.5401	-0.9820	-0.0981	***
Low - High	-0.5100	-0.9520	-0.0681	***
Low - Control	0.0296	-0.4123	0.4716	
Control - VeryHigh	-0.6960	-1.1379	-0.2540	***
Control - Medium	-0.5697	-1.0116	-0.1277	***
Control - High	-0.5397	-0.9816	-0.0977	***
Control - Low	-0.0296	-0.4716	0.4123	

15:51 Tuesday, July 3, 2012

The GLM Procedure

t Tests (LSD) for y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.112222
Critical Value of t	2.08596
Least Significant Difference	0.442

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	1.3235	5	VeryHigh
A			
A	1.1972	5	Medium
A			
A	1.1672	5	High
B	0.6572	5	Low
B			
B	0.6275	5	Control

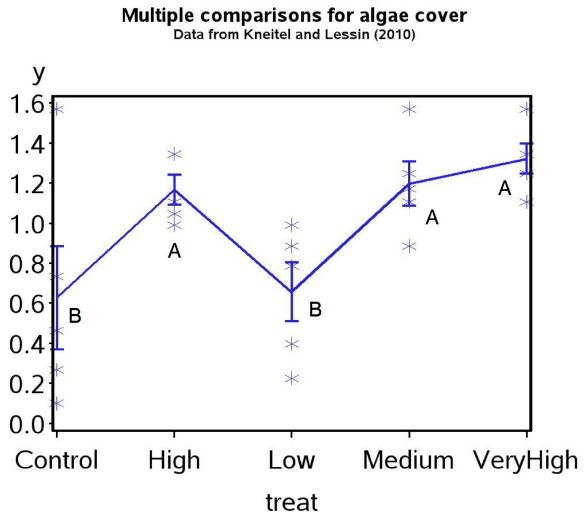

Figure 13.2: Algae cover vs. nutrient addition treatment for data from Kneitel and Lessin (2010). Means with different letters are significantly different (least significant difference method).

We will now calculate the value of LSD for this example to show how it is used to construct confidence intervals and tests. From the ANOVA output for `proc glm`, we see that $MS_{within} = 0.1122$ with 20 degrees of freedom. From Table T (Chapter 22), using $\alpha = 0.05$ we see that $c_{0.05,20} = 2.086$. There are also $n = 5$ replicates per treatment. We then have

$$LSD = c_{\alpha,a(n-1)}\sqrt{\frac{2MS_{within}}{n}} = 2.086\sqrt{\frac{2(0.1122)}{5}} = 0.4419. \tag{13.14}$$

Note that SAS also displays the value of LSD in the output. We next calculate a 95% confidence interval for $\mu_{\text{medium}} - \mu_{\text{control}}$. Recall that the formula for the interval is

$$\left(\bar{Y}_i - \bar{Y}_j - LSD, \bar{Y}_i - \bar{Y}_j + LSD\right). \tag{13.15}$$

Inserting the estimated means for these two treatments (see SAS output) in this formula, and the LSD value, we obtain

$$(1.1972 - 0.6275 - 0.4419, 1.1972 - 0.6275 + 0.4419) \tag{13.16}$$

or $(0.1278, 1.0116)$. This confidence interval and the LSD value are quite close to the values obtained by SAS.

We now show how the LSD value is used to test $H_0 : \mu_{\text{medium}} - \mu_{\text{control}} = 0$ or equivalently $H_0 : \mu_{\text{medium}} = \mu_{\text{control}}$. We would reject H_0 if $|\bar{Y}_i - \bar{Y}_j| \geq LSD$. Inserting the estimated means for these two treatments, we see that $|1.1972 - 0.6275| = 0.5687 \geq 0.4419$, and so this pair of means is significantly different.

13.3.3 The Tukey procedure

The Tukey method for multiple comparisons is similar to the least significant difference procedure, except that it uses the **studentized range distribution** in place of the t distribution. The studentized range distribution is designed to control the EER rate for all pairwise comparisons among group means (Hsu 1996). Another advantage is that the confidence intervals constructed using this distribution are **simultaneous confidence intervals**. This means that the overall probability the confidence intervals include the true value of $\mu_i - \mu_j$, for all pairs of groups, is equal to $1 - \alpha$ for some specified α. The overall probability α is also the EER for the family of all pairwise tests.

The Tukey procedure makes use of a quantity called the honestly significant difference (HSD), defined as

$$HSD = q_{\alpha,a,a(n-1)}\sqrt{\frac{MS_{within}}{n}}. \tag{13.17}$$

The quantity $q_{\alpha,a,a(n-1)}$ is obtained from the studentized range distribution, and depends on α (the desired EER), the number of groups a, as well as the degrees of freedom for MS_{within}.

To test $H_0 : \mu_i = \mu_j$ or $H_0 : \mu_i - \mu_j = 0$, we accept H_0 if $|\bar{Y}_i - \bar{Y}_j| < HSD$, and reject it $|\bar{Y}_i - \bar{Y}_j| \geq HSD$. This same rule applies to any pair of groups, because HSD would take the same value. Any pair of means that equals or exceeds this value is declared to be significantly different. The Tukey confidence intervals are of the form

$$\left(\bar{Y}_i - \bar{Y}_j - HSD, \bar{Y}_i - \bar{Y}_j + HSD\right). \tag{13.18}$$

13.3.4 Tukey procedure - SAS demo

Implementing the Tukey procedure requires only a small change in our previous SAS program. The `means` statement within `proc glm` becomes

```
means treat / tukey cldiff lines;
```

Confidence intervals for $\mu_i - \mu_j$ and $\mu_j - \mu_i$ are given for every pair of groups, as well as a diagram indicating which treatments are significantly different. See a section of the SAS output below. For this example, the Tukey finds fewer significant comparisons than the least significant difference procedure. We see there are only two significant comparisons, very high vs. low and very high vs. control treatments. This is a common pattern observed with multiple comparison tests, a few significant differences but also substantial overlap among treatments or groups.

SAS Output

Multiple comparisons for algae cover 4
Data from Kneitel and Lessin (2010)
11:45 Thursday, July 5, 2012

The GLM Procedure

Tukey's Studentized Range (HSD) Test for y

NOTE: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.112222
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.634

Comparisons significant at the 0.05 level are indicated by ***.

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
VeryHigh - Medium	0.1263	-0.5077	0.7603	
VeryHigh - High	0.1563	-0.4777	0.7903	
VeryHigh - Low	0.6663	0.0323	1.3003	***
VeryHigh - Control	0.6960	0.0620	1.3300	***
Medium - VeryHigh	-0.1263	-0.7603	0.5077	
Medium - High	0.0300	-0.6040	0.6640	
Medium - Low	0.5401	-0.0939	1.1741	
Medium - Control	0.5697	-0.0643	1.2037	
High - VeryHigh	-0.1563	-0.7903	0.4777	
High - Medium	-0.0300	-0.6640	0.6040	
High - Low	0.5100	-0.1239	1.1440	
High - Control	0.5397	-0.0943	1.1737	
Low - VeryHigh	-0.6663	-1.3003	-0.0323	***
Low - Medium	-0.5401	-1.1741	0.0939	
Low - High	-0.5100	-1.1440	0.1239	
Low - Control	0.0296	-0.6044	0.6636	
Control - VeryHigh	-0.6960	-1.3300	-0.0620	***
Control - Medium	-0.5697	-1.2037	0.0643	

```
Control  - High          -0.5397    -1.1737  0.0943
Control  - Low           -0.0296    -0.6636  0.6044
```

```
                Multiple comparisons for algae cover                    5
                Data from Kneitel and Lessin (2010)
                                        11:45 Thursday, July 5, 2012

                          The GLM Procedure

                Tukey's Studentized Range (HSD) Test for y

NOTE: This test controls the Type I experimentwise error rate, but it generally
              has a higher Type II error rate than REGWQ.


              Alpha                                   0.05
              Error Degrees of Freedom                  20
              Error Mean Square                   0.112222
              Critical Value of Studentized Range  4.23186
              Minimum Significant Difference         0.634


        Means with the same letter are not significantly different.

  Tukey Grouping          Mean      N    treat

               A        1.3235      5    VeryHigh
               A
          B    A        1.1972      5    Medium
          B    A
          B    A        1.1672      5    High
          B
          B             0.6572      5    Low
          B
          B             0.6275      5    Control
```

We will now calculate the value of HSD for this example, to show how it is used to construct confidence intervals and tests. As before, we have $MS_{within} = 0.1122$ with 20 degrees of freedom. The SAS output gives the value of $q_{0.05,5,20} = 4.2319$, and there are $n = 5$ replicates per treatment. We then have

$$HSD = q_{\alpha,a,a(n-1)}\sqrt{\frac{MS_{within}}{n}} = 4.2319\sqrt{\frac{(0.1122)}{5}} = 0.6339. \qquad (13.19)$$

This value agrees with the SAS output labeled `Minimum Significant Difference`. We now calculate a 95% confidence interval for $\mu_{\text{medium}} - \mu_{\text{control}}$. The formula for the confidence interval is

$$\left(\bar{Y}_i - \bar{Y}_j - HSD, \bar{Y}_i - \bar{Y}_j + HSD\right). \qquad (13.20)$$

Inserting the estimated means for these two treatments (see SAS output) in this formula, and the HSD value, we obtain

$$(1.1972 - 0.6275 - 0.6339, 1.1972 - 0.6275 + 0.6339). \qquad (13.21)$$

or $(-0.0642, 1.2036)$. This confidence interval is close to the value provided by SAS.

How does this procedure control the EER as well as provide simultaneous confidence intervals? **The Tukey procedure basically controls the EER by making each pairwise test more conservative, through the use of the studentized range distribution.** Notice that $HSD > LSD$ for the same data set (0.6339 vs. 0.4419). This means that the Tukey procedure requires a larger difference between groups before declaring they are significantly different, and the confidence intervals are also broader. As a consequence, there is lower power to detect differences among groups when they do exist. This is the price paid for controlling the EER.

13.3.5 Multiple range tests - REGW

The multiple comparison procedures we have examined so far yield both tests and confidence intervals. Another type of multiple comparison procedure are multiple range tests. These procedures provide only tests, but are also more powerful procedures because they essentially conduct fewer overall tests than the methods we studied earlier. There are a number of

different multiple range tests, but we will only examine the REGW (Ryan-Einot-Gabriel-Welsch) procedure because it controls the EER (Hsu 1996).

The test works as follows (Hsu 1996). Suppose we order the sample means of the a different groups from smallest to largest:

$$\bar{Y}_{[1]} \leq \bar{Y}_{[2]} \leq \ldots \bar{Y}_{[a-1]}, \leq \bar{Y}_{[a]} \tag{13.22}$$

where $\bar{Y}_{[1]}$ is the smallest and $\bar{Y}_{[a]}$ the largest sample mean.

We then examine the range (difference) between the largest and smallest sample mean, namely $\bar{Y}_{[a]} - \bar{Y}_{[1]}$. If

$$\bar{Y}_{[a]} - \bar{Y}_{[1]} < q_a \sqrt{\frac{MS_{within}}{n}} \tag{13.23}$$

then we stop and declare there are no significant differences among groups. Otherwise, we assert that these two groups are significantly different and continue the process. We next examine the next innermost ranges $\bar{Y}_{[a-1]} - \bar{Y}_{[1]}$ and $\bar{Y}_{[a]} - \bar{Y}_{[2]}$. If

$$\bar{Y}_{[a-1]} - \bar{Y}_{[1]]} < q_{a-1} \sqrt{\frac{MS_{within}}{n}} \tag{13.24}$$

and

$$\bar{Y}_{[a1]} - \bar{Y}_{[2]]} < q_{a-1} \sqrt{\frac{MS_{within}}{n}} \tag{13.25}$$

then we stop the testing process. Otherwise, we assert that one or both groups are significantly different. This process is continued until no more significant differences are found.

The values of q are not the same for every step of the test. They are constructed so that $q_a > q_{a-1} > \ldots > q_2$, meaning that the largest range is tested using the largest value of q, the next largest two ranges with a smaller value of q, and so forth. This implies that the largest range must have the largest difference in means to be judged significant, while later tests allow for smaller differences. The values of q are chosen so that the experimentwise error rate has a specified value, usually $\alpha = 0.05$ (Hsu 1996). The studentized range distribution is involved in this process. The value of q_a used in the first step of the procedure is the same as that used by the Tukey procedure, as well as the difference in the means judged to be significant. The two procedures diverge after this point.

13.3.6 REGW procedure - SAS demo

Implementing the REGW procedure requires only a small change in our previous SAS programs. The `means` statement within `proc glm` becomes

```
means treat / regwq;
```

Here the `regwq` option requests the REGW procedure. SAS then generates a diagram indicating which groups are significantly different. See a section of the SAS output below, using the same data as our previous examples. For this example, the REGW procedure gives the same pattern of significant differences among groups as the Tukey method. The REGW procedure may become liberal (not fully control the EER) when the data are unbalanced, and SAS prints a warning note in this situation.

SAS Output

```
                    Multiple comparisons for algae cover                    4
                    Data from Kneitel and Lessin (2010)
                                          11:45 Thursday, July 5, 2012

                              The GLM Procedure

                Ryan-Einot-Gabriel-Welsch Multiple Range Test for y

              NOTE: This test controls the Type I experimentwise error rate.


                               Alpha                          0.05
                               Error Degrees of Freedom         20
                               Error Mean Square          0.112222


Number of Means              2             3             4             5
Critical Range       0.5340892     0.5871678     0.5930101     0.6339938

              Means with the same letter are not significantly different.

  REGWQ Grouping          Mean      N    treat

                A        1.3235      5    VeryHigh
                A
            B   A        1.1972      5    Medium
            B   A
            B   A        1.1672      5    High
            B
            B            0.6572      5    Low
            B
            B            0.6275      5    Control
```

13.4 Comparisons with a control - Dunnett procedure

Many studies include some sort of control group or treatment, and the experimenter may only be interested in comparing the control group with each of the other $a-1$ groups. For example, the control could represent a standard medical treatment for a disease while the other treatments represent alternative forms of therapy. The physician only wants to know if the alternative forms are better or worse than the standard method.

In this situation, there are only $a-1$ comparisons to be made rather than the full $a(a-1)/2$ comparisons of all pairs of means. The Dunnett procedure is designed to control the EER for just these $a-1$ comparisons, and hence has more power than other pairwise methods (Hsu 1996). The calculations are similar to the Tukey method, but use the quantity

$$DSD = d_{\alpha,a,a(n-1)}\sqrt{\frac{2MS_{within}}{n}}, \tag{13.26}$$

where DSD stands for Dunnett's significant difference. The values of $d_{\alpha,a,a(n-1)}$ are obtained from a distribution analogous to the studentized range distribution, except that it controls the EER for $a-1$ comparisons. The value of d depends on α (the desired EER), the number of groups a, and the degrees of freedom for MS_{within}.

Let μ_c be the mean of the control group, while μ_i is any other group. Dunnett's procedure can be used to test for $H_0 : \mu_i = \mu_c$ or equivalently $H_0 : \mu_i - \mu_c = 0$. We would accept H_0 if $|\bar{Y}_i - \bar{Y}_c| < DSD$. Conversely, we would reject H_0 if $|\bar{Y}_i - \bar{Y}_c| \geq DSD$. This same rule applies to all comparisons with the control group.

Confidence intervals for $\mu_i - \mu_c$ have the form

$$\left(\bar{Y}_i - \bar{Y}_c - DSD, \bar{Y}_i - \bar{Y}_c + DSD\right). \tag{13.27}$$

13.4.1 Dunnett's procedure - SAS demo

Using Dunnett's procedure requires only a small change to our program. The `means` statement within `proc glm` becomes

```
means treat / dunnett('Control');
```

The control group in our data set is coded as `Control`, and the `('Control')` portion of the statement informs SAS of this fact. Confidence intervals for $\mu_i - \mu_c$ are given in the SAS output, with the symbol *** indicating which comparisons of the control are significantly different. We see that the very high and medium treatments are significantly different from control.

SAS Output

```
                    Multiple comparisons for algae cover                    4
                    Data from Kneitel and Lessin (2010)
                                        11:45 Thursday, July 5, 2012

                              The GLM Procedure

                           Dunnett's t Tests for y

NOTE: This test controls the Type I experimentwise error for comparisons of all
                        treatments against a control.


                    Alpha                             0.05
                    Error Degrees of Freedom            20
                    Error Mean Square             0.112222
                    Critical Value of Dunnett's t  2.65103
                    Minimum Significant Difference  0.5617


          Comparisons significant at the 0.05 level are indicated by ***.


                                  Difference      Simultaneous
                 treat               Between     95% Confidence
              Comparison             Means         Limits

            VeryHigh - Control        0.6960     0.1343  1.2576  ***
            Medium   - Control        0.5697     0.0080  1.1314  ***
            High     - Control        0.5397    -0.0220  1.1013
            Low      - Control        0.0296    -0.5320  0.5913
```

13.5 Bonferroni and Sidak corrections

One way of controlling the EER in a set of comparisons is to use a distribution designed to control it, such as the studentized range distribution. These procedures control the EER by essentially making the per comparison rate for each test more conservative. This adjustment of the per comparison error rate is built into the studentized range distribution.

The Bonferroni correction provides another way of controlling the EER, by explicitly reducing the per comparison error rate and then using a simple t test (like the least significant difference procedure) to compare group means. Suppose that we are interested in k possible comparisons, either all $a(a-1)/2$ pairwise comparisons or $a-1$ comparisons with a control, where a is the number of groups. The Bonferroni correction adjusts the per comparison error rate as follows. Let α be the per comparison error rate, while α' is the desired EER. If we conduct each comparison at the per comparison rate of

$$\alpha = \frac{\alpha'}{k}, \tag{13.28}$$

then it can be shown the EER will not exceed α' (Hsu 1996). For example, suppose we are interested in all $k = a(a-1)/2$ pairwise comparison among groups. We would then conduct each test at the

$$\alpha = \frac{\alpha'}{k} = \frac{\alpha'}{a(a-1)/2} \tag{13.29}$$

level. We would use the same t test as in the least significant difference procedure, but adjust the value α according to this formula. We then have

$$BSD = c_{\frac{\alpha'}{a(a-1)/2}, a(n-1)} \sqrt{\frac{2MS_{within}}{n}}, \tag{13.30}$$

where BSD is the difference judged to be significant given the Bonferroni correction. We would accept $H_0 : \mu_i = \mu_j$ (or $H_0 : \mu_i - \mu_j = 0$) if $\bar{Y}_i - \bar{Y}_j$ falls inside the interval $(-BSD, BSD)$, or equivalently if $|\bar{Y}_i - \bar{Y}_j| < BSD$. Conversely, we would reject H_0 if $|\bar{Y}_i - \bar{Y}_j| \geq BSD$. A confidence interval for $\mu_i - \mu_j$ based on the Bonferroni correction would have the form

$$\left(\bar{Y}_i - \bar{Y}_j - BSD, \bar{Y}_i - \bar{Y}_j + BSD\right). \tag{13.31}$$

To make things more concrete, we can calculate the value of BSD for the algae cover example (Kneitel & Lessin 2010). From our previous output, we

have $a = 5$ groups, $n = 5$ replicates per group, and $MS_{within} = 0.1122$. If we set the EER to be $\alpha' = 0.05$, by the above formula we have

$$\alpha = \frac{\alpha'}{a(a-1)/2} = \frac{0.05}{5(5-1)/2} = \frac{0.05}{10} = 0.005. \tag{13.32}$$

For $\alpha = 0.005$, we have $c_{0.005,20} = 3.1534$, and so

$$BSD = c_{\frac{\alpha'}{a(a-1)/2},a(n-1)}\sqrt{\frac{2MS_{within}}{n}} = 3.1534\sqrt{\frac{2(0.1122)}{5}} = 0.6681. \tag{13.33}$$

Note that the value of $BSD = 0.6681$ is larger than $HSD = 0.6339$ value for the Tukey procedure. Thus, the Bonferroni method requires a greater difference among means before declaring they are significantly different, implying it has lower power than the Tukey procedure. It would also generate larger confidence intervals and so provides less precision in estimation.

Given these drawbacks, why would the Bonferroni correction be used? The Bonferroni procedure is quite general and can be used to control the EER for other testing procedures, not just comparisons among means in ANOVA. For example, it is common to have a collection of statistical tests that address a particular question. We might have a single experiment in which a number of different Y variables are measured, with a separate ANOVA conducted on each variable. If enough variables are examined it is possible that some could be significant by chance, and we could control the EER for all these tests using the Bonferroni correction, with k being the number of Y variables. There is also a version of this procedure similar in spirit to REGW, called the **sequential Bonferroni method** (Rice 1989). The sequential Bonferroni alleviates to some extent the lack of power in the standard Bonferroni correction. This procedure is implemented in `proc multtest` in SAS.

The Sidak correction is another procedure used to control the EER, which provides slightly more power than the Bonferroni method. Let α be the per comparison error rate, while α' is the desired EER. If we conduct each comparison at the per comparison rate of

$$\alpha = 1 - (1 - \alpha')^{1/k}, \tag{13.34}$$

then the actual EER will not exceed α'. For example, suppose we are interested in all $k = a(a-1)/2$ pairwise comparison among groups. We would then conduct each test at the

$$\alpha = 1 - (1 - \alpha')^{1/k} = 1 - (1 - \alpha')^{1/[a(a-1)/2]} \tag{13.35}$$

level. For $\alpha' = 0.05$ and $a = 5$ groups, we obtain

$$\alpha = 1 - (1 - \alpha')^{1/[a(a-1)/2]} = 1 - (1 - 0.05)^{1/10} = 0.0051. \quad (13.36)$$

We would then compare pairs of means using the same test as for the Bonferroni correction, except that we would use $\alpha = 0.0051$ rather than $\alpha = 0.005$. This value of α is a bit larger than the corresponding Bonferroni one, making the Sidak correction slightly more powerful.

SAS implements both the Bonferroni and Sidak corrections in the `means` statement with the options `bon` or `sidak`, similar to using the `tukey` option.

13.6 Vascular plant cover - SAS demo

Kneitel & Lessin (2010) also examined vascular plant cover in their study of the effect of eutrophication on vernal pools in California. Vascular plant cover (`cover`) was derived by subtracting algal cover (`algae`) from total cover (`total`), then arcsine-square root transformed before analysis (see Chapter 15). See `data` step in the SAS program below.

The `proc glm` code compares all possible pairs of group means using the Tukey procedure, and also compares the `Control` treatment with the other treaments using Dunnett's procedure. This was done to provide more examples of these procedures. **In practice, you should choose one procedure for comparing the means.**

The diagram generated by the Tukey procedure indicates two significant differences among treatments. Reading the diagram, we see the control vs. high and control vs. very high comparisons are significant, because they have different letters. No other pairs of groups are significantly different. Fig. 13.3 indicates how these results could be graphically displayed using letters. We see that vascular plant cover actually decreases with increased nutrient levels, likely due to inhibition from the algal mats that form (Kneitel and Lessin 2010).

We can also determine which groups are significantly different by examining the confidence intervals generated by the Tukey procedure. Confidence intervals that do not include zero indicate a significant difference among groups, because of the duality between confidence intervals and tests (see Chapter 10). The significant tests are indicated by *** in the SAS output. The SAS output for Dunnett's procedure shows that the high and very high treatments are significantly different from the control group.

SAS Program

```
* Kneitel_2010_cover2.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Multiple comparisons for vascular plant cover';
title2 'Data from Kneitel and Lessin (2010)';
data kneitel;
        input treat $ richness total algae;
        * Apply transformations here;
        vcover = total-algae;
        y = arsin(sqrt(vcover/100));
        datalines;
Control   8   78    1
Control   5   84    7
Control  10         115   45
Control   7         200  100
Control   6   72   20
Low       8   73   15
Low       7  124   70
Low       8  116   50
Low       8   92    5
Low       7  138   60
Medium    7  124   85
Medium    8  116   80
Medium    8  145   60
Medium    6  154  100
Medium    7  129   90
High      6  134   95
High      7  138   95
High      8  103   70
High      8  119   75
High          6  132   80
VeryHigh  6  148   95
VeryHigh  5  134   95
VeryHigh  5  119  100
VeryHigh  5  117   90
VeryHigh  5  129   80
;
run;
* Print data set;
proc print data=kneitel;
* Plot means, standard errors, and observations;
proc gplot data=kneitel;
        plot y*treat=1 / vaxis=axis1 haxis=axis1;
```

```
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* One-way anova with comparisons;
proc glm order=data data=kneitel;
        class treat;
        model y = treat;
        output out=resids p=pred r=resid;
        * Tukey procedure - controls the EER;
        means treat / tukey cldiff lines;
        * Dunnett's procedure - controls EER for comparisons with a control;
        means treat / dunnett('Control');
run;
goptions reset=all;
title "Diagnostic plots to check anova assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
        plot resid*pred=1 / vaxis=axis1 haxis=axis1;
        symbol1 v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
        qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

Multiple comparisons for vascular plant cover 1
Data from Kneitel and Lessin (2010)
11:45 Thursday, July 5, 2012

Obs	treat	richness	total	algae	vcover	y
1	Control	8	78	1	77	1.07062
2	Control	5	84	7	77	1.07062
3	Control	10	115	45	70	0.99116
4	Control	7	200	100	100	1.57080
5	Control	6	72	20	52	0.80540
6	Low	8	73	15	58	0.86574
7	Low	7	124	70	54	0.82544
8	Low	8	116	50	66	0.94826
9	Low	8	92	5	87	1.20193
10	Low	7	138	60	78	1.08259
11	Medium	7	124	85	39	0.67449
12	Medium	8	116	80	36	0.64350
13	Medium	8	145	60	85	1.17310
14	Medium	6	154	100	54	0.82544
15	Medium	7	129	90	39	0.67449
16	High	6	134	95	39	0.67449
17	High	7	138	95	43	0.71517
18	High	8	103	70	33	0.61194
19	High	8	119	75	44	0.72525
20	High	6	132	80	52	0.80540
21	VeryHigh	6	148	95	53	0.81542
22	VeryHigh	5	134	95	39	0.67449
23	VeryHigh	5	119	100	19	0.45103
24	VeryHigh	5	117	90	27	0.54640
25	VeryHigh	5	129	80	49	0.77540

Multiple comparisons for vascular plant cover 2
Data from Kneitel and Lessin (2010)
11:45 Thursday, July 5, 2012

The GLM Procedure

Class Level Information

Class	Levels	Values

```
          treat             5    Control Low Medium High VeryHigh


                   Number of Observations Read          25
                   Number of Observations Used          25


                Multiple comparisons for vascular plant cover                3
                     Data from Kneitel and Lessin (2010)
                                              11:45 Thursday, July 5, 2012

                              The GLM Procedure

Dependent Variable: y

                                      Sum of
 Source                       DF       Squares    Mean Square   F Value   Pr > F

 Model                         4    0.71900305     0.17975076      4.93   0.0063

 Error                        20    0.72959178     0.03647959

 Corrected Total              24    1.44859482


              R-Square     Coeff Var      Root MSE        y Mean

              0.496345      22.50344      0.190996      0.848743


 Source                       DF      Type I SS    Mean Square   F Value   Pr > F

 treat                         4    0.71900305     0.17975076      4.93   0.0063


 Source                       DF    Type III SS    Mean Square   F Value   Pr > F

 treat                         4    0.71900305     0.17975076      4.93   0.0063


                Multiple comparisons for vascular plant cover                4
                     Data from Kneitel and Lessin (2010)
                                              11:45 Thursday, July 5, 2012

                              The GLM Procedure
```

Tukey's Studentized Range (HSD) Test for y

NOTE: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.03648
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.3615

Comparisons significant at the 0.05 level are indicated by ***.

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Control - Low	0.1169	-0.2445	0.4784	
Control - Medium	0.3035	-0.0580	0.6650	
Control - High	0.3953	0.0338	0.7567	***
Control - VeryHigh	0.4492	0.0877	0.8106	***
Low - Control	-0.1169	-0.4784	0.2445	
Low - Medium	0.1866	-0.1749	0.5481	
Low - High	0.2783	-0.0831	0.6398	
Low - VeryHigh	0.3322	-0.0292	0.6937	
Medium - Control	-0.3035	-0.6650	0.0580	
Medium - Low	-0.1866	-0.5481	0.1749	
Medium - High	0.0918	-0.2697	0.4532	
Medium - VeryHigh	0.1457	-0.2158	0.5071	
High - Control	-0.3953	-0.7567	-0.0338	***
High - Low	-0.2783	-0.6398	0.0831	
High - Medium	-0.0918	-0.4532	0.2697	
High - VeryHigh	0.0539	-0.3076	0.4154	
VeryHigh - Control	-0.4492	-0.8106	-0.0877	***
VeryHigh - Low	-0.3322	-0.6937	0.0292	
VeryHigh - Medium	-0.1457	-0.5071	0.2158	
VeryHigh - High	-0.0539	-0.4154	0.3076	

The GLM Procedure

Tukey's Studentized Range (HSD) Test for y

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.03648
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.3615

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	1.1017	5	Control
A			
B A	0.9848	5	Low
B A			
B A	0.7982	5	Medium
B			
B	0.7065	5	High
B			
B	0.6525	5	VeryHigh

Multiple comparisons for vascular plant cover 6
Data from Kneitel and Lessin (2010)
11:45 Thursday, July 5, 2012

The GLM Procedure

Dunnett's t Tests for y

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05

```
Error Degrees of Freedom            20
Error Mean Square                0.03648
Critical Value of Dunnett's t    2.65103
Minimum Significant Difference    0.3202

Comparisons significant at the 0.05 level are indicated by ***.

                          Difference      Simultaneous
          treat             Between   95% Confidence
       Comparison             Means        Limits

Low      - Control          -0.1169    -0.4372  0.2033
Medium   - Control          -0.3035    -0.6237  0.0167
High     - Control          -0.3953    -0.7155 -0.0750  ***
VeryHigh - Control          -0.4492    -0.7694 -0.1289  ***
```

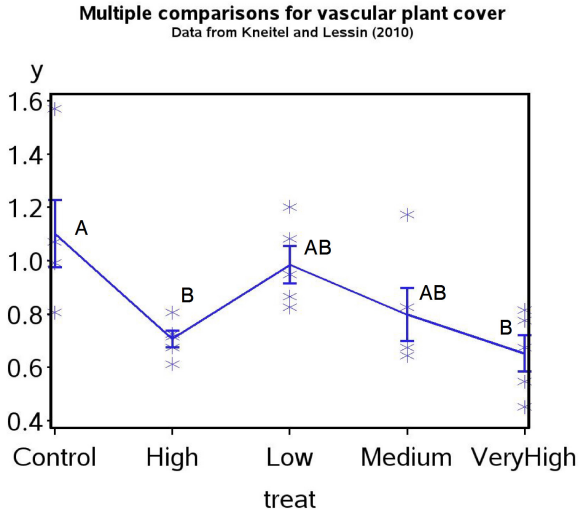

Figure 13.3: Vascular plant cover vs. nutrient addition treatment for simulated data patterned after Kneitel and Lessin (2010). Means with different letters are significantly different (Tukey procedure).

13.7 False discovery rate method

The multiple comparison procedures we have examined control the EER, but at the cost of power. This is especially true for studies with many treatments or groups. For example, suppose we have $a = 5$ treatments and want to conduct all pairwise comparisons using the Bonferroni method, with an EER of $\alpha' = 0.05$. There are $k = a(a-1)/2 = 5(4)/2 = 10$ pairwise comparisons, and so we would conduct each comparison at the $\alpha = \alpha'/k = 0.05/10 = 0.005$ level. For $a = 10$ treatments, a similar calculation suggests that each comparison should be conducted at the $\alpha = 0.0011$ level, yielding a much more conservative test. As the number of treatments increases, this makes it less likely significant differences will be found, and so the power to detect differences among treatments decreases. The number of treatments has similar effects on other multiple comparison procedures that control the EER.

The **false discovery rate** method provides an alternative approach to multiple comparisons and tests. This method controls the **proportion** of Type I errors in a set of comparisons, known as the false discovery rate or FDR (Benjamini & Hochberg 1995). This differs substantially from methods that control the EER, which are concerned with keeping the **number** of Type I errors low. One will have more Type I errors using the FDR, but the proportion of them is controlled, and the power to detect differences among treatments will be higher than EER methods. This approach seems particularly useful for studies that screen many treatments or groups, possibly for future work, and it is more important to identify possible effects than controlling the number of Type I errors (Verhoeven et al. 2005).

The FDR method for multiple comparisons works as as follows (Benjamini & Hochberg 1995). Suppose you have k pairwise comparisons, and obtain a P value for each one using the LSD procedure. Let $P_{[1]} \leq P_{[2]} \leq \ldots \leq P_{[k]}$ be the P values for these tests, ordered from smallest to largest, with $P_{[i]}$ the ith one. Let α^* be the specified false discovery rate. We then examine the ordered P values from largest to smallest (from $i = k$ to 1), examining at each step whether

$$P_{[i]} \leq \frac{i}{k}\alpha^*. \tag{13.37}$$

We can see that the right side of this equation decreases from α^* to α^*/k as i decreases. The first time this inequality is true, we declare that this pairwise comparison and all further ones are significantly different. Benjamini &

Hochberg (1995) show that this procedure controls the false discovery rate. The same method can also be used in other multiple testing scenarios, not just multiple comparisons among means.

As an example of this procedure, consider the algae cover example we examined earlier (Kneitel and Lessin 2010). There are ten pairwise comparisons among the different nutrient treatments. We first obtain the P values for each comparison using the LSD method (see SAS demo below), and order these from largest to smallest (Table 13.1). We then compare the P values with the right side of Eq. 13.37, beginning at the top of the table. We see that first comparison that satisfies Eq. 13.37 is high vs. low, and so we declare this comparison and all further ones to be significant. Thus, the the FDR procedure finds six of ten pairwise comparisons to be significant, similar to the LSD procedure. The Tukey and REGW procedures, which control the EER, found only two significant comparisons.

Table 13.1: Ordered P values for LSD comparisons of algae cover in different nutrient treatments (Kneitel and Lessin 2010). The last column calculates the right side of Eq. 13.37 for $\alpha^* = 0.05$ and $k = 10$ pairwise comparisons.

Comparison	i	$P_{[i]}$	$\frac{i}{k}\alpha^*$
control–low	10	0.8902	0.0500
medium–high	9	0.8887	0.0450
medium–very high	8	0.5578	0.0400
high–very high	7	0.4693	0.0350
high–low	6	0.0258	0.0300
control–high	5	0.0192	0.0250
low–medium	4	0.0191	0.0200
control–medium	3	0.0141	0.0150
low–very high	2	0.0051	0.0100
control–very high	1	0.0037	0.0010

13.7.1 False discovery rate - SAS demo

The FDR procedure can be implemented in two steps using SAS. We first need to obtain the P values for the LSD procedure. This can be accomplished by adding an `lsmeans` statement to our previous program, with a `pdiff` option:

```
lsmeans treat / adjust=t pdiff;
```

The result is a table of P values for each comparison, shown below.

SAS Output

```
                    Multiple comparisons for algae cover                    4
                    Data from Kneitel and Lessin (2010)
                                          14:39 Monday, May 23, 2016

                            The GLM Procedure
                           Least Squares Means

                                                    LSMEAN
                    treat            y LSMEAN       Number

                    Control        0.62753783            1
                    High           1.16721374            2
                    Low            0.65716894            3
                    Medium         1.19723297            4
                    VeryHigh       1.32351133            5


                    Least Squares Means for effect treat
                    Pr > |t| for H0: LSMean(i)=LSMean(j)

                           Dependent Variable: y

  i/j             1             2             3             4             5

    1                       0.0192        0.8902        0.0141        0.0037
    2        0.0192                       0.0258        0.8887        0.4693
    3        0.8902        0.0258                       0.0191        0.0051
    4        0.0141        0.8887        0.0191                      0.5578
    5        0.0037        0.4693        0.0051        0.5578
```

We then use `proc multtest` to carry out the FDR procedure. The P values for each comparison are supplied in a SAS data set, labeled as `raw_p`. The data set is specified using the `inpvalues` option, while the FDR procedure is requested using the `fdr` option. The output consists of the original and adjusted P values, with the adjustment made according to the FDR procedure. Adjusted P values less than 0.05 are judged to be significant. See program and output below. We observe that six of ten pairwise comparisons have an adjusted P value less than 0.05, and so these are judged significant by the FDR procedure.

SAS Program

```
* Kneitel_2010_algae_fdr2.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Multiple comparisons for algae cover';
title2 'False discovery rate (Benjamini and Hochberg 1995)';
data pvalues;
        input comparison :$18. raw_p;
        datalines;
Control-High     0.0192
Control-Low      0.8902
Control-Medium   0.0141
Control-VeryHigh 0.0037
High-Low         0.0258
High-Medium      0.8887
High-VeryHigh    0.4693
Low-Medium       0.0191
Low-VeryHigh     0.0051
Medium-VeryHigh  0.5578
;
* Multiple comparisons using fdr;
proc multtest inpvalues=pvalues fdr;
run;
quit;
```

SAS Output

Multiple comparisons for algae cover 1
False discovery rate (Benjamini and Hochberg 1995)
14:39 Monday, May 23, 2016

The Multtest Procedure

P-Value Adjustment Information

P-Value Adjustment False Discovery Rate

p-Values

Test	Raw	False Discovery Rate
1	0.0192	0.0384
2	0.8902	0.8902
3	0.0141	0.0384
4	0.0037	0.0255
5	0.0258	0.0430
6	0.8887	0.8902
7	0.4693	0.6704
8	0.0191	0.0384
9	0.0051	0.0255
10	0.5578	0.6973

13.8 References

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Verhoeven, K. J. F., Simonsen, K. L. & McIntyre, L. M. (2005) Implementing false discovery rate control: increasing your power. *Oikos* 108: 643-647.

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13.9 Problems

1. White-tailed deer are voracious consumers of landscaping plants. A frustrated homeowner/professor is interested in testing whether different repellents actually reduce deer herbivory. Replicate plots of houseplants are established and four different treatments applied to the plots: (1) a control with no treatment, (2) hot pepper oil repellent, (3) rotten egg repellent, and (4) livestock blood repellent. There were 4 replicate plots per treatment. The amount of herbivory (percentage of plants eaten) after one month are given in the following table.

Control	Hot pepper	Rotten eggs	Blood
61.1	54.4	32.0	36.2
64.9	67.9	28.5	38.3
61.6	54.6	21.6	31.1
67.8	58.1	38.8	44.1

   (a) Test whether there is an overall effect of treatment on the percentage of plants eaten, using one-way anova and SAS. Report your results using P values and discuss the significance of the test.

   (b) Use the Tukey procedure to compare the different treatments, and interpret your results. Which pairs of treatments are significantly different? Do the treatments fall into particular groups?

   (c) Suppose the homeowner is only interested in treatments that are different from the control. Use the Dunnett method to compare the three treatments with the control one. Which treatments are significantly different from the control?

2. PCB concentrations were measured in the sediment of Crab Orchard Lake, at 11 different sites (Kohler et al. 1990). Three samples were taken at each site, yielding the data shown in the table below. Site 10 is near an abandoned dump site for a manufacturer of electrical transformers.

Site	PCB (mg/kg), sample 1-3
1	0.0453, 0.0626, 0.527
2	0.0395, 0.0494, 0.0416
3	0.0234, 0.0451, 0.0541
4	0.033, 0.0643, 0.0517
5	0.0394, 0.0810, 0.0266
6	0.0294, 0.0425, 0.0538
7	0.0255, 0.0440, 0.0427
8	0.0323, 0.0382, 0.0360
9	0.0533, 0.0407, 0.0626
10	0.160, 0.437, 0.343
11	0.135, 0.142, 0.0592

(a) Test whether there is an overall effect of site on PCB concentration, using one-way ANOVA and SAS. Treat site as a fixed effect. Report your results using P values and discuss the significance of the test. A log transformation should be applied before analysis.

(b) Use the REGW procedure to compare the different sites, and interpret your results. Which pairs of sites are significantly different? Do the sites fall into particular groups?

3. An entomologist wants to compare the attractiveness of nine different baits (A-I) for bark beetles. There were three replicate traps for each bait treatment. The table below lists the number of beetles captured in each trap.

Bait	Beetles, trap 1-3
A	27, 36, 26
B	25, 19, 37
C	8, 16, 12
D	15, 8, 12
E	68, 42, 57
F	43, 32, 47
G	10, 12, 19
H	71, 62, 53
I	19, 11, 21

(a) Test whether there is an overall effect of bait on beetle captures, using one-way ANOVA and SAS. Report your results using P

values and discuss the significance of the test. Apply a log transformation before analysis.

(b) Use the FDR procedure to compare the different baits, and interpret your results. Which baits are significantly different?

Chapter 14

Analysis of Variance (Two-Way)

Two-way ANOVA examines how two different factors, such as different experimental treatments, affect the means of the different groups. For example, we might be interested in how different baits, as well as trap color, affect the number of insects caught in the traps. If we conducted an experiment where traps were deployed with different combinations of bait and trap color, this would be a **two-way factorial design**, where the word 'factorial' implies all possible combinations of the two factors. If there were three different baits (A, B, and C) and two trap colors (black, white), a factorial design implies there would be six different treatment combinations in the experiment (A-black, A-white, B-black, B-white, C-black, C-white). There would be one or more traps deployed with each treatment combination. It is customary to call one of the factors in a two-way design 'Factor A', while the other is 'Factor B'.

Similar to one-way ANOVA designs, the factors in two-way ANOVA can be either fixed or random. In the insect trapping experiment discussed above, both bait and trap color would be fixed effects because they were selected by the investigator. There are then F tests for each factor in the design, and potentially a test for the **interaction** of the two factors. **An interaction between two factors implies there is a joint effect of the two factors beyond that predicted by each factor operating additively.** For example, insects might be strongly attracted to A-black traps, more than would be predicted by the bait and trap color effects observed in the rest of the treatments. We will focus some effort on the analysis of this design

because it is one of the more common ones.

There are other possible two-way designs, including one fixed and one random effect, or more rarely both effects are random. We will examine one common design where one factor is fixed and the other random, called a **randomized block design**. There is an F test for the fixed effect in this design, and this test is often the primary goal of the analysis. With respect to the random effects, it is common to simply estimate the variance components associated with these effects and not conduct any tests, although these are still available. This design is ubiquitous in field studies because it helps control for certain forms of spatial or temporal heterogeneity in the observations, permitting a more powerful test of any treatment or group effects.

What do the data look like for a two-way ANOVA design? We will first examine a simplified data set from a trapping study of the bark beetle predator *T. dubius* (Reeve et al. 2009). These predators feed on bark beetles which attack and kill pine trees, and are attracted to the pheromones of the bark beetles as well as odors emitted by damaged pines. Visual cues may also play a role in their behavior, in particular the dark vertical silhouette provided by the bole of the tree. Three different baits were used: frontalin + turpentine (FRT), ipsdienol + turpentine (IDT), and ipsenol + turpentine (IST). Frontalin, ipsdienol, and ipsenol are bark beetle pheromones, while turpentine contains volatiles similar to those in pine resin. The traps were also painted two different colors, black vs. white, to manipulate their appearance to the predators. Thus, there were a total of six treatments (three baits, two colors) in the design. The different treatments were randomly assigned to trapping locations along transects in a pine forest, with four replicates per treatment. The number of predators caught in each trap were counted after several weeks of trapping (Table 14.1). The fourth column in the table shows the values after applying a log transformation, which is commonly used with count data (see Chapter 15).

We will use the notation Y_{ijk} to reference the observations in two-way ANOVA designs. The i subscript refers to the group or treatment within Factor A (bait), j the group or treatment within Factor B (trap color), while k refers to the observation within the treatment. For example, Y_{123} refers to the third observation in the FRT bait - W color treatment, which is 0.903.

We will also examine data from an experiment that examined how nutrient and water availability, as well as resource heterogeneity in space or time, affect biomass production in grassland plants (Maestre & Reynolds

2007). Plants from a grassland community were seeded in small containers in the greenhouse, with the treatments consisting of different levels of nitrogen and watering. There were three nitrogen and three watering levels in the experiment, for a total of nine treatments, with four replicate containers per treatment. The experiment also included treatments were the nitrogen was heterogeneously distributed in the container and watering was pulsed in time, but we will defer analysis of these other factors to Chapter 19. The total biomass of the plants was then determined after 100 d of growth (Table 14.2).

The data sets presented in this chapter are balanced designs with the same number of replicates per group, because this simplifies the formulas. They can be extended to unbalanced designs, but we will let SAS handle the details of the calculations in this case. We will later see how unbalanced data sets can influence the tests in two-way ANOVA.

Table 14.1: Example 1 - Effect of bait and trap color on catches of *T. dubius*, a bark beetle predator (Reeve et al. 2009). The baits used were frontalin + turpentine (FRT), ipsdienol + turpentine (IDT), and ipsenol + turpentine (IST), and the traps were painted either black (B) or white (W). Also shown are the means for each treatment group ($\bar{Y}_{ij.}$) and preliminary calculations to find SS_{within}

Bait	Color	*T. dubius*	$Y_{ijk} = \log_{10}(T.dubius + 1)$	i	j	k	$\bar{Y}_{ij.}$	$(Y_{ijk} - \bar{Y}_{ij.})^2$
FRT	B	18	1.279	1	1	1	1.150	1.664×10^{-2}
FRT	B	12	1.114	1	1	2		1.296×10^{-3}
FRT	B	22	1.362	1	1	3		4.494×10^{-2}
FRT	B	6	0.845	1	1	4		9.303×10^{-2}
FRT	W	12	1.114	1	2	1	0.980	1.796×10^{-2}
FRT	W	15	1.204	1	2	2		5.018×10^{-2}
FRT	W	7	0.903	1	2	3		5.929×10^{-3}
FRT	W	4	0.699	1	2	4		7.896×10^{-2}
IDT	B	0	0.000	2	1	1	0.369	1.363×10^{-1}
IDT	B	2	0.477	2	1	2		1.161×10^{-2}
IDT	B	1	0.301	2	1	3		4.658×10^{-3}
IDT	B	4	0.699	2	1	4		1.087×10^{-1}
IDT	W	2	0.477	2	2	1	0.314	2.665×10^{-2}
IDT	W	1	0.301	2	2	2		1.626×10^{-4}
IDT	W	2	0.477	2	2	3		2.665×10^{-2}
IDT	W	0	0.000	2	2	4		9.844×10^{-2}

Bait	Color	*T. dubius*	$Y_{ijk} = \log_{10}(T.dubius + 1)$	i	j	k	$\bar{Y}_{ij.}$	$(Y_{ijk} - \bar{Y}_{ij.})^2$
IST	B	2	0.477	3	1	1	0.725	6.126×10^{-2}
IST	B	2	0.477	3	1	2		6.126×10^{-2}
IST	B	10	1.041	3	1	3		1.002×10^{-1}
IST	B	7	0.903	3	1	4		3.186×10^{-2}
IST	W	1	0.301	3	2	1	0.719	1.745×10^{-1}
IST	W	4	0.699	3	2	2		3.901×10^{-4}
IST	W	14	1.176	3	2	3		2.091×10^{-1}
IST	W	4	0.699	3	2	4		3.901×10^{-4}

Table 14.2: Example 2 - Effect of nutrient and water availability on the total biomass of grassland plants grown in microcosms (Maestre & Reynolds 2007).

N (mg)	Water (ml/week)	Y_{ijk} = Biomass	i	j	k
40	125	4.372	1	1	1
40	125	4.482	1	1	2
40	125	4.221	1	1	3
40	125	3.977	1	1	4
40	250	7.400	1	2	1
40	250	8.027	1	2	2
40	250	7.883	1	2	3
40	250	7.769	1	2	4
40	375	7.226	1	3	1
40	375	8.126	1	3	2
40	375	6.840	1	3	3
40	375	7.901	1	3	4
80	125	5.140	2	1	1
80	125	3.913	2	1	2
80	125	4.669	2	1	3
80	125	4.306	2	1	4
80	250	9.099	2	2	1
80	250	9.711	2	2	2
80	250	9.123	2	2	3
80	250	9.709	2	2	4
80	375	10.701	2	3	1
80	375	11.552	2	3	2
80	375	11.356	2	3	3
80	375	9.759	2	3	4

N (mg)	Water (ml)	Y_{ijk} = Biomass	i	j	k
120	125	5.021	3	1	1
120	125	4.970	3	1	2
120	125	5.055	3	1	3
120	125	4.862	3	1	4
120	250	9.029	3	2	1
120	250	10.791	3	2	2
120	250	9.115	3	2	3
120	250	10.319	3	2	4
120	375	12.189	3	3	1
120	375	14.381	3	3	2
120	375	13.153	3	3	3
120	375	14.066	3	3	4

14.1 Random assignment of treatments

A essential step in executing ANOVA designs is the random assignment of treatments to experimental units. For example, in the Example 2 experiment we would want to randomly assign nitrogen and watering levels to the microcosms. This avoids any bias on the part of the experimenter in assigning the treatments to the containers, and also ensures that the replicates for each treatment are spread and intermingled throughout the greenhouse. What could happen if the treatments are not randomly assigned? Suppose that all the replicates for a given treatment in Example 2 are placed next to each other in the greenhouse, perhaps because this is convenient when applying the treatments. If a particular location happens to be warmer or receive more sunlight than another location, then the plants may be larger in that location and so bias the results of the experiment. We may falsely conclude a particular treatment has an effect on biomass because of this location effect. The random assignment of treatments avoids biases of this sort and also ensures independence of the observations, a basic assumption of most statistical models (Hurlbert 1984; Potvin 1993). Experiments with this feature are also known as **completely randomized designs**. We will illustrate the random assignment of treatments using a SAS program below.

14.1.1 Random assignment of treatments - SAS Demo

The program below shows one way of randomly assigning treatments to containers for the Example 2 experiment. We first input the different treatment combinations using a `data` step, with one line in the data set for each replicate. The `data` step also assigns a random number to each observation. The program uses a uniform random variable generated by the `ranuni` function, but any continuous random variable would work. We then use `proc sort` to sort the observations in ascending order by this random variable, thereby randomly shuffling the treatments. We would then assign to the first container the first treatment combination in the shuffled observations, the second container the second treatment combination, and so forth.

SAS Program

```
* Rand_treatments.sas;
options pageno=1 linesize=80;
title "Random assignment of Example 2 treatments";
data treat;
    input nitrogen water;
    * Generate a uniform random variable;
    u = ranuni(0);
    datalines;
 40  125
 40  125
 40  125
 40  125
 40  250
 40  250
 40  250
 40  250

etc.

;
run;
title2 "Original order of treatments";
proc print data=treat;
run;
* Sort treatments by value of u;
proc sort out=shuffled data=treat;
    by u;
run;
title2 "Randomly shuffled treatments";
```

```
proc print data=shuffled;
run;
quit;
```

SAS Output

```
                    Random assignment of Example 2 treatments                    1
                           Original order of treatments
                                                13:37 Friday, July 13, 2012

                       Obs    nitrogen    water       u

                         1        40        125     0.42081
                         2        40        125     0.87644
                         3        40        125     0.39786
                         4        40        125     0.59752
                         5        40        250     0.61762
                         6        40        250     0.13813
                         7        40        250     0.89420
                         8        40        250     0.09823

etc.

                    Random assignment of Example 2 treatments                    2
                           Randomly shuffled treatments
                                                13:37 Friday, July 13, 2012

                       Obs    nitrogen    water       u

                         1       120        250     0.07799
                         2        40        250     0.09823
                         3        80        375     0.10110
                         4       120        375     0.10234
                         5        80        375     0.12797
                         6        40        375     0.12981
                         7        40        250     0.13813
                         8        80        250     0.15527

etc.
```

14.2 Two-way fixed effects model

Suppose that we want to model the observations in studies like Example 1 or 2, where there are two factors that are manipulated and are fixed effects. Let Factor A be one treatment (such as bait type) while Factor B is the other treatment (trap color). Let the symbol Y_{ijk} stand for the kth observation ($k = 1, 2, \ldots, n$) in the ith Factor A treatment and jth Factor B treatment. For example, with the Example 1 data set we have $Y_{111} = 1.279$ while $Y_{222} = 0.301$ (see Table 14.1). One commonly used model for such a design (Searle 1971) is

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}. \tag{14.1}$$

Here μ is the grand mean of the observations, while α_i is the deviation from μ caused by the ith treatment in Factor A, while β_j is the deviation caused by the jth treatment in Factor B. These terms are called the **main effects** in the model. The term $(\alpha\beta)_{ij}$ represents an **interaction** between Factors A and B, implying a shift in the mean for a particular treatment combination beyond the effects of Factor A and B. An interaction between two factors A and B is often symbolized as 'A $\times$ B.' It is also considered a fixed effect when both A and B are fixed effects. The ϵ_{ijk} term represents random departures from the mean value predicted by the main effects and interaction due to natural variability among the observations, and are also assumed to be independent. The model also assumes that $\sum \alpha_i = 0, \sum \beta_j = 0$, and $\sum(\alpha\beta)_{ij} = 0$, but this does not affect its generality. The same model can also be used to describe the observations for studies where there are a groups or levels for Factor A, and b for Factor B, with any number of replicates (n) per treatment combination, as well as unbalanced designs with different numbers of replicates.

It follows for the ith level of Factor A and jth of Factor B that $E[Y_{ijk}] = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij}$ and $Var[Y_{ijk}] = \sigma^2$, using the rules for expected values and variances. Thus, for the ith and jth level we have $Y_{ijk} \sim N(\mu + \alpha_i + \beta_j + (\alpha\beta)_{ij}, \sigma^2)$. We can illustrate how the different parameters work in this model by plotting the distribution of the data for different parameter values. The behavior of the model is described for four different scenarios below. We will model an experiment similar to Example 1, where there are three levels for Factor A ($a = 3$) and two for Factor B ($b = 2$).

14.2.1 Factor A effect

Suppose that Factor A has a strong effect on Y_{ijk}, but there is only a minimal effect of Factor B and no interaction between the two factors. To make things concrete, let $\mu = 1.5$, $\alpha_1 = 0.5, \alpha_2 = 0, \alpha_3 = -0.5, \beta_1 = 0.1, \beta_2 = -0.1$, $(\alpha\beta)_{ij} = 0$ for all i and j, and $\sigma^2 = 0.05$. Fig. 14.1 shows the distribution of the observations in each treatment group. Note that the mean for treatment 1 under Factor A is shifted upward from μ while treatment 3 is shifted downward, for both levels of Factor B. The distribution for each treatment combination has the same variance, namely $\sigma^2 = 0.05$.

14.2.2 Factor B effect

Suppose the reverse situation is now true, with Factor B having a strong effect on Y_{ijk} while Factor A has a minimal effect, again with no interaction. This could be modeled using $\alpha_1 = 0.1, \alpha_2 = 0, \alpha_3 = -0.1, \beta_1 = 0.5, \beta_2 = -0.5$, and $(\alpha\beta)_{ij} = 0$ for all i and j. Fig. 14.2 shows the pattern that results. Note that the mean for treatment 1 under Factor B is shifted upward from μ, while treatment 2 is shifted downward, for all three levels of Factor A.

14.2.3 Factor A and B effect

If both factors have an effect on Y_{ijk}, we would expect to see a combination of the previous patterns, with the treatment groups shifted away from each other (Fig. 14.3). This figure uses $\alpha_1 = 0.5, \alpha_2 = 0, \alpha_3 = -0.5, \beta_1 = 0.3, \beta_2 = -0.3$, and $(\alpha\beta)_{ij} = 0$ for all i and j.

14.2.4 Interaction effect

We now examine how an A $\times$ B interaction influences the model. Suppose that $\alpha_1 = 0.5, \alpha_2 = 0, \alpha_3 = -0.5, \beta_1 = 0.3$ and $\beta_2 = -0.3$ as in the previous figure, but now $(\alpha\beta)_{11} = 0.2, (\alpha\beta)_{12} = -0.2, (\alpha\beta)_{21} = 0, (\alpha\beta)_{22} = 0, (\alpha\beta)_{31} = -0.2$, and $(\alpha\beta)_{32} = 0.2$. We see that Factor B has a substantial effect under treatment 1 for Factor A, and smaller effect under treatment 2, and almost no effect under treatment 3 (Fig. 14.4). Note that the distributions under the different treatment combinations no longer move in parallel as in Fig. 14.3. This pattern is diagnostic of an interaction in the analysis of

real data. We will later examine a data set where there is strong interaction between the two factors.

The objective in two-way ANOVA is to test whether Factor A, B, or both have an effect on the group means, and whether there is interaction between the two factors. For Factor A this amounts to testing H_0 : all $\alpha_i = 0$, while for Factor B we would test H_0 : all $\beta_j = 0$. For interaction between the two factors, we would test H_0 : all $(\alpha\beta)_{ij} = 0$. The corresponding alternative hypotheses are H_1 : some $\alpha_i \neq 0$, H_1 : some $\beta_j \neq 0$, and H_1 : some $(\alpha\beta)_{ij} \neq 0$. We will discuss how these null hypotheses are tested in the next section.

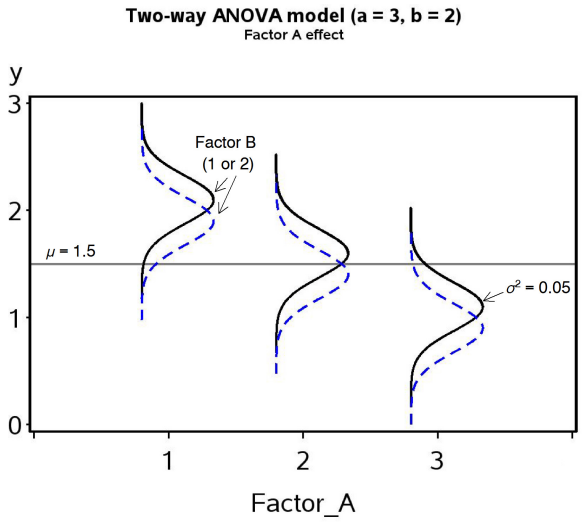

Figure 14.1: Fixed effects model for two-way ANOVA showing a Factor A effect.

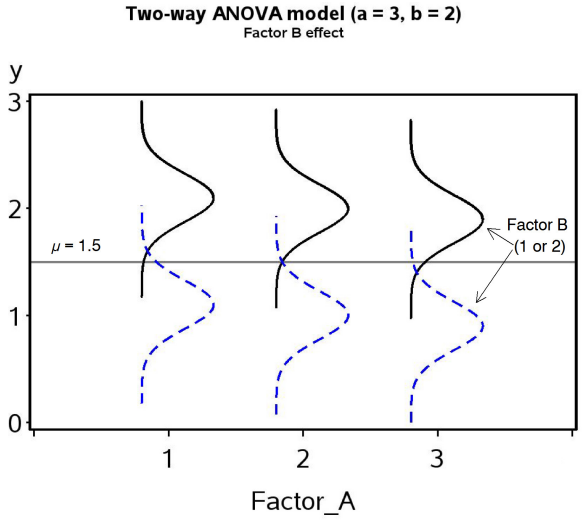

Figure 14.2: Fixed effects model for two-way ANOVA showing a Factor B effect.

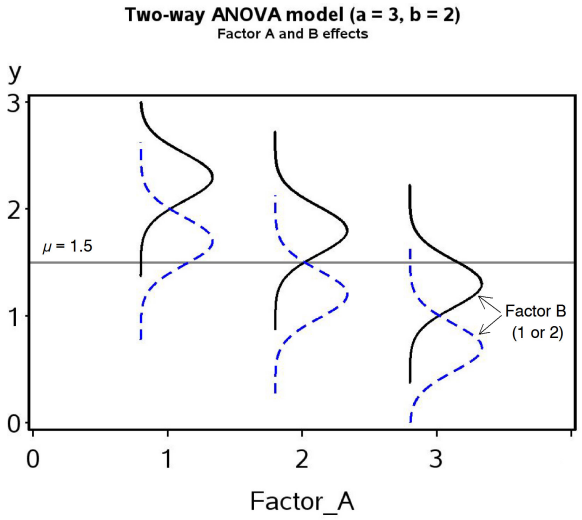

Figure 14.3: Fixed effects model for two-way ANOVA showing both Factor A and B effects.

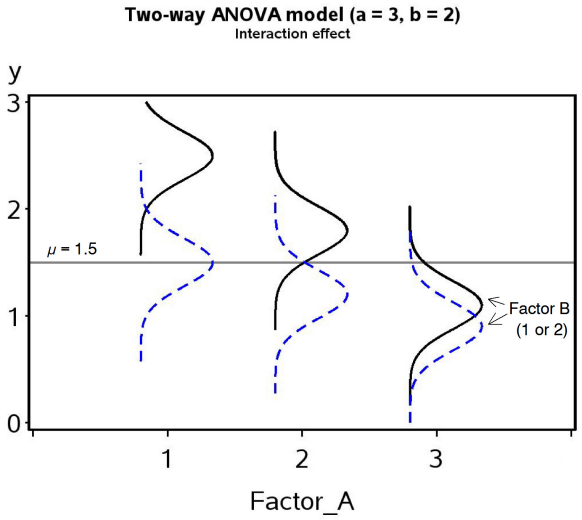

Figure 14.4: Fixed effects model for two-way ANOVA showing an A $\times$ B interaction between the two factors.

14.3 Hypothesis testing for two-way ANOVA

We now develop statistical tests for each of the null hypotheses listed above. All work in a similar fashion to the F test for one-way ANOVA. For Factor A and B in the model, as well as the interaction term, there is a corresponding sum of squares and mean square term. There is also an overall sum of squares and mean square within groups. These quantities are used to construct three different F tests, one for Factor A, Factor B, and the A $\times$ B interaction. These three tests are also examples of likelihood ratio tests, in which the fit is compared between the null and alternative models (Searle 1971). We will illustrate the calculations for these tests using the Example 1 data set, with Factor A being bait while Factor B is trap color.

14.3.1 Sum of squares and mean squares

We begin by calculating the group means for each treatment combination. For the Example 1 data, this amounts to calculating a group mean for each combination of bait and trap color. These group means are shown in Table 14.1 and labeled as $\bar{Y}_{ij\cdot}$. Here the '$\cdot$' notation implies the mean was calculated using all the observations in that group ($k = 1, 2, \ldots, n$). A grand mean can then be calculated as the mean of these group means, or equivalently by summing all the observations and dividing by their total number. We label this grand mean as $\bar{\bar{Y}}$. It can be generally calculated using the formula

$$\bar{\bar{Y}} = \frac{\sum_{i=1}^{a} \sum_{j=1}^{b} \bar{Y}_{ij\cdot}}{ab}. \tag{14.2}$$

For the Example 1 data set, we have

$$\bar{\bar{Y}} = \frac{1.150 + 0.980 + 0.369 + 0.314 + 0.725 + 0.719}{6} = 0.709. \tag{14.3}$$

We next calculate a mean corresponding to each level of Factor A by averaging across the levels of Factor B, which we denote as $\bar{\bar{Y}}_{i\cdot\cdot}$. It can be calculated using the formula

$$\bar{\bar{Y}}_{i\cdot\cdot} = \frac{\sum_{j=1}^{b} \bar{Y}_{ij\cdot}}{b}. \tag{14.4}$$

For the Example 1 data set, we have

$$\bar{\bar{Y}}_{1\cdot\cdot} = \frac{1.150 + 0.980}{2} = 1.065, \tag{14.5}$$

$$\bar{\bar{Y}}_{2\cdot\cdot} = \frac{0.369 + 0.314}{2} = 0.342, \tag{14.6}$$

and

$$\bar{\bar{Y}}_{3\cdot\cdot} = \frac{0.725 + 0.719}{2} = 0.722. \tag{14.7}$$

The difference $\bar{\bar{Y}}_{i\cdot\cdot} - \bar{\bar{\bar{Y}}}$ is a measure of the shift generated by Factor A in the observations, as well as an estimate of α_i for each level of Factor A. We can obtain a single measure of these shifts by squaring and summing them across all groups to obtain a sum of squares for Factor A, or SS_A. It can be calculated using the general formula

$$SS_A = nb \sum_{i=1}^{a} (\bar{\bar{Y}}_{i\cdot\cdot} - \bar{\bar{\bar{Y}}})^2. \tag{14.8}$$

SS_A has $a-1$ degrees of freedom. We can calculate a mean square for Factor A using the formula

$$MS_A = \frac{SS_A}{a-1}. \tag{14.9}$$

Note the factor nb in the expression for SS_A, which scales MS_A so that it estimates σ^2 if H_0 : all $\alpha_i = 0$ is true (no Factor A effect). If H_1 is true, implying some $\alpha_i \neq 0$, then MS_A will become larger. For the Example 1 data, we have

$$SS_A = 4(2)\left[(1.065 - 0.709)^2 + (0.342 - 0.709)^2 + (0.722 - 0.709)^2\right] \tag{14.10}$$

$$= 8\left[1.265 \times 10^{-1} + 1.353 \times 10^{-1} + 1.501 \times 10^{-4}\right] = 2.096. \tag{14.11}$$

and

$$MS_A = \frac{2.096}{3-1} = 1.048. \tag{14.12}$$

We can similarly calculate a mean corresponding to each level of Factor B, averaging across levels of Factor A. The general formula for these means is

$$\bar{\bar{Y}}_{\cdot j\cdot} = \frac{\sum_{i=1}^{a} \bar{Y}_{ij\cdot}}{a}. \tag{14.13}$$

For the Example 1 data set, we have

$$\bar{\bar{Y}}_{\cdot 1\cdot} = \frac{1.150 + 0.369 + 0.725}{3} = 0.748 \tag{14.14}$$

and

$$\bar{\bar{Y}}_{\cdot 2\cdot} = \frac{0.980 + 0.314 + 0.719}{3} = 0.671. \tag{14.15}$$

The difference $\bar{\bar{Y}}_{\cdot j\cdot} - \bar{\bar{\bar{Y}}}$ is a measure of the shift generated by Factor B in the observations, as well as an estimate of β_j for each level of Factor B. Squaring and summing them across all groups, we obtain a sum of squares for Factor B, or SS_B. It can be calculated using the general formula

$$SS_B = na \sum_{j=1}^{b} (\bar{\bar{Y}}_{\cdot j\cdot} - \bar{\bar{\bar{Y}}})^2. \tag{14.16}$$

SS_B has $b - 1$ degrees of freedom. We can then calculate a mean square for Factor B using the formula

$$MS_B = \frac{SS_B}{b-1}. \tag{14.17}$$

For the Example 1 data, we have

$$SS_B = 4(3)\left[(0.748 - 0.709)^2 + (0.671 - 0.709)^2\right] \tag{14.18}$$

$$= 12\left[1.485 \times 10^{-3} + 1.485 \times 10^{-3}\right] = 3.565 \times 10^{-2} \tag{14.19}$$

and

$$MS_B = \frac{3.565 \times 10^{-2}}{2-1} = 3.565 \times 10^{-2}. \tag{14.20}$$

We can also calculate a sum of squares and mean square to test for the A × B interaction. The sum of squares for interaction, SS_{AB}, is calculated in general using the formula

$$SS_{AB} = n \sum_{i=1}^{a} \sum_{j=1}^{b} (\bar{Y}_{ij\cdot} - \bar{\bar{Y}}_{i\cdot\cdot} - \bar{\bar{Y}}_{\cdot j\cdot} + \bar{\bar{\bar{Y}}})^2. \tag{14.21}$$

The terms within this expression estimate $(\alpha\beta)_{ij}$, and are measures of the difference between the means for each treatment combination and the values predicted by the model without any interaction. SS_{AB} has $(a-1)(b-1)$ degrees of freedom. Its associated mean square is defined by the formula

$$MS_{AB} = \frac{SS_{AB}}{(a-1)(b-1)}. \tag{14.22}$$

For the Example 1 data, we have

$$SS_{AB} = 4[(1.150 - 1.065 - 0.748 + 0.709)^2 \tag{14.23}$$
$$+(0.980 - 1.065 - 0.671 + 0.709)^2 \tag{14.24}$$
$$+(0.369 - 0.342 - 0.748 + 0.709)^2 \tag{14.25}$$
$$+(0.314 - 0.342 - 0.671 + 0.709)^2 \tag{14.26}$$
$$+(0.725 - 0.722 - 0.748 + 0.709)^2 \tag{14.27}$$
$$+(0.719 - 0.722 - 0.671 + 0.709)^2 \tag{14.28}$$
$$= 5[2.111 \times 10^{-3} + \cdots + 2.836 \times 10^{-2}] = 2.836 \times 10^{-2}. \tag{14.29}$$

and

$$MS_{AB} = \frac{2.836 \times 10^{-2}}{(3-1)(2-1)} = 1.418 \times 10^{-2}. \tag{14.30}$$

These sum of squares and mean squares measure how Factor A, B, and the A $\times$ B interaction influence the means of each treatment combination. What about variability within each group? We can calculate SS_{within} using the general formula

$$SS_{within} = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(Y_{ijk} - \bar{Y}_{ij\cdot})^2 \tag{14.31}$$

which has $ab(n-1)$ degrees of freedom. The associated mean square is calculated as

$$MS_{within} = \frac{SS_{within}}{ab(n-1)}. \tag{14.32}$$

The last column of Table 14.1 shows the preliminary calculations for SS_{within}. Adding this column across all the treatment groups yields

$$SS_{within} = 1.644 \times 10^{-2} + \cdots + 3.901 \times 10^{-4} = 1.361 \tag{14.33}$$

and

$$MS_{within} = \frac{1.361}{(3)(2)(4-1)} = 7.561 \times 10^{-2}. \tag{14.34}$$

There is one more sum of squares that is often calculated in two-way ANOVA, the total sum of squares. It is defined as

$$SS_{total} = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(Y_{ijk} - \bar{\bar{\bar{Y}}})^2. \tag{14.35}$$

It measures the variability of the observations around the grand mean of the data ($\bar{\bar{\bar{Y}}}$) and has $abn - 1$ degrees of freedom. An interesting feature of the sum of squares is that they add to the total sum of squares when the design is balanced, as do the degrees of freedom. In particular, we have

$$SS_A + SS_B + SS_{AB} + SS_{within} = SS_{total} \tag{14.36}$$

and

$$(a-1) + (b-1) + (a-1)(b-1) + ab(n-1) = abn - 1. \tag{14.37}$$

Thus, the sum of squares and degrees of freedom can be partitioned into components corresponding to every source of variation in the study. For Example 1, we have $SS_{total} = 3.521$ with $3(2)(4) - 1 = 23$ degrees of freedom.

14.3.2 ANOVA tables and tests

We can organize the different sum of squares and mean squares into an ANOVA table. It lists the different sources of variation in the data (Factor A, B, A $\times$ B interaction, within groups, and total) and their degrees of freedom. Table 14.3 shows the general layout of such a table for two-way ANOVA designs.

Also shown in the table are F statistics used test to whether Factor A, Factor B, and their interaction have an effect on the observations. The numerator of the test statistic is the mean square for each factor (MS_A, MS_B, or MS_{AB}), while the denominator is always MS_{within}. Thus, we use $F_s = MS_A/MS_{within}$ to test for the effect of Factor A. Under H_0 : all $\alpha_i = 0$ this statistic has an F distribution with $df_1 = a-1$ and $df_2 = ab(n-1)$. Similarly, we use $F_s = MS_B/MS_{within}$ to test for an effect of Factor B. Under H_0 : all $\beta_j = 0$ it has an F distribution with $df_1 = b-1$ and $df_2 = ab(n-1)$. Finally, we use $F_s = MS_{AB}/MS_{within}$ to test for an interaction between A and B. Under H_0 : all $(\alpha\beta)_{ij} = 0$ it has an F distribution with $df_1 = (a-1)(b-1)$ and $df_2 = ab(n-1)$.

All these tests are examples of likelihood ratio tests. For example, consider the test for the A $\times$ B interaction. To construct the likelihood ratio test for the interaction, we first find the maximum likelihood estimates of various parameters under H_1 vs. H_0. Recall that the observations in the two-way ANOVA model are described as

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}. \tag{14.38}$$

where μ is the grand mean, α_i is the effect of the ith level of Factor A, β_j is the effect of the jth level of Factor B, $(\alpha\beta)_{ij}$ is effect of the interaction, and $\epsilon_{ijk} \sim N(0, \sigma^2)$. This is the statistical model under the alternative hypothesis H_1 : some $(\alpha\beta)_{ij} \neq 0$, implying an interaction effect. Under H_0 : all $(\alpha\beta)_{ij} = 0$, the model reduces to

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \epsilon_{ijk}. \tag{14.39}$$

We would need to find the maximum likelihood estimates under both H_1 and H_0, as well as L_{H_0} and L_{H_1}, the maximum height of the likelihood function under H_0 and H_1. We would then use the likelihood ratio test statistic

$$\lambda = \frac{L_{H_0}}{L_{H_1}}. \tag{14.40}$$

It can be shown that there is a one-to-one correspondence between $-2\ln(\lambda)$ and F_s for the interaction effect, and so the F test is actually a likelihood ratio test (Searle 1971), as are the tests for the other effects. Large values of the test statistic $-2\ln(\lambda)$ or F_s indicate a lower value of the likelihood under H_0 relative to H_1, and thus a poorer fit of the H_0 model.

Table 14.4 shows the results for the Example 1 data set, including the F statistics and P values obtained using Table F. **In examining the test results, it is customary to examine the test for the interaction first, followed by the main effects.** If the interaction is nonsignificant this suggests the two main effects have a simple additive effect on the observations, provided they are significant. If the interaction is significant the interpretation requires more attention. If one or more of the main effects are significant, it suggests the observations are driven by both interaction and main effects. Fig. 14.4 shows a theoretical example where an interaction, Factor A, and Factor B all influence the observations.

For the bait × trap color interaction, we see that $F_s = 0.19$ with $df_1 = 2$ and $df_2 = 18$, and from Table F find that $P > 0.100$. Thus, the interaction is nonsignificant for these data ($F_{2,18} = 0.19, P > 0.100$). The color effect is also nonsignificant ($F_{1,18} = 0.47$, $P > 0.100$), but the bait effect is highly significant ($F_{2,18} = 13.86, P < 0.001$). Each bait represents a different bark beetle pheromone, and apparently some baits are more attractive than others for *T. dubius*.

Table 14.3: General ANOVA table for two-way designs with replication, showing formulas for different mean squares and F tests.

Source	*df*	Sum of squares	Mean square	F_s
Factor A	$a-1$	SS_A	$MS_A = SS_A/(a-1)$	MS_A/MS_{within}
Factor B	$b-1$	SS_B	$MS_B = SS_B/(b-1)$	MS_B/MS_{within}
AB interaction	$(a-1)(b-1)$	SS_{AB}	$MS_{AB} = SS_{AB}/(a-1)(b-1)$	MS_{AB}/MS_{within}
Within	$ab(n-1)$	SS_{within}	$MS_{within} = SS_{within}/ab(n-1)$	
Total	$abn-1$	SS_{total}		

Table 14.4: ANOVA table for the Example 1 data set, including P values for the tests.

Source	*df*	Sum of squares	Mean square	F_s	P
Bait	2	2.096	1.048	13.86	< 0.001
Color	1	3.565×10^{-2}	3.565×10^{-2}	0.47	> 0.100
Bait $\times$ Color	2	2.836×10^{-2}	1.418×10^{-2}	0.19	> 0.100
Within	18	1.361	7.561×10^{-2}		
Total	23	3.521			

14.3.3 Two-way ANOVA for Example 1 - SAS demo

The same calculations for the Example 1 study can be carried out using `proc glm` (SAS Institute Inc. 2014a). This procedure is primarily intended for fixed effects ANOVA models, and this study has two fixed effects, bait type and trap color, plus the interaction is also considered a fixed effect.

The first step in the program (see below) is to read in the observations using a `data` step, with one variable denoting the bait treatment (`bait`), another the trap color (`color`), and the third the number of *T. dubius* captured per trap (`Tdubius`). These numbers are then log-transformed using a SAS function to yield the variable `y = log10(Tdubius+1)`. We add one to the observations before taking the log to avoid problems with zeroes.

The data are then plotted using `proc gplot`, with the `bait` treatment on the x-axis and separate lines drawn for each color (SAS Institute Inc. 2014b). This is accomplished with the command `plot y*bait=color`. The rest of the `gplot` statements control the appearance of the axes.

The next section of the program conducts the two-way ANOVA using `proc glm`. The `class` statement tells SAS that both `bait` and `color` are used to classify the observations into the six treatment groups. The `model` statement tells SAS the form of the ANOVA model. Recall that the model for fixed effects two-way ANOVA is given by the equation

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk} \tag{14.41}$$

The α_i, β_j, and $(\alpha\beta)_{ij}$ terms in this model equate directly with the `bait`, `color`, and `bait*color` entries in the `model` statement. The `lsmeans` statement causes `glm` to calculate quantities called least squares means for each level of `bait` and `color`. When the data are balanced these are equivalent to the means for each treatment group, but least squares means have some advantages for unbalanced data and other statistical models. The option `adjust=tukey` requests multiple comparisons among treatments using the Tukey method. This is useful for comparing the different `bait` treatments, but for color there is only one comparison (black vs. white) and in this case would be equivalent to the F test for `color`.

The SAS output provides information similar to that summarized in an ANOVA table. The degrees of freedom, sum of squares, mean squares, F statistics and P values for the bait, color, and bait $\times$ color interaction are listed near the bottom of the output under `Type III SS`. The degrees of freedom, sum of squares, and mean square for the variation within groups

are labeled as `Error` above this section (this terminology will be explained in Chapter 15). The output labeled `Type I SS` is produced by sequentially fitting the different terms in the model, in the order listed in the `model` statement. Type III sums of squares are more generally useful than Type I for ANOVA designs, although the results are the same when the design is balanced. The output labeled `Model` refers to the combined variation due to bait, color, plus their interaction. The associated F statistic tests whether any or all of these effects influence the observations vs. the null hypothesis that they have no effect. This particular test is not used much with ANOVA designs.

We now examine the results of the tests generated by SAS, examining the interaction first. We see that the bait $\times$ color interaction is nonsignificant ($F_{2,18} = 0.19, P = 0.8311$). The color effect was also nonsignificant ($F_{1,18} = 0.47, P = 0.5011$), while bait was highly significant ($F_{2,18} = 13.85, P = 0.0002$). Examining the graph (Fig. 14.5) and Tukey results generated by SAS, we see that predator densities for the FRT and IST treatments are significantly higher than for IDT. Note that the lines connecting the different treatments are roughly parallel, further indicating an absence of interaction. The effect of trap color appears minimal in this study, although trap catches were somewhat higher for black traps.

SAS Program

```
* Tdubius_bait_color.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Two-way ANOVA for T. dubius trapping";
title2 "Data from Reeve et al. (2009)";
data Tdubius;
    input bait $ color $ Tdubius;
    * Apply transformations here;
    y = log10(Tdubius+1);
    datalines;
FRT         B         18
FRT         B         12
FRT         B         22
FRT         B         6
FRT         W         12
FRT         W         15
FRT         W         7
FRT         W         4

etc.
```

```
;
run;
* Print data set;
proc print data=Tdubius;
run;
* Plot means, standard errors, and observations;
proc gplot data=Tdubius;
    plot y*bait=color / vaxis=axis1 haxis=axis1 legend=legend1;
    symbol1 i=std1mjt v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
    legend1 label=(height=2) value=(height=2);
run;
* Two-way ANOVA with all fixed effects;
proc glm data=Tdubius;
    class bait color;
    model y = bait color bait*color;
    lsmeans bait color / adjust=tukey cl lines;
    output out=resids p=pred r=resid;
run;
goptions reset=all;
title "Diagnostic plots to check anova assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
    plot resid*pred=1 / vaxis=axis1 haxis=axis1;
    symbol1 v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
    qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

```
                    Two-way ANOVA for T. dubius trapping                    1
                        Data from Reeve et al. 2009
                                              08:57 Wednesday, July 11, 2012

                  Obs    bait    color    Tdubius       y

                    1    FRT       B         18      1.27875
                    2    FRT       B         12      1.11394
                    3    FRT       B         22      1.36173
                    4    FRT       B          6      0.84510
```

5	FRT	W	12	1.11394
6	FRT	W	15	1.20412
7	FRT	W	7	0.90309
8	FRT	W	4	0.69897
9	IDT	B	0	0.00000

etc.

Two-way ANOVA for T. dubius trapping 2
Data from Reeve et al. 2009
08:57 Wednesday, July 11, 2012

The GLM Procedure

Class Level Information

Class	Levels	Values
bait	3	FRT IDT IST
color	2	B W

Number of Observations Read 24
Number of Observations Used 24

Two-way ANOVA for T. dubius trapping 3
Data from Reeve et al. 2009
08:57 Wednesday, July 11, 2012

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2.15900779	0.43180156	5.71	0.0025
Error	18	1.36120842	0.07562269		
Corrected Total	23	3.52021621			

R-Square	Coeff Var	Root MSE	y Mean
0.613317	38.76405	0.274996	0.709409

Source	DF	Type I SS	Mean Square	F Value	Pr > F
bait	2	2.09508772	1.04754386	13.85	0.0002
color	1	0.03564427	0.03564427	0.47	0.5011
bait*color	2	0.02827579	0.01413790	0.19	0.8311

Source	DF	Type III SS	Mean Square	F Value	Pr > F
bait	2	2.09508772	1.04754386	13.85	0.0002
color	1	0.03564427	0.03564427	0.47	0.5011
bait*color	2	0.02827579	0.01413790	0.19	0.8311

Two-way ANOVA for T. dubius trapping 4
Data from Reeve et al. 2009
08:57 Wednesday, July 11, 2012

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

bait	y LSMEAN	LSMEAN Number
FRT	1.06495577	1
IDT	0.34154922	2
IST	0.72172331	3

Least Squares Means for effect bait
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: y

i/j	1	2	3
1		0.0001	0.0558
2	0.0001		0.0326

```
         3        0.0558        0.0326


 bait        y LSMEAN      95% Confidence Limits

 FRT         1.064956        0.860692     1.269219
 IDT         0.341549        0.137286     0.545813
 IST         0.721723        0.517460     0.925987


        Least Squares Means for Effect bait

              Difference          Simultaneous 95%
                 Between       Confidence Limits for
 i    j            Means       LSMean(i)-LSMean(j)

 1    2         0.723407         0.372489     1.074324
 1    3         0.343232        -0.007685     0.694150
 2    3        -0.380174        -0.731091    -0.029257


    Tukey Comparison Lines for Least Squares Means of bait

LS-means with the same letter are not significantly different.

                                        LSMEAN
                    y LSMEAN    bait    Number

              A     1.06495577  FRT          1
              A


            Two-way ANOVA for T. dubius trapping                    5
                Data from Reeve et al. 2009
                                  08:57 Wednesday, July 11, 2012

                    The GLM Procedure
                  Least Squares Means
        Adjustment for Multiple Comparisons: Tukey

    Tukey Comparison Lines for Least Squares Means of bait

LS-means with the same letter are not significantly different.

                                        LSMEAN
```

	y LSMEAN	bait	Number
A	0.72172331	IST	3
B	0.34154922	IDT	2

Two-way ANOVA for T. dubius trapping 6
Data from Reeve et al. 2009
08:57 Wednesday, July 11, 2012

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

color	y LSMEAN	H0:LSMean1=LSMean2 Pr > |t|
B	0.74794744	0.5011
W	0.67087142	

color	y LSMEAN	95% Confidence Limits	
B	0.747947	0.581167	0.914728
W	0.670871	0.504091	0.837652

Least Squares Means for Effect color

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	0.077076	-0.158787	0.312939

Tukey Comparison Lines for Least Squares Means of color

LS-means with the same letter are not significantly different.

	y LSMEAN	color	LSMEAN Number

```
A      0.74794744     B              1
A
A      0.67087142     W              2
```

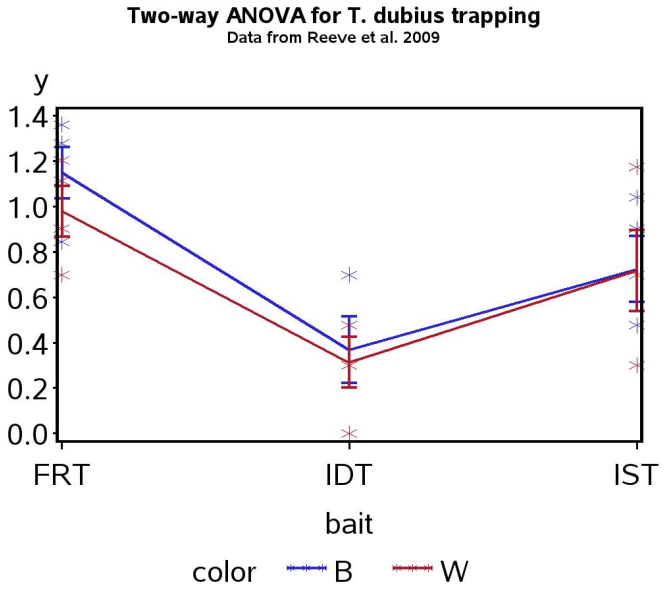

Figure 14.5: Means ± standard errors and individual data points for the Example 1 experiment, where $Y = \log_{10}(T.dubius + 1)$.

14.3.4 Two-way ANOVA for Example 2 - SAS demo

We next analyze the Example 2 data set using SAS. These data involve the total biomass of grass plants grown in small containers, where the treatments are nitrogen or water availability. The SAS program is similar to the previous example with some changes in the variable names. We see that the nitrogen $\times$ water interaction is highly significant ($F_{4,27} = 11.31, P < 0.0001$). The interaction can be observed in Fig. 14.6, which shows that the lines connecting the treatments are not parallel. Note that the greatest response of biomass to nitrogen occurred at the highest water level, while the response was minimal at the lowest level (Maestre & Reynolds (2007). Thus, low water levels apparently prevent growth even when nitrogen is abundant.

The SAS analysis also found highly significant main effects of nitrogen ($F_{2,27} = 64.28, P < 0.0001$) and water ($F_{2,27} = 456.46, P < 0.0001$) on biomass. We can judge the relative strength of these effects by examining Fig. 14.6 as well as their sum of squares values, which are a measure of the amount of variation explained by each effect. They suggest that watering had the most effect on biomass, followed by nitrogen and the nitrogen $\times$ water interaction.

SAS Program

```
* Maestre_biomass.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Two-way ANOVA for total biomass";
title2 "Data from Maestre and Reynolds (2007)";
data maestre;
    input nitrogen water biomass;
    * Apply transformations here;
    y = log10(biomass);
    datalines;
 40  125   4.372
 40  125   4.482
 40  125   4.221
 40  125   3.977
 40  250   7.400
 40  250   8.027
 40  250   7.883
 40  250   7.769

etc.

;
run;
* Print data set;
proc print data=maestre;
run;
* Plot means, standard errors, and observations;
proc gplot data=maestre;
    plot y*nitrogen=water / vaxis=axis1 haxis=axis1 legend=legend1;
    symbol1 i=std1mjt v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
    legend1 label=(height=2) value=(height=2);
run;
* Two-way ANOVA with all fixed effects;
proc glm data=maestre;
    class nitrogen water;
    model y = nitrogen water nitrogen*water;
    lsmeans nitrogen water / adjust=tukey cl lines;
    output out=resids p=pred r=resid;
run;
goptions reset=all;
title "Diagnostic plots to check anova assumptions";
* Plot residuals vs. predicted values;
```

```
proc gplot data=resids;
    plot resid*pred=1 / vaxis=axis1 haxis=axis1;
    symbol1 v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
    qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

```
                          Two-way ANOVA for total biomass                        1
                        Data from Maestre and Reynolds 2007
                                                13:37 Friday, July 13, 2012

                  Obs    nitrogen    water    biomass       y

                    1        40       125       4.372    0.64068
                    2        40       125       4.482    0.65147
                    3        40       125       4.221    0.62542
                    4        40       125       3.977    0.59956
                    5        40       250       7.400    0.86923
                    6        40       250       8.027    0.90455
                    7        40       250       7.883    0.89669
                    8        40       250       7.769    0.89037
```

etc.

```
                          Two-way ANOVA for total biomass                        2
                        Data from Maestre and Reynolds 2007
                                                13:37 Friday, July 13, 2012

                                The GLM Procedure

                             Class Level Information

                             Class         Levels    Values

                             nitrogen           3    40 80 120

                             water              3    125 250 375
```

```
                    Number of Observations Read          36
                    Number of Observations Used          36


                       Two-way ANOVA for total biomass                        3
                     Data from Maestre and Reynolds 2007
                                                13:37 Friday, July 13, 2012

                             The GLM Procedure

Dependent Variable: y

                                      Sum of
 Source                     DF        Squares    Mean Square   F Value   Pr > F

 Model                       8     1.01770131     0.12721266    135.84   <.0001

 Error                      27     0.02528594     0.00093652

 Corrected Total            35     1.04298725


              R-Square     Coeff Var      Root MSE        y Mean

              0.975756      3.499961      0.030603      0.874368


 Source                     DF      Type I SS    Mean Square   F Value   Pr > F

 nitrogen                    2     0.12039036     0.06019518     64.28   <.0001
 water                       2     0.85496135     0.42748068    456.46   <.0001
 nitrogen*water              4     0.04234959     0.01058740     11.31   <.0001


 Source                     DF    Type III SS    Mean Square   F Value   Pr > F

 nitrogen                    2     0.12039036     0.06019518     64.28   <.0001
 water                       2     0.85496135     0.42748068    456.46   <.0001
 nitrogen*water              4     0.04234959     0.01058740     11.31   <.0001


                       Two-way ANOVA for total biomass                        4
                     Data from Maestre and Reynolds 2007
                                                13:37 Friday, July 13, 2012
```

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

nitrogen	y LSMEAN	LSMEAN Number
40	0.79828979	1
80	0.88642108	2
120	0.93839425	3

Least Squares Means for effect nitrogen
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: y

i/j	1	2	3
1		<.0001	<.0001
2	<.0001		0.0008
3	<.0001	0.0008	

nitrogen	y LSMEAN	95% Confidence Limits	
40	0.798290	0.780164	0.816416
80	0.886421	0.868295	0.904547
120	0.938394	0.920268	0.956521

Least Squares Means for Effect nitrogen

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	-0.088131	-0.119108	-0.057155
1	3	-0.140104	-0.171081	-0.109128
2	3	-0.051973	-0.082950	-0.020997

Tukey Comparison Lines for Least Squares Means of nitrogen

LS-means with the same letter are not significantly different.

	y LSMEAN	nitrogen	LSMEAN Number
A	0.93839425	120	3

Two-way ANOVA for total biomass 5
Data from Maestre and Reynolds 2007
13:37 Friday, July 13, 2012

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Tukey Comparison Lines for Least Squares Means of nitrogen

LS-means with the same letter are not significantly different.

	y LSMEAN	nitrogen	LSMEAN Number
B	0.88642108	80	2
C	0.79828979	40	1

Two-way ANOVA for total biomass 6
Data from Maestre and Reynolds 2007
13:37 Friday, July 13, 2012

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

water	y LSMEAN	LSMEAN Number
125	0.65929804	1
250	0.95137559	2
375	1.01243148	3

Least Squares Means for effect water

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: y

i/j	1	2	3
1		<.0001	<.0001
2	<.0001		0.0001
3	<.0001	0.0001	

water	y LSMEAN	95% Confidence Limits	
125	0.659298	0.641172	0.677424
250	0.951376	0.933249	0.969502
375	1.012431	0.994305	1.030558

Least Squares Means for Effect water

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	-0.292078	-0.323054	-0.261101
1	3	-0.353133	-0.384110	-0.322157
2	3	-0.061056	-0.092032	-0.030079

Tukey Comparison Lines for Least Squares Means of water

LS-means with the same letter are not significantly different.

	y LSMEAN	water	LSMEAN Number
A	1.01243148	375	3

The GLM Procedure

Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Tukey Comparison Lines for Least Squares Means of water

LS-means with the same letter are not significantly different.

	y LSMEAN	water	LSMEAN Number
B	0.95137559	250	2
C	0.65929804	125	1

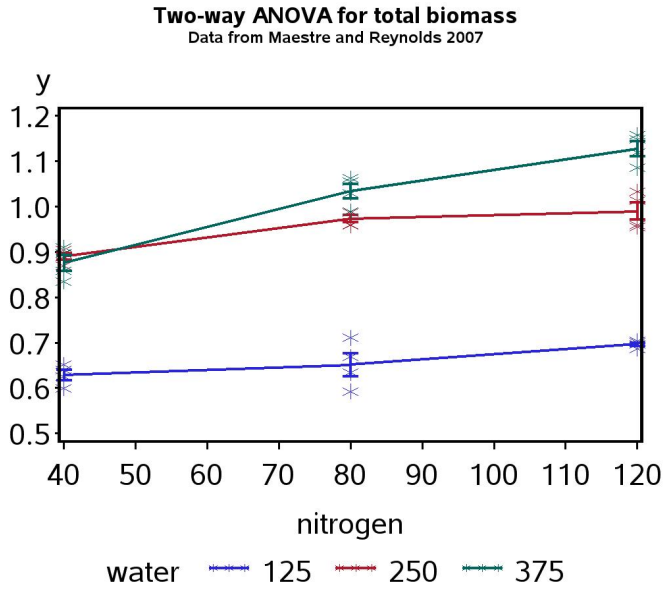

Figure 14.6: Means ± standard errors and individual data points for the Example 2 experiment, where $Y = \log_{10}(\text{Biomass})$.

14.3.5 Tests for main effects with interaction

There is disagreement among statisticians on whether tests of the main effects are appropriate when there is significant interaction. Two different procedures have been developed. The SAS one involves fitting models with and without a given main effect, but always including interaction terms, yielding what SAS calls Type III sums of squares and tests (Speed et al. 1978, Shaw & Mitchell-Olds 1993, SAS Institute Inc. 2014). This has the benefit of generating tests for the interaction and main effects in a single pass (see preceding SAS demo). However, there are authors that believe tests of the main effects are questionable in the presence of interaction (e.g., Cox 1984, Winer et al. 1991, Stewart-Oaten 1995). One issue is whether a model with interaction but lacking a main effect is even plausible (Stewart-Oaten 1995). These considerations motivate a different procedure. The first step is to examine the test for interaction using the full two-way ANOVA model. If interaction appears weak or absent, there are two alternate ways of testing the main effects. One is to drop the interaction and rerun the model, examining the main effects in the usual fashion. Another method is to use what SAS calls Type II sums of squares, obtained using the option `\ss2` in the `model` statement. The tests based on these sums of squares assume there is no interaction. If the interaction is significant the main effects tests are ignored, although one can still test for Factor A effects at each level of Factor B, or vice versa (Winer et al. 1991). These are called tests of **simple effects**, and can be conducted using the SAS `slice` option for `lsmeans`.

The modified SAS code to implement these procedures is listed below, along with the output, for the Example 2 data set. We see that the nitrogen $\times$ water interaction is highly significant ($F_{4,27} = 11.31, P < 0.0001$), and so we skip the tests of the main effects. Note that the main effects sum of squares are identical to our previous ones using SAS Type III tests, but this would only be true for the special case of balanced designs with equal n for each treatment (see next section for unbalanced designs). The `slice` option is used to test for a nitrogen effect at every level of water, and vice versa. We see that the effect of nitrogen is significant at the lowest water level, while highly significant at the other two levels. It appears the nitrogen effect is less significant at low water levels (see also Fig. 14.6). The water effect is highly significant at every level of nitrogen.

SAS Program

```
* Two-way ANOVA with interaction;
title3 "MODEL WITH INTERACTION - USE THIS OUTPUT IF INTERACTION SIGNIFICANT";
proc glm data=maestre;
        class nitrogen water;
        model y = nitrogen water nitrogen*water / ss2;
        lsmeans nitrogen*water / slice=water slice=nitrogen;
        output out=resids p=pred r=resid;
run;
* Two-way ANOVA without interaction;
title3 "MODEL WITHOUT INTERACTION - USE THIS OUTPUT IF INTERACTION NS";
proc glm data=maestre;
        class nitrogen water;
        model y = nitrogen water / ss2;
        lsmeans nitrogen water / adjust=tukey cl lines;
run;
```

SAS Output

```
                          Two-way ANOVA for biomass                          3
                      Data from Maestre and Reynolds (2007)
      MODEL WITH INTERACTION - USE THIS OUTPUT IF INTERACTION SIGNIFICANT
                                              09:09 Thursday, October 31, 2013

                                The GLM Procedure

Dependent Variable: y

                                      Sum of
 Source                       DF       Squares    Mean Square   F Value   Pr > F

 Model                         8    1.01770131     0.12721266    135.84   <.0001

 Error                        27    0.02528594     0.00093652

 Corrected Total              35    1.04298725


               R-Square     Coeff Var      Root MSE        y Mean

               0.975756      3.499961      0.030603      0.874368


 Source                       DF    Type II SS    Mean Square   F Value   Pr > F
```

nitrogen	2	0.12039036	0.06019518	64.28	<.0001
water	2	0.85496135	0.42748068	456.46	<.0001
nitrogen*water	4	0.04234959	0.01058740	11.31	<.0001

Two-way ANOVA for biomass 4
Data from Maestre and Reynolds (2007)
MODEL WITH INTERACTION - USE THIS OUTPUT IF INTERACTION SIGNIFICANT
09:09 Thursday, October 31, 2013

The GLM Procedure
Least Squares Means

nitrogen	water	y LSMEAN
40	125	0.62928074
40	250	0.89021041
40	375	0.87537823
80	125	0.65169272
80	250	0.97339245
80	375	1.03417806
120	125	0.69692068
120	250	0.99052391
120	375	1.12773815

Two-way ANOVA for biomass 5
Data from Maestre and Reynolds (2007)
MODEL WITH INTERACTION - USE THIS OUTPUT IF INTERACTION SIGNIFICANT
09:27 Monday, November 25, 2013

The GLM Procedure
Least Squares Means

nitrogen*water Effect Sliced by water for y

water	DF	Sum of Squares	Mean Square	F Value	Pr > F
125	2	0.009497	0.004749	5.07	0.0135
250	2	0.023034	0.011517	12.30	0.0002
375	2	0.130209	0.065104	69.52	<.0001

Two-way ANOVA for biomass 6

Data from Maestre and Reynolds (2007)
MODEL WITH INTERACTION - USE THIS OUTPUT IF INTERACTION SIGNIFICANT
09:27 Monday, November 25, 2013

The GLM Procedure
Least Squares Means

nitrogen*water Effect Sliced by nitrogen for y

nitrogen	DF	Sum of Squares	Mean Square	F Value	Pr > F
40	2	0.171824	0.085912	91.74	<.0001
80	2	0.337974	0.168987	180.44	<.0001
120	2	0.387512	0.193756	206.89	<.0001

14.4 Unbalanced designs and two-way ANOVA

The examples we have examined so far are balanced designs, with equal numbers of observations in each treatment combination. For these designs, the various sums of squares are independent and additive ($SS_A + SS_B + SS_{AB} + SS_{within} = SS_{total}$), the different methods of calculating the sum of squares (Type I, II, and III) yield the same results, and the resulting tests are the same. This is not the case for unbalanced two-way (or higher) designs, which occur frequently in practice. These are designs where there are fewer observations in some treatments than others, possible only a single observation. These designs can be analyzed using the same SAS procedures and programs as before, but the various sums of squares are no longer additive, and the tests are not independent (Shaw & Mitchell-Olds 1993). For this reason, if the lack of balance is severe the analysis should be interpreted with some caution.

We will use the Example 2 data set, with nine observations removed, to illustrate the analysis of unbalanced designs (see Table 14.5). The number of observations varies from $n = 1$ to 4 across treatments. These data can be analyzed using the same program as before. To show the results for both Type II and III sums of squares, the option `\ ss2 ss3` was added to the `model` statement. Examining the output, we see that the bait $\times$ trap color interaction is nonsignificant ($F_{2,9} = 0.29, P = 0.7563$). The color effect is also nonsignificant (Type II: $F_{1,9} = 0.94, P = 0.3576$, Type III: $F_{1,9} = 0.98, P = 0.3475$), but the bait effect is highly significant (Type II: $F_{2,9} = 8.11, P < 0.0097$, Type III: $F_{2,9} = 8.15, P = 0.0096$). This is basically the same result as we obtained earlier for this study, despite the lack of balance. We can also see that the sums of squares are no longer additive. For example, with Type III sums of squares we have $SS_A + SS_B + SS_{AB} + SS_{within} = 0.9106 + 0.0549 + 0.0322 + 0.5028 = 1.5005$. This does not equal $SS_{total} = 1.4562$.

Table 14.5: Example 2 - Unbalanced design.

Bait	Color	*T. dubius*
FRT	B	18
FRT	W	12
FRT	W	15
FRT	W	7
FRT	W	4
IDT	B	2
IDT	B	1
IDT	B	4
IDT	W	2
IDT	W	1
IST	B	2
IST	B	2
IST	B	10
IST	B	7
IST	W	4

SAS Output

Two-way ANOVA for T. dubius counts 3
Data from Reeve et al. (2009)
12:58 Tuesday, May 31, 2016

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.95343427	0.19068685	3.41	0.0526
Error	9	0.50280246	0.05586694		
Corrected Total	14	1.45623672			

R-Square	Coeff Var	Root MSE	y Mean
0.654725	32.07997	0.236362	0.736790

Source	DF	Type II SS	Mean Square	F Value	Pr > F
bait	2	0.90584753	0.45292376	8.11	0.0097
color	1	0.05252717	0.05252717	0.94	0.3576
bait*color	2	0.03219452	0.01609726	0.29	0.7563

Source	DF	Type III SS	Mean Square	F Value	Pr > F
bait	2	0.91062846	0.45531423	8.15	0.0096
color	1	0.05488645	0.05488645	0.98	0.3475
bait*color	2	0.03219452	0.01609726	0.29	0.7563

14.5 Two-way ANOVA without replication

The designs we have examined so far assume there are multiple observations for each treatment combination, implying $n > 1$ for each group. However, it is possible to analyze studies where there is only replicate per group ($n = 1$) although this requires a change in the model. With so little data, it is not possible to estimate the interaction terms nor easily conduct a test for the interaction. However, we can fit a simplified model of the form

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}. \tag{14.42}$$

Note that the interaction term is absent. In addition, we no longer need the third subscript k for the observations because there is only one observation per treatment group. One can visualize the behavior of this model using the same figures as for the two-way model with replication (see Fig. 14.1-14.2), except that the model does not incorporate interaction.

It is important to realize that interaction could still be present in the data, even though we cannot test for it using this model. If interaction is present it will reduce the power to detect main effects, because it adds variability to the observations in a way not accounted for by the model. Even if interaction is absent, this design will obviously have less power than a design with replication.

For these designs, we will be interested in testing whether Factor A or B have an effect on the groups means. For Factor A, this amounts to testing H_0 : all $\alpha_i = 0$, while for Factor B we would test H_0 : all $\beta_j = 0$. No test of this type is possible for the interaction.

14.5.1 Hypothesis testing

The sums of squares, mean squares, and other quantities for two-way ANOVA without replication are similar to those for designs with replication. We will illustrate the calculations using another data set for the insect predator *T. dubius* (Example 3, Table 14.6). This predator is most abundant during cool periods of the year in the southern USA, possibly because it cannot tolerate high temperatures. A study was conducted to see how temperature (which we call Factor A) and relative humidity (Factor B) affect the mortality rate of its eggs in the laboratory (Reeve 2000). Eggs and environmental chambers were in short supply, however, so only a single replicate was conducted at each temperature and humidity combination. Six temperatures

(15°, 20°, 25°, 30°, 35°, and 37.5°C) and three relative humidity treatments (55%, 75%, and 100%) were used. This corresponds to $a = 6$ and $b = 3$ in the formulas below. An arcsine-square root transformation was applied to the mortality rate observations, a common practice for data in the form of proportions.

We begin by calculating a mean corresponding to each level of Factor A by averaging across the levels of Factor B, which we denote as $\bar{Y}_{i\cdot}$. It can be calculated using the formula

$$\bar{Y}_{i\cdot} = \frac{\sum_{j=1}^{b} Y_{ij}}{b}. \tag{14.43}$$

For example, we have

$$\bar{Y}_{1\cdot} = \frac{0.379 + 0.325 + 0.615}{3} = 0.440 \tag{14.44}$$

for the first temperature treatment (15°C) in Example 3. The means for other temperature values are given in Table 14.6. We similarly can find means corresponding to each level of Factor B by averaging across the levels of Factor A. The general formula is

$$\bar{Y}_{\cdot j} = \frac{\sum_{i=1}^{a} Y_{ij}}{a}. \tag{14.45}$$

For the first humidity treatment in Example 3, we have

$$\bar{Y}_{\cdot 1} = \frac{0.379 + 0.439 + 0.358 + 0.466 + 0.970 + 1.571}{6} = 0.697. \tag{14.46}$$

The means for the other humidity treatments are $\bar{Y}_{\cdot 2} = 0.731$ and $\bar{Y}_{\cdot 3} = 0.719$. A grand mean $\bar{\bar{Y}}$ can then be calculated by averaging across the values of $\bar{Y}_{i\cdot}$ or equivalently by summing all the observations and dividing by their total number. It can be generally calculated using the formula

$$\bar{\bar{Y}} = \frac{\sum_{i=1}^{a} \bar{Y}_{i\cdot}}{a}. \tag{14.47}$$

For the Example 3 data set, we have

$$\bar{\bar{Y}} = \frac{0.440 + 0.502 + 0.454 + 0.521 + 0.806 + 1.571}{6} = 0.716. \tag{14.48}$$

We next develop sums of squares and means squares for this design. The difference $\bar{Y}_{i\cdot} - \bar{\bar{Y}}$ is a measure of the shift generated by Factor A in the observations, and also estimates α_i. Squaring and summing them across all the levels of Factor A, we obtain SS_A. It is calculated using the general formula

$$SS_A = b\sum_{i=1}^{a}(\bar{Y}_{i\cdot} - \bar{\bar{Y}})^2. \tag{14.49}$$

SS_A has $a-1$ degrees of freedom. Its mean square is calculated using the formula

$$MS_A = \frac{SS_A}{a-1}. \tag{14.50}$$

Note the factor b in the expression for SS_A, which as usual scales MS_A so that it estimates σ^2 under H_0. For the Example 3 data, we have

$$SS_A = 3\left[(0.440-0.716)^2 + (0.502-0.716)^2 + \cdots + (1.571-0.716)^2\right] \tag{14.51}$$

$$= 3\left[0.076176 + 0.045796 + 0.068644 + 0.038025 + 0.008100 + 0.731025\right] \tag{14.52}$$

$$= 2.903298 \tag{14.53}$$

$$\tag{14.54}$$

and

$$MS_A = \frac{2.903298}{6-1} = 0.580660. \tag{14.55}$$

We similarly define SS_B using the general formula

$$SS_B = a\sum_{j=1}^{b}(\bar{Y}_{\cdot j} - \bar{\bar{Y}})^2. \tag{14.56}$$

SS_B has $b-1$ degrees of freedom. We can then calculate a mean square for Factor B using the formula

$$MS_B = \frac{SS_B}{b-1}. \tag{14.57}$$

For the Example 3 data, we have

$$SS_B = 6\left[(0.697-0.716)^2 + (0.731-0.716)^2 + (0.719-0.716)^2\right] \tag{14.58}$$

$$= 6\left[0.000361 + 0.000225 + 0.000009\right] \tag{14.59}$$

$$= 0.003570. \tag{14.60}$$

and

$$MS_B = \frac{0.003570}{3-1} = 0.001785. \tag{14.61}$$

We now need a measure of the variability of the observations. We previously used SS_{within} for this purpose, which measured the variability of the observations within each treatment group. However, in two-way designs without replication there is only a single observation in these groups ($n = 1$). If we assume there is no interaction, however, we can use an interaction-like sum of squares as a measure of variability. In particular, we have

$$SS_{within} = \sum_{i=1}^{a}\sum_{j=1}^{b}(Y_{ij} - \bar{Y}_{i\cdot} - \bar{Y}_{\cdot j} + \bar{\bar{Y}})^2. \tag{14.62}$$

The squared terms within this expression measure the difference between the one observation for each treatment combination and the values predicted by the model without any interaction. Note the similarity to SS_{AB} for designs with replication. SS_{within} has $(a-1)(b-1)$ degrees of freedom, and the associated mean square is defined by the formula

$$MS_{within} = \frac{SS_{within}}{(a-1)(b-1)}. \tag{14.63}$$

The last column of Table 14.6 shows the preliminary calculations for SS_{within}. Adding this column across all the treatment groups yields

$$SS_{within} = 0.131334 \tag{14.64}$$

and

$$MS_{within} = \frac{0.131334}{(6-1)(3-1)} = 0.013133. \tag{14.65}$$

The total sum of squares is given by the formula

$$SS_{total} = \sum_{i=1}^{a}\sum_{j=1}^{b}(Y_{ij} - \bar{\bar{Y}})^2 \tag{14.66}$$

and has $ab-1$ degrees of freedom. For Example 3, we calculate that $SS_{total} = 3.038202$ with 17 degrees of freedom.

As before, we can organize the different sum of squares and mean squares into an ANOVA table. Table 14.7 shows the general layout of such a table

for two-way designs without replication. We use $F_s = MS_A/MS_{within}$ to test for the effect of Factor A. Under H_0 : all $\alpha_i = 0$ this statistic has an F distribution with $df_1 = a - 1$ and $df_2 = (a - 1)(b - 1)$. Similarly, we use $F_s = MS_B/MS_{within}$ to test for an effect of Factor B. Under H_0 : all $\beta_j = 0$ it has an F distribution with $df_1 = b - 1$ and $df_2 = (a - 1)(b - 1)$.

Table 14.8 shows the results for the Example 3 data set, including the F statistics and P values obtained using Table F. The temperature effect is highly significant ($F_{5,10} = 44.214, P < 0.001$) while humidity is nonsignificant ($F_{2,10} = 0.136, P > 0.100$). Examining the data in Table 14.6, we see that mortality rates sharply increase as temperature increases.

Table 14.6: Example 3 - Effect of temperature and relative humidity on the mortality rate of *T. dubius* eggs. Also shown are the means for each temperature level ($\bar{Y}_{i\cdot}$) and preliminary calculations to find SS_{within}

Temp. (°C)	Humidity (%)	Mortality	$Y_{ij} = \sin^{-1}(\sqrt{\text{Mortality}})$	i	j	$\bar{Y}_{i\cdot}$	$(Y_{ij} - \bar{Y}_{i\cdot} - \bar{Y}_{\cdot j} + \bar{\bar{Y}})^2$
15	55	0.137	0.379	1	1		0.001764
15	75	0.102	0.325	1	2	0.440	0.016900
15	100	0.333	0.615	1	3		0.029584
20	55	0.181	0.439	2	1		0.001936
20	75	0.337	0.619	2	2	0.502	0.010404
20	100	0.188	0.448	2	3		0.003249
25	55	0.123	0.358	3	1		0.005929
25	75	0.259	0.534	3	2	0.454	0.004225
25	100	0.205	0.470	3	3		0.000169
30	55	0.202	0.466	4	1		0.001296
30	75	0.321	0.602	4	2	0.521	0.004356
30	100	0.226	0.495	4	3		0.000841
35	55	0.680	0.970	5	1		0.033489
35	75	0.447	0.732	5	2	0.806	0.007921
35	100	0.431	0.716	5	3		0.008649
37.5	55	1.000	1.571	6	1		0.000361
37.5	75	1.000	1.571	6	2	1.571	0.000225
37.5	100	1.000	1.571	6	3		0.000036

Table 14.7: General ANOVA table for two-way designs without replication, showing formulas for different mean squares and F tests.

Source	*df*	Sum of squares	Mean square	F_s
Factor A	$a-1$	SS_A	$MS_A = SS_A/(a-1)$	MS_A/MS_{within}
Factor B	$b-1$	SS_B	$MS_B = SS_B/(b-1)$	MS_B/MS_{within}
Within	$(a-1)(b-1)$	SS_{within}	$MS_{within} = SS_{within}/(a-1)(b-1)$	
Total	$ab-1$	SS_{total}		

Table 14.8: ANOVA table for the Example 3 data set, including P values for the tests.

Source	*df*	Sum of squares	Mean square	F_s	P
Temperature	5	2.903298	0.580660	44.214	< 0.001
Humidity	2	0.003570	0.001785	0.136	> 0.100
Within	10	0.131334	0.013133		
Total	17	3.038198			

14.5.2 Two-way ANOVA no replication - SAS demo

We now analyze these same data using SAS. The program is similar to previous ones for two-way designs with replication, except that the interaction term needs to be deleted from the `model` statement. Because there are several levels of temperature (`temp`) and relative humidity (`rh`) in the experimental design, it seems reasonable to use multiple comparisons to compare the different groups using an `lsmeans` statement. See SAS program and output below.

We see there was a highly significant effect of temperature on egg mortality ($F_{5,10} = 44.31, P < 0.0001$), while the effect of humidity was nonsignificant ($F_{2,10} = 0.13, P = 0.8777$). The results are similar to the manual calculations in Table 14.6. Examining the results for the Tukey multiple comparison procedure, we see that 37.5°C was significantly different from all the other temperatures, while 35°C was significantly different from 15°C and 25°C. No other differences were significant. There were no significant differences among the humidity treatments. Examining Fig. 14.7, we see that mortality appears level up to 30°C, then rapidly increases.

SAS Program

```
* Clerid_eggs_th.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Two-way ANOVA for T. dubius egg mortality";
title2 "No replication";
data mortality;
    input temp rh mortrate;
    * Apply transformations here;
    y = arsin(sqrt(mortrate));
    datalines;
15  55  0.137
15  75  0.102
15 100  0.333
20  55  0.181
20  75  0.337
20 100  0.188
25  55  0.123
25  75  0.259
25 100  0.205
30  55  0.202
30  75  0.321
30 100  0.226
```

```
35  55  0.680
35  75  0.447
35 100  0.431
37.5 55 1.000
37.5 75 1.000
37.5 100 1.000
;
run;
* Print data set;
proc print data=mortality;
run;
* Plot means, standard errors, and observations;
proc gplot data=mortality;
    plot y*temp=rh / vaxis=axis1 haxis=axis1 legend=legend1;
    symbol1 i=j v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
    legend1 label=(height=2) value=(height=2);
run;
* Two-way ANOVA with all fixed effects;
proc glm data=mortality;
    class temp rh;
    model y = temp rh;
    lsmeans temp rh / adjust=tukey cl lines;
    output out=resids p=pred r=resid;
run;
goptions reset=all;
title "Diagnostic plots to check anova assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
    plot resid*pred=1 / vaxis=axis1 haxis=axis1;
    symbol1 v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
    qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

```
Two-way ANOVA for T. dubius egg mortality                    1
                No replication   08:30 Sunday, October 24, 2010

 Obs    temp     rh    mortrate       y

   1    15.0     55       0.137     0.37915
   2    15.0     75       0.102     0.32507
   3    15.0    100       0.333     0.61513
   4    20.0     55       0.181     0.43945
   5    20.0     75       0.337     0.61936
   6    20.0    100       0.188     0.44847
   7    25.0     55       0.123     0.35833
   8    25.0     75       0.259     0.53393
   9    25.0    100       0.205     0.46987
  10    30.0     55       0.202     0.46614
  11    30.0     75       0.321     0.60234
  12    30.0    100       0.226     0.49541
  13    35.0     55       0.6125    0.96953
  14    35.0     75       0.447     0.73230
  15    35.0    100       0.431     0.71618
  16    37.5     55       1.000     1.57080
  17    37.5     75       1.000     1.57080
  18    37.5    100       1.000     1.57080
```

```
Two-way ANOVA for T. dubius egg mortality                    2
                No replication   08:30 Sunday, October 24, 2010

                    The GLM Procedure

             Class Level Information

Class         Levels    Values

temp               6    15 20 25 30 35 37.5

rh                 3    55 75 100


  Number of Observations Read          18
  Number of Observations Used          18
```

Two-way ANOVA for T. dubius egg mortality 3
No replication 08:30 Sunday, October 24, 2010

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.90510830	0.41501547	31.68	<.0001
Error	10	0.13098482	0.01309848		
Corrected Total	17	3.03609312			

R-Square	Coeff Var	Root MSE	y Mean
0.956857	15.99058	0.114449	0.715725

Source	DF	Type I SS	Mean Square	F Value	Pr > F
temp	5	2.90164653	0.58032931	44.31	<.0001
rh	2	0.00346178	0.00173089	0.13	0.8777

Source	DF	Type III SS	Mean Square	F Value	Pr > F
temp	5	2.90164653	0.58032931	44.31	<.0001
rh	2	0.00346178	0.00173089	0.13	0.8777

Two-way ANOVA for T. dubius egg mortality 4
No replication 08:30 Sunday, October 24, 2010

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

temp	y LSMEAN	LSMEAN Number
15	0.43978326	1

20	0.50242839	2
25	0.45404396	3
30	0.52129702	4
35	0.80600259	5
37.5	1.57079633	6

Least Squares Means for effect temp
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: y

i/j	1	2	3	4	5	6
1		0.9815	1.0000	0.9450	0.0254	<.0001
2	0.9815		0.9941	0.9999	0.0703	<.0001
3	1.0000	0.9941		0.9749	0.0320	<.0001
4	0.9450	0.9999	0.9749		0.0953	<.0001
5	0.0254	0.0703	0.0320	0.0953		0.0001
6	<.0001	<.0001	<.0001	<.0001	0.0001	

temp	y LSMEAN	95% Confidence Limits	
15	0.439783	0.292555	0.587012
20	0.502428	0.355200	0.649657
25	0.454044	0.306815	0.601273
30	0.521297	0.374068	0.668526
35	0.806003	0.658774	0.953231
37.5	1.570796	1.423568	1.718025

Least Squares Means for Effect temp

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	-0.062645	-0.387216	0.261926
1	3	-0.014261	-0.338832	0.310310
1	4	-0.081514	-0.406085	0.243057
1	5	-0.366219	-0.690790	-0.041648
1	6	-1.131013	-1.455584	-0.806442
2	3	0.048384	-0.276186	0.372955

```
Two-way ANOVA for T. dubius egg mortality                    5
              No replication   08:30 Sunday, October 24, 2010

            The GLM Procedure
          Least Squares Means
Adjustment for Multiple Comparisons: Tukey

   Least Squares Means for Effect temp

          Difference          Simultaneous 95%
             Between       Confidence Limits for
i    j           Means       LSMean(i)-LSMean(j)

2    4       -0.018869       -0.343440     0.305702
2    5       -0.303574       -0.628145     0.020997
2    6       -1.068368       -1.392939    -0.743797
3    4       -0.067253       -0.391824     0.257318
3    5       -0.351959       -0.676530    -0.027388
3    6       -1.116752       -1.441323    -0.792181
4    5       -0.284706       -0.609276     0.039865
4    6       -1.049499       -1.374070    -0.724928
5    6       -0.764794       -1.089365    -0.440223


Tukey Comparison Lines for Least Squares Means of temp

LS-means with the same letter are not significantly different.

                                          LSMEAN
                    y LSMEAN    temp      Number

          A     1.57079633    37.5           6

          B     0.80600259    35             5
          B
   C      B     0.52129702    30             4
   C      B
   C      B     0.50242839    20             2
   C
   C           0.45404396    25             3
   C
   C           0.43978326    15             1
```

Two-way ANOVA for T. dubius egg mortality 6
No replication 08:30 Sunday, October 24, 2010

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

rh	y LSMEAN	LSMEAN Number
55	0.69723464	1
75	0.73063222	2
100	0.71930892	3

Least Squares Means for effect rh
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: y

i/j	1	2	3
1		0.8704	0.9407
2	0.8704		0.9840
3	0.9407	0.9840	

rh	y LSMEAN	95% Confidence Limits	
55	0.697235	0.593128	0.801341
75	0.730632	0.626526	0.834739
100	0.719309	0.615203	0.823415

Least Squares Means for Effect rh

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	-0.033398	-0.214534	0.147739
1	3	-0.022074	-0.203211	0.159062
2	3	0.011323	-0.169813	0.192460

```
     Tukey Comparison Lines for Least Squares Means of rh

LS-means with the same letter are not significantly different.

                                        LSMEAN
                  y LSMEAN    rh        Number

               A  0.73063222  75             2
               A
               A  0.71930892  100            3


          Two-way ANOVA for T. dubius egg mortality                    7
                       No replication   08:30 Sunday, October 24, 2010

                     The GLM Procedure
                    Least Squares Means
          Adjustment for Multiple Comparisons: Tukey

     Tukey Comparison Lines for Least Squares Means of rh

LS-means with the same letter are not significantly different.

                                        LSMEAN
                  y LSMEAN    rh        Number

               A
               A  0.69723464  55             1
```

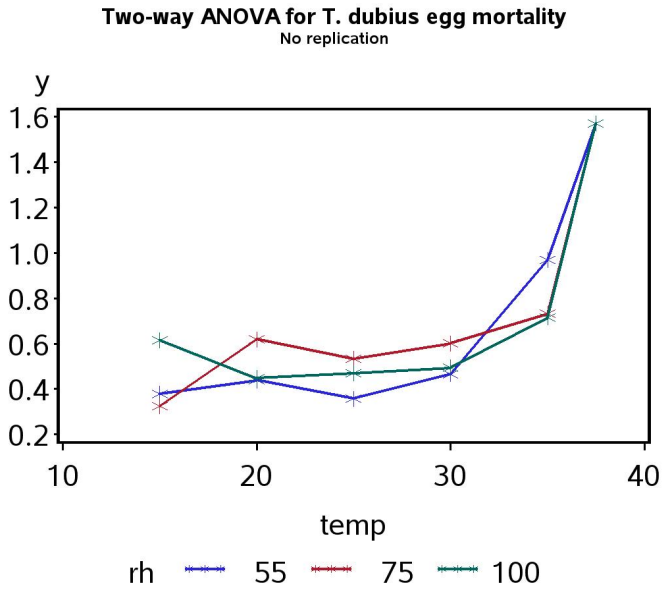

Figure 14.7: Mortality of *T. dubius* eggs at different temperatures and humidities (Table 14.6). Mortality rates were arcsine-square root transformed.

14.6 Randomized block designs

Suppose that we are interested in the yield of five different strains (A, B, C, D, and E) of corn, with five replicates per strain. One possible design would be to randomly assign the strain treatments to 30 small plots scattered throughout a large field, in a completely randomized design (Fig. 14.8). The resulting data from this design could be analyzed using one-way ANOVA (Chapter 11), with strain as the treatment. One problem with this design is soil fertility, moisture, and other factors could vary across this large field. This spatial heterogeneity would make it more difficult to see any treatment effects because it would increase the variance among replicate plots.

A common two-way design, the **randomized block design**, provides a possible solution to this spatial heterogeneity problem. Suppose that soil fertility and moisture are more homogeneous on smaller spatial scales, as often seems to be true. We could then select six plots within this field, called **blocks**, and within sections of each block plant the five corn strains (see Fig. 14.9). The order of the different treatments within each block would be randomized, hence the name randomized blocks. This ensures that the sequence of treatments varies across blocks, and that each treatment has different strains for neighbors in each block. The resulting data would then be analyzed using a two-way model with a fixed treatment effect and a random block effect, which helps account and control for spatial heterogeneity in the system. The block is considered a random effect because the blocks are usually selected from a potentially large collection of possible blocks. **A statistical model with both fixed and random effects is called a mixed model.**

Another example of a randomized block design could be insect traps baited with different attractants, say A, B, C, D, and E. Different stands in the forest would be the blocks. Five traps would be deployed in each stand along a transect, with baits randomly assigned to the traps within the transect. In another type of randomized block design, the blocks are different times rather than locations in space. For example, suppose that we want to test six different diets for rearing fish in ponds, but only have six ponds available. We could randomly assign the diets to the ponds and conduct the experiment, obtaining one replicate of each treatment. We would then repeat the study several more times using the same ponds, with the treatments randomly assigned each time. Each time would be treated as a separate block in the analysis.

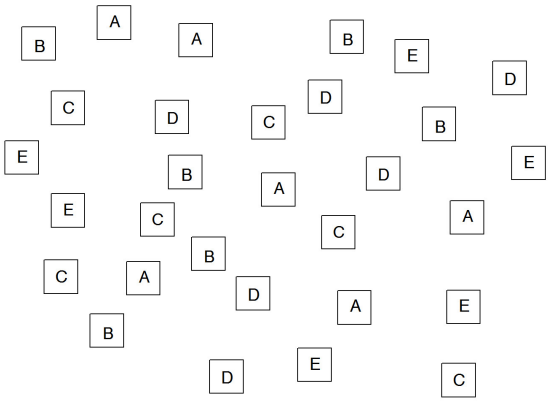

Figure 14.8: Completely randomized design with five treatments (A, B, C, D, and E) and six replicates per treatment.

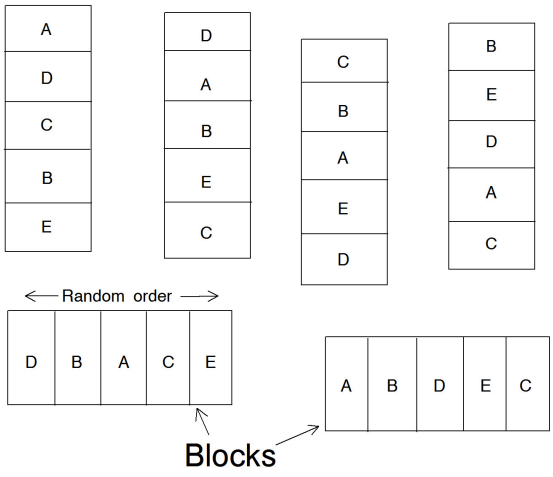

Figure 14.9: Randomized block design with five treatments (A, B, C, D, and E) and six blocks.

14.6.1 Randomized block models

There are two effects in a randomized block design, a fixed treatment and a random block effect, usually denoted as Factor A and B. The model commonly used to analyze these designs has the form

$$Y_{ij} = \mu + \alpha_i + B_j + \epsilon_{ij}. \tag{14.67}$$

Here μ, α_i, and ϵ_{ij} are defined as in previous models, while $B_j \sim N(0, \sigma_B^2)$. The model thus has two variance components, the variance among blocks (σ_B^2) and the variance of ϵ_{ij} (σ^2).

Note that there is no interaction term in this model, although there could be interaction in the data. A randomized block design has just one observation per combination of treatment and block, and so there are insufficient data to estimate an A $\times$ B interaction. However, there are variants of the randomized block design that have two or more replicates of each Factor A treatment per block. In this, case, we could fit a model with interaction of the form

$$Y_{ij} = \mu + \alpha_i + B_j + (\alpha B)_{ij} + \epsilon_{ij}. \tag{14.68}$$

Here $(\alpha B)_{ij} \sim N(0, \sigma_{AB}^2)$. The interaction term in these designs is considered to be a random effect because it involves the random block effect. This model has three variance components, the interaction variance (σ_{AB}^2), the block variance (σ_B^2), and the variance of ϵ_{ij} (σ^2).

14.6.2 Hypothesis testing and variance components

We will use `proc mixed` in SAS to analyze the data for randomized block designs (SAS Institute Inc. 2014a). The default method in SAS estimates the variance components in the model using a method called restricted maximum likelihood, or REML. This process involves separating the fixed effects from the likelihood function, then estimating the variance components of the random effects by maximizing this restricted likelihood (hence the name). Once these are determined, the fixed effects parameters are estimated and F tests generated for those effects (Littell et al. 1996, McCulloch & Searle 2001). For a randomized block design, the null hypothesis tested for Factor A would be H_0 : all $\alpha_i = 0$. However, there is no ANOVA table nor related quantities like sum of squares and mean squares. The emphasis in `proc mixed` is on the estimation of variance components rather than tests on them, although tests can be constructed if necessary (see below).

14.6.3 Randomized block design - SAS demo

We will illustrate a `proc mixed` analysis for the randomized block design using a different trapping study of *T. dubius* (Reeve et al. 2009). Six different stands were located in the forest and considered to be blocks. Five traps were placed in a line at 30 m intervals within each stand, and then a bait treatment randomly assigned to each trap. There were five such treatments: blank trap (`BLANK`), α-pinene (`AP`), frontalin + α-pinene (`FRAP`), ipsdienol + α-pinene (`IDAP`), and ipsenol + α-pinene (`ISAP`). As mentioned earlier, frontalin, ipsdienol, and ipsenol are bark beetle pheromones while α-pinene is a major component of pine resin. The number of predators caught in each trap was then counted. See SAS program with data below.

The count data were manipulated in two ways before analysis. A log transformation was applied to predator counts to ensure the observations meet the assumptions of ANOVA (see Chapter 15). All observations for the `BLANK` treatment were also removed using the statement

```
if treat="BLANK" then delete;
```

because this treatment caught no insects. The `proc mixed` portion of the program basically implements the model for randomized block designs. We first need to tell SAS the variables categorizing the groups in the data, using a `class` statement. For the trapping study, the variables `treat` and `block` identify the treatment and block variables, so we use the statement

```
class treat block;
```

Next, recall that the randomized block model has the form

$$Y_{ij} = \mu + \alpha_i + B_j + \epsilon_{ij}. \tag{14.69}$$

Here, the SAS variable `treat` corresponds to α_i, the fixed effect in the model, while `block` corresponds to B_j, the random effect. One feature of `proc mixed` is the separation of fixed and random effects in the model – all fixed effects are placed in the `model` statement while random effects are included in the `random` statement. Thus, the `model` statement for the trapping data would be

```
model y = treat / ddfm=kr outp=resids;
```

while the `random` statement is

```
random block;
```

The `ddfm=kr` option specifies the Kenward-Rogers method of calculating the degrees of freedom (SAS Institute Inc. 2014), a general method for calculating the degrees of freedom that works in a variety of circumstances. An `lsmeans` statement of the form

```
lsmeans treat / pdiff=all adjust=tukey adjdfe=row;
```

is also used to compare the different bait treatments using the Tukey method. See complete program listing and output below.

We see that there is a highly significant effect of bait treatment on the number of predators trapped ($F_{3,13.9} = 54.68, P < 0.0001$). Note the non-integer degrees of freedom for this F statistic. This has occurred because the data are unbalanced (one observation is a missing value) and `proc mixed` is adjusting the test. Examining the Tukey results, we see that every pair of bait treatments is significantly different except for IDAP vs. ISAP. The graph (Fig. 14.10) and least squares means show that `FRAP` caught the most insects, IDAP and ISAP were intermediate, while AP caught the fewest.

The `proc mixed` output also provides estimates of the two variance components in the model, the block variance (σ_B^2) and the variance of ϵ_{ij} (σ^2). They are listed under the `Covariance Parameter Estimates` in the SAS output, labeled as `block` and `Residual`, along with confidence intervals for these estimates. We see that the block variance $\sigma_B^2 = 0.3332$ is large relative to $\sigma^2 = 0.1831$. The block variance can be directly observed in Fig. 14.10 as the vertical spread between different blocks. In most cases, we are primarily interested in testing the fixed effects in the model, with the random effects and their associated variance components of less importance. They are included in the model and analysis to account and control for spatial heterogeneity in the observations. We will examine a likelihood ratio test for the block variance in the next section.

SAS Program

```
* TrapRCBD_clerids.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Randomized block ANOVA for trapping experiment data";
data trapexp;
    input block $ treat $ count;
    * Apply transformations here;
    sqrtcount = sqrt(count);
    logcount = log(count+1);
    * Choose which variable is used for plots and anova;
    y = logcount;
    * Delete blank traps;
    if treat="BLANK" then delete;
    datalines;
1        AP        4
1        BLANK       0
1        FRAP       79
1        IDAP        7
1        ISAP       10
2        AP        1
2        BLANK       0
2        FRAP      124
2        IDAP       13
2        ISAP       20
3        AP        0
3        BLANK       0
3        FRAP       14
3        IDAP        .
3        ISAP        2
4        AP        0
4        BLANK       0
4        FRAP       15
4        IDAP       11
4        ISAP        7
5        AP        0
5        BLANK       0
5        FRAP       29
5        IDAP        7
5        ISAP        7
6        AP        2
6        BLANK       0
6        FRAP       70
6        IDAP       14
```

```
6        ISAP        20
;
run;
* Print data set;
proc print data=trapexp;
run;
* Plot means, standard errors, and observations;
proc gplot data=trapexp;
    plot y*treat=block / vaxis=axis1 haxis=axis1;
    symbol1 i=j v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
proc mixed cl data=trapexp;
    class treat block;
    model y = treat / ddfm=kr outp=resids;
    random block;
    lsmeans treat / pdiff=all adjust=tukey adjdfe=row;
run;
goptions reset=all;
title "Diagnostic plots to check anova assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
    plot resid*pred=1 / vaxis=axis1 haxis=axis1;
    symbol1 v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
    qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

Randomized block ANOVA for trapping experiment data 1
09:28 Tuesday, October 26, 2010

Obs	block	treat	count	sqrtcount	logcount	y
1	1	AP	4	2.0000	1.60944	1.60944
2	1	FRAP	79	8.8882	4.38203	4.38203
3	1	IDAP	7	2.6458	2.07944	2.07944
4	1	ISAP	10	3.1623	2.39790	2.39790
5	2	AP	1	1.0000	0.69315	0.69315
6	2	FRAP	124	11.1355	4.82831	4.82831
7	2	IDAP	13	3.6056	2.63906	2.63906
8	2	ISAP	20	4.4721	3.04452	3.04452
9	3	AP	0	0.0000	0.00000	0.00000
10	3	FRAP	14	3.7417	2.70805	2.70805
11	3	IDAP	.	.	.	.
12	3	ISAP	2	1.4142	1.09861	1.09861
13	4	AP	0	0.0000	0.00000	0.00000
14	4	FRAP	15	3.8730	2.77259	2.77259
15	4	IDAP	11	3.3166	2.48491	2.48491
16	4	ISAP	7	2.6458	2.07944	2.07944
17	5	AP	0	0.0000	0.00000	0.00000
18	5	FRAP	29	5.3852	3.40120	3.40120
19	5	IDAP	7	2.6458	2.07944	2.07944
20	5	ISAP	7	2.6458	2.07944	2.07944
21	6	AP	2	1.4142	1.09861	1.09861
22	6	FRAP	70	8.3666	4.26268	4.26268
23	6	IDAP	14	3.7417	2.70805	2.70805
24	6	ISAP	20	4.4721	3.04452	3.04452

Randomized block ANOVA for trapping experiment data 2
09:28 Tuesday, October 26, 2010

The Mixed Procedure

Model Information

Data Set	WORK.TRAPEXP
Dependent Variable	y
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile

```
Fixed Effects SE Method      Kenward-Roger
Degrees of Freedom Method    Kenward-Roger


                Class Level Information

  Class    Levels    Values

  treat         4    AP FRAP IDAP ISAP
  block         6    1 2 3 4 5 6


                     Dimensions

          Covariance Parameters            2
          Columns in X                     5
          Columns in Z                     6
          Subjects                         1
          Max Obs Per Subject             24


                Number of Observations

       Number of Observations Read          24
       Number of Observations Used          23
       Number of Observations Not Used       1


                   Iteration History

Iteration    Evaluations    -2 Res Log Like       Criterion

        0              1        47.44629548
        1              2        38.98690259      0.00950955
        2              1        38.96571169      0.00025308
        3              1        38.96519017      0.00000021
        4              1        38.96518975      0.00000000


              Convergence criteria met.
```

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate	Alpha	Lower	Upper
block	0.3332	0.05	0.1159	3.1475
Residual	0.1831	0.05	0.09789	0.4576

Fit Statistics

-2 Res Log Likelihood	39.0
AIC (smaller is better)	43.0
AICC (smaller is better)	43.7
BIC (smaller is better)	42.5

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
treat	3	13.9	54.68	<.0001

Least Squares Means

Effect	treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	AP	0.5669	0.2933	8.59	1.93	0.0869
treat	FRAP	3.7258	0.2933	8.59	12.70	<.0001
treat	IDAP	2.2417	0.3069	9.83	7.30	<.0001
treat	ISAP	2.2907	0.2933	8.59	7.81	<.0001

Differences of Least Squares Means

Effect	treat	_treat	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
treat	AP	FRAP	-3.1589	0.2470	13.9	-12.79	<.0001	Tukey-Kramer

Differences of Least

Squares Means

Effect	treat	_treat	Adj P
treat	AP	FRAP	<.0001

Randomized block ANOVA for trapping experiment data 4
09:28 Tuesday, October 26, 2010

The Mixed Procedure

Differences of Least Squares Means

Effect	treat	_treat	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
treat	AP	IDAP	-1.6748	0.2630	14	-6.37	<.0001	Tukey-Kramer
treat	AP	ISAP	-1.7239	0.2470	13.9	-6.98	<.0001	Tukey-Kramer
treat	FRAP	IDAP	1.4841	0.2630	14	5.64	<.0001	Tukey-Kramer
treat	FRAP	ISAP	1.4351	0.2470	13.9	5.81	<.0001	Tukey-Kramer
treat	IDAP	ISAP	-0.04903	0.2630	14	-0.19	0.8548	Tukey-Kramer

Differences of Least Squares Means

Effect	treat	_treat	Adj P
treat	AP	IDAP	<.0001
treat	AP	ISAP	<.0001
treat	FRAP	IDAP	0.0003
treat	FRAP	ISAP	0.0002
treat	IDAP	ISAP	0.9976

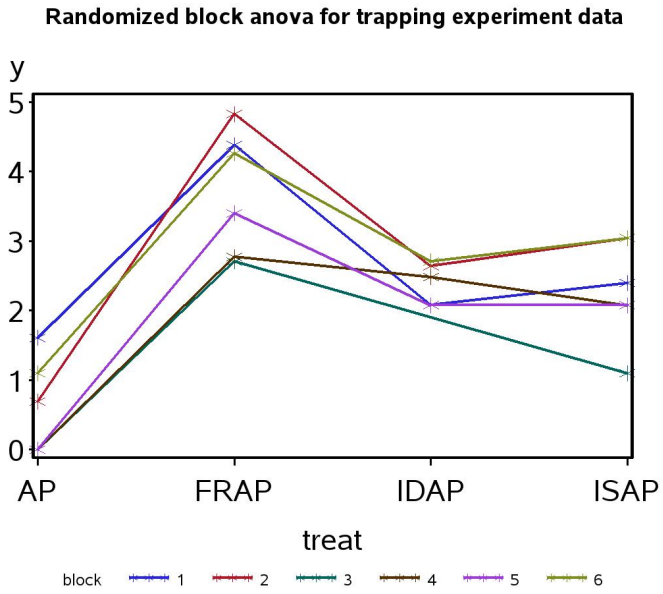

Figure 14.10: Log-transformed trap catches of *T. dubius* for four different bait treatments. Different line colors denote different blocks.

14.6.4 Likelihood ratio test for the block effect

In the preceding example, the block variance $\sigma_B^2 = 0.3332$ appears large relative to $\sigma^2 = 0.1831$, the variance due to ϵ_{ij}. The block effect is also clearly visible in Fig. 14.10. A further step would be a test of $H_0 : \sigma_B^2 = 0$ vs. $H_1 : \sigma_B^2 > 0$. If the test is significant it provides further evidence for variability among blocks in the density of insects. Littell et al. (1996) recommend a likelihood ratio test for this purpose.

We can construct this test by fitting two different models to the data, corresponding to H_0 vs. H_1. Under $H_0 : \sigma_B^2 = 0$ the statistical model for a randomized block design reduces to

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \tag{14.70}$$

because $B_j = 0$ for all j under H_0. The statistical model under $H_1 : \sigma_B^2 > 0$ is just the full model for randomized block designs:

$$Y_{ij} = \mu + \alpha_i + B_j + \epsilon_{ij} \tag{14.71}$$

We now need to find maximum likelihood estimates of the model parameters under both H_1 and H_0, as well as L_{H_0} and L_{H_1}, the maximum height of the likelihood function under H_0 and H_1. We would then use the likelihood ratio test statistic

$$-2\ln(\lambda) = 2\ln(L_{H_1}) - 2\ln(L_{H_0}). \tag{14.72}$$

The SAS program below finds the likelihoods for both models using `proc mixed`. Two separate calls to `proc mixed` are required, one for each model. The likelihoods are labeled `-2 Res Log Likelihood` in the output, which is almost the form required above except for the sign. Examining the output, we see that $-2\ln(L_{H_0}) = 47.4$ and $-2\ln(L_{H_1}) = 39.0$. We then have

$$-2\ln(\lambda) = -39.0 - (-47.4) = -39.0 + 47.4 = 8.4 \tag{14.73}$$

How do we obtain a P value for this test statistic? For any likelihood ratio test, the quantity $-2\ln(\lambda)$ has approximately a χ^2 distribution under H_0. The degrees of freedom for the test are equal to the difference in the number of parameters for the two models (H_1 vs. H_0). There is a difference in one parameter between the two models here, because H_1 has the block variance σ_B^2 while under H_0 this is assumed to be zero. We therefore have $df = 1$, and from Table C find that $P < 0.005$. We are actually conducting a one-tailed

test, however, because H_1 is a one-tailed alternative. Thus, the P value is half this quantity, or $P < 0.0025$. It appears the variance due to blocks is highly significant.

We can calculate the P value more exactly using a simple SAS program (see below). In the `data` step, the program reads in the values of $-2\ln(L_{H_0})$, $-2\ln(L_{H_1})$, and df, then calculates the P value using the SAS function `probchi`. We find that $P = 0.0019$.

SAS Program

```
* TrapRCBD_clerids_block_test.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Randomized block ANOVA for trapping experiment data";
data trapexp;
    input block $ treat $ count;
    * Apply transformations here;
    sqrtcount = sqrt(count);
    logcount = log(count+1);
    * Choose which variable is used for plots and anova;
    y = logcount;
    * Delete blank traps;
    if treat="BLANK" then delete;
    datalines;
1        AP                4
1        BLANK        0
1        FRAP        79
1        IDAP        7
1        ISAP        10

etc.

6        AP                2
6        BLANK        0
6        FRAP        70
6        IDAP        14
6        ISAP        20
;
run;
title2 "H0 true - no block effect";
proc mixed cl data=trapexp;
    class treat;
    model y = treat / ddfm=kr;
run;
title2 "H1 true - there is a block effect";
```

```
proc mixed cl data=trapexp;
    class treat block;
    model y = treat / ddfm=kr;
    random block;
run;
quit;
```

SAS Output

```
            Randomized block ANOVA for trapping experiment data             1
                        H0 true - no block effect
                                        13:18 Wednesday, October 27, 2010

                           The Mixed Procedure

                            Model Information

Data Set                      WORK.TRAPEXP
Dependent Variable            y
Covariance Structure          Diagonal
Estimation Method             REML
Residual Variance Method      Profile
Fixed Effects SE Method       Model-Based
Degrees of Freedom Method     Residual


                        Class Level Information

  Class    Levels    Values

  treat         4    AP FRAP IDAP ISAP


                            Dimensions

                Covariance Parameters            1
                Columns in X                     5
                Columns in Z                     0
                Subjects                         1
                Max Obs Per Subject             24


                        Number of Observations

            Number of Observations Read             24
```

Number of Observations Used	23
Number of Observations Not Used	1

Covariance Parameter Estimates

Cov Parm	Estimate	Alpha	Lower	Upper
Residual	0.4925	0.05	0.2848	1.0506

Fit Statistics

-2 Res Log Likelihood	47.4
AIC (smaller is better)	49.4
AICC (smaller is better)	49.7
BIC (smaller is better)	50.4

Randomized block anova for trapping experiment data 2
H0 true - no block effect
13:18 Wednesday, October 27, 2010

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
treat	3	19	20.43	<.0001

Randomized block ANOVA for trapping experiment data 3
H1 true - there is a block effect
13:18 Wednesday, October 27, 2010

The Mixed Procedure

Model Information

Data Set	WORK.TRAPEXP
Dependent Variable	y
Covariance Structure	Variance Components
Estimation Method	REML

Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
treat	4	AP FRAP IDAP ISAP
block	6	1 2 3 4 5 6

Dimensions

Covariance Parameters	2
Columns in X	5
Columns in Z	6
Subjects	1
Max Obs Per Subject	24

Number of Observations

Number of Observations Read	24
Number of Observations Used	23
Number of Observations Not Used	1

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	47.44629548	
1	2	38.98690259	0.00950955
2	1	38.96571169	0.00025308
3	1	38.96519017	0.00000021
4	1	38.96518975	0.00000000

Convergence criteria met.

Randomized block anova for trapping experiment data 4
H1 true - there is a block effect

13:18 Wednesday, October 27, 2010

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate	Alpha	Lower	Upper
block	0.3332	0.05	0.1159	3.1475
Residual	0.1831	0.05	0.09789	0.4576

Fit Statistics

-2 Res Log Likelihood	39.0
AIC (smaller is better)	43.0
AICC (smaller is better)	43.7
BIC (smaller is better)	42.5

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
treat	3	13.9	54.68	<.0001

SAS Program

```
* lrtpvalue.sas;
options pageno=1 linesize=80;
title "P-value for likelihood ratio test";
data values;
    *Data are -2lnL values under H0 and H1, plus degrees of freedom;
    input m2lnLH1 m2lnLH0 df;
    m2lnl = -m2lnLH1 - (-m2lnLH0);
    * Find P-value;
    Pvalue = (1 - probchi(m2lnl,df))/2;
    datalines;
39.0 47.4 1
;
run;
proc print data=values;
run;
```

SAS Output

```
                    P-value for likelihood ratio test                    1
                                          13:18 Wednesday, October 27, 2010

                    m2ln    m2ln
              Obs    LH1     LH0    df    m2lnl        Pvalue

               1      39     47.4     1     8.4     .001876105
```

14.7 References

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14.8 Problems

1. An entomologist is interested in how bark beetles respond to traps baited with two treatments, their own pheromone (P) vs. the pheromone plus a repellent chemical (PR). They also want to see if trap color (black vs. white) affects the response of the beetles. They conduct an experiment in which these two factors are randomly assigned to traps in one section of the forest, with five replicate traps for each treatment. The counts of bark beetles responding to each trap are listed below.

Bait	Trap color	Counts for five replicate traps
P	Black	138, 569, 196, 139, 726
PR	Black	96, 168, 25, 36, 152
P	White	174, 99, 293, 67, 122
PR	White	52, 27, 11, 57, 93

   (a) Write an appropriate ANOVA model for this design, and state which effects are fixed or random. Is it possible to include an interaction term in the model?

   (b) Use SAS to analyze these data using your ANOVA model, log transforming the observations. Interpret the results of all the tests. Attach your SAS program and output.

2. A research group is interested in the effects of diet and temperature on the growth rate of fish in aquaculture. They conduct an experiment with three different diet treatments (A, B and C) crossed with three rearing temperatures (15, 20 and 25°C). Two fish tanks are assigned to each treatment combination and the growth rate (g/week) determined for each tank. The following data were obtained:

Diet	Temp	Growth rate (two tanks)
A	15	24.7, 22.3
A	20	31.9, 28.9
A	25	32.6, 31.3
B	15	19.6, 14.2
B	20	30.5, 26.5
B	25	25.5, 32.8
C	15	21.1, 21.3
C	20	23.4, 23.4
C	25	28.2, 25.8

(a) Write an appropriate ANOVA model for this design, and state which effects are fixed or random. Is it possible to include an interaction term in the model?

(b) Use SAS to analyze these data using your ANOVA model. You may use any method for dealing with interactions. Interpret the results of all the tests.

(c) Use the Tukey method to compare the different diet treatments, and then the temperature treatments. Interpret the results.

Chapter 15

Assumptions and Transformations

Analysis of variance as well as regression analysis (see Chapter 17) make a number of assumptions about the nature of the observations. These assumptions are embodied in the statistical model used in the analysis. For example, recall the model for fixed effects one-way ANOVA:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}. \tag{15.1}$$

Here μ is the grand mean while α_i is the deviation from μ caused by the ith level of Factor A. The ϵ_{ij} term represents random departures from the mean value predicted by Factor A due to natural variability. It is assumed that $\epsilon_{ij} \sim N(0, \sigma^2)$ and that these random variables are also independent of one another. We examine these assumptions in more detail below and discuss how their violation can affect the validity of the statistical analyses. We then describe how **variance-stabilizing transformations** are used to fix certain violations of these assumptions. We also present a common method for identifying these violations known as **residual analysis**.

15.1 ANOVA assumptions

15.1.1 Independence of observations

One key assumption embodied in the above model is that the error terms ϵ_{ij} are independent, implying that the observations Y_{ij} are also independent.

How would a lack of independence influence the results of ANOVA? The consensus is that a lack of independence can greatly influence the validity of ANOVA, including the Type I error rate and power of the F test, as well as the estimation of group effects (Glass et al. 1972).

As an example of an experimental design where the observations are not independent, suppose that we conduct an insect trapping experiment with two bait types, A and B. We place all of the bait A traps in one location and bait B ones in a second location. If location influences the abundance of insects, then we would expect the trap catches at a particular location to be high or low for this reason, separate of any treatment effect. Thus, the observations at a particular location are related to one another and so are not independent. We would be more likely to find a treatment effect if these data were analyzed using one-way ANOVA, because of the location effect on insect abundance, even if there was no effect of bait type on trap catches. Thus, the Type I error rate of the F test would be higher. This combination of poor experimental design and an inappropriate statistical analysis has been called **pseudoreplication** (Hurlbert 1984). While there are multiple traps within each location, they are not true replicates because the observations are not independent, and treatment and location effects cannot be separated. This design basically has only one replicate per treatment, one for each location.

Fortunately, the assumption of independence will usually be satisfied by good experimental design and execution (Hurlbert 1984). In the insect bait experiment, a better experimental design would randomly allocate bait types to traps at both locations, and the analysis could also include a location (block) effect in the statistical model. Randomization also helps ensure that estimates of the treatment effects are unbiased. For example, bait type A might be messier to use than B, and the experimenter might be tempted to do those replicates last or place them in a different location. This potential source of bias by the experimenter is avoided by randomization of the treatments.

15.1.2 Homogeneity of variances

Another key assumption of ANOVA is that the variance is similar among treatment groups, also known as the **homogeneity of variances** assumption or **homoscedasticity**. This follows from the assumption that ϵ_{ij} has a variance of σ^2 regardless of the treatment group. We can also see this from a graphical presentation of the one-way ANOVA model, where each treatment

group has the same distribution with the same variance except for shifts due to Factor A (see Fig. 11.1 in Chapter 11). The condition of unequal variances is also called **heteroscedasticity**.

If the homogeneity of variances assumption is not satisfied this can strongly affect the validity of the F test in ANOVA, especially when the design is unbalanced (Glass et al. 1972). If the treatments with higher variances have smaller sample sizes, then the actual Type I error rate will be higher than its nominal value (say $\alpha = 0.05$). Conversely, if the treatments with higher variances have larger sample sizes, the actual Type I error rate will be smaller than its nominal value. We will see later in this chapter how **variance-stabilizing transformations** can be used to equalize the variance among groups, making the observations better conform to this assumption.

15.1.3 Normality

A further assumption of ANOVA is that the error term ϵ_{ij} is normally distributed, and as a consequence so are the observations (Y_{ij} values). The assumption of normality appears to be less important for the validity of ANOVA than homogeneity of variances. Many studies indicate that the ANOVA F test has the nominal Type I error rate ($\alpha = 0.05$) even when the observations have distributions quite different from the normal, although power may be increased or decreased relative to the normal (see Table 16, Glass et al. 1972). For large values of n per group, ANOVA is likely to be a valid procedure regardless of the distribution of the observations due to the central limit theorem (Chapter 7). In practice, a transformation that equalizes the variance among groups also seems to normalize the observations, solving both problems.

15.1.4 Absence of outliers

An assumption of ANOVA related to normality is the absence of outliers. **Outliers are observations that lie far from the other observations in a particular study.** The source of the outlier could be a rare biological event, or simply a data entry error or bad measurement with an instrument. Because it lies far from the other observations, an outlier will increase the size of MS_{within} and alter the estimated effect of its treatment group. If the outlier is a data error then there is justification for deleting it from the observations. If the source is unclear or the outlier is a valid observation, then

one common approach is to conduct the statistical analysis with and without the outlier and present both results. Outliers can be often be identified using residual analysis (see below).

15.1.5 Additivity

ANOVA models are known as additive models because the observations are modeled as the sum of several factors. For example, the model for two-way fixed effects ANOVA without replication is

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}. \tag{15.2}$$

Thus, the Y_{ij} values are modeled as the sum of the grand mean, the effects of Factor A and B, and a random term representing variability among the observations. Additivity of effects is a basic assumption of ANOVA.

However, some biological processes like survival and reproduction are inherently multiplicative processes. For example, suppose our observations are the number of offspring surviving to maturity from a single female. This number will be the product of the fecundity of the female and the survival rate of the offspring. We now apply a number of treatments that could potentially influence both these factors. The resulting observations could be described using the model

$$Y_{ij} = \lambda s_i f_j \gamma_{ij}, \tag{15.3}$$

where λ is the average number of offspring surviving to maturity, while s_i and f_j are the differential effects of the survival and fecundity treatments. The term γ_{ij} is a multiplicative error term with a distribution that takes only positive values, and it is typically required that $E[\gamma_{ij}] = 1$. Note that these must all be positive quantities in order for the number of offspring (Y_{ij}) to be positive.

Can data of this type be analyzed using ANOVA? The answer is yes, because we can use a log transformation to make the data additive. Taking the log of both sides of this model, we obtain

$$\log Y_{ij} = \log \lambda + \log s_i + \log f_j + \log \gamma_{ij}. \tag{15.4}$$

The result is an additive model the same as for unreplicated two-way ANOVA, and the data can be analyzed using standard ANOVA methods. This is one reason why studies of reproduction and survival as well as population dynamics routinely use the log transformation.

15.2 Variance-stabilizing transformations

Variance-stabilizing transformations are often used by statisticians to equalize the variance of observations across different treatment groups, so that the homogeneity of variances assumption is better satisfied. We have already employed these transformations in some of our analyses, including the log and arcsine-square root transformations.

The different transformations are derived as follows. Suppose we have a random variable Y that describes the data, and there is a functional relationship between its variance $Var[Y] = v$ and its mean $E[Y] = m$. More specifically, suppose that we have

$$v = f(m) \tag{15.5}$$

where f is some function. For example, with the Poisson distribution for parameter λ we have $Var[Y] = E[Y] = \lambda$ (Chapter 7), and so $v = m$ is the functional relationship. It can then be shown that a function g that satisfies the equation

$$g(m) = \int \frac{\theta dm}{\sqrt{f(m)}}, \tag{15.6}$$

where θ is a constant, will be a variance-stabilizing transformation (Bartlett 1947). To see how this process works, suppose that a random variable Y has a Poisson distribution. We find that

$$g(m) = \int \frac{\theta dm}{\sqrt{m}} = \theta \frac{m^{1/2}}{1/2} + C = 2\theta\sqrt{m} + C \propto \sqrt{m}. \tag{15.7}$$

Thus, the variance-stabilizing transformation for Poisson data is $\sqrt{Y}$.

As another example, suppose that $v = m^2$ so that the variance increases with the square of the mean. Negative binomial data will have this form for large m, because $v = m + m^2/k$ for this distribution (Chapter 7). For this relationship between v and m, we have

$$g(m) = \int \frac{\theta dm}{\sqrt{m^2}} = \int \frac{\theta dm}{m} = \theta \log m + C \propto \log m, \tag{15.8}$$

implying that $\log Y$ is the variance-stabilizing transformation. Either natural or base 10 log transformations can be used and will yield identical test results. The $\log Y$ transformation is a 'stronger' transformation than the $\sqrt{Y}$ because it corrects for a stronger relationship between v and m.

A variance-stabilizing transformation is also needed for proportions, because the variance of a proportion depends on its mean. To see this, suppose that we observe l different individuals from some population and record their sex. Let Y be the number of individuals in the sample that are female. The variable Y would be a binomial random variable with parameters l and p, where p is the proportion of females in the population, and so $E[Y] = lp$ and $Var[Y] = lp(1-p)$ (see Chapter 5). Then, a **binomial proportion** would be Y/l, the proportion of females in the sample. For this proportion, we have $E[Y/l] = lp/l = p$ while $Var[Y/l] = lp(1-p)/l^2 = p(1-p)/l$. If we set $m = p$, then $v = Var[Y/l] = m(1-m)/l$ and so v is a function of m. Using the same method as above, we find that the variance-stabilizing transformation for binomial proportions is $\sin^{-1}(\sqrt{Y})$ or $\arcsin(\sqrt{Y})$. This transformations maps proportions from 0 to 1 to the interval 0 to $\pi/2$. The largest effect of the transformation is on proportions close to 0 or 1.

Table 15.1 lists the commonly used variance-stabilizing transformations. Also listed are variants of the transformations that are useful when the data include zeroes, as often occurs in count data. In the next section, we will illustrate the use of these transformations, and how the appropriate transformation can be determined through residual analysis.

Table 15.1: Variance-stabilizing transformations for various $v = f(m)$ and the data for which they are useful.

$v = f(m)$	Transformation	Comments
$v = m$	$\sqrt{Y}$, $\sqrt{Y + 1/2}$ (zeroes)	Poisson data
$v = m^2$	$\log Y, \log(Y+1)$ (zeroes)	Overdispersed count data, many other types
$v = m(1-m)/l$	$\arcsin\sqrt{Y}$	Proportions

15.3 Residual analysis

We will present the details of residual analysis in this section. We begin by defining predicted and residual values using one-way ANOVA as an example, for both fixed and random effects (similar results hold for more complex designs). We then illustrate residual analysis and the use of variance-stabilizing

transformations with some examples.

15.3.1 Models, estimates, and predictors

ANOVA is based on statistical models that contain a number of parameters. For example, the statistical model for fixed effects one-way ANOVA has the form

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}, \tag{15.9}$$

where μ is the grand mean, α_i is the deviation from the μ caused by the ith treatment, and $\epsilon_{ij} \sim N(0, \sigma^2)$. We saw earlier how likelihood methods could be used to estimate the parameters μ, α_i, and σ^2 for this model. For the random effects version, the model contained a random variable $A_i \sim N(0, \sigma_A^2)$, and is written as

$$Y_{ij} = \mu + A_i + \epsilon_{ij}. \tag{15.10}$$

The parameters in this model are μ, σ_A^2, and σ^2, and these quantities can also be estimated using likelihood methods. It is also possible to estimate the random variable A_i itself, more specifically the value realized in a particular group and study. Estimators of A_i are often called **predictors** in this context, because they concern random variables rather than model parameters (Searle et al. 1992).

15.3.2 Predicted and residual values

We can use these estimates to generate a **predicted value** for each observation Y_{ij} in the data set. For the fixed effects model listed above, the predicted value of Y_{ij} is $\hat{Y}_{ij} = \hat{\mu} + \hat{\alpha}_i$, where $\hat{\mu}$ and $\hat{\alpha}_i$ are the estimated values of μ and α_i. Note that all observations in the ith group would have the same predicted value.

What actually are the predicted values here? Recall that for the fixed effects model, the maximum likelihood estimates of these parameters are

$$\hat{\mu} = \bar{\bar{Y}} \tag{15.11}$$

and

$$\hat{\alpha}_i = \bar{Y}_{i\cdot} - \bar{\bar{Y}}. \tag{15.12}$$

Thus,

$$\hat{Y}_{ij} = \hat{\mu} + \hat{\alpha}_i = \bar{\bar{Y}} + \bar{Y}_{i\cdot} - \bar{\bar{Y}} = \bar{Y}_{i\cdot}. \tag{15.13}$$

So, the predicted value for the *ith* group is just the mean of that group.

Similarly, for the random effects model the predicted value of Y_{ij} is $\hat{Y}_{ij} = \hat{\mu} + \hat{A}_i$, where $\hat{\mu} = \bar{\bar{Y}}$ and $\hat{A}_i$ is the predictor of A_i. It turns out that the best predictor for the realized value of A_i is 'shrunk' relative to α_i and has the form

$$\hat{A}_i = \frac{\sigma_A^2}{\sigma_A^2 + \sigma^2/n}(\bar{Y}_{i\cdot} - \bar{\bar{Y}}) \tag{15.14}$$

(Searle et al. 1992). It depends on σ_A^2 and σ^2 as well as $\bar{Y}_{i\cdot}$ and $\bar{\bar{Y}}$. It follows that

$$\hat{Y}_{ij} = \hat{\mu} + \hat{A}_i = \bar{\bar{Y}} + \frac{\sigma_A^2}{\sigma_A^2 + \sigma^2/n}(\bar{Y}_{i\cdot} - \bar{\bar{Y}}) \tag{15.15}$$

for the random effects model. Thus, $\hat{Y}_{ij}$ is not equal to $\bar{Y}_{i\cdot}$ in this situation but lies closer to the grand mean $\bar{\bar{Y}}$, unless n is large. In practice, estimates of the two variance components are used to generate the predicted value.

In assessing the validity of our statistical models, we will also be interested in the **residuals** of the observations, which are defined as the difference $Y_{ij} - \hat{Y}_{ij}$. The residuals essentially provide an estimate of the error term ϵ_{ij} for each observation, which we can call $\hat{\epsilon}_{ij}$. Why is this so? The model for one-way ANOVA can be expressed as

$$Y_{ij} - (\mu + \alpha_i) = \epsilon_{ij}. \tag{15.16}$$

If we insert estimates for μ and α_i in this equation, we obtain an estimate of ϵ_{ij}:

$$Y_{ij} - (\hat{\mu} + \hat{\alpha}_i) = Y_{ij} - \hat{Y}_i = \hat{\epsilon}_{ij}. \tag{15.17}$$

There is an interesting relationship between these residual values and MS_{within}. Suppose that we use the sample variance of the $\hat{\epsilon}_{ij}$ values to estimate the variance of ϵ_{ij}, namely σ^2. The sum of squares associated with this sample variance is

$$SS = \sum_{i=1}^{a}\sum_{j=1}^{n}(\hat{\epsilon}_{ij})^2 = \sum_{i=1}^{a}\sum_{j=1}^{n}\left(Y_{ij} - (\hat{\mu} + \hat{\alpha}_i)\right)^2, \tag{15.18}$$

and the degrees of freedom are $a(n-1)$. Dividing SS by its degrees of freedom, we obtain an estimator of σ^2 based on the residuals:

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^{a}\sum_{j=1}^{n}\left(Y_{ij} - (\hat{\mu} + \hat{\alpha}_i)\right)^2}{a(n-1)}. \tag{15.19}$$

How is this quantity related to MS_{within}, our other estimate of σ^2? If we plug $\hat{\mu} = \bar{\bar{Y}}$ and $\hat{\alpha}_i = \bar{Y}_{i\cdot} - \bar{\bar{Y}}$ into this equation, we obtain

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^{a}\sum_{j=1}^{n}\left(Y_{ij} - (\bar{\bar{Y}} + \bar{Y}_{i\cdot} - \bar{\bar{Y}})\right)^2}{a(n-1)} \tag{15.20}$$

$$= \frac{\sum_{i=1}^{a}\sum_{j=1}^{n}\left(Y_{ij} - \bar{Y}_{i\cdot})\right)^2}{a(n-1)} \tag{15.21}$$

$$= MS_{within}. \tag{15.22}$$

Thus, MS_{within} can be expressed in terms of the residuals from the ANOVA estimation process. This relationship is true for all ANOVA models (and regression as well). Because MS_{within} can be expressed using the residual or error terms, MS_{within} is also called $MS_{residual}$ or MS_{error}, and SS_{within} similarly named $SS_{residual}$ or SS_{error}. This terminology is used in SAS output as well.

It is also possible to express MS_{among} in terms of the maximum likelihood estimates of the parameters. Because $\hat{\alpha}_i = \bar{Y}_{i\cdot} - \bar{\bar{Y}}$, we have

$$MS_{among} = \frac{n\sum_{i=1}^{a}(\bar{Y}_{i\cdot} - \bar{\bar{Y}})^2}{a-1} = \frac{n\sum_{i=1}^{a}\hat{\alpha}_i^2}{a-1}. \tag{15.23}$$

From this result, it is clear that MS_{among} is an increasing function of the values of $\hat{\alpha}_i$, the estimated treatment effects (Winer et al. 1991).

15.3.3 Evaluating ANOVA assumptions

Residuals play a key role in determining if a particular data set satisfies the assumptions of ANOVA. They can be used to evaluate three of the assumptions: (1) homogeneity of variances among groups, (2) absence of outliers, and (3) normality of the error terms.

We can evaluate the homogeneity of variances assumption through a plot of the residuals vs. predicted values. **If the variances are homogeneous among groups, the points should be equally scattered for each group.** This is because the residuals are estimates of the ϵ_{ij} values and are supposed to have the same variance across groups. If the residual vs. predicted plot shows a definite pattern, such as a increase or decrease in the scatter as the predicted values increase, this suggests a variance-stabilizing

transformation may be needed. This type of plot is also useful for detecting any outliers in the data. **If an outlier is present it will have a very large residual value.** The normality assumption can be evaluated using a normal quantile plot of the residuals. **If the residuals are normal, then this plot will be a straight diagonal line.**

15.3.4 Residual analysis and transformations - SAS demo

We will illustrate residual analysis and the use of transformations with data from a trapping study of the predatory insect *Thanasiumus dubius* (Reeve et al. 2009). This study used a randomized block design with five bait treatments and six blocks, previously analyzed in Chapter 14. Note that the model for this design contains both fixed and random effects, but predicted values and residuals can still be generated through a more complex process (Searle et al. 1992)

The complete program for this example is listed below for reference. We will concentrate here on the steps necessary to generate a residual vs. predicted plot, and a normal quantile plot, in order to examine the homogeneity of variances and normality assumptions. The `outp=resids` option in the `model` statement sends the residual and predicted values for each observation to an output data file called `resids` (SAS Institute Inc. 2014). They are given the names `resid` and `pred` in this file. The subsequent `proc gplot` portion of the program plots the residuals vs. predicted values, with residuals on the y-axis and predicted values on the x-axis. A normal quantile plot of the residuals is generated using `proc univariate`.

We first analyze the data using no transformation by setting `y = count` in the `data` step. Examining the residual vs. predicted plot, we see an increase in the scatter of the residuals as the predicted values increase (Fig. 15.1), especially for the largest predicted values. This implies that the variance of the observations increases with their mean (v is some function of m). In addition, the normal quantile plot does not appear to be a straight diagonal line (Fig. 15.2). Neither assumption appears to be satisfied in this analysis.

We next analyze the data using a square root transformation by setting `y = sqrtcount` in the `data` step. The residual vs. predicted plot shows less scatter of the residuals for larger predicted values, although there is still some spread (Fig. 15.3). The normal quantile plot is now a straight diagonal

line (Fig. 15.4).

We next try a log transformation of the data, setting `y = logcount` in the `data` step. The residual vs. predicted plot shows the same scatter across the range of predicted values (Fig. 15.5), and the normal quantile plot is a straight diagonal line (Fig. 15.6). This is the desired outcome with the data now satisfying the homogeneity of variances and normality assumptions. There also appear to be no outliers (extreme residual values) in these observations. **We can then proceed to interpret the rest of the analysis, such as the F test and multiple comparisons. They should be valid at this point because the ANOVA assumptions are satisfied.** See Chapter 14 for the interpretation of this analysis.

SAS Program

```
* TrapRCBD_clerids.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Randomized block anova for trapping experiment data";
data trapexp;
    input block $ treat $ count;
    * Apply transformations here;
    sqrtcount = sqrt(count);
    logcount = log(count+1);
    * Choose which variable is used for plots and anova;
    y = logcount;
    * Delete blank traps;
    if treat="BLANK" then delete;
    datalines;
1   AP      4
1   BLANK   0
1   FRAP    79
1   IDAP    7
1   ISAP    10
2   AP      1
2   BLANK   0
2   FRAP    124
2   IDAP    13
2   ISAP    20
3   AP      0
3   BLANK   0
3   FRAP    14
3   IDAP    .
3   ISAP    2
4   AP      0
4   BLANK   0
4   FRAP    15
4   IDAP    11
4   ISAP    7
5   AP      0
5   BLANK   0
5   FRAP    29
5   IDAP    7
5   ISAP    7
6   AP      2
6   BLANK   0
6   FRAP    70
6   IDAP    14
```

```
6   ISAP    20
;
run;
* Print data set;
proc print data=trapexp;
run;
* Plot means, standard errors, and observations;
proc gplot data=trapexp;
    plot y*treat=block / vaxis=axis1 haxis=axis1;
    symbol1 i=j v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Mixed model analysis;
proc mixed cl data=trapexp;
    class treat block;
    model y = treat / ddfm=kr outp=resids;
    random block;
    lsmeans treat / pdiff=all adjust=tukey;
run;
goptions reset=all;
title "Diagnostic plots to check anova assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
    plot resid*pred=1 / vaxis=axis1 haxis=axis1;
    symbol1 v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
    qqplot resid / normal waxis=3 height=4;
run;
quit;
```

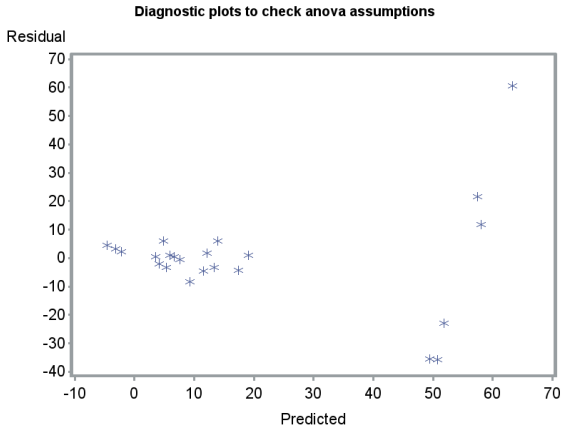

Figure 15.1: Residual vs. predicted plot for a trapping experiment with no transformation of the data.

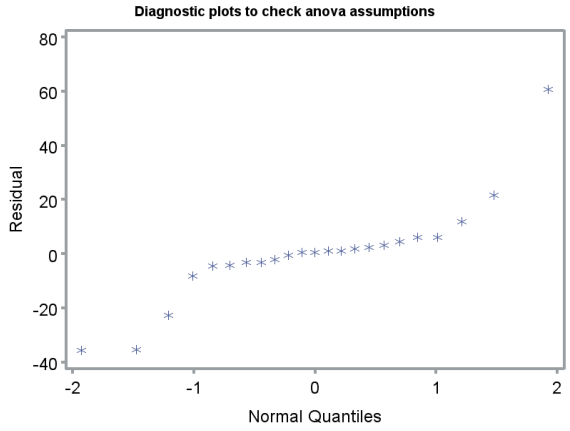

Figure 15.2: Normal quantile plot of the residuals for a trapping experiment with no transformation of the data.

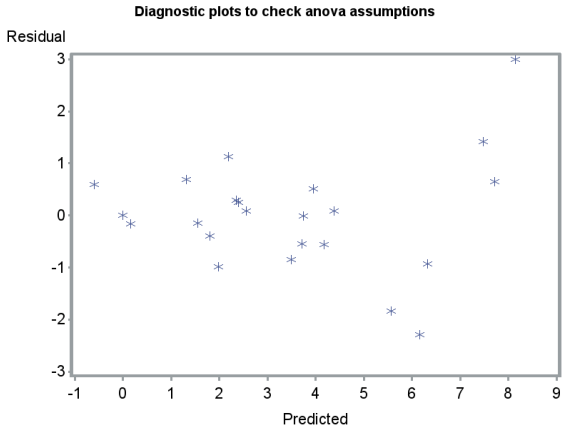

Figure 15.3: Residual vs. predicted plot for a trapping experiment with a $\sqrt{Y}$ transformation of the data.

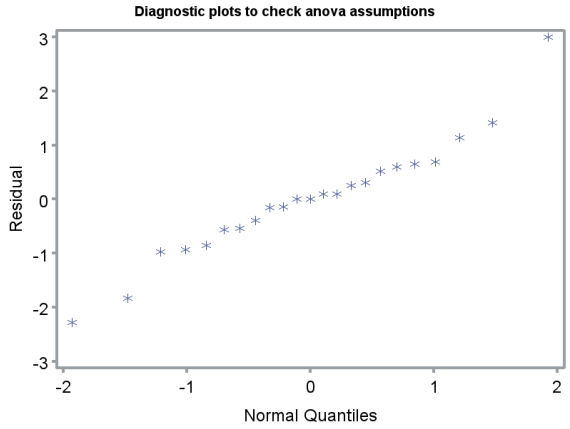

Figure 15.4: Normal quantile plot of the residuals for a trapping experiment with a $\sqrt{Y}$ transformation of the data.

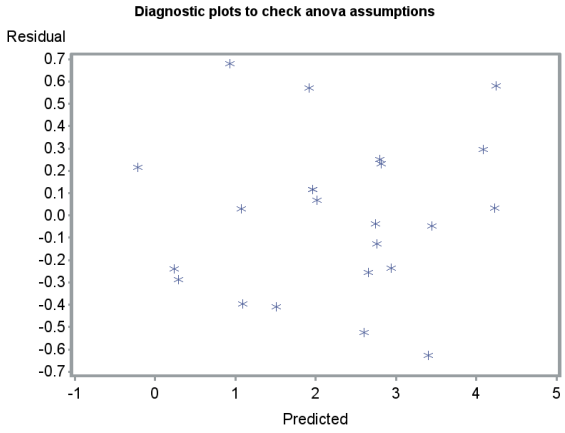

Figure 15.5: Residual vs. predicted plot for a trapping experiment with a $\log Y$ transformation of the data.

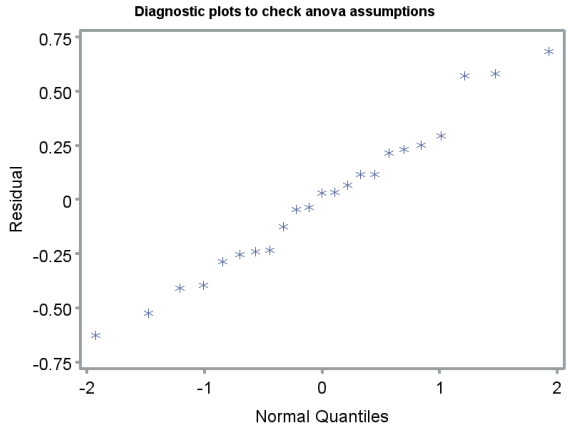

Figure 15.6: Normal quantile plot of the residuals for a trapping experiment with a $\log Y$ transformation of the data.

15.3.5 $\arcsin(\sqrt{Y})$ transformation - SAS demo

As another example of residual analysis and transformation, we will analyze the observations from an experiment involving an insect predator and the survival of a pest insect on which it feeds. Plots are established each containing 20 pest insects, and a predator treatment (0, 10, or 20 predators) randomly assigned to each plot. There were $n = 10$ plots per predator treatment. The proportion of pest insects surviving was determined for each plot. See SAS program below.

We first analyze these data using untransformed proportions, using `y = prop` in the `data` step, where `prop` is the proportion of surviving pest insects. A one-way ANOVA is then conducted using `proc glm` with `predator` as the treatment (a fixed effect). Examining the residual vs. predicted plot (Fig. 15.7), we see that the variability of the observations for one treatment is smaller. This is the 0 predator treatment and has a very high survival rate. The normal quantile plot is a straight diagonal line, so this assumption is apparently satisfied (Fig. 15.8).

We then analyze the experiment using the transformation $\arcsin(\sqrt{Y})$ where Y is the proportion, using `y = arsin(sqrt(prop))` in the `data` step. The residual vs. predicted plot shows an equal scatter of the residuals across the predicted values, suggesting the homogeneity of variances assumption is satisfied (Fig. 15.9). The normal quantile plot is a straight diagonal line once more (Fig. 15.10). What has happened here? The transformation has spread out the survival rates for the 0 predator treatment, thus equalizing the variances among the treatment groups.

Examining the SAS output, we see there was a highly significant effect of the predator treatment on the survival rate of the pest insect ($F_{2,27} = 21.26, P < 0.0001$). Pest survival decreased as the number of predators increased (Fig. 15.11).

SAS Program

```
* arcsine.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'One-way ANOVA for proportions';
data arcsine;
    input predators survivors;
    prop = survivors/20;
    * Apply transformations here;
    y = arsin(sqrt(prop));
    datalines;
0 18
0 18
0 18
0 16
0 19
0 19
0 17
0 18
0 20
0 17
1 14
1 17
1 15
1 10
1 17
1 14
1 13
1 17
1 14
1 15
2 12
2 16
2 16
2 12
2 6
2 12
2 13
2 10
2 9
2 10
;
run;
* Print data set;
```

```
proc print data=arcsine;
run;
* Plot means, standard errors, and observations;
proc gplot data=arcsine;
    plot y*predators=1 / vaxis=axis1 haxis=axis1;
    symbol1 i=std1mjt v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* One-way anova with all fixed effects;
proc glm data=arcsine;
    class predators;
    model y = predators;
    output out=resids p=pred r=resid;
run;
goptions reset=all;
title "Diagnostic plots to check ANOVA assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
    plot resid*pred=1 / vaxis=axis1 haxis=axis1;
    symbol1 v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
    qqplot resid / normal waxis=3 height=4;
run;
quit;
```

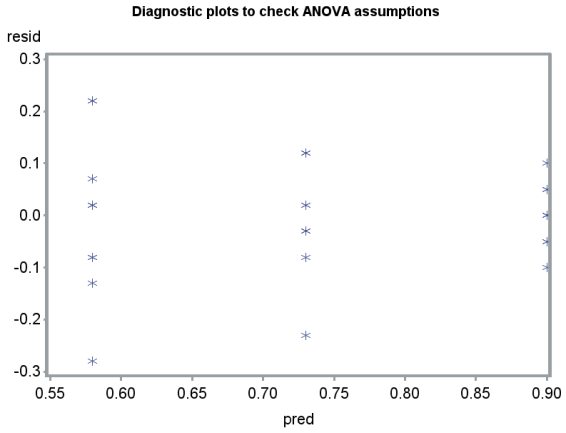

Figure 15.7: Residual vs. predicted plot for a predation experiment with no transformation of the data.

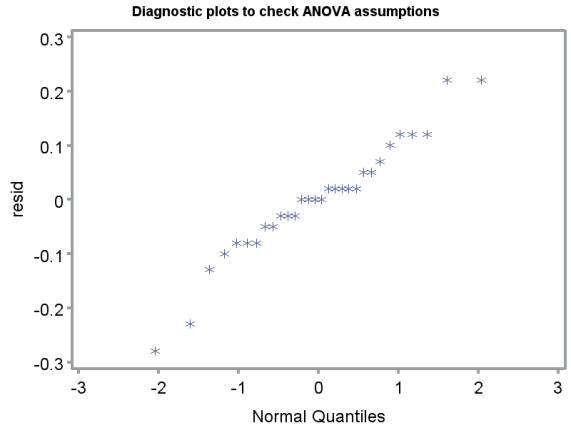

Figure 15.8: Normal quantile plot of the residuals for a predation experiment with no transformation of the data.

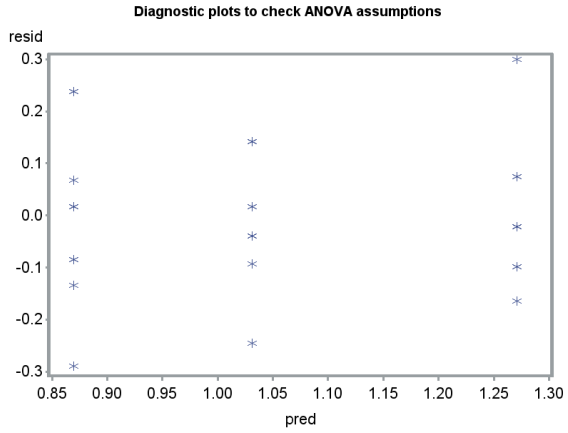

Figure 15.9: Residual vs. predicted plot for a predation experiment with a $\arcsin(\sqrt{Y})$ transformation of the data.

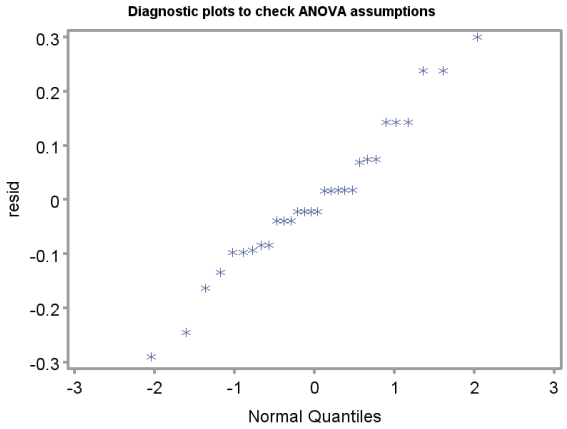

Figure 15.10: Normal quantile plot of the residuals for a predation experiment with a $\arcsin(\sqrt{Y})$ transformation of the data.

SAS Output

One-way ANOVA for proportions 1
13:58 Monday, November 9, 2015

Obs	predators	survivors	prop	y
1	0	18	0.90	1.24905
2	0	18	0.90	1.24905
3	0	18	0.90	1.24905
4	0	16	0.80	1.10715
5	0	19	0.95	1.34528
6	0	19	0.95	1.34528
7	0	17	0.85	1.17310
8	0	18	0.90	1.24905
9	0	20	1.00	1.57080
10	0	17	0.85	1.17310
11	1	14	0.70	0.99116
12	1	17	0.85	1.17310
13	1	15	0.75	1.04720
14	1	10	0.50	0.78540
15	1	17	0.85	1.17310
16	1	14	0.70	0.99116
17	1	13	0.65	0.93774
18	1	17	0.85	1.17310
19	1	14	0.70	0.99116
20	1	15	0.75	1.04720
21	2	12	0.60	0.88608
22	2	16	0.80	1.10715
23	2	16	0.80	1.10715
24	2	12	0.60	0.88608
25	2	6	0.30	0.57964
26	2	12	0.60	0.88608
27	2	13	0.65	0.93774
28	2	10	0.50	0.78540
29	2	9	0.45	0.73531
30	2	10	0.50	0.78540

One-way ANOVA for proportions 2
13:58 Monday, November 9, 2015

The GLM Procedure

Class Level Information

```
                    Class          Levels    Values

                    predators           3    0 1 2


                Number of Observations Read          30
                Number of Observations Used          30


                     One-way ANOVA for proportions                          3
                                                  13:58 Monday, November 9, 2015

                          The GLM Procedure

Dependent Variable: y

                                      Sum of
Source                        DF       Squares    Mean Square   F Value   Pr > F

Model                          2    0.81626150     0.40813075     21.26   <.0001

Error                         27    0.51834395     0.01919792

Corrected Total               29    1.33460544


             R-Square     Coeff Var      Root MSE        y Mean

             0.611613      13.10549      0.138557      1.057240


Source                        DF      Type I SS    Mean Square   F Value   Pr > F

predators                      2    0.81626150     0.40813075     21.26   <.0001


Source                        DF    Type III SS    Mean Square   F Value   Pr > F

predators                      2    0.81626150     0.40813075     21.26   <.0001
```

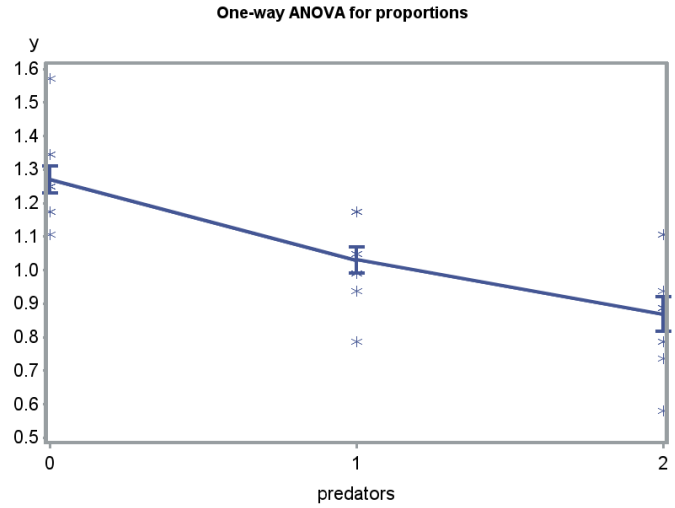

Figure 15.11: Transformed survival rates vs. predator treatment.

15.3.6 Transformations when data are limited

In many real studies, we will have insufficent data to determine the appropriate variance-stabilizing transformation using residual analysis. For example, we may not have enough points to determine if the variance is related to the mean, or whether the normality assumption is satisfied. In this situation you may have to guess the appropriate transformation. For count data you would use the $\sqrt{Y}$ or $\log Y$ transformation. Most count data are more overdispersed or clumped than the Poisson distribution, however, and so the $\log Y$ transformation will usually be a better choice than $\sqrt{Y}$. You would use the $\arcsin(\sqrt{Y})$ transformation for proportion data, especially if there are some proportions near 0 or 1.

15.4 References

Bartlett, M. S. (1947). The use of transformations. *Biometrics* 3: 39-52.

Glass, G. V., Peckham, P. D., & Sanders, J. R. (1972) Consequences of failure to meet assumptions underlying fixed effects analysis of variance and covariance. *Review of Educational Research* 42: 237-288.

Hurlbert, S. H. (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* 54: 187-211.

SAS Institute Inc. (2014) *SAS/STAT 13.2 Users Guide.* SAS Institute Inc., Cary, NC.

Searle, S. R., Casella, G., & McCulloch, C. E. (1992) *Variance Components*. John Wiley & Sons, Inc., New York, NY.

Reeve, J. D., Strom, B. L., Rieske-Kinney, L. K., Ayres, B. D. Ayres, & Costa, A. (2009) Geographic variation in prey preference in bark beetle predators. *Ecological Entomology* 34: 183-192.

Winer, B. J., Brown, D. R. & Michels, K. M. (1991) *Statistical Principles in Experimental Design*, 3rd edition. McGraw-Hill, Inc., Boston, MA.

Chapter 16

Nonparametric Tests

The statistical tests we have examined so far are called **parametric tests**, because they assume the data have a known distribution, such as the normal, and test hypotheses about the parameters of this distribution. Examples of such tests are the F test in ANOVA, and one- or two-sample t tests. Parametric tests can also be constructed for other distributions, such as the Poisson and binomial.

While ANOVA and other procedures are derived assuming the data are normal, they can also be validly applied to non-normal data provided sample sizes are large, due to the central limit theorem (Glass et al. 1972). For example, the means used in the ANOVA F tests are assumed to have a normal distribution, which will be true for normal data. This will also hold for non-normal data, provided the sample sizes are sufficiently large for the central limit theorem to operate (Chapter 7). Thus, the tests used in ANOVA will still be valid for large sample sizes, regardless of the distribution of the data. Valid in this context means the tests have the correct Type I error rate (such as $\alpha = 0.05$) and power levels.

There are conditions where parametric procedures are less than ideal. For example, suppose that the data appear non-normal and sample sizes are relatively small. We cannot rely on the central limit theorem here, and so parametric tests based on the normal distribution might be invalid. **Nonparametric tests** are often useful in this situation. These procedures do not assume a particular probability distribution for the data, and are therefore applicable for any distribution. For this reason they are also known as **distribution-free** methods. Nonparametric tests can be more powerful than parametric tests for non-normal data (Conover 1999; Hollander et al.

2014). The increase in power can be substantial for distributions with heavy tails compared to the normal distribution, which implies that extreme observations are more common. While nonparametric tests are less powerful than parametric ones for normal data, the loss of power is often quite minimal.

We will examine three types of nonparametric tests for one-way designs. The first are tests based on ranks. These replace the data values with their rank values, obtained by ordering the data from smallest to largest. They then utilize test statistics that are functions of these ranks rather than the original data values. We will cover rank tests for two or more groups, in particular the Wilcoxon and Kruskal-Wallis tests (Conover 1999; Hollander et al. 2014). They are used to test whether the distributions for each group differ in location, and serve a function similar to parametric tests like one-way ANOVA. We will also examine the two-sample Kolmogorov-Smirnov test, which can detect differences in both the shape and location of two distributions (Conover 1999; Hollander et al. 2014). It makes use of the empirical distribution function for each group, the empirical counterpart of the distribution function for continuous random variables (Chapter 6). The last type of nonparametric test we will consider are randomization tests. These tests examine whether the data are consistent with a null hypothesis of randomness (Hinkelmann & Kempthorne 1994; Manly 1997). The behavior of a test statistic (often a parametric one like an F statistic) is examined under this null hypothesis, in a process that involves randomly permuting or rearranging observations across the groups many times.

We will use data from a study of chitons (a kind of mollusk) in the intertidal zone (Flores-Campaña et al. 2012) to illustrate the use of nonparametric tests. Populations of *Chiton albolineatus* were sampled from three islands in Mazatlan Bay, Mexico. For each island, samples were taken from sites that were exposed or sheltered from wave action, and the body length of the chitons measured. The authors found that the distribution of chiton length was non-normal, and so used the nonparametric Kruskal-Wallis test to compare the lengths of chitons across islands and sites. They found significant differences in length among various combinations of island and site, and a tendency for chiton to be larger in exposed sites. We will use a small subset of these data in our calculations, shown in Tables 16.1 and 16.2.

Table 16.1: Example 1 - Body lengths of *Chiton albolineatus* in the intertidal zone of the island of Venados (Flores-Campaña et al. 2012). Chitons were sampled from sites sheltered or exposed to wave action. Also shown are the rank values (R_{ij}) for each observation, and the sum of the ranks for each groups ($\sum_{j=1}^{n_i} R_{ij}$, where n_i is the sample size for each group.)

Site	Y_{ij} = Length (mm)	R_{ij}	i	j	$\sum_{j=1}^{n_i} R_{ij}$
Sheltered	44.39	20	1	1	
Sheltered	22.30	3	1	2	
Sheltered	21.31	2	1	3	
Sheltered	23.80	5	1	4	
Sheltered	26.23	8	1	5	70
Sheltered	27.98	10	1	6	
Sheltered	28.10	11	1	7	
Sheltered	24.39	6	1	8	
Sheltered	22.32	4	1	9	
Sheltered	15.16	1	1	10	
Exposed	30.20	16	2	1	
Exposed	29.36	14	2	2	
Exposed	28.88	12	2	3	
Exposed	32.23	19	2	4	
Exposed	26.54	9	2	5	140
Exposed	24.85	7	2	6	
Exposed	30.54	17	2	7	
Exposed	31.36	18	2	8	
Exposed	28.98	13	2	9	
Exposed	29.49	15	2	10	

Table 16.2: Example 2 - Body length of *C. albolineatus* on the sheltered side of three islands, located in Mazatlan Bay, Mexico (Flores-Campaña et al. 2012). Also shown are the rank values (R_{ij}) for each observation, and the sum of the ranks for each group ($\sum_{j=1}^{n_i} R_{ij}$)

Site	Y_{ij} = Length (mm)	R_{ij}	i	j	$\sum_{j=1}^{n_i} R_{ij}$
Lobos	23.86	16	1	1	
Lobos	20.20	6	1	2	
Lobos	29.32	27	1	3	
Lobos	23.56	13	1	4	
Lobos	24.32	17	1	5	157
Lobos	22.33	12	1	6	
Lobos	23.69	14	1	7	
Lobos	26.78	21	1	8	
Lobos	27.32	23	1	9	
Lobos	21.22	8	1	10	
Pajaros	32.90	29	2	1	
Pajaros	32.73	28	2	2	
Pajaros	26.94	22	2	3	
Pajaros	29.09	26	2	4	
Pajaros	12.32	1	2	5	142
Pajaros	15.25	5	2	6	
Pajaros	25.87	19	2	7	
Pajaros	20.21	7	2	8	
Pajaros	13.96	3	2	9	
Pajaros	12.48	2	2	10	
Venados	44.39	30	3	1	
Venados	22.30	10	3	2	
Venados	21.31	9	3	3	
Venados	23.80	15	3	4	
Venados	26.23	20	3	5	166
Venados	27.98	24	3	6	
Venados	28.10	25	3	7	
Venados	24.39	18	3	8	
Venados	22.32	11	3	9	
Venados	15.16	4	3	10	

16.1 Wilcoxon two-sample test

The Wilcoxon test provides a nonparametric alternative to a two-sample t test or a one-way ANOVA for two groups (see Chapter 11). It does not assume any particular distribution of the data, except that it is a continuous one (see Chapter 6). The null and alternative hypotheses for the Wilcoxon test are expressed in terms of the cumulative distribution for the two groups, say $F_1(y)$ and $F_2(y)$. Under the null hypothesis the two distribution are supposed to be identical, which can be expressed as $H_0 : F_2(y) = F_1(y)$ for all y (Fig. 16.1). Under the alternative, one distribution is shifted from the other by a distance Δ, but they otherwise have the same shape (Conover 1999; Hollander et al. 2014). This can be expressed as $H_1 : F_2(y) = F_1(y-\Delta)$ (Fig. 16.2).

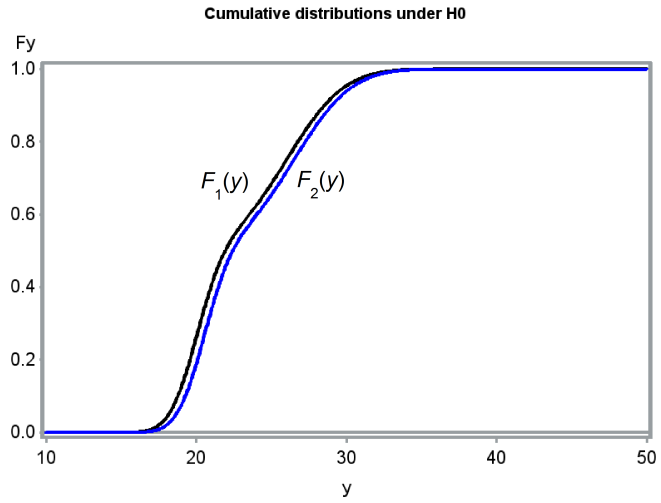

Figure 16.1: Cumulative distributions for two groups under $H_0 : \Delta = 0$.

The Wilcoxon test statistic W is based on the ranks of the observations. The observations are first assigned ranks from the smallest to the largest across the two groups. The test statistic is then the sum of the ranks for one of the groups. Typically the one with the smallest sample size is chosen, or if the sample sizes are equal, one is arbitrarily selected (SAS uses group order). For the Example 1 data the sample sizes are equal, so we could use

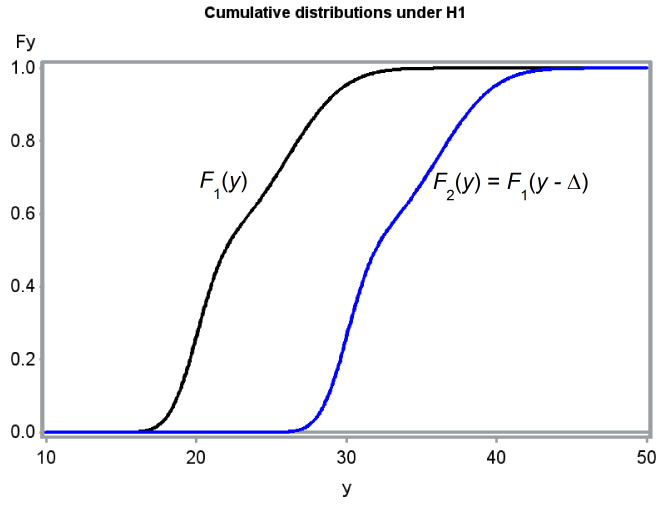

Figure 16.2: Cumulative distributions for two groups under $H_1 : \Delta = 10$.

the summed ranks for the Sheltered chiton group, namely

$$W = \sum_{j=1}^{n_1} R_{1j} = 70 \tag{16.1}$$

(Conover 1999; Hollander et al. 2014). We would expect small values of this statistic when F_1 is located to the left of F_2 ($\Delta > 0$), because this implies that values of Y_{1j} are more likely to be small relative to Y_{2j} ones. Conversely, large values of the statistic would occur when F_1 is to the right of F_2 ($\Delta < 0$). W is also sensitive to differences in the expected values (means) of the two distributions, because of the relationship between expected values and distributions. For a two-tailed test, we would reject H_0 if W is sufficiently large, or sufficiently small. An exact P value for both one- and two-tailed tests can be calculated using the distribution of W. We will let SAS handle the calculations for exact tests.

For large sample sizes, the distribution of W under H_0 approaches the normal distribution with mean and variance given by

$$E_{H_0}[W] = \frac{n_1(n_1 + n_2 + 1)}{2} \tag{16.2}$$

and

$$Var_{H_0}[W] = \frac{n_1 n_2(n_1 + n_2 + 1)}{12}. \tag{16.3}$$

The expected value formula assumes W is calculated using the first group. We then have

$$Z = \frac{W - E_{H_0}[W]}{\sqrt{Var_{H_0}[W]}} \sim N(0,1) \tag{16.4}$$

for large sample sizes. We can use this approximation to find P values for both one- and two-tailed tests (Hollander et al. 2014).

The Wilcoxon statistic W can be derived starting with a two-sample t test (see Chapter 11), and simply replacing the observations with their rank values (Bickel & Doksum 1977). It is also equivalent to the Mann-Whitney U test, another common nonparametric test. Modifications of the Wilcoxon test are also available to deal with the problem of tied observations. The tied observations are assigned the average of the tied ranks, and the variance equation is modified to account for the number of ties (Hollander et al. 2014).

Sample calculation

For the Example 1 data, we see that $W = 70$ for the Sheltered chitons (see Table 16.1). We will use the normal approximation for this statistic to obtain a two-tailed P value for the test. We have $E_{H_0}[W] = 10(10+10+1)/2 = 105$ and $Var_{H_0}[W] = 10 \cdot 10(10+10+1)/12 = 175$, and so

$$Z = \frac{70 - 105}{\sqrt{175}} = -2.646. \tag{16.5}$$

From Table Z, we find that $P[Z < -2.646] = 1 - P[Z < 2.646] \approx 1 - 0.9960 = 0.0040$. The two-tailed P value is then twice this value, or $P = 2(0.0040) = 0.0080$.

16.1.1 Wilcoxon test for Example 1 - SAS demo

We now conduct the Wilcoxon test using the Example 1 data and the SAS procedure `npar1way`, which implements a number of nonparametric procedures for one-way (single factor) designs (SAS Institute Inc. 2014a). See program listing below. The Wilcoxon test is invoked by adding the `wilcoxon` option in the `proc npar1way` statement. The `class` statement identifies the group variable, while `var` selects the dependent variable. The `exact wilcoxon` line generates exact P values for the test. The program also includes `proc gplot` code to plot the group means (SAS Institute Inc. 2014b). For purposes

of comparison, a one-way ANOVA is also conducted using `proc glm`. See program and output below.

We find that the Wilcoxon two-tailed test is highly significant, for both the exact test ($W = 70, P = 0.0068$) and the normal approximation ($Z = -2.6080, P = 0.0091$). The value of Z calculated by SAS differs slightly from our earlier result, because it includes a correction that improves the normal approximation. From the summed ranks for each group, as well as the graph, we see that the Sheltered chitons are smaller than the Exposed ones. Note that the parametric one-way ANOVA for these data was non-significant ($F_{1,18} = 2.13, P = 0.1619$). This likely occurred because of one very large and one small chiton at the Sheltered site, which would be outliers in the ANOVA. In the analysis using ranks, these are simply the largest and smallest rank values, only one step away from the next ones.

SAS Program

```
* WKWtest_chitons_Venados.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Wilcoxon and Kruskal-Wallis tests for chiton length';
data chitons;
        input site :$10. length;
        datalines;
Sheltered        44.39
Sheltered        22.30
Sheltered        21.31
Sheltered        23.80
Sheltered        26.23

etc.

;
run;
* Print data set;
proc print data=chitons;
run;
* Plot means, standard error, and observations;
proc gplot data=chitons;
        plot length*site / vaxis=axis1 haxis=axis1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Kruskal-Wallis/Wilcoxon tests;
proc npar1way wilcoxon data=chitons;
        class site;
        var length;
        exact wilcoxon;
run;
* One-way ANOVA for comparison;
proc glm data=chitons;
        class site;
        model length = site;
        output out=resids p=pred r=resid;
run;
quit;
```

SAS Output

Wilcoxon and Kruskal-Wallis tests for chiton length 1
13:00 Wednesday, November 18, 2015

Obs	site	length
1	Sheltered	44.39
2	Sheltered	22.30
3	Sheltered	21.31
4	Sheltered	23.80
5	Sheltered	26.23

etc.

Wilcoxon and Kruskal-Wallis tests for chiton length 2
13:00 Wednesday, November 18, 2015

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable length
Classified by Variable site

site	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Sheltered	10	70.0	105.0	13.228757	7.0
Exposed	10	140.0	105.0	13.228757	14.0

Wilcoxon Two-Sample Test

Statistic (S)	70.0000
Normal Approximation	
Z	-2.6080
One-Sided Pr < Z	0.0046
Two-Sided Pr > |Z|	0.0091
t Approximation	
One-Sided Pr < Z	0.0086
Two-Sided Pr > |Z|	0.0173
Exact Test	
One-Sided Pr <= S	0.0034

```
Two-Sided Pr >= |S - Mean|   0.0068

Z includes a continuity correction of 0.5.


Kruskal-Wallis Test

Chi-Square         7.0000
DF                      1
Pr > Chi-Square    0.0082


Wilcoxon and Kruskal-Wallis tests for chiton length              3
                                  13:00 Wednesday, November 18, 2015

The GLM Procedure

Class Level Information

Class         Levels    Values

site               2    Exposed Sheltered

 Number of Observations Read          20
 Number of Observations Used          20


Wilcoxon and Kruskal-Wallis tests for chiton length              4
                                  13:00 Wednesday, November 18, 2015

The GLM Procedure

Dependent Variable: length

                                  Sum of
 Source                    DF       Squares    Mean Square   F Value   Pr > F

 Model                      1    66.4301250     66.4301250      2.13   0.1619

 Error                     18   562.0077700     31.2226539

 Corrected Total           19   628.4378950
```

R-Square	Coeff Var	Root MSE	length Mean
0.105707	20.37791	5.587723	27.42050

Source	DF	Type I SS	Mean Square	F Value	Pr > F
site	1	66.43012500	66.43012500	2.13	0.1619

Source	DF	Type III SS	Mean Square	F Value	Pr > F
site	1	66.43012500	66.43012500	2.13	0.1619

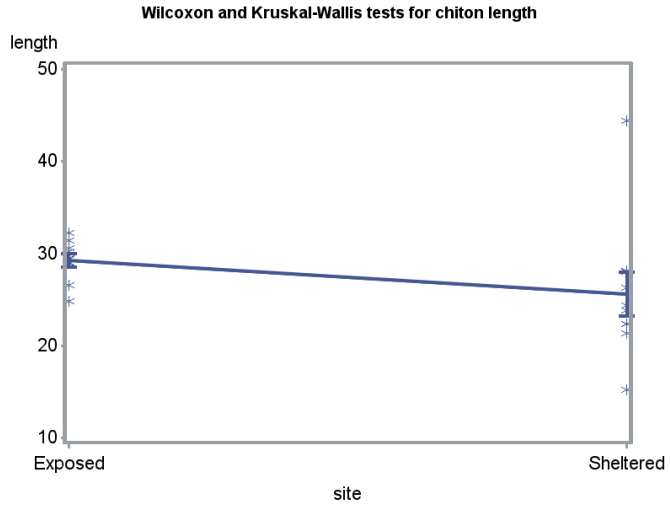

Figure 16.3: Means ± standard errors and individual data points for the Example 1 data.

16.2 Kruskal-Wallis test

The Kruskal-Wallis test is an extension of rank methods to one-way designs with three or more groups. The null and alternative hypotheses are similar to the Wilcoxon test, with the cumulative distributions for the different groups the same under H_0, and differing by shift parameters under H_1. The Kruskal-Wallis test is sensitive to these shifts as well as differences among the means of the groups.

The Kruskal-Wallis test statistic H is calculated using the ranks of the observations across all groups. Suppose we have a different groups, and for simplicity assume the same sample size n for each group. The Kruskal-Wallis test statistic is

$$H = \frac{12n}{an(an+1)} \sum_{i=1}^{a} \left(\frac{\sum_{j=1}^{n} R_{ij}}{n} - \frac{an+1}{2} \right)^2 \tag{16.6}$$

(Conover 1999; Hollander et al. 2014). Note that the left term in parentheses is the mean rank for each group, while the right one is the mean rank across all the groups. This implies that H will become large when the mean rank differs among groups, similar to the way differences in the group means affect the F statistic for one-way ANOVA. In fact, the Kruskal-Wallis statistic can be derived from the F test by substituting ranks for the observations (Bickel & Doksum 1977). A more complex form of H is used when sample sizes are unequal, or when there are ties in the data. Under H_0, H has approximately a χ^2 distribution with $a-1$ degrees of freedom.

Sample calculations

We will illustrate the Kruskal-Wallis test using both the Example 1 and 2 data sets. For Example 1, we have two groups with ten observations each, so $a = 2$ and $n = 10$. The summed ranks for the two groups are 70 (Sheltered)

and 140 (Exposed). It follows that

$$\begin{aligned} H &= \frac{12 \cdot 10}{2 \cdot 10(2 \cdot 10 + 1)} \left[\left(\frac{70}{10} - \frac{2 \cdot 10 + 1}{2} \right)^2 + \left(\frac{140}{10} - \frac{2 \cdot 10 + 1}{2} \right)^2 \right] \\ &= \frac{120}{420} \left[(7 - 10.5)^2 + (14 - 10.5)^2 \right] \\ &= 0.2857 \left[12.25 + 12.25 \right] \\ &= 7.00. \end{aligned}$$

The degrees of freedom are $a - 1 = 2 - 1 = 1$. From Table C, we find that $P < 0.01$, and so the Exposed and Sheltered chitons are significantly different in length ($H = 7.00, df = 1, P < 0.01$).

The Example 2 data involves chitons collected from three different islands ($a = 3$), with ten chitons sampled per island ($n = 10$). The summed ranks for the three islands are 157, 142, and 166. From this information, we calculate that

$$\begin{aligned} H &= \frac{12 \cdot 10}{3 \cdot 10(3 \cdot 10 + 1)} \\ &\quad \cdot \left[\left(\frac{157}{10} - \frac{3 \cdot 10 + 1}{2} \right)^2 + \left(\frac{142}{10} - \frac{3 \cdot 10 + 1}{2} \right)^2 + \left(\frac{166}{10} - \frac{3 \cdot 10 + 1}{2} \right)^2 \right] \\ &= \frac{120}{930} \left[(15.7 - 15.5)^2 + (14.2 - 15.5)^2 + (16.6 - 15.5)^2 \right] \\ &= 0.129 \left[0.04 + 1.69 + 1.21 \right] \\ &= 0.38. \end{aligned}$$

The degrees of freedom are $a - 1 = 3 - 1 = 2$. From Table C, we find that $P < 0.9$. There was no significant difference in length among the three islands ($H = 0.38, df = 2, P < 0.9$).

16.2.1 Kruskal-Wallis test for Example 1 - SAS demo

The Kruskal-Wallis test is automatically calculated when the `wilcoxon` option for `proc npar1way` is used (see previous SAS output). We see there is a highly significant difference in length betwee the Sheltered and Exposed sites ($H = 7.00, df = 1, P = 0.0082$).

16.2.2 Kruskal-Wallis test for Example 2 - SAS demo

The Kruskal-Wallis test for the Example 2 data is shown below. There was no significant difference in length among the three islands ($H = 0.38, df = 2, P = 0.8272$). Note that an exact version of this test is also provided ($P = 0.8386$).

SAS Program

```
* KWtest_chitons_3islands.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Kruskal-Wallis test for chiton length';
data chitons;
        input island $ length;
        datalines;
Lobos                23.86
Lobos                20.20
Lobos                29.32
Lobos                23.56
Lobos                24.32

etc.

;
run;
* Print data set;
proc print data=chitons;
run;
* Plot means, standard error, and observations;
proc gplot data=chitons;
        plot length*island / vaxis=axis1 haxis=axis1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Kruskal-Wallis/Wilcoxon tests;
proc npar1way wilcoxon data=chitons;
        class island;
        var length;
        exact wilcoxon;
run;
quit;
```

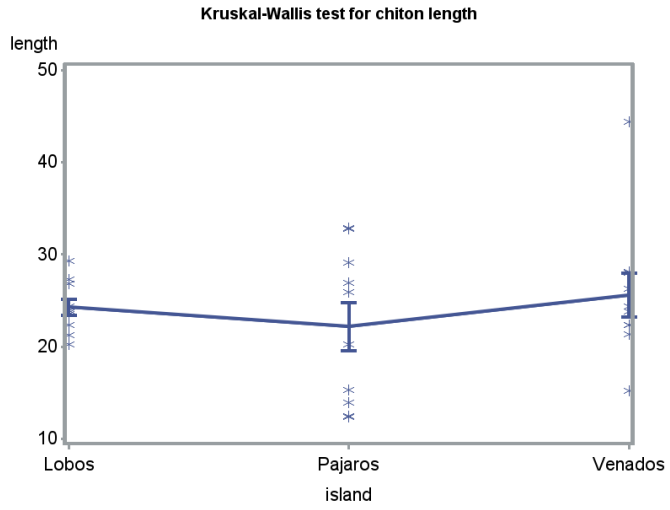

Figure 16.4: Means ± standard errors and individual data points for the Example 2 data.

SAS Output

Kruskal-Wallis test for chiton length 1
10:24 Wednesday, January 7, 2015

Obs	island	length	length Rank
1	Lobos	23.86	16
2	Lobos	20.20	6
3	Lobos	29.32	27
4	Lobos	23.56	13
5	Lobos	24.32	17

etc.

Kruskal-Wallis test for chiton length 2
10:24 Wednesday, January 7, 2015

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable length
Classified by Variable island

island	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Lobos	10	157.0	155.0	22.730303	15.70
Pajaros	10	142.0	155.0	22.730303	14.20
Venados	10	166.0	155.0	22.730303	16.60

Kruskal-Wallis Test

Chi-Square		0.3794
DF		2
Asymptotic Pr >	Chi-Square	0.8272
Exact	Pr >= Chi-Square	0.8386

16.3 Kolmogorov-Smirnov test

The Kolmogorov-Smirnov test is a nonparametric procedure used to compare the distributions of two samples. Let $F_1(y)$ be the cumulative distribution function for the first group, while $F_2(y)$ is the second. The null hypothesis for the Kolmogorov-Smirnov test is $H_0 : F_2(y) = F_1(y)$, which means that the two groups have the same distribution. The alternative hypothesis is $H_1 : F_2(y) \neq F_1(y)$ for some y, implying there is some difference in the distributions, which could involve their location, general shape, variance, and so forth. This is a broader alternative hypothesis than the rank tests we examined earlier, where the distributions had the same shape but differed by location.

The Kolmogorov-Smirnov test statistic is calculated using the empirical distribution functions of the two samples. These are the empirical counterparts of the distribution functions defined for distributions like the normal (see Chapter 6). For a sample with n_i observations, the empirical distribution function is defined as

$$G_i(y) = \frac{\text{Number of } Y_{ij} \text{ values} \leq y}{n_i}. \tag{16.7}$$

$G_i(y)$ increases in a step-like fashion as y increases, with a jump occurring at every value of Y_{ij} (Conover 1999; Hollander et al. 2014). Fig. 16.5 shows these functions for the two samples in Example 1. The Kolmogorov-Smirnov test uses the maximum vertical distance between the two functions as the test statistic. The distance is defined using the formula

$$D = \max_y |G_1(y) - G_2(y)| \tag{16.8}$$

(Conover 1999; Hollander et al. 2014). D is the largest distance between $G_1(y)$ and $G_2(y)$ over all values of y, with the absolute value making it a positive quantity. We would then reject H_0 for sufficiently large values of D. The P value for the test can calculated exactly for small sample sizes, and there is also a large sample approximation for the test. We will let SAS handle the details. This test can also be used when there ties in the observations, in which case it is conservative, meaning it is less likely to reject H_0 (Hollander et al. 2014).

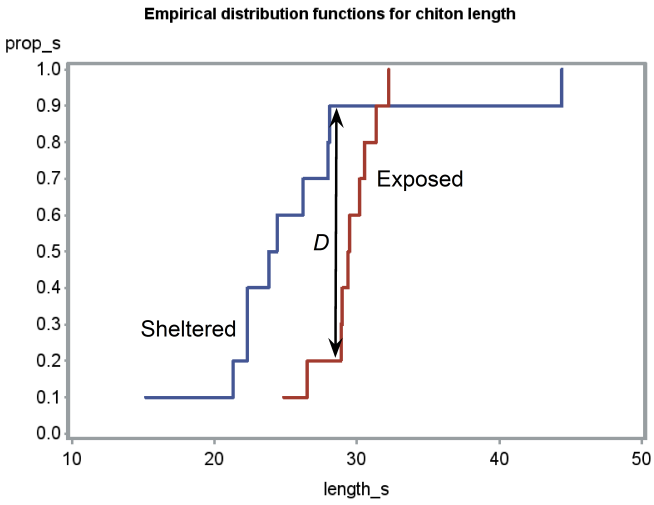

Figure 16.5: Empirical distribution functions for the Example 1 data. Also shown is the maximum value of D for the two samples.

16.3.1 Kolmogorov-Smirnov test for Example 1 - SAS demo

The SAS procedure `npar1way` can also be used for the Kolmogorov-Smirnov test (SAS Institute Inc. 2014a). It is invoked by adding the `edf` option in the `proc npar1way` statement (see program below). An exact version of test can also be generated using the line `exact ks`. The program also includes `proc gchart` code to generate histograms of the two groups (SAS Institute Inc. 2014b). This seems more appropriate for the Kolmogorov-Smirnov test than plotting the means, because this test can detect differences in both shape and location. Examining the SAS output, we see that $D = 0.7$ (see also Fig. 16.5). The P value for the exact version of the test is significant ($P = 0.0123$), implying there is some difference in the distributions of the two samples. The graph generated by `proc gchart` illustrates these differences (Fig. 16.6). See program and output below.

SAS Program

```
* KStest_chitons_Venados.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Kolmogorov-Smirnov test for chiton length';
data chitons;
        input site :$10. length;
        datalines;
Sheltered        44.39
Sheltered        22.30
Sheltered        21.31
Sheltered        23.80
Sheltered        26.23

etc.

;
run;
* Print data set;
proc print data=chitons;
run;
* Histograms for the two groups;
proc gchart data=chitons;
        vbar length / group=site axis=axis1 gaxis=axis1 maxis=axis2;
        axis1 label=(height=2) value=(height=2) width=3 minor=none;
        axis2 label=(height=1.5) value=(height=1.5) width=1.5;
run;
* Kolmogorov-Smirnov test;
proc npar1way edf data=chitons;
        class site;
        var length;
        exact ks;
run;
quit;
```

SAS Output

```
                Kolmogorov-Smirnov test for chiton length                  1
                                        10:24 Wednesday, January 7, 2015

                        Obs      site       length

                          1    Sheltered     44.39
                          2    Sheltered     22.30
                          3    Sheltered     21.31
                          4    Sheltered     23.80
                          5    Sheltered     26.23
etc.

                Kolmogorov-Smirnov test for chiton length                  2
                                        10:24 Wednesday, January 7, 2015

                          The NPAR1WAY Procedure

                    Kolmogorov-Smirnov Test for Variable length
                            Classified by Variable site

                                      EDF at    Deviation from Mean
            site            N       Maximum        at Maximum

            Sheltered      10         0.900          1.106797
            Exposed        10         0.200         -1.106797
            Total          20         0.550

                 Maximum Deviation Occurred at Observation 7
                      Value of length at Maximum = 28.10

                         KS  0.3500    KSa  1.5652


                        Kolmogorov-Smirnov Two-Sample Test

                          D = max |F1 - F2|      0.7000
                          Asymptotic Pr >  D     0.0149
                          Exact      Pr >= D     0.0123

                          D+ = max (F1 - F2)     0.7000
                          Asymptotic Pr >  D+    0.0074
                          Exact      Pr >= D+    0.0062
```

```
D- = max (F2 - F1)     0.1000
Asymptotic Pr >  D-    0.9048
Exact      Pr >= D-    0.9091
```

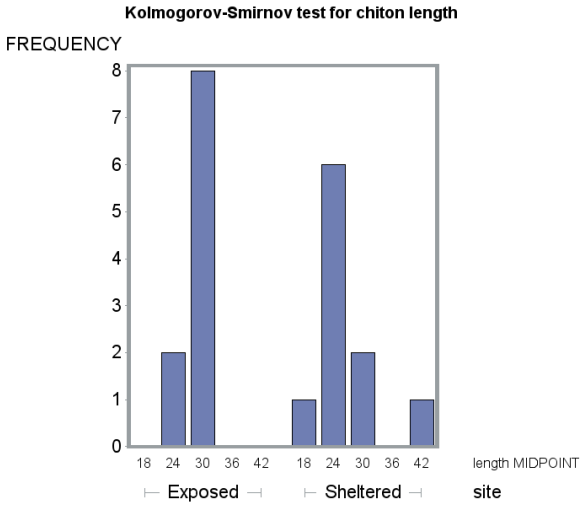

Figure 16.6: Histograms showing the distribution of lengths for the Example 1 data.

16.4 Randomization tests

Randomization tests are another common kind of nonparametric test used for one-way designs, as well as more complex ones (Hinkelmann & Kempthorne 1994; Manly 1997). The null hypothesis for these tests is different from other tests we have considered, which involved statements about probability distributions and their parameters. For randomization tests, the null hypothesis is that all possible permutations (rearrangements) of the data among groups are equally likely, given no treatment or group effects, with the observed data being one such arrangement (Hinkelmann & Kempthorne 1994; Manly 1997). These tests commonly employ a parametric test statistic to examine the null hypothesis, one that is sensitive to potential differences among groups. For one-way designs, the F_s statistic from one-way ANOVA (Chapter 11) is often

used to detect differences in the group means. To conduct a randomization test using this statistic, we first calculate the value of $F_s(obs)$ for the observed data. Similar to one-way ANOVA, we then need to determine if $F_s(obs)$ is sufficiently large to consider rejecting H_0. This is accomplished by permuting or rearranging the observations many times across groups, and calculating the value of F_s for each permutation. The justification for this procedure follows directly from the definition of H_0. The P value for the test is defined as the proportion of the F_s values greater than or equal to $F_s(obs)$, including $F_s(obs)$ as one of the values.

For small data sets it may be possible to carry out all possible permutations, but for larger data sets this may be impractical. Instead, the observations are randomly rearranged across groups a large number of times, in effect drawing a random sample from all possible permutations. The collection of F_s values obtained by this process is called the **randomization distribution**. How many of these randomizations are needed to generate an accurate P value for the test? Some guidance is provided by Manly (1997), who suggests that 1000 randomizations should be sufficient for $P \approx 0.05$, and 5000 for $P \approx 0.01$.

An interesting feature of randomization tests is that the randomization distribution of F_s under H_0 can be approximated by the parametric F distribution (Hinkelmann & Kempthorne 1974) under some conditions. This provides another justification for the use of F tests when the normality assumption of these tests is violated.

We will use data on nematode intensities for male vs. female bobcats (*Lynx rufus*) to illustrate randomization tests. The sampled bobcats were recent roadkill collected from the Southern Illinois region (Francisco A. Jimenez-Ruiz and Eliot A. Zieman, unpublished data). The guts were examined for nematodes as well as other parasites, and the total number counted (Table 16.3). These data have many zeroes as well as large values, as is common for parasite intensity data. The data are clearly non-normal and so a nonparametric test seems warranted.

Table 16.3: Example 3 - Number of nematode parasites found in the gut of male and female bobcats collected from Southern Illinois .

Sex	Nematodes	Sex	Nematodes	Sex	Nematodes	Sex	Nematodes
F	0	F	0	M	6	M	8
F	8	F	5	M	10	M	0
F	0	F	0	M	1	M	60
F	0	F	0	M	0	M	25
F	0	F	0	M	5	M	1
F	0	F	11	M	59	M	0
F	0	F	0	M	2	M	74
F	1	F	5	M	3	M	3
F	2	F	11	M	0	M	1
F	1	F	0	M	44	M	15
F	1	F	24	M	1	M	0
F	6	F	13	M	1	M	7
F	1	F	2	M	0	M	0
F	6			M	2	M	0
F	2			M	17		
F	1			M	5		
F	13			M	3		
F	0			M	26		
F	0			M	20		
F	7			M	3		

16.4.1 Randomization test for Example 3 - SAS demo

We will analyze the bobcat data using both one-way ANOVA and the analogous randomization test, comparing the parasite intensities for male vs. female cats. The SAS program below first generates a graph showing the mean intensities for both sexes, then conducts a standard one-way ANOVA. We see that the mean intensity for male bobcats is higher than females (Fig. 16.7), and the ANOVA shows this difference is significant ($F_{1,65} = 5.50, P = 0.0221$).

The program then uses two SAS macro programs to conduct the randomization test (Cassell 2002). SAS macros are chunks of code that are used to carry out custom calculations, ones not available in standard SAS procedures (SAS Institute Inc. 2014c). They are inserted into a main program through the use of `%include` statements, which point to the file locations of the macros. Note that the percent sign (%) in tells SAS that a particular line contains macro code. The first macro, `%rand_gen.sas`, is used to generate the desired number of random permutations of the data. Once the macro is included in the program, it can be called using the following arguments. The input data set is specified using the `indata=parasites` statement, while the output data set specified by `outdata=outrand` contains all the randomizations. The statement `numreps=5000` sets the number of randomizations, with the dependent variable specified by `depvar=nematodes`.

The next step in the randomization test is to conduct a one-way ANOVA for each one of the randomizations, as well as the original data set. This is accomplished using `proc glm` with a `by replicate` statement. The variable `replicate` is generated by the `rand_gen` macro to number the different randomizations. In addition, a data file containing the statistical output of the ANOVA is specified using the statement `outstat=outstat1`. The ANOVA for the original data corresponds to a `replicate = 0` in this output file. The `noprint` option is used to suppress the printing of each ANOVA.

The last step in the randomization test uses the second macro, `%rand_anl.sas`, to determine the P value for the test. The data file containing the statistical output from `proc glm` is specified using a `randdata=outstat1` argument. The `where=_source_='sex' and _type_='SS3'` argument tells the macro which part of the statistical output to use, in particular the test associated with the sex effect and Type III sum of squares. The `testprob=prob` statement tells the macro to use the P value for this F test in calculating the P value for the randomization test. The macro uses the P rather than F_s value to

provide some additional flexibility for other kinds of tests (Cassell 2002). As the F_s and P value for the ANOVA are related, it yields the same result. The P value for the randomization test is provided in the SAS log. The `testlabel=Model F test` argument provides some labeling for this output. Examining the SAS log, we find that the randomization test is significant ($P = 0.0172$). The P value for this test is smaller than the one found using one-way ANOVA, and makes no assumptions about the distribution of the data.

The remaining portion of the program generates a graph of the randomization distribution of F_s, and displays the value of this statistic for the original distribution. We see that the original value of F_s lies far above most of the randomizations. This illustrates the pattern for a significant randomization test. For a non-significant test, we would see an F_s value that is more central within the randomization distribution.

SAS Program

```
* Randtest_bobcat_parasites.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Randomization test for bobcat parasites';
data parasites;
        input nematodes sex $;
        datalines;
0        F
8        F
0        F
0        F
0        F

etc.

;
run;
* Print data set;
proc print data=parasites;
run;
* Plot means, standard error, and observations;
proc gplot data=parasites;
        plot nematodes*sex / vaxis=axis1 haxis=axis1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* One-way ANOVA;
proc glm data=parasites;
        class sex;
        model nematodes = sex;
run;
* Include two macros for randomization test;
%include "C:\Users\John\Documents\My Documents\Nonparametric\rand_gen.sas";
%include "C:\Users\John\Documents\My Documents\Nonparametric\rand_anl.sas";
* One-way ANOVA as a randomization test;
%rand_gen(indata=parasites,outdata=outrand,
depvar=nematodes,numreps=5000)
proc glm data=outrand noprint outstat=outstat1;
        by replicate;
        class sex;
        model nematodes = sex;
run;
%rand_anl(randdata=outstat1,
```

```
where=_source_='sex' and _type_='SS3',
testprob=prob,testlabel=Model F test)
* Extract F values from outstat1 for null distribution graph;
data nulldist;
        set outstat1;
        if _type_="SS3";
        * Assign original F value to macro variable;
        if replicate=0 then call symput('F',F);
run;
* Null distribution;
title2 "Null distribution";
proc univariate data=nulldist noprint;
        var F;
        histogram F / vscale=count wbarline=3 waxis=3 height=4 href=&F whref=3
        hreflabel="F";
run;
quit;
```

SAS Output

```
Randomization test for bobcat parasites                    1
                                  10:24 Friday, January 9, 2015

        Obs    nematodes    sex

          1         0        F
          2         8        F
          3         0        F
          4         0        F
          5         0        F


Randomization test for bobcat parasites                    3
                                  10:24 Friday, January 9, 2015

                The GLM Procedure

           Class Level Information

     Class         Levels    Values

     sex                2    F M


Number of Observations Read          67
```

Number of Observations Used 67

Randomization test for bobcat parasites 4
10:24 Friday, January 9, 2015

The GLM Procedure

Dependent Variable: nematodes

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1122.49709	1122.49709	5.50	0.0221
Error	65	13274.57754	204.22427		
Corrected Total	66	14397.07463			

R-Square	Coeff Var	Root MSE	nematodes Mean
0.077967	183.4248	14.29071	7.791045

Source	DF	Type I SS	Mean Square	F Value	Pr > F
sex	1	1122.497087	1122.497087	5.50	0.0221

Source	DF	Type III SS	Mean Square	F Value	Pr > F
sex	1	1122.497087	1122.497087	5.50	0.0221

SAS Log

```
Randomization test for Model F test where _source_='sex' and _type_='SS3'
 has significance level of 0.0172
```

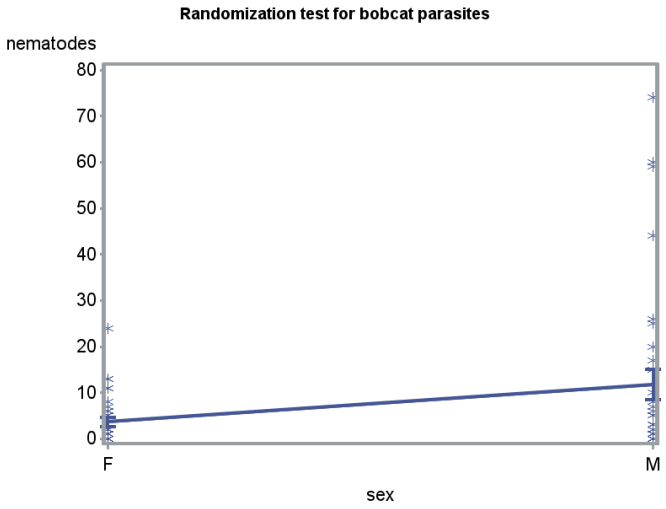

Figure 16.7: Means ± standard errors and individual data points for the Example 1 data.

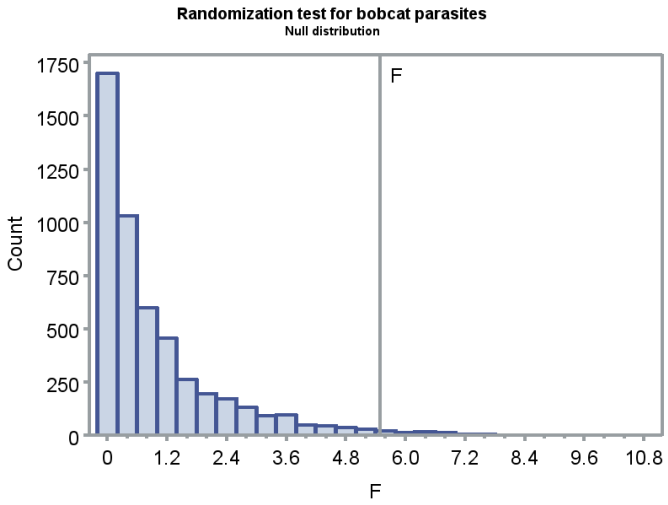

Figure 16.8: Distribution of F under the null hypothesis, obtained through randomization.

16.5 Limitations of nonparametric tests

While nonparametric tests can be useful for non-normal data, they do have some drawbacks. One is that the number of designs that have nonparametric tests are fairly limited. We have seen tests nonparametric tests analogous to one-way ANOVA and two-sample t tests. There is also a rank test for randomized block designs called Friedman's test, as well as procedures for multiple comparisons (Hollander et al. 2014). Unfortunately, for more complex designs there are few available procedures.

Although nonparametric tests are not based on a particular distribution, they do make some assumptions. Consider the null and alternative hypotheses for the Wilcoxon test. The two groups are assumed to have the same cumulative distribution function, differing only by a shift parameter Δ. This implies the two groups have the same variance under both hypotheses, similar to parametric ones. When the variances are unequal as well as the sample sizes, both parametric and nonparametric tests may not be valid (Stewart-Oaten 1995). In particular, they may not have the correct Type I error rate.

Table 16.4 illustrates how unequal variances and sample sizes can affect the Type I error rate. It summarizes a simulation study comparing the validity of several different methods of comparing samples from two groups, including parametric and nonparametric methods. The first six columns give the theoretical mean, variance, and the sample sizes for the two groups. The simulated data were normally distributed with these parameters. Each data set was then analyzed using a two-sample t test, a Welch t test that implements a correction for unequal variances, the Wilcoxon test, and a randomization test. Any significant differences detected by these tests are Type I errors, because the two groups have the same mean. A total of 5000 simulated data sets were generated and analyzed. The proportion of simulated data sets showing significant results is an estimate of the Type I error rate (α) for each test. If the test is conducted using $\alpha = 0.05$, for example, we would expect this proportion of the simulations to be significant.

Regardless of differences in the variance between the two groups, when the sample sizes are equal all methods yield a Type I error rate near the nominal $\alpha = 0.05$ level. When sample sizes are unequal, the t test, Wilcoxon test, and the randomization test all yield Type I error rates higher or lower than $\alpha = 0.05$. Note that the pattern depends on which group (high or low variance) has the smaller sample size. Thus, the validity of these procedures

depends on equal variances, especially when sample sizes are unequal across groups. This assumption needs to be carefully examined within applying both parametric and nonparametric tests.

The only valid test in this scenario was the Welch t test, which employs a correction for unequal variances. The correction alters the degrees of freedom for the test, based on the sample sizes and variances of the two groups (Stuart et al. 1999). It is conducted automatically by `proc ttest` in SAS, with the output labeled `Satterthwaite` (see Chapter 11). There is also a similar procedure for one-way designs called Welch ANOVA. It can conducted under `proc glm` using the `welch` option for the `means` statement.

Table 16.4: Effect of unequal variances and sample sizes on the estimated Type I error rate for common parametric and nonparametric tests, using $\alpha = 0.05$ for all tests. See text for further details.

μ_1	σ_1^2	n_1	μ_2	σ_2^2	n_2	t	Welch	Wilcoxon	Randomization
10	1	10	10	1	10	0.0474	0.0454	0.0422	0.0484
10	1	10	10	2	10	0.0516	0.0504	0.0514	0.0524
10	1	5	10	2	15	0.0208	0.0510	0.0236	0.0214
10	1	15	10	2	5	0.0956	0.0578	0.0662	0.0954
10	1	10	10	4	10	0.0510	0.0452	0.0464	0.0510
10	1	5	10	4	15	0.0104	0.0494	0.0170	0.0108
10	1	15	10	4	5	0.1588	0.0574	0.0836	0.1598

16.6 Problems

1. Using the Example 3 data, conduct a Wilcoxon test comparing parasite intensity in male vs. female bobcats. How do the results compare to the randomization test for these data in the text?

2. Data were also collected on the number of cestode parasites found in the bobcats from Example 3 (see below). Cestodes are another common type of gut parasite. Conduct a randomization test comparing the cestode intensity for male vs. female bobcats.

Sex	Cestodes	Sex	Cestodes	Sex	Cestodes	Sex	Cestodes
F	1	F	0	M	9	M	3
F	7	F	7	M	31	M	2
F	9	F	6	M	5	M	2
F	0	F	33	M	0	M	0
F	1	F	2	M	10	M	3
F	1	F	1	M	6	M	7
F	8	F	18	M	0	M	2
F	0	F	6	M	0	M	5
F	0	F	1	M	6	M	1
F	32	F	14	M	9	M	1
F	11	F	12	M	6	M	4
F	4	F	6	M	18	M	0
F	3	F	0	M	4	M	3
F	13			M	9	M	1
F	2			M	6		
F	2			M	5		
F	12			M	17		
F	4			M	4		
F	1			M	8		
F	3			M	11		

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Chapter 17

Linear Regression

Linear regression is a statistical method for examining the relationship between two continuous variables, typically called Y and X. It is usually assumed there is a causal relationship between Y and X, with different values of X causing changes in Y. For this reason, Y is often called the **dependent variable** while X is the **independent variable** in the analysis. The variable X is sometimes under the control of the investigator, similar to a fixed effect in ANOVA, but can also be a random variable. For example, we might be interested in the effect of temperature on the growth rate of fish. Temperature might cause an increased growth rate, but clearly growth rate cannot influence temperature. This causal relationship is a distinguishing feature of regression as opposed to **correlation** analysis. Correlation is used to examine the **association** between two continuous variables and no causal direction is assumed (see Chapter 18). For example, we might be interested in the relationship between fish length and weight but there is no obvious causal relationship between the two variables.

Although linear regression assumes a different statistical model than ANOVA, there are a number of similarities in the estimation process and statistical tests for the two types. For example, both ANOVA and linear regression models use likelihood methods for parameter estimation and test construction, and employ F statistics to test various hypotheses. Both are examples of **general linear models**, in which the model parameters and error terms enter the model in an additive (linear) fashion.

What do the data look like for linear regression? As an example, we will use data from study on the southern pine beetle, *Dendroctonus frontalis* (Reeve et al. 1998). The study used cages to experimentally manipulate the

density of *D. frontalis* attacking pine trees. The independent or X variable in the study was the number of beetles added to the cages, while the dependent or Y variable was the number of attacks the beetles made through the bark into the tree (Table 17.1). Besides establishing the relationship between the two variables, there was also some interest in predicting the attack density as a function of the number of beetles added to the cage, for use in future studies. The notation Y_i and X_i refers to the values for the ith pair of numbers. For example, $Y_2 = 2.660$ and $X_2 = 1.000$. Fig. 17.2 shows there is a positive relationship between the two variables, with attack density (Y) increasing as more beetles are added to the cages (X).

Table 17.1: Example 1 - Observations from an experiment in which different numbers of the bark beetle *D. frontalis* were introduced into cages and the resulting attack density recorded (Reeve et al. 1998). Here Y is the attack density (attacks per 100 cm^2 of bark) while X is the number of beetles added ($\times 10^3$). Also shown are some preliminary calculations for the regression analysis.

i	Y_i	X_i	$(X_i - \bar{X})^2$	$(Y_i - \bar{Y})(X_i - \bar{X})$	$\hat{Y}_i = \hat{\alpha} + \hat{\beta} X_i$	$Y_i - \hat{Y}_i$	$(Y_i - \hat{Y}_i)^2$	$(\hat{Y}_i - \bar{Y})^2$	$(Y_i - \bar{Y})^2$
1	1.250	0.100	0.740	2.779	2.206	-0.956	0.914	5.176	10.440
2	2.660	1.000	0.002	-0.073	4.586	-1.926	3.711	0.011	3.316
3	7.330	2.000	1.081	2.962	7.231	0.099	0.010	7.563	8.116
4	1.600	1.250	0.084	-0.835	5.248	-3.648	13.305	0.588	8.301
5	2.620	0.500	0.212	0.856	3.264	-0.644	0.415	1.481	3.464
6	1.000	0.200	0.578	2.646	2.471	-1.471	2.162	4.042	12.118
7	4.340	1.500	0.291	-0.076	5.909	-1.569	2.461	2.038	0.020
8	5.230	0.750	0.044	-0.157	3.925	1.305	1.702	0.309	0.561
9	2.500	0.250	0.504	1.407	2.603	-0.103	0.011	3.528	3.925
10	3.250	0.500	0.212	0.567	3.264	-0.014	0.000	1.481	1.516
11	6.000	2.000	1.081	1.579	7.231	-1.231	1.516	7.563	2.307
12	4.750	1.500	0.291	0.145	5.909	-1.159	1.343	2.038	0.072
13	2.500	0.250	0.504	1.407	2.603	-0.103	0.011	3.528	3.925
14	8.750	2.000	1.081	4.439	7.231	1.519	2.307	7.563	18.223
15	6.000	1.000	0.002	0.060	4.586	1.414	1.998	0.011	2.307
16	5.000	0.500	0.212	-0.239	3.264	1.736	3.014	1.481	0.269
17	7.150	1.000	0.002	0.106	4.586	2.564	6.572	0.011	7.123
18	6.750	1.500	0.291	1.225	5.909	0.841	0.708	2.038	5.158
19	7.500	1.500	0.291	1.630	5.909	1.591	2.532	2.038	9.114
20	2.500	0.500	0.212	0.912	3.264	-0.764	0.584	1.481	3.925
21	5.000	2.000	1.081	0.540	7.231	-2.231	4.979	7.563	0.269
22	2.250	0.250	0.504	1.585	2.603	-0.353	0.124	3.528	4.978

i	Y_i	X_i	$(X_i - \bar{X})^2$	$(Y_i - \bar{Y})(X_i - \bar{X})$	$\hat{Y}_i = \hat{\alpha} + \hat{\beta} X_i$	$Y_i - \hat{Y}_i$	$(Y_i - \hat{Y}_i)^2$	$(\hat{Y}_i - \bar{Y})^2$	$(Y_i - \bar{Y})^2$
23	1.250	0.125	0.698	2.699	2.272	-1.022	1.045	4.879	10.440
24	4.750	1.000	0.002	0.011	4.586	0.164	0.027	0.011	0.072
25	4.500	0.250	0.504	-0.013	2.603	1.897	3.599	3.528	0.000
26	9.560	2.000	1.081	5.281	7.231	2.329	5.423	7.563	25.795
27	5.000	0.500	0.212	-0.239	3.264	1.736	3.014	1.481	0.269
$\sum$			11.798	31.203			63.486	82.528	146.014

17.1 Linear regression model

Suppose that we want to model the observations in studies like Example 1, where Y is observed for a number of X values. Let Y_i and X_i stand for the ith pair of values. The linear regression model takes the form

$$Y_i = \alpha + \beta X_i + \epsilon_i, \tag{17.1}$$

where α is the intercept and β the slope of a line, while $\epsilon_i \sim N(0, \sigma^2)$ (Searle 1971). Thus, the linear regression model represents the relationship between Y_i and X_i as a line on which random deviations due to natural variability (ϵ_i) are imposed.

For the ith pair of values, we have $E[Y_i] = \alpha + \beta X_i$ and $Var[Y_i] = \sigma^2$ using the rules for expected values and variances. Thus, $Y_i \sim N(\alpha + \beta X_i, \sigma^2)$ for any X_i value. The behavior of the linear regression model can be illustrated by plotting this distribution across a range of X_i values . When β is positive, the mean of Y_i will increase as X_i increases (Fig. 17.1), while if β is negative the mean would decrease (not shown). The variance remains the same for all X_i. Note that the linear regression model has assumptions similar to the ANOVA models – the observations are assumed be normal and have the same variance.

The usual objectives in linear regression are to estimate the model parameters, especially the slope β, and then test whether the slope is different from zero. In particular, we will be interested in testing $H_0 : \beta = 0$. If a test of this hypothesis is significant this suggests there is some relationship (positive or negative) between Y and X. The alternative hypothesis can be written as $H_1 : \beta \neq 0$. It is also possible to test whether the intercept differs from zero although this is less common. We will discuss how these parameters are estimated and hypotheses tested in the next section.

17.2 Linear regression and likelihood

The maximum likelihood method can be used to estimate the parameters for regression models, similar to ANOVA models. Suppose we have n observations conforming to the linear regression model

$$Y_i = \alpha + \beta X_i + \epsilon_i. \tag{17.2}$$

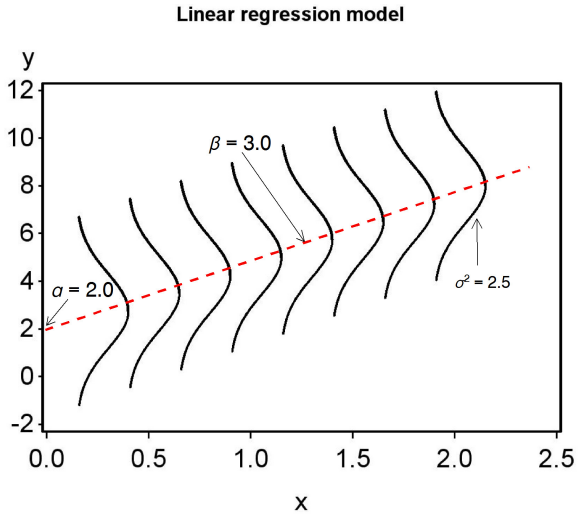

Figure 17.1: The linear regression model plotted across a range of X values, with $\alpha = 2.0$, $\beta = 3.0$, and $\sigma^2 = 2.5$.

This model has three parameters to estimate, namely α, β, and σ^2 (the variance of ϵ_i). What would the likelihood function be for these data? Consider the first observation in the *D. frontalis* cage experiment, for which $Y_1 = 1.250$ and $X_1 = 0.100$. For this observation, the model states that $Y_1 \sim N(\alpha + \beta X_1, \sigma^2)$, and so the likelihood would be

$$L_1 = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(Y_1-(\alpha+\beta X_1))^2}{\sigma^2}} \qquad = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{(1.250-(\alpha+\beta 0.100))^2}{\sigma^2}} \tag{17.3}$$

The likelihood L_i for the ith observation would be similar, and the overall likelihood is defined as their product:

$$L(\alpha, \beta, \sigma^2) = L_1 \times L_2 \times \ldots \times L_n. \tag{17.4}$$

Finding the maximum likelihood estimates involves maximizing this quantity with respect to the parameters α, β, and σ^2. Using some calculus to find the maximum, it can be shown that estimators of these parameters are

$$\hat{\beta} = \frac{\sum_{i=1}^{n}(Y_i - \bar{Y})(X_i - \bar{X})}{\sum_{i=1}^{n}(X_i - \bar{X})^2}, \tag{17.5}$$

$$\hat{\alpha} = \bar{Y} - \hat{\beta}\bar{X} \tag{17.6}$$

and

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^{n}(Y_i - (\hat{\alpha} + \hat{\beta}X_i))^2}{n-2} = \frac{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}{n-2}. \tag{17.7}$$

Here $\hat{Y}_i = \hat{\alpha} + \hat{\beta}X_i$, the value of Y_i predicted by the model at X_i.

We can gain some insight into the estimation process by rearranging the likelihood function. It can be written in the form

$$L(\alpha, \beta, \sigma^2) = \left(\frac{1}{\sqrt{2\pi\sigma^2}}\right)^n e^{-\frac{1}{2}\frac{\sum_{i=1}^{n}(Y_i-(\alpha+\beta X_i))^2}{\sigma^2}}. \tag{17.8}$$

Now examine the terms in the sum, which are of the form $(Y_i - (\alpha + \beta X_i))^2$. Values of α and β that minimize these terms will make the overall likelihood larger, because of the negative sign in the exponent. The likelihood will reach its maximum when this sum is smallest. Thus, values of α and β that minimize

$$\sum_{i=1}^{n}(Y_i - (\alpha + \beta X_i))^2 \tag{17.9}$$

are the maximum likelihood estimates. These estimates are also called **least squares** estimates because they minimize the sum of these squared terms. In fact, we could directly estimate α and β using this method without recourse to likelihood (Searle 1971). The two methods yield the same results when the data have a normal distribution.

A likelihood ratio test for linear regression can be constructed as follows. Suppose we want to test $H_0 : \beta = 0$ vs. $H_1 : \beta \neq 0$, the latter implying a linear relationship between Y and X. The statistical model under H_0 would be

$$Y_i = \alpha + \beta X_i + \epsilon_i \tag{17.10}$$

$$= \alpha + \epsilon_i \tag{17.11}$$

because $\beta = 0$ under H_0. The statistical model under H_1 would be the full model including a slope term, namely

$$Y_i = \alpha + \beta X_i + \epsilon_i. \tag{17.12}$$

We would need to find the maximum likelihood estimates under both H_1 (see previous section) and H_0, as well as L_{H_0} and L_{H_1}, the maximum height of

the likelihood function under H_0 and H_1. We would then use the likelihood ratio test statistic

$$\lambda = \frac{L_{H_0}}{L_{H_1}}. \tag{17.13}$$

There is a one-to-one correspondence between $-2\ln(\lambda)$ and the statistic F_s used to test this null hypothesis (McCulloch & Searle 2001).

We can gain further insight into this test by defining various sum of squares and mean squares used to calculate F_s. In particular, we will define SS_{error}, $SS_{regression}$, and SS_{total} and their associated mean squares, which have functions similar to those in ANOVA. We will also summarize the calculations in an ANOVA table.

SS_{error} describes variation in the data around the regression line, or variation not explained by the model. It is defined as

$$SS_{error} = \sum_{i=1}^{n} \left(Y_i - (\hat{\alpha} + \hat{\beta}X_i)\right)^2 = \sum_{i=1}^{n} (Y_i - \hat{Y}_i)^2. \tag{17.14}$$

SS_{error} has $n-2$ degrees of freedom. We can therefore define

$$MS_{error} = \frac{SS_{error}}{n-2} = \hat{\sigma}^2. \tag{17.15}$$

Thus, MS_{error} is equivalent to $\hat{\sigma}^2$, the maximum likelihood estimate of σ^2, the same relationship as found in ANOVA. SS_{error} and MS_{error} will be small if the data lie on a straight line and large if the data are scattered around the line.

$SS_{regression}$ describes variation in the data explained by the regression model. It is defined as

$$SS_{regression} = \sum_{i=1}^{n} (\hat{Y}_i - \bar{Y})^2 \tag{17.16}$$

and has one degree of freedom. We therefore have

$$MS_{regression} = \frac{SS_{regression}}{1} = SS_{regression}. \tag{17.17}$$

$SS_{regression}$ and $MS_{regression}$ will be large if the data have a strong positive or negative slope. To see this, recall that $\hat{Y}_i = \hat{\alpha} + \hat{\beta}X_i$. If the estimated slope

$\hat{\beta}$ is large, the values of $\hat{Y}_i$ will vary strongly as X_i changes and so generate a large sum of squares.

The total sum of squares is defined (as in ANOVA) to be

$$SS_{total} = \sum_{i=1}^{n} (Y_i - \bar{Y})^2 \tag{17.18}$$

and has $n-1$ degrees of freedom. There is also a familiar relationship among the different sums of squares, namely

$$SS_{regression} + SS_{error} = SS_{total}. \tag{17.19}$$

The likelihood ratio statistic used to test $H_0 : \beta = 0$ is defined as

$$F_s = \frac{MS_{regression}}{MS_{error}}. \tag{17.20}$$

Under H_0, F_s has an F distribution with $df_1 = 1$ and $df_2 = n-2$ the degrees of freedom. Given the definitions of $MS_{regression}$ and MS_{error}, we can see that F_s tends to be large when the data have a strong slope (the numerator of this expression) relative to the amount of scatter in the data (the denominator).

We can organize the different sum of squares and mean squares into an ANOVA table for linear regression. It lists the different sources of variation in the data (regression, error, and total), their degrees of freedom, as well as the F test. Table 17.2 shows the general layout for linear regression.

Table 17.2: General ANOVA table for linear regression, showing formulas for different mean squares and the F test.

Source	*df*	Sum of squares	Mean square	F_s
Regression	1	$SS_{regression}$	$MS_{regression} = SS_{regression}/1$	$MS_{regression}/MS_{error}$
Error	$n-2$	SS_{error}	$MS_{error} = SS_{within}/(n-2)$	
Total	$n-1$	SS_{total}		

Table 17.3: ANOVA table for the Example 1 data set, including a P value for the test.

Source	*df*	Sum of squares	Mean square	F_s	P
Regression	1	82.528	82.528	32.504	< 0.001
Error	25	63.486	2.539		
Total	26	146.014			

17.2.1 Sample calculation - $\hat{\beta}$, $\hat{\alpha}$, and F test

We will illustrate the above calculations using the Example 1 data set, where Y is *D. frontalis* attack density and X is the number of beetles added to the cage. We are interested in estimating the slope and intercept (β and α) of the relationship between the two variables, and then testing whether the slope is significantly different from zero ($H_0 : \beta = 0$).

The first step is to calculate the sample mean for both Y and X, and we obtain $\bar{Y} = 4.481$ and $\bar{X} = 0.960$. We then calculate $(X_i - \bar{X})^2$ for each value of X_i (see Table 17.1) and sum these values to obtain

$$\sum_{i=1}^{n}(X_i - \bar{X})^2 = 11.798. \tag{17.21}$$

We then calculate the $(Y_i - \bar{Y})(X_i - \bar{X})$ for each pair of numbers and sum these to obtain

$$\sum_{i=1}^{n}(Y_i - \bar{Y})(X_i - \bar{X}) = 31.203. \tag{17.22}$$

The estimate of β can then be calculated, and we find

$$\hat{\beta} = \frac{\sum_{i=1}^{n}(Y_i - \bar{Y})(X_i - \bar{X})}{\sum_{i=1}^{n}(X_i - \bar{X})^2} = \frac{31.203}{11.798} = 2.645. \tag{17.23}$$

We can then estimate α using the formula

$$\hat{\alpha} = \bar{Y} - \hat{\beta}\bar{X} = 4.481 - 2.645(0.960) = 1.942. \tag{17.24}$$

The next step is to calculate the predicted values of Y_i using the formula $\hat{Y}_i = \hat{\alpha} + \hat{\beta}X_i$, for each value of X_i (see Table 17.1). We then calculate $Y_i - \hat{Y}_i$ in another column, which contains the residuals for each observation. Squaring and summing the residuals, we find

$$SS_{error} = \sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2 = 63.486, \tag{17.25}$$

and

$$MS_{error} = \frac{SS_{error}}{n-2} = \frac{63.486}{27-2} = 2.539. \tag{17.26}$$

We next calculate a column consisting of $(\hat{Y}_i - \bar{Y})^2$ for each observation, then sum these values to obtain

$$SS_{regression} = \sum_{i=1}^{n} (\hat{Y}_i - \bar{Y})^2 = 82.528, \tag{17.27}$$

and so

$$MS_{regression} = SS_{regression}/1 = 82.528. \tag{17.28}$$

We are now in a position to calculate F_s, the statistic used to test $H_0 : \beta = 0$. We have

$$F_s = \frac{MS_{regression}}{MS_{error}} = \frac{82.528}{2.539} = 32.504. \tag{17.29}$$

Under H_0, F_s has an F distribution with $df_1 = 1$ and $df_2 = 27 - 2 = 25$ degrees of freedom. Using Table F, we find the $P < 0.001$. There is a highly significant effect of beetles numbers on the attack density of *D. frontalis* ($F_{1,25} = 32.504, P < 0.001$).

The last column in Table 17.1 calculates $(Y_i - \bar{Y})^2$, the components of SS_{total}. Summing these components we obtain $SS_{total} = 146.014$. It can also be calculated using the formula $SS_{regression} + SS_{error} = SS_{total}$. Table 17.3 shows the completed ANOVA table.

The observations for Example 1 and the fitted linear regression model are shown in Fig. 17.2. The estimation procedure (maximum likelihood or least squares) finds values of α and β that minimize the sum of the squared differences between the data points and the line. In particular, it minimizes the sum of the squared residuals, where the residuals are $Y_i - \hat{Y}_i = Y_i - (\hat{\alpha} + \hat{\beta} X_i)$.

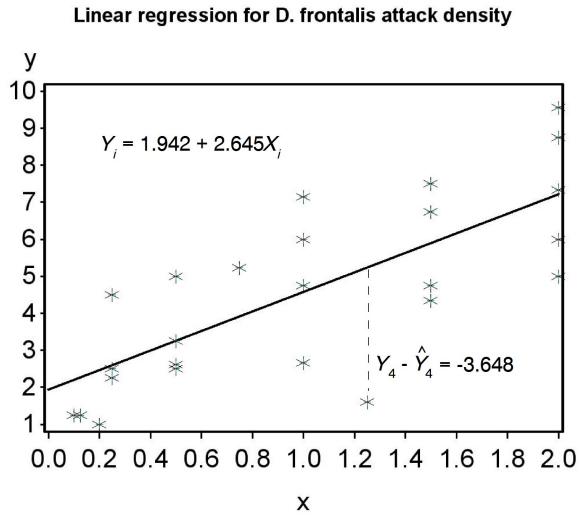

Figure 17.2: Linear regression model fitted to the Example 1 data, where Y is attack density and X is beetles added to the cages. The vertical dashed line shows the residual $Y_4 - \hat{Y}_4 = -3.648$ for the $i = 4$ observation.

17.3 Confidence and prediction intervals

In this section, we will examine confidence intervals for the parameters of the regression model, and for the mean value of Y_i at a given value of X_i. Like other confidence intervals, they provide a measure of the accuracy or reliability of an estimate, with wider intervals indicating lower accuracy (Chapter 9). Another type of interval for linear regression are **prediction intervals**. These are used to set limits for future Y_i values given some value of X_i. See Draper & Smith (1981) for further details.

The confidence interval for the slope β is based on $\hat{\beta}$, the maximum likelihood estimate of β, and the standard error of this estimate $s_{\hat{\beta}}$, given by the formula

$$s_{\hat{\beta}} = \sqrt{\frac{\hat{\sigma}^2}{\sum_{i=1}^{n}(X_i - \bar{X})^2}}, \tag{17.30}$$

where $\hat{\sigma}^2 = MS_{error}$. Note that $s_{\hat{\beta}}$ depends on the scatter of the data around the line ($\hat{\sigma}^2$) as well as the amount of variability in X_i. **A study using a larger range of X_i values will thus provide a more accurate estimate of** β, because it reduces $s_{\hat{\beta}}$. **Increasing the sample size n would also increase the accuracy**, by increasing the sum of squares in the denominator for $s_{\hat{\beta}}$.

It can be shown that the quantity

$$\frac{\hat{\beta} - \beta}{s_{\hat{\beta}}} \tag{17.31}$$

has a t distribution with $n - 2$ degrees of freedom, the same as for MS_{error}. This fact can be used to derive a confidence interval for β. Using Table T, we first find a value of $c_{\alpha,n-2}$ for $n-2$ degrees of freedom such that the following equation is true:

$$P\left[-c_{\alpha,n-2} < \frac{\hat{\beta} - \beta}{s_{\hat{\beta}}} < c_{\alpha,n-2}\right] = 1 - \alpha. \tag{17.32}$$

Rearranging this equation we obtain

$$P\left[\hat{\beta} - c_{\alpha,n-2}s_{\hat{\beta}} < \beta < \hat{\beta} + c_{\alpha,n-2}s_{\hat{\beta}}\right] = 1 - \alpha. \tag{17.33}$$

It follows that the interval

$$(\hat{\beta} - c_{\alpha,n-2}s_{\hat{\beta}}, \hat{\beta} + c_{\alpha,n-2}s_{\hat{\beta}}) \tag{17.34}$$

is a $100(1-\alpha)\%$ confidence interval for β. The center of the confidence interval would be $\hat{\beta}$.

We may also want to test various null hypotheses concerning β. For example, we may want to test $H_0 : \beta = \beta_0$ vs. $H_1 : \beta \neq \beta_0$, where β_0 takes some value of interest. Similar to the approach in Chapter 10, we would use the test statistic

$$T_s = \frac{\hat{\beta} - \beta_0}{s_{\hat{\beta}}}. \tag{17.35}$$

Under H_0, T_s has a t distribution with $n-2$ degrees of freedom, and we would reject H_0 for sufficiently large values of this statistic. For $\beta_0 = 0$, this test is equivalent to the F test we developed earlier for $H_0 : \beta = 0$, and in fact $T_s^2 = F_s$. The t test is more general, however, because we can also test $H_0 : \beta = \beta_0$ for any value of β_0.

It is possible to derive similar t tests and confidence intervals for the intercept parameter α. The t test is most commonly used to test $H_0 : \alpha = 0$. If the test is significant this implies an intercept different from zero. We will let SAS handle the calculations here.

We can also derive a confidence interval for the theoretical mean of Y_i at a given X_i value. Recall that according to the linear regression model, $E[Y_i] = \alpha + \beta X_i$. Thus, Y_i has a mean of $\mu = \alpha + \beta X_i$ for any X_i value. The confidence interval is based on $\hat{Y}_i = \hat{\alpha} + \hat{\beta} X_i$, the predicted value of Y_i at X_i. It also depends on the standard error $s_{\hat{Y}}$ of $\hat{Y}$, which is given by the formula

$$s_{\hat{Y}} = \sqrt{\hat{\sigma}^2 \left[\frac{1}{n} + \frac{(X_i - \bar{X})^2}{\sum_{i=1}^{n} (X_i - \bar{X})^2} \right]}. \tag{17.36}$$

Note that the standard error $s_{\hat{Y}}$ depends on the value of $(X_i - \bar{X})^2$, which is the squared distance of X_i from $\bar{X}$. The farther X_i is from $\bar{X}$, the larger the value of $s_{\hat{Y}}$.

Using methods similar to the confidence interval for β, it can be shown that a $100(1-\alpha)$ confidence interval for $\mu = \alpha + \beta X_i$ has the form

$$(\hat{Y}_i - c_{\alpha,n-2} s_{\hat{Y}}, \hat{Y}_i + c_{\alpha,n-2} s_{\hat{Y}}). \tag{17.37}$$

The interval will be broader for values of X_i far from $\bar{X}$ because $s_{\hat{Y}}$ will be larger. In other words, the precision of the confidence interval decreases with the distance from $\bar{X}$.

Another type of interval associated with regression are **prediction intervals**. Here, we are trying to find an interval that contains a defined percentage of future Y_i values for a given value of X_i, hence the name prediction interval. These are similar in form to the intervals for the theoretical mean $\mu = \alpha + \beta X_i$, but are always wider because you are trying to enclose a single future observation rather than a mean value.

The prediction interval is based on $\hat{Y}_i = \hat{\alpha} + \hat{\beta} X_i$, the predicted value of Y_i at X_i, and the standard error $s_{\hat{Y}(1)}$ of $\hat{Y}_i$, which is given by the formula

$$s_{\hat{Y}(1)} = \sqrt{\hat{\sigma}^2 \left[1 + \frac{1}{n} + \frac{(X_i - \bar{X})^2}{\sum_{i=1}^{n} (X_i - \bar{X})^2} \right]}. \tag{17.38}$$

Note the additional term (1+) within the square brackets, which makes this standard error larger than $s_{\hat{Y}}$). It also depends on the value of $(X_i - \bar{X})^2$, and so the farther X_i is from $\bar{X}$, the larger the value of $s_{\hat{Y}(1)}$. It can be shown that a $100(1-\alpha)$ prediction interval for a single future Y_i has the form

$$(\hat{Y}_i - c_{\alpha,n-2} s_{\hat{Y}(1)}, \hat{Y}_i + c_{\alpha,n-2} s_{\hat{Y}(1)}). \tag{17.39}$$

17.3.1 Sample calculation - confidence and prediction intervals

We now illustrate the calculations for confidence intervals using the Example 1 data. We earlier found that $\hat{\beta} = 2.645$ and $\hat{\alpha} = 1.942$. To find a confidence interval for β, we first need to calculate $s_{\hat{\beta}}$. From Table 17.1, we see that $\sum_{i=1}^{n} (X_i - \bar{X})^2 = 11.798$, and we earlier calculated that $\hat{\sigma}^2 = MS_{error} = 2.539$. Inserting these quantities into the formula for $s_{\hat{\beta}}$, we find

$$s_{\hat{\beta}} = \sqrt{\frac{\hat{\sigma}^2}{\sum_{i=1}^{n} (X_i - \bar{X})^2}} = \sqrt{\frac{2.539}{11.798}} = 0.464. \tag{17.40}$$

For a 95% confidence interval and $\alpha = 0.05$, the confidence interval for β has the form

$$(\hat{\beta} - c_{0.05,n-2} s_{\hat{\beta}}, \hat{\beta} + c_{0.05,n-2} s_{\hat{\beta}}) \tag{17.41}$$

From Table T, with $\alpha = 0.05$ and $df = n - 2 = 27 - 2 = 25$, we find that $c_{0.05,25} = 2.060$. Inserting this value, $\hat{\beta} = 2.645$, and $s_{\hat{\beta}} = 0.464$ in this formula, we obtain

$$(2.645 - 2.060(0.464), 2.645 + 2.060(0.464)) \tag{17.42}$$

or

$$(1.689, 3.601). \tag{17.43}$$

We next find a confidence interval for the theoretical mean $\mu = \alpha + \beta X_i$ at $X_i = 0.5$. For this value of X_i, we have

$$\hat{Y}_i = \hat{\alpha} + \hat{\beta} X_i = 1.942 + 2.645(0.5) = 3.265. \tag{17.44}$$

From Table 17.1 we have $\sum_{i=1}^{n}(X_i - \bar{X})^2 = 11.798$, and earlier found that $\bar{X} = 0.960$ and $\hat{\sigma}^2 = MS_{error} = 2.539$. Inserting these quantities into the formula for $s_{\hat{Y}}$, we find that

$$s_{\hat{Y}} = \sqrt{\hat{\sigma}^2 \left[\frac{1}{n} + \frac{(X_i - \bar{X})^2}{\sum_{i=1}^{n}(X_i - \bar{X})^2} \right]} \tag{17.45}$$

$$= \sqrt{2.539 \left[\frac{1}{27} + \frac{(0.5 - 0.960)^2}{11.798} \right]} \tag{17.46}$$

$$= \sqrt{2.539 \left[0.037 + \frac{0.212}{11.798} \right]} \tag{17.47}$$

$$= 0.374. \tag{17.48}$$

For a 95% confidence interval and $\alpha = 0.05$, the confidence interval for the theoretical mean $\mu = \alpha + \beta X_i$ has the form

$$(\hat{Y} - c_{0.05,n-2} s_{\hat{Y}}, \hat{Y} + c_{0.05,n-2} s_{\hat{Y}}) \tag{17.49}$$

From Table T with $\alpha = 0.05$ and $df = n - 2 = 27 - 2 = 25$, we find that $c_{0.05,25} = 2.060$. Inserting this value, $\hat{Y} = 3.265$, and $s_{\hat{Y}} = 0.374$ in this formula, we find

$$(3.265 - 2.060(0.374), 3.265 + 2.060(0.374)) \tag{17.50}$$

or

$$(2.495, 4.035). \tag{17.51}$$

Lastly, we calculate a prediction interval for a single future observation Y_i at $X_i = 0.5$. For this value of X_i, we earlier calculated that

$$\hat{Y}_i = \hat{\alpha} + \hat{\beta} X_i = 1.942 + 2.645(0.5) = 3.265. \tag{17.52}$$

We again have $\sum_{i=1}^{n}(X_i - \bar{X})^2 = 11.798$, $\bar{X} = 0.960$ and $\hat{\sigma}^2 = MS_{error} = 2.539$. Inserting these quantities into the formula for $s_{\hat{Y}(1)}$, we obtain

$$s_{\hat{Y}(1)} = \sqrt{\hat{\sigma}^2 \left[1 + \frac{1}{n} + \frac{(X_i - \bar{X})^2}{\sum_{i=1}^{n}(X_i - \bar{X})^2}\right]} \tag{17.53}$$

$$= \sqrt{2.539 \left[1 + \frac{1}{27} + \frac{(0.5 - 0.960)^2}{11.798}\right]} \tag{17.54}$$

$$= \sqrt{2.539 \left[1 + 0.037 + \frac{0.212}{11.798}\right]} \tag{17.55}$$

$$= 1.637. \tag{17.56}$$

For a 95% prediction interval and $\alpha = 0.05$, the interval has the form

$$(\hat{Y} - c_{0.05,n-2}s_{\hat{Y}(1)}, \hat{Y} + c_{0.05,n-2}s_{\hat{Y}(1)}) \tag{17.57}$$

From Table T with we have $c_{0.05,25} = 2.060$. Inserting $c_{0.05,25} = 2.060$, $\hat{Y} = 3.265$, and $s_{\hat{Y}(1)} = 1.637$ in this formula, we obtain

$$(3.265 - 2.060(1.637), 3.265 + 2.060(1.637)) \tag{17.58}$$

or

$$(-0.107, 6.637). \tag{17.59}$$

Note this interval is much wider than the interval for the theoretical mean $\mu = \alpha + \beta X_i$, which was $(2.495, 4.035)$. This is because you are trying to enclose a single future observation, a random variable Y_i, rather than a theoretical mean.

17.4 R^2 values

R^2 values are a measure of how well a statistical model explains the data. Recall that the following relationship holds among the sum of squares in linear regression:

$$SS_{regression} + SS_{error} = SS_{total}. \tag{17.60}$$

We can think of the different sum of squares as partitioning the variability in the data into different sources. $SS_{regression}$ represents variability explained by

the regression line, SS_{error} represents variability of the observations around the regression line, while SS_{total} is the total amount of variability in the data. The R^2 value for a linear regression model is the proportion of total variability explained by the model, or

$$R^2 = \frac{SS_{regression}}{SS_{total}} = \frac{SS_{regression}}{SS_{regression} + SS_{error}}. \tag{17.61}$$

It is clear from this formula that R^2 must range between 0 and 1 ($0 \leq R^2 \leq 1$). For the Example 1 data, we have

$$R^2 = 82.528/146.014 = 0.565. \tag{17.62}$$

Thus, 56.5% of the variation is explained by the regression model for these data. Small R^2 values indicate there is substantial variability in the data not explained by the model, while large ones indicate the model explains most of the variation.

More generally, we can define an R^2 value for both ANOVA and regression models as

$$R^2 = \frac{SS_{model}}{SS_{total}} = \frac{SS_{model}}{SS_{model} + SS_{error}}. \tag{17.63}$$

For example, we have $SS_{model} = SS_{among}$ for one-way ANOVA while $SS_{error} = SS_{within}$. The R^2 value here is the proportion of the variation explained by the one-way ANOVA model, in particular the variation among the group means. The SAS output for `proc glm` provides an R^2 for ANOVA models of this form.

17.5 Linear regression for Example 1 - SAS demo

The linear regression analysis can be conducted using `proc glm` and a program similar in structure to ANOVA ones (see SAS program and output below). We first input the observations using a `data` step, applying transformations if necessary. The dependent variable Y is defined as the SAS variable `y`, while the independent variable X is defined as `x`. **It is important to realize that the actual names of these variables are not important - it is their position in `proc gplot` and `proc glm` that determines which one is the dependent variable, and which is the independent one.**

The dependent variable always goes first. Note also the additional observation at end of the data set, for which `x` = 0.5 but `y` is a missing value. The purpose of this observation is to make `proc glm` calculate a confidence interval for the mean, as well as a prediction interval, at that particular value of `x`.

The data are then plotted along with the fitted line plus confidence and prediction intervals. This accomplished using the following `proc gplot` code (SAS Institute Inc. 2014a). The three `y*x` statements in the `plot` command plot the same data in three different ways, which are then combined into one graph using the `overlay` option. The first plot, using the `symbol1` command, draws the data points. The second plot, using the `symbol2` command, draws a regression line through the points and also plots 95% confidence intervals for the mean of Y_i at X_i, or $\mu = \alpha + \beta X_i$, across the range of X_i values. The third plot, using the `symbol3` command, plots 95% prediction intervals for a single future observation, again across the range of X_i values.

The regression analysis is conducted using `proc glm` as shown below (SAS Institute Inc. 2014b). There is no `class` statement because the independent variable `X` is a continuous variable and does not fall into discrete groups like ANOVA. Note the similarity of the `model` statement to the linear regression model. The option `clparm` is used to generate 95% confidence intervals for α and β, while `clm` generates a 95% confidence interval for the mean of Y_i at each value of X_i. If we want prediction intervals it is necessary to run `proc glm` a second time using the `cli` option in the `model` statement (see below). This is necessary because `proc glm` cannot generate both types of intervals at the same time.

The data points, regression line, and confidence or prediction intervals are shown in Fig. 17.3. The prediction intervals are much wider than the confidence intervals, because the prediction intervals are for single future Y_i while the confidence intervals enclose a mean. Note that both types of interval increase in width as you move away from the center of the X values. This follows from the fact that the standard errors involved in these calculations are a function of $(X_i - \bar{X})^2$, which increases as X_i moves away from $\bar{X}$.

Examining the output for `proc glm`, first note that the slope β is labeled as `x` while the intercept α is `Intercept`. We see that attack density `y` increases with beetle numbers `x`, because $\hat{\beta} = 2.645$ and is positive. The effect of beetle numbers on attack density was highly significant ($F_{1,25} = 32.5, P < 0.0001$). There are several F tests to chose from in the output, but all give the same result for simple linear regression. Alternately, we could report the t test for

β ($t_{25} = 5.70, P < 0.0001$), which also tests $H_0 : \beta = 0$. We see that $R^2 = 0.565$, indicating that 56.5% of the variation is explained by the regression model.

The `proc glm` output also provides 95% confidence intervals for α and β. A 95% confidence interval for the mean of Y_i at $X_i = 0.5$ is also given, and labeled as `95% Confidence Limits for Mean Predicted Value`. The second set of output for `proc glm` contains a 95% prediction interval for a single future Y_i at $X_i = 0.5$, labeled as `95% Confidence Limits for Individual Predicted Value`.

Note that the estimated intercept is some distance from zero ($\hat{\alpha} = 1.942$), and in fact the t test of $H_0 : \alpha = 0$ reported by SAS is highly significant ($t_{25} = 3.59, P = 0.0014$). This cannot really be true because the addition of zero beetles should give you an attack density of zero. A more accurate (and possibly non-linear) model would require that the intercept be zero.

This is a potential pitfall when using linear regression. Many biological phenomenon are approximately linear over some range of the data but the approximation breaks down for more extreme values. A linear regression does not take this possibility into account and so cannot provide a general explanation of some phenomena.

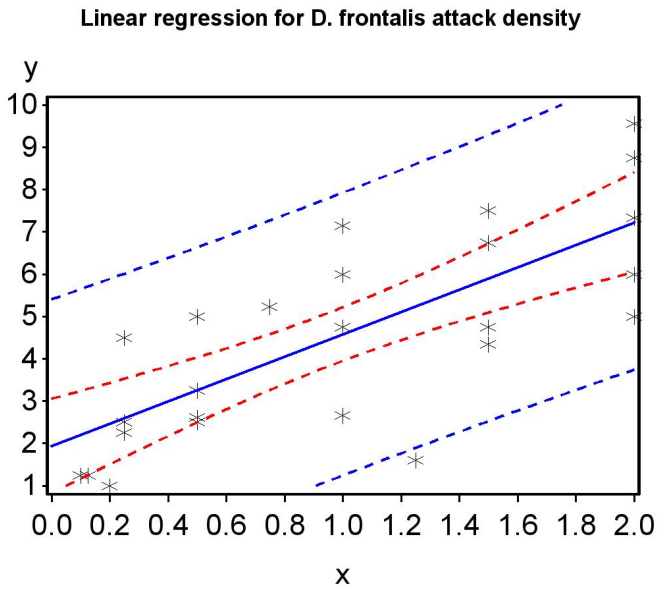

Figure 17.3: Linear regression model fitted to the Example 1 data, where Y is attack density and X is beetles added to the cages. Also shown are 95% confidence intervals for the mean, and prediction intervals for a single future observation.

SAS Program

```
* SPBattack.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Linear regression for D. frontalis attack density';
data frontalis;
    input attacks beetles;
    * Apply transformations here;
    y = attacks;
    x = beetles;
    datalines;
1.25 0.100
2.66 1.000
7.33 2.000
1.60 1.250
2.62 0.500

etc.

5.00 0.500
.    0.500
;
run;
* Print data set;
proc print data=frontalis;
run;
* Plot data and regression line;
proc gplot data=frontalis;
    plot y*x y*x y*x / overlay vaxis=axis1 haxis=axis1;
    symbol1 i=none v=star c=black height=2 width=3;
    symbol2 i=rlclm v=none c=red height=2 width=3;
    symbol3 i=rlcli v=none c=blue height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Regression analysis with confidence intervals;
proc glm data=frontalis;
    model y = x / clparm clm;
    output out=resids p=pred r=resid;
run;
* Regression analysis with prediction intervals;
proc glm data=frontalis;
    model y = x / clparm cli;
run;
goptions reset=all;
```

```
title "Diagnostic plots to check ANOVA assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
    plot resid*pred=1 / vaxis=axis1 haxis=axis1;
    symbol1 v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
    qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

Linear regression for D. frontalis attack density 1
08:45 Sunday, November 14, 2010

Obs	attacks	beetles	y	x
1	1.25	0.100	1.25	0.100
2	2.66	1.000	2.66	1.000
3	7.33	2.000	7.33	2.000
4	1.60	1.250	1.60	1.250
5	2.62	0.500	2.62	0.500

etc.

27	5.00	0.500	5.00	0.500
28	.	0.500	.	0.500

Linear regression for D. frontalis attack density 2
08:45 Sunday, November 14, 2010

The GLM Procedure

Number of Observations Read	28
Number of Observations Used	27

Linear regression for D. frontalis attack density 3
08:45 Sunday, November 14, 2010

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	82.5283492	82.5283492	32.50	<.0001
Error	25	63.4855174	2.5394207		
Corrected Total	26	146.0138667			

R-Square	Coeff Var	Root MSE	y Mean
0.565209	35.56163	1.593556	4.481111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
x	1	82.52834922	82.52834922	32.50	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
x	1	82.52834922	82.52834922	32.50	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1.941567811	0.54083158	3.59	0.0014
x	2.644847410	0.46394486	5.70	<.0001

Parameter	95% Confidence Limits	
Intercept	0.827704323	3.055431300
x	1.689335080	3.600359740

The GLM Procedure

Observation	Observed	Predicted	Residual
1	1.25000000	2.20605255	-0.95605255
2	2.66000000	4.58641522	-1.92641522
3	7.33000000	7.23126263	0.09873737
4	1.60000000	5.24762707	-3.64762707
5	2.62000000	3.26399152	-0.64399152

etc.

27	5.00000000	3.26399152	1.73600848
28 *	.	3.26399152	.

Observation	95% Confidence Limits for Mean Predicted Value	
1	1.16947580	3.24262930
2	3.95365127	5.21917917
3	6.05393677	8.40858849
4	4.55796883	5.93728532
5	2.49438766	4.03359537

etc.

27	2.49438766	4.03359537
28 *	2.49438766	4.03359537

* Observation was not used in this analysis

Sum of Residuals	-0.00000000
Sum of Squared Residuals	63.48551745
Sum of Squared Residuals - Error SS	0.00000000
PRESS Statistic	73.72506348
First Order Autocorrelation	0.45535896
Durbin-Watson D	1.02741345

etc.

Linear regression for D. frontalis attack density 8
08:45 Sunday, November 14, 2010

The GLM Procedure

Observation	Observed	Predicted	Residual
1	1.25000000	2.20605255	-0.95605255
2	2.66000000	4.58641522	-1.92641522
3	7.33000000	7.23126263	0.09873737
4	1.60000000	5.24762707	-3.64762707
5	2.62000000	3.26399152	-0.64399152

etc.

```
27          5.00000000        3.26399152        1.73600848
28 *         .                3.26399152         .

                          95% Confidence Limits for
  Observation             Individual Predicted Value

            1             -1.23574200       5.64784710
            2              1.24398368       7.92884676
            3              3.74449413      10.71803113
            4              1.89395940       8.60129475
            5             -0.10702442       6.63500745

etc.

           27             -0.10702442       6.63500745
           28 *           -0.10702442       6.63500745


* Observation was not used in this analysis


                Sum of Residuals                        -0.00000000
                Sum of Squared Residuals                63.48551745
                Sum of Squared Residuals - Error SS      0.00000000
                PRESS Statistic                         73.72506348
                First Order Autocorrelation              0.45535896
                Durbin-Watson D                          1.02741345
```

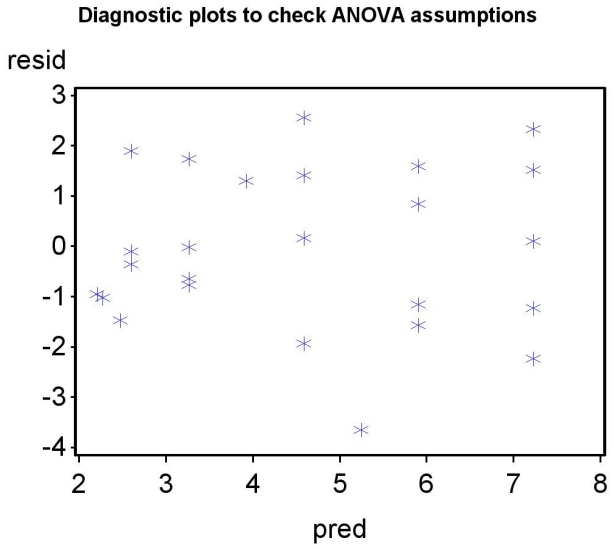

Figure 17.4: Residual vs. predicted plot for the Example 1 analysis.

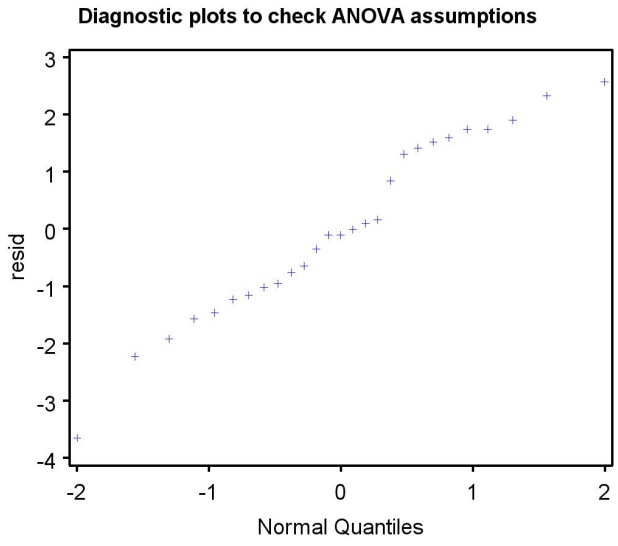

Figure 17.5: Normal quantile plot for the Example 1 analysis.

17.6 Assumptions and transformations

Linear regression makes the same assumptions as ANOVA, including homogeneity of variances and normality, and the same types of plots can be used to assess them. If the homogeneity of variances assumption is satisfied, the points in a residual vs. predicted plot should be equally scattered across the range of predicted values. Outliers can also be identified using this plot. The normality assumption can be evaluated using a normal quantile plot of the residuals, with a straight diagonal line indicating this assumption is satisfied.

Examining the residuals from the Example 1 analysis, we see no obvious pattern in the residual vs. predicted plot, suggesting the homogeneity of variances assumption is satisfied (Fig. 17.4). No outliers were present. The normal quantile plot suggests the normality assumption is satisfied (Fig. 17.5).

Linear regression makes another key assumption, namely that the relationship between Y and X is linear. This assumption can be checked by examining a plot of Y vs. X as well as the residual vs. predicted plot (see examples below). What can be done if the relationship seems nonlinear? We can sometimes fix this problem by applying a transformation to Y, X, or both Y and X, so that linear regression can be applied to the transformed data. **This use of transformations greatly extends the utility of linear regression.** Some commonly used transformations are $\log Y$ vs. X, $\log Y$ vs. $\log X$, Y vs. $\log X$, and $1/Y$ vs. X. A transformation that linearizes the data sometimes corrects for problems with the homogeneity of variances and normality assumptions.

A transformation may be selected based on prior information about the data and system. For example, a conservation biologist may be interested in the relationship between island area A and the number of species S on the island, and previous studies suggest the relationship between $\log_{10} S$ and $\log_{10} A$ will be linear (MacArthur & Wilson 1967). Another approach is to try a number of transformations and chose the one that makes the data most linear. We will illustrate each approach with an example below.

In cases where no transformation can linearize the data, another possibility would be **nonlinear regression** (Juliano 1993). This type of analysis requires that the user specify a model $Y = f(X, \theta_1, \theta_2, \ldots) + \epsilon$ for the data, where f is a function with parameters $\theta_1, \theta_2, \ldots$ to be estimated. SAS implements this type of nonlinear regression in `proc nlin`, while `proc nlmixed` allows

for nonlinear functions as well as random effects and nonnormal distributions.

17.6.1 Species-area data - SAS demo

For many organisms there is a relationship between a defined area of habitat, such as an island, and the number of species found there. If S is the number of species, and A the area of habitat, then the model $S = cA^z$ seems to describe many data sets (MacArthur & Wilson 1967). Taking the $\log_{10}$ of both sides of this equation, we obtain

$$\log_{10} S = \log_{10} c + z \log_{10} A. \tag{17.64}$$

This form of the model is linear and suggests linear regression could be used to analyze species-area data. The SAS program listed below shows how these transformations can be applied to the bird fauna on archipelagos and islands of varying areas. The data are the number of species vs. island area (square miles) for 23 islands. The data were simulated to resemble Fig. 9 in MacArthur & Wilson (1967). An extra observation is included with a missing value for the number of species, but an island area of 5000 square miles, to make `proc glm` calculate a confidence interval for the mean of this island.

We first conduct the analysis without any transformation and examine the `gplot` graph of Y vs. X, where Y is the number of species and X is island area (Fig. 17.6). Note the nonlinear nature of the relationship between the number of species and island area. This pattern is also reflected in the residual vs. predicted plot (Fig. 17.7), which appears to be hump-shaped. Both plots suggest that a transformation is required for these data in order to linearize the relationship between the two variables.

The picture improves after a $\log_{10}$ transformation is applied to both species and area. We see that the graph of the transformed variables is linear (Fig. 17.8) and residual vs. predicted plot is featureless (Fig. 17.9). The normal quantile plot is also well-behaved (Fig. 17.10). Now that the various assumptions are satisfied we can interpret the rest of the SAS output (see below). We see that the number of species increases with island area ($\hat{\beta} = 0.241$) and the effect is highly significant ($F_{1,21} = 148.16, P < 0.0001$). In terms of the original model, where $S = cA^z$, we see that $\hat{\beta} = 0.241$ is also an estimate of z. The R^2 value is 0.876, indicating that 87.6% of the variation is explained by the regression model. Confidence intervals are also provided for the intercept and slope.

The `proc glm` output also generates a predicted value $\hat{Y}_i = 1.800$ at $X_i = 3.699$ ($\log_{10} 5000 = 3.699$). We need to convert this to the original scale measurement using antilogs. We have $\hat{S}_i = 10^{\hat{Y}_i} = 10^{1.800} = 63.10$ species. So, we predict there would be 63 species on an island of 5000 square miles. The confidence interval for the mean is $(1.746, 1.855)$, which we can similarly convert to $(10^{1.745}, 10^{1.855})$ or $(55.72, 71.61)$.

SAS Program

```
* SAprob2.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Linear regression for species-area data';
data sa;
    input species area;
    * Apply transformations here;
    y = log10(species);
    x = log10(area);
    datalines;
 15      28
104  113480
165  380358
116   33252
 35    1010
 33     305
 78   37620
 93    4762
 50     213
 76    2976
 18      23
 28     186
 20     423
121  108512
 53     364
 22     269
102   11163
 28     487
158  445409
 19      70
111   38309
152  100873
 55    1354
  .    5000
;
run;
* Print data set;
proc print data=sa;
run;
* Plot data and regression line;
proc gplot data=sa;
    plot y*x=1 y*x=2 y*x=3 / overlay vaxis=axis1 haxis=axis1;
    symbol1 i=none v=star c=black height=2 width=3;
```

```
    symbol2 i=rlclm v=none c=red height=2 width=3;
    symbol3 i=rlcli v=none c=blue height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Regression analysis with confidence intervals;
proc glm data=sa;
    model y = x / clparm clm;
    output out=resids p=pred r=resid;
run;
* Regression analysis with prediction intervals;
proc glm data=sa;
    model y = x / clparm cli;
run;
goptions reset=all;
title "Diagnostic plots to check ANOVA assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
    plot resid*pred=1 / vaxis=axis1 haxis=axis1;
    symbol1 v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
    qqplot resid / normal waxis=3 height=4;
run;
quit;
```

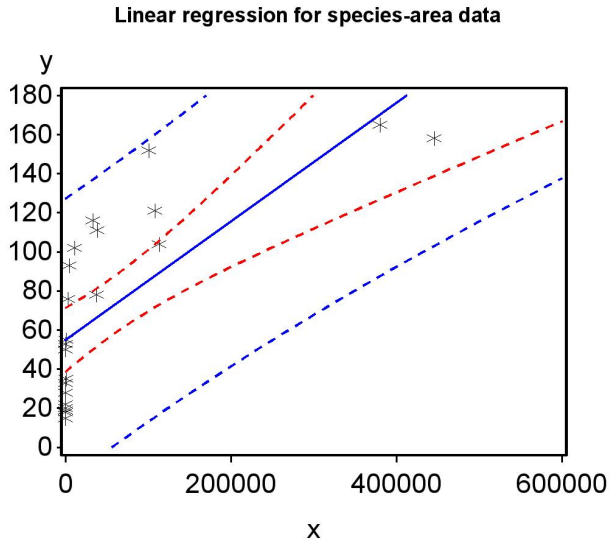

Figure 17.6: Linear regression model fitted to the species-area data, where Y is the number of species and X is island area.

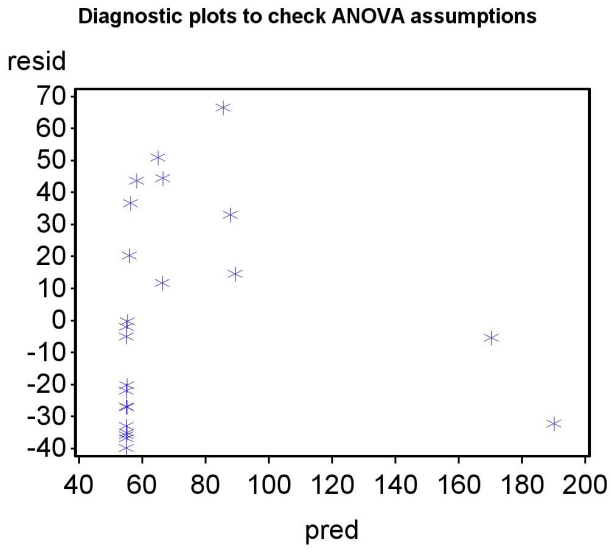

Figure 17.7: Residual vs. predicted plot for the species-area data.

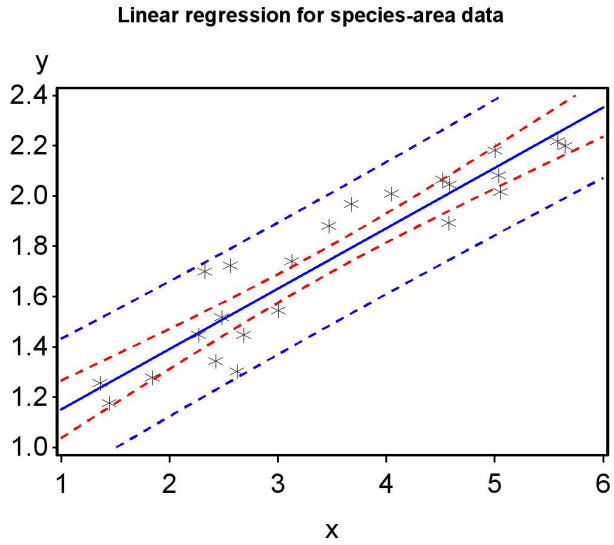

Figure 17.8: Linear regression model fitted to the species-area data, where Y is log-transformed species and X is log-transformed area.

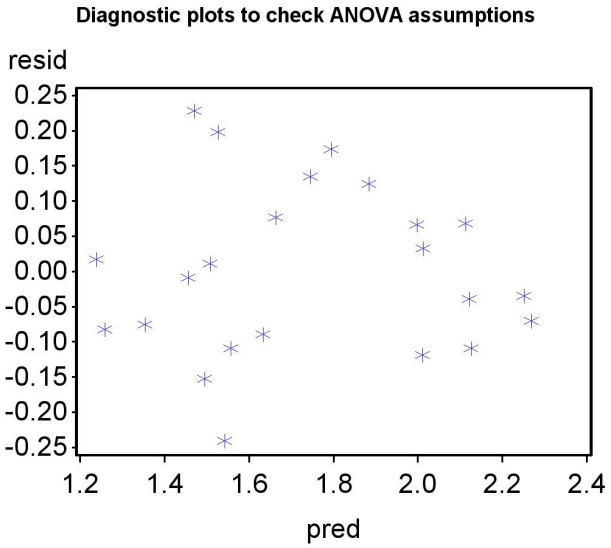

Figure 17.9: Residual vs. predicted plot for the log-transformed species-area data.

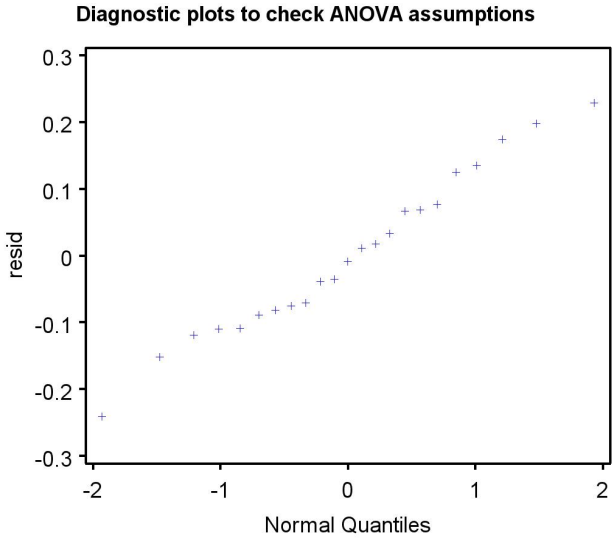

Figure 17.10: Normal quantile plot for the log-transformed species-area data.

SAS Output

Linear regression for species-area data 1
11:32 Tuesday, November 16, 2010

Obs	species	area	y	x
1	15	28	1.17609	1.44716
2	104	113480	2.01703	5.05492
3	165	380358	2.21748	5.58019
4	116	33252	2.06446	4.52182
5	35	1010	1.54407	3.00432

etc.

23	55	1354	1.74036	3.13162
24	.	5000	.	3.69897

Linear regression for species-area data 2
11:32 Tuesday, November 16, 2010

The GLM Procedure

Number of Observations Read	24
Number of Observations Used	23

Linear regression for species-area data 3
11:32 Tuesday, November 16, 2010

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.25182542	2.25182542	148.16	<.0001
Error	21	0.31916133	0.01519816		
Corrected Total	22	2.57098675			

R-Square	Coeff Var	Root MSE	y Mean
0.875860	7.083042	0.123281	1.740507

Source	DF	Type I SS	Mean Square	F Value	Pr > F
x	1	2.25182542	2.25182542	148.16	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
x	1	2.25182542	2.25182542	148.16	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	0.9102215097	0.07289411	12.49	<.0001
x	0.2405722961	0.01976395	12.17	<.0001

Parameter	95% Confidence Limits	
Intercept	0.7586299190	1.0618131004
x	0.1994709127	0.2816736795

Linear regression for species-area data 4
11:32 Tuesday, November 16, 2010

The GLM Procedure

Observation		Observed	Predicted	Residual
1		1.17609126	1.25836764	-0.08227638
2		2.01703334	2.12629506	-0.10926172
3		2.21748394	2.25266125	-0.03517730
4		2.06445799	1.99804559	0.06641240
5		1.54406804	1.63297800	-0.08890996

etc.

23		1.74036269	1.66360220	0.07676049
24	*	.	1.80009122	.

Observation	95% Confidence Limits for Mean Predicted Value	
1	1.16016869	1.35656659
2	2.04142998	2.21116013
3	2.15012264	2.35519985
4	1.92880841	2.06728278
5	1.57645124	1.68950477

etc.

23	1.60855303	1.71865138
24 *	1.74567238	1.85451005

* Observation was not used in this analysis

Sum of Residuals	-0.00000000
Sum of Squared Residuals	0.31916133
Sum of Squared Residuals - Error SS	0.00000000
PRESS Statistic	0.36922092
First Order Autocorrelation	0.04242134
Durbin-Watson D	1.87548592

etc.

17.6.2 Population growth rates - SAS demo

As another example of transformations, consider a study of the population growth of phytophagous mites on leaf sections. An experiment is conducted in which leaf sections are inoculated with a range of mite densities and the number of offspring recorded one generation later. The number of offspring per initial mite is the finite growth of the population, usually symbolized as λ. The SAS program listed below gives the mite densities and the λ values for this experiment.

We first conduct the analysis without any transformation. Looking at the plot of Y (λ) vs. X (density), we see a curvilinear relationship (Fig. 17.11) that also appears in the residual vs. predicted plot (Fig. 17.12). A transformation is clearly needed, but which one? A natural log transformation usually a good starting point for population data, both for growth rates and numbers. We begin by log-tranforming the dependent variable λ and examining the plots (see program below). The graph after transformation is linear (Fig. 17.13) and the residual vs. predicted plot shows no pattern (Fig. 17.14). The normal quantile plot is also adequate (Fig. 17.15).

Interpreting the SAS output (see below), we see that λ decreases with mite density ($\hat{\beta} = -0.020$) and the effect is highly significant ($F_{1,15} = 1695.22, P < 0.0001$). The R^2 value is 0.991, indicating that almost all the variation in the data is explained by the regression line. It appears that the growth rate of the mites is adversely affected by their density, probably through competition for resources or other intraspecific interactions.

SAS Program

```
* logistic.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Linear regression for growth rate-density data';
data grd;
    input lambda density;
    * Apply transformations here;
    y = log(lambda);
    x = density;
    datalines;
  7.32   5
  4.82  15
  4.69  25
  3.90  35
  2.65  45
  2.52  55
  1.70  65
  1.68  75
  1.43  85
  1.07  95
  0.74 105
  0.72 115
  0.64 125
  0.47 135
  0.40 145
  0.38 155
  0.25 165
;
run;
* Print data set;
proc print data=grd;
run;
* Plot data and regression line;
proc gplot data=grd;
    plot y*x=1 y*x=2 y*x=3 / overlay vaxis=axis1 haxis=axis1;
    symbol1 i=none v=star c=black height=2 width=3;
    symbol2 i=rlclm v=none c=red height=2 width=3;
    symbol3 i=rlcli v=none c=blue height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Regression analysis with confidence intervals;
proc glm data=grd;
    model y = x / clparm clm;
```

```
    output out=resids p=pred r=resid;
run;
* Regression analysis with prediction intervals;
proc glm data=grd;
    model y = x / clparm cli;
run;
goptions reset=all;
title "Diagnostic plots to check ANOVA assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
    plot resid*pred=1 / vaxis=axis1 haxis=axis1;
    symbol1 v=star height=2 width=3;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
    qqplot resid / normal waxis=3 height=4;
run;
quit;
```

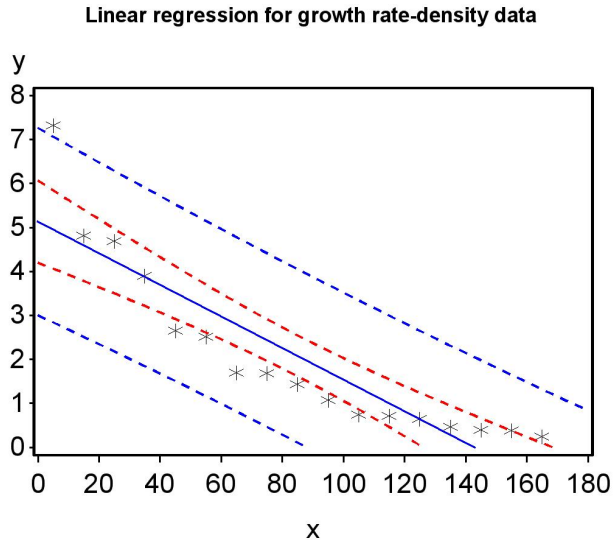

Figure 17.11: Linear regression model fitted to the λ-density data, where Y is λ and X is initial mite density.

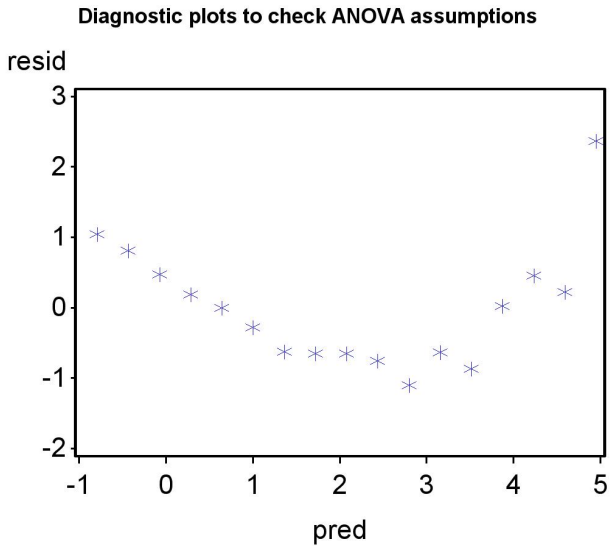

Figure 17.12: Residual vs. predicted plot for the λ-density data.

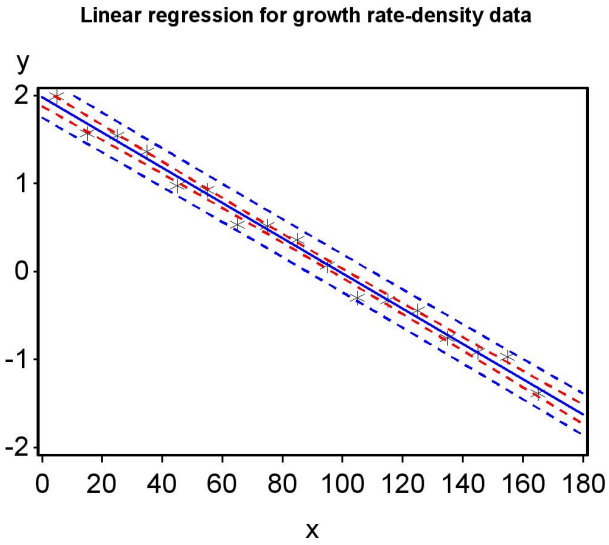

Figure 17.13: Linear regression model fitted to the λ-density data, where Y is $\log \lambda$ and X is initial mite density.

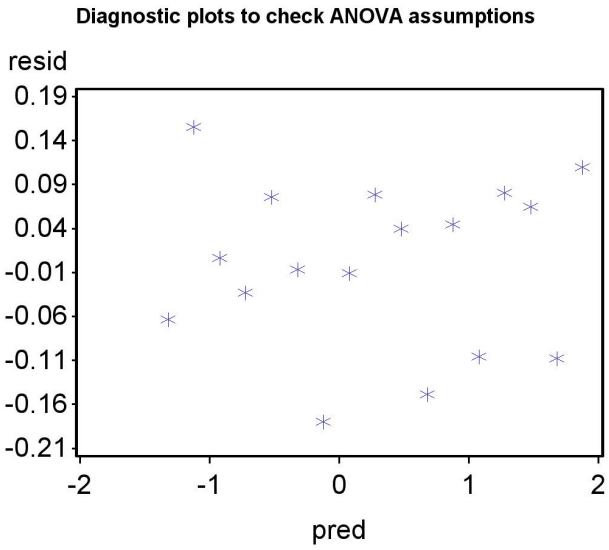

Figure 17.14: Residual vs. predicted plot for the transformed λ-density data.

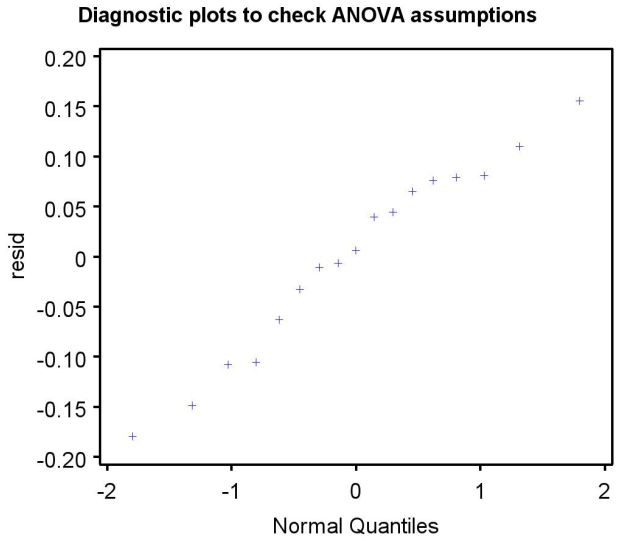

Figure 17.15: Normal quantile plot for the transformed λ-density data.

SAS Output

Linear regression for growth rate-density data 1
18:39 Tuesday, November 16, 2010

Obs	lambda	density	y	x
1	7.32	5	1.99061	5
2	4.82	15	1.57277	15
3	4.69	25	1.54543	25
4	3.90	35	1.36098	35
5	2.65	45	0.97456	45

etc.

Linear regression for growth rate-density data 2
18:39 Tuesday, November 16, 2010

The GLM Procedure

Number of Observations Read	17
Number of Observations Used	17

Linear regression for growth rate-density data 3
18:39 Tuesday, November 16, 2010

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	16.36176928	16.36176928	1695.22	<.0001
Error	15	0.14477544	0.00965170		
Corrected Total	16	16.50654472			

R-Square	Coeff Var	Root MSE	y Mean
0.991229	35.21791	0.098243	0.278958

Source	DF	Type I SS	Mean Square	F Value	Pr > F
x	1	16.36176928	16.36176928	1695.22	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
x	1	16.36176928	16.36176928	1695.22	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1.981131688	0.04771689	41.52	<.0001
x	-0.020025578	0.00048638	-41.17	<.0001

Parameter	95% Confidence Limits	
Intercept	1.879425551	2.082837825
x	-0.021062263	-0.018988893

Linear regression for growth rate-density data 4
18:39 Tuesday, November 16, 2010

The GLM Procedure

Observation	Observed	Predicted	Residual
1	1.99061033	1.88100380	0.10960653
2	1.57277393	1.68074802	-0.10797410
3	1.54543258	1.48049225	0.06494033
4	1.36097655	1.28023647	0.08074008
5	0.97455964	1.07998070	-0.10542106

etc.

Observation	95% Confidence Limits for Mean Predicted Value	
1	1.78375413	1.97825347
2	1.59217362	1.76932242
3	1.40019098	1.56079352

```
                    4          1.20766853      1.35280442
                    5          1.01441498      1.14554641

etc.


          Linear regression for growth rate-density data                5
                                        18:39 Tuesday, November 16, 2010

                              The GLM Procedure

          Sum of Residuals                          -0.00000000
          Sum of Squared Residuals                   0.14477544
          Sum of Squared Residuals - Error SS       -0.00000000
          PRESS Statistic                            0.18945485
          First Order Autocorrelation               -0.31722141
          Durbin-Watson D                            2.52386773

etc.
```

17.7 Problems

1. An experiment was conducted to measure the effect of density on the rate of egg laying in cowpea weevils. Ten different densities were used in the experiment, and the rate of egg laying determined for each density. The following data were obtained:

Density	Eggs per day
100	7.629
200	4.530
500	3.820
700	2.718
1200	2.403
1500	1.756
1700	1.772
2000	1.508
2200	1.518
2500	1.359

   (a) Plot the rate of egg laying (Y) vs. density (X), and observe the nonlinear relationship between Y and X. Find a transformation of Y and/or X that linearizes this relationship using SAS.

   (b) For the transformed data, use SAS to plot a 95% confidence interval for the mean of Y_i and a 95% prediction interval for a single value of Y_i. Label the intervals (confidence or prediction) on the `gplot` graph.

   (c) Analyze the transformed data set using linear regression and SAS. In your SAS output, label the 95% confidence intervals for the intercept (α) and slope (β) in your SAS printout.

   (d) Interpret the results of the regression analysis. Is there a significant effect of density on the rate of egg production? What direction is the effect?

2. A zoologist wants to establish the relationship between the length of an animal and its weight. He wants to use length to predict weight in future studies, because length is easier to measure. The lengths and weights of a random sample of 20 animals were determined, yielding the following data:

Length (mm)	Weight (g)
14.7	1.65
19.9	4.86
15.8	2.04
19.0	3.53
8.4	0.32
10.2	0.46
13.5	1.68
22.1	6.24
16.2	1.85
8.2	0.28
10.1	0.48
19.8	4.18
20.6	4.77
22.0	6.10
18.1	2.78
22.4	5.26
10.5	0.55
14.5	1.56
11.9	1.07
14.7	1.74

(a) Plot the weight (Y) vs. length (X) using SAS, and observe the nonlinear rela-tionship between Y and X. Attach your graph of this relationship. Then, find a transformation of Y and/or X that linearizes this relationship using SAS or R. What transformation did you use? Attach your graph showing the transformed relationship.

(b) Analyze the transformed data using linear regression and SAS. Briefly interpret your results using P values. Is there a significant effect of length on weight? What direction is the effect? Attach your program and output.

(c) For animals that are 21 mm long, find a 95% confidence interval for the mean weight.

17.8 References

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Reeve, J. D., Rhodes, D. J. & Turchin, P. (1998) Scramble competition in southern pine beetle (Coleoptera: Scolytidae). *Ecological Entomology* 23: 433-443.

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Chapter 18

Correlation

Correlation is a statistical technique used to examine the **association** between two continuous variables. Unlike regression, correlation does not assume a particular direction to the relationship among the variables, and there is no dependent or independent variable. Instead, there are two random variables Y_1 and Y_2 that could be related in some way. Correlation may be used to examine the relationship between just two variables, or as a screening tool to examine the pairwise relationships among many variables.

We will use a classic data set to illustrate correlation, the iris flowers examined by Fisher (1936). The data set contains measurements of iris flowers for three different *Iris* species, but we will only examine *I. setosa*. The variables measured were sepal length and width, and petal length and width. A total of 50 flowers were measured, but we will only use the first ten observations to illustrate the calculations, and only sepal length and width (Table 18.1). The notation Y_{1i} and Y_{2i} refer to the values for the ith pair of numbers. For example, $Y_{11} = 5.1$ and $Y_{21} = 3.5$. Figure 18.9 shows there is a positive association between the two variables, with sepal length (Y_{1i}) and width (Y_{2i}) appearing to increase together.

Table 18.1: Example 1 - Sepal length and width measurements for ten flowers of *I. setosa* (Fisher 1936), showing some preliminary calculations for the correlation analysis. See Chapter 21 for the full data set.

i	Y_{1i} = Sepal length	Y_{2i} = Sepal width	$(Y_{1i} - \bar{Y}_1)(Y_{2i} - \bar{Y}_2)$	$(Y_{1i} - \bar{Y}_1)^2$	$(Y_{2i} - \bar{Y}_2)^2$
1	5.1	3.5	4.56×10^{-2}	5.76×10^{-2}	3.61×10^{-2}
2	4.9	3.0	-1.24×10^{-2}	1.60×10^{-3}	9.61×10^{-2}
3	4.7	3.2	1.76×10^{-2}	2.56×10^{-2}	1.21×10^{-2}
4	4.6	3.1	5.46×10^{-2}	6.76×10^{-2}	4.41×10^{-2}
5	5.0	3.6	4.06×10^{-2}	1.96×10^{-2}	8.41×10^{-2}
6	5.4	3.9	3.19×10^{-1}	2.92×10^{-1}	3.48×10^{-1}
7	4.6	3.4	-2.34×10^{-2}	6.76×10^{-2}	8.10×10^{-3}
8	5.0	3.4	1.26×10^{-2}	1.96×10^{-2}	8.10×10^{-3}
9	4.4	2.9	1.89×10^{-1}	2.12×10^{-1}	1.68×10^{-1}
10	4.9	3.1	-8.40×10^{-3}	1.60×10^{-3}	4.41×10^{-2}
$\sum$	-	-	6.34×10^{-1}	7.64×10^{-1}	8.49×10^{-1}

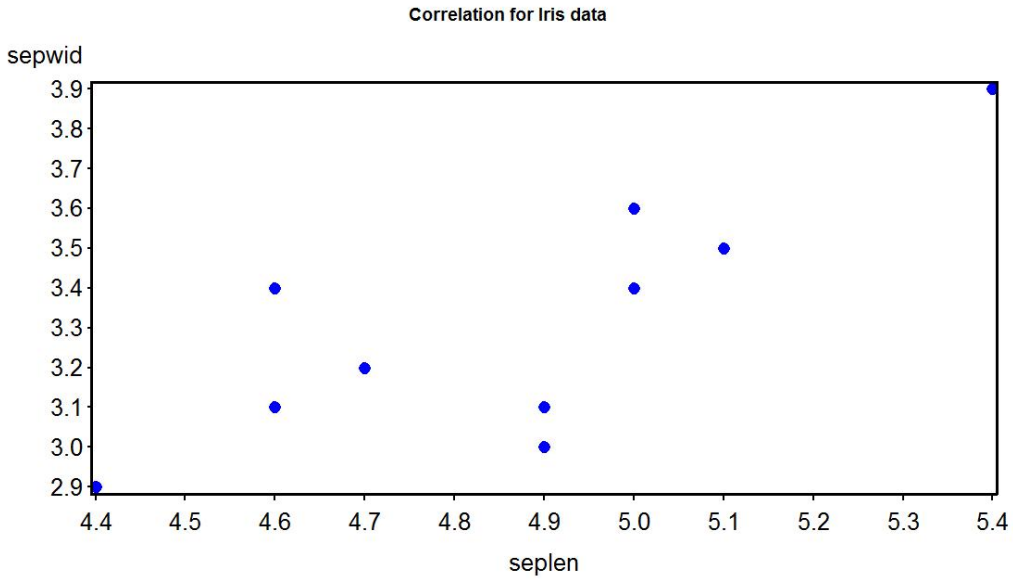

Figure 18.1: Scatterplot of *I. setosa* sepal length and width.

18.1 Correlation model

The statistical model for correlation is the **bivariate normal distribution**. This is an extension of the normal distribution to two random variables Y_1 and Y_2. The bivariate normal distribution has five parameters, the mean and standard deviation for Y_1 and Y_2 $(\mu_1, \sigma_1, \mu_2, \sigma_2)$ and the parameter ρ, which describes the association between them (Stuart et al. 1999). If $\rho > 0$ then the two variables are positively related, as in Fig. 18.9, while if the $\rho < 0$ they are inversely related. If $\rho = 0$ the two variables are independent of one another. The probability density for the bivariate normal distribution is given by the function

$$f(y_1, y_2) = \frac{1}{2\pi\sigma_1\sigma_2\sqrt{1-\rho^2}} \times \exp\left[-\frac{1}{2(1-\rho^2)}\left\{\left(\frac{y_1-\mu_1}{\sigma_1}\right)^2 - 2\rho\frac{y_1-\mu_1}{\sigma_1}\frac{y_2-\mu_2}{\sigma_2} + \left(\frac{y_2-\mu_2}{\sigma_2}\right)^2\right\}\right]. \tag{18.1}$$

A interesting property of this distribution is that each Y variable, when considered alone, also has a normal distribution. In particular, $Y_1 \sim N(\mu_1, \sigma_1^2)$ and $Y_2 \sim N(\mu_2, \sigma_2^2)$. These are known as the **marginal distributions** of Y_1 and Y_2.

Figure 18.2 and Fig. 18.3 shows this distribution as a surface or contour plot, for $\rho = 0.7$. This value of ρ implies a strong positive relationship between the two variables, and so the probability density has a ridge-like shape because Y_1 and Y_2 are likely to increase or decrease together. Fig. 18.4 shows a sample data set generated for the same parameter values of this distribution. Note the relationship between Y_1 and Y_2 and the elliptical cloud of points.

Figure 18.5 shows the distribution for a strong negative relationship between the variables ($\rho = -0.7$). A sample data set for the same parameter values is shown in Fig. 18.6. Figure 18.7 and Fig. 18.8 show the patterns when the two variables are unassociated or independent ($\rho = 0$).

The usual goal in correlation is to estimate the value of ρ and then test $H_0 : \rho = 0$. This null hypothesis means the two variables are independent, and if we can reject this suggests the two variables are associated or dependent. It is also possible to test null hypotheses of the form $H_0 : \rho = \rho_0$, where ρ_0 is any value.

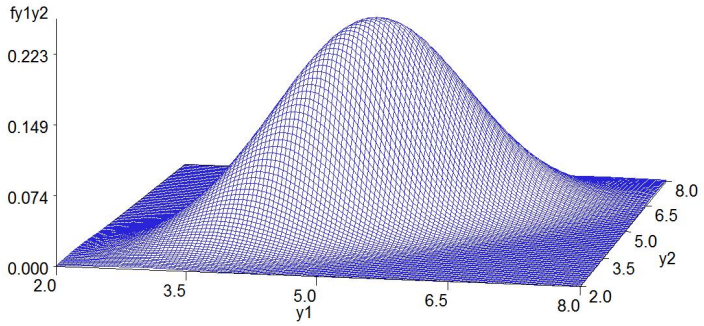

Figure 18.2: Surface plot of the bivariate normal distribution for $\mu_1 = \mu_2 = 5, \sigma_1^2 = \sigma_2^2 = 1$, and $\rho = 0.7$.

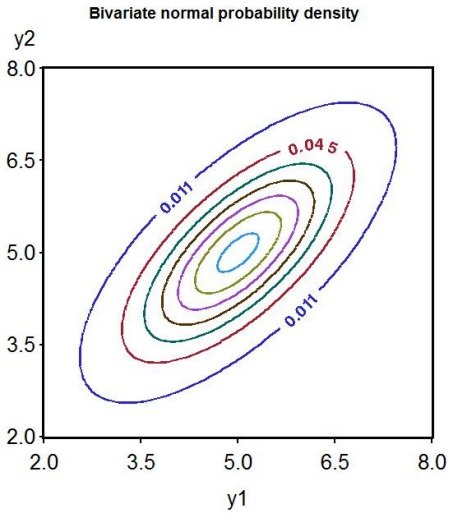

Figure 18.3: Contour plot of the bivariate normal for the same parameter values as Fig. 18.3

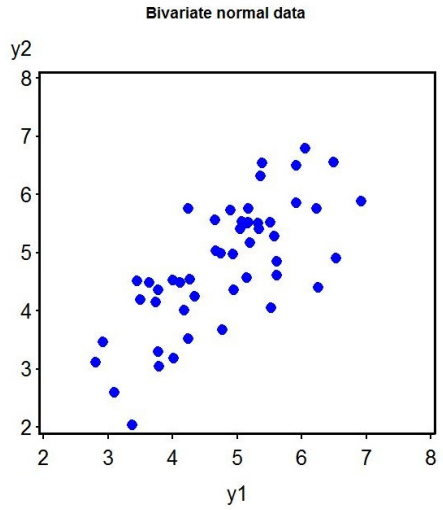

Figure 18.4: Simulated data for the bivariate normal distribution with $\mu_1 = \mu_2 = 5, \sigma_1^2 = \sigma_2^2 = 1$, and $\rho = 0.7$.

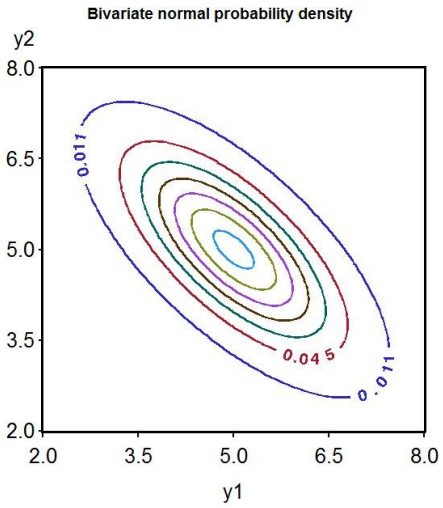

Figure 18.5: Contour plot of the bivariate normal for $\mu_1 = \mu_2 = 5, \sigma_1^2 = \sigma_2^2 = 1$, and $\rho = -0.7$.

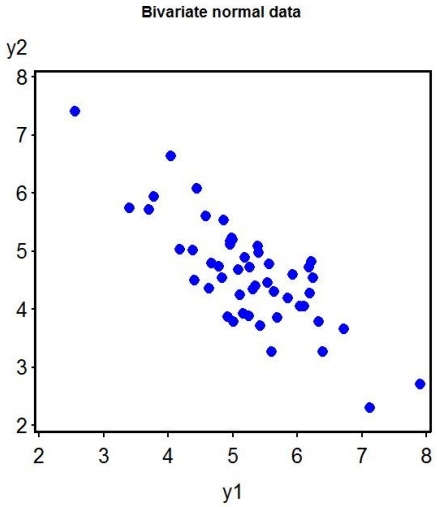

Figure 18.6: Simulated data for the bivariate normal distribution with $\mu_1 = \mu_2 = 5, \sigma_1^2 = \sigma_2^2 = 1$, and $\rho = -0.7$.

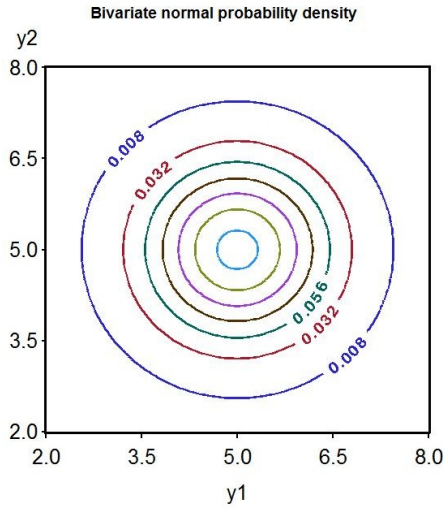

Figure 18.7: Contour plot of the bivariate normal for $\mu_1 = \mu_2 = 5, \sigma_1^2 = \sigma_2^2 = 1$, and $\rho = 0$.

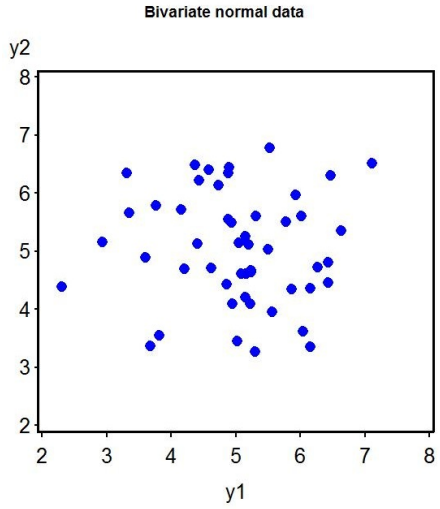

Figure 18.8: Simulated data for the bivariate normal distribution with $\mu_1 = \mu_2 = 5, \sigma_1^2 = \sigma_2^2 = 1$, and $\rho = 0$.

18.2 Correlation and maximum likelihood

Maximum likelihood can be used to estimate the parameters for the bivariate normal distribution, using methods like those for simpler distributions. It turns out that the sample mean $\bar{Y}$ and standard deviation s can be used to estimate $\mu_1, \sigma_1, \mu_2, \text{and} \sigma_2$ for this distribution. For the Example 1 data set, we have $\bar{Y}_1 = 4.86, s_1 = 0.29136, \bar{Y}_2 = 3.31, \text{and} s_2 = 0.30714$. The maximum likelihood estimator of ρ is the sample **correlation coefficient**, r, given by the formula

$$r = \frac{\sum_{i=1}^{n}(Y_{1i} - \bar{Y}_1)(Y_{2i} - \bar{Y}_2)}{\sqrt{\sum_{i=1}^{n}(Y_{1i} - \bar{Y}_1)^2 \sum_{i=1}^{n}(Y_{2i} - \bar{Y}_2)^2}} \tag{18.2}$$

(Stuart et al. 1999). Note that the sign of r depends on the numerator of this expression. If Y_1 and Y_2 are positively or negatively associated, the numerator will be positive or negative. For the Example 1 data, we have

$$\sum_{i=1}^{n}(Y_{1i} - \bar{Y}_1)(Y_{2i} - \bar{Y}_2) = 0.634, \tag{18.3}$$

$$\sum_{i=1}^{n}(Y_{1i} - \bar{Y}_1)^2 = 0.764, \tag{18.4}$$

$$\text{and} \sum_{i=1}^{n}(Y_{2i} - \bar{Y}_2)^2 = 0.849. \tag{18.5}$$

Using these values, the correlation coefficient can then be calculated:

$$r = \frac{6.34 \times 10^{-1}}{\sqrt{7.64 \times 10^{-1} \times 8.49 \times 10^{-1}}} = 0.787. \tag{18.6}$$

The equation for r can also be expressed using the standard deviations of the two variables, and a quantity called the **sample covariance**. The sample covariance for two variables is given by the formula

$$s_{12} = \frac{\sum_{i=1}^{n}(Y_{1i} - \bar{Y}_1)(Y_{2i} - \bar{Y}_2)}{n-1}. \tag{18.7}$$

Dividing the top and bottom of the equation for r by $n-1$, we have

$$r = \frac{\frac{1}{n-1}\sum_{i=1}^{n}(Y_{1i} - \bar{Y}_1)(Y_{2i} - \bar{Y}_2)}{\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(Y_{1i} - \bar{Y}_1)^2 \frac{1}{n-1}\sum_{i=1}^{n}(Y_{2i} - \bar{Y}_2)^2}} \tag{18.8}$$

$$= \frac{s_{12}}{\sqrt{s_1^2 s_2^2}} = \frac{s_{12}}{s_1 s_2} \tag{18.9}$$

Thus, r can be expressed as the sample covariance s_{12} scaled by the standard deviation s_1 and s_2 for each variable. This quantity is also known as the **Pearson correlation coefficient**.

The square of the correlation coefficient is called the **coefficient of determination**, and provides an indication of the amount of variability in Y_1 explained by Y_2, or vice versa. It is typically written as R^2 like in linear regression or ANOVA. The value of R^2 ranges from zero to one, with values near one implying a strong relationship (positive or negative) between Y_1 and Y_2, while values near zero imply a weak one. For the Example 1 data, we have $R^2 = 0.787^2 = 0.619$. About 62% of the variability in Y_1 is explained by Y_2, or vice versa.

There is also a likelihood ratio test for $H_0 : \rho = 0$ vs. $H_1 : \rho \neq 0$, equivalent to testing whether Y_1 is independent of Y_2. Under H_0, the test statistic

$$T_s = r\sqrt{\frac{n-2}{1-r^2}} \tag{18.10}$$

has a t distribution with $n-2$ degrees of freedom, and we would reject H_0 for sufficiently large values (Stuart et al. 1999). For the Example 1 data, we have

$$T_s = 0.787\sqrt{\frac{10-2}{1-0.787^2}} = 3.608. \tag{18.11}$$

Using Table T with $10-2 = 8$ degrees of freedom, we see that $P < 0.01$. The correlation between sepal length and width is highly significant ($t_8 = 3.608, P < 0.01$), and so the two variables appear dependent, not independent.

There is an approximate test for $H_0 : \rho = \rho_0$ vs. $H_0 : \rho \neq \rho_0$, for values ρ_0 different from zero. It uses a special transformation for r, the inverse hyperbolic tangent function:

$$\text{arctanh}(r) = \frac{1}{2}\ln\left(\frac{1+r}{1-r}\right), \tag{18.12}$$

defined for $-1 < r < 1$. The effect of this transformation is to spread out the distribution of r and make it more normal. Under H_0, we have $E[\text{arctanh}(r)] \approx \text{arctanh}(\rho_0)$ and $Var[\text{arctanh}(r)] \approx 1/(n-3)$, and so

$$Z_s = \frac{\text{arctanh}(r) - \text{arctanh}(\rho_0)}{\sqrt{1/(n-3)}} \tag{18.13}$$

$$= \sqrt{n-3}\,[\text{arctanh}(r) - \text{arctanh}(\rho_0)] \sim N(0,1) \tag{18.14}$$

for large n (Stuart et al. 1999). As an example of this test, suppose we want to test $H_0 : \rho = 0.5$ for the Example 1 data set. We have

$$Z_s = \sqrt{10-3}\,[\text{arctanh}(0.787) - \text{arctanh}(0.5)] \tag{18.15}$$

$$= 2.646(1.064 - 0.549) = 1.363. \tag{18.16}$$

Using the last row of Table T ($df = \infty$) to find the P value for the standard normal distribution, we find that $P < 0.2$. The correlation coefficient was not significantly different from 0.5 ($Z_s = 1.363, P < 0.2$).

18.2.1 Correlation for Example 1 - SAS demo

We can conduct a correlation analysis using `proc corr` in SAS. We first input the observations using a `data` step, and then generate a scatterplot using `proc gplot` (SAS Institute Inc. 2014a). The correlation analysis is conducted using `proc corr` as shown below, with the variables to be analyzed listed in the `var` statement (SAS Institute Inc. 2014b). The `ods graphics on` and `off` statements enable `proc corr` to generate more sophisticated plots of the data, using the `plots=(scatter matrix)` option (SAS Institute Inc. 2014a). These commands will generate pairwise scatterplots of all the variables, and a scatterplot matrix of all the graphs together.

From the `proc corr` output, we see that the correlation between sepal length and width is highly significant ($r = 0.787, P = 0.0069$). The scatterplot generated by `proc corr` for these two variables is also shown (Fig. 18.9).

SAS Program

```
* Iris.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Correlation for Iris data";
data iris;
        input seplen sepwid;
        datalines;
5.1 3.5
4.9 3.0
4.7 3.2
4.6 3.1
5.0 3.6
5.4 3.9
4.6 3.4
5.0 3.4
4.4 2.9
4.9 3.1
;
run;
* Print data set;
proc print data=iris;
run;
* Correlation analysis and scatterplots;
ods graphics on;
proc corr data=iris plots=(scatter matrix);
        var seplen sepwid;
run;
ods graphics off;
quit;
```

SAS Output

```
                          Correlation for Iris data                    1
                                        09:23 Thursday, June 5, 2014

 Obs    seplen    sepwid

   1      5.1       3.5
   2      4.9       3.0
   3      4.7       3.2
   4      4.6       3.1
   5      5.0       3.6
   6      5.4       3.9
```

```
 7      4.6       3.4
 8      5.0       3.4
 9      4.4       2.9
10      4.9       3.1


                    Correlation for Iris data                          2
                                          09:23 Thursday, June 5, 2014

                       The CORR Procedure

                    2  Variables:    seplen   sepwid


                       Simple Statistics

Variable         N        Mean     Std Dev         Sum     Minimum     Maximum

seplen          10     4.86000     0.29136    48.60000     4.40000     5.40000
sepwid          10     3.31000     0.30714    33.10000     2.90000     3.90000


                 Pearson Correlation Coefficients, N = 10
                       Prob > |r| under H0: Rho=0

                                 seplen        sepwid

                    seplen       1.00000       0.78721
                                               0.0069

                    sepwid       0.78721       1.00000
                                  0.0069
```

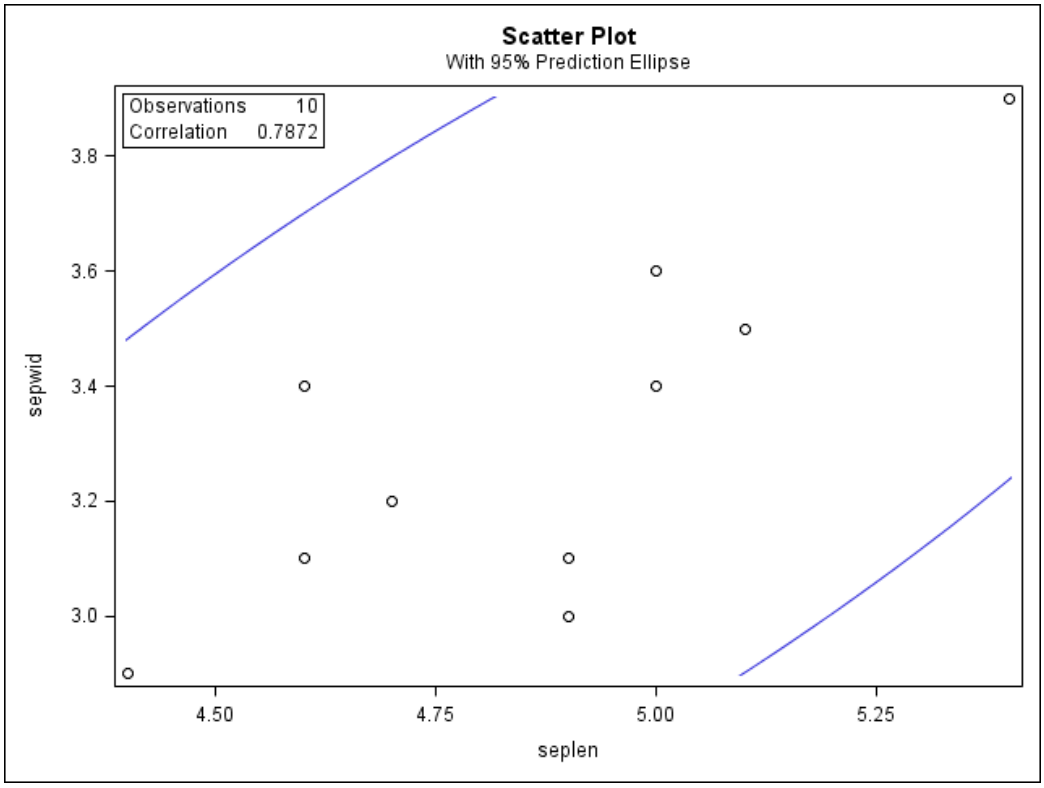

Figure 18.9: Scatterplot of *I. setosa* sepal length and width.

18.2.2 Testing $H_0 : \rho = \rho_0$ - SAS demo

We can use a short SAS program to test $H_0 : \rho = 0.5$ vs. $H_1 : \rho \neq 0.5$ for the Example 1 data (see program and output below). The program calculates the P value for this two-tailed alternative (`pvalue2`) as well as both one-tailed ones (`p_val_gt`,`p_val_lt`). We see that the correlation between sepal length and width is not significantly different from 0.5 ($Z_s = 1.360, P = 0.174$).

SAS Program

```
* rhocalc.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Test Ho: rho = rho_0 where rho_0 is non-zero';
data rhocalc;
        * Input sample size, rho, and rho_0;
        n = 10;
        r = 0.787;
        rho_0 = 0.5;
        zs = sqrt(n-3)*(artanh(r)-artanh(rho_0));
        * P-value for two-tailed test;
        p_value2 = 2*(1 - probnorm(abs(zs)));
        * P-values for one-tailed tests;
        * Ho: rho = rho_0 vs. H1: rho > rho_0;
        p_val_gt = 1 - probnorm(zs);
        * Ho: rho = rho_0 vs. H1: rho < rho_0;
        p_val_lt = probnorm(zs);
run;
* Print test results;
proc print data=rhocalc;
run;
```

SAS Output

```
                Test Ho: rho = rho_0 where rho_0 is non-zero                1
                                                  11:03 Wednesday, June 11, 2014

  Obs     n      r      rho_0       zs      p_value2    p_val_gt    p_val_lt

   1      10    0.787     0.5     1.36043     0.17369    0.086847     0.91315
```

18.2.3 Correlation for *I. setosa*, all data - SAS demo

We now analyze the full data set for *I. setosa*, as listed in Chapter 21. We will examine the correlation between sepal length, sepal width, petal length, and petal width for all 50 flowers. The SAS program is similar to the Example 1 analysis, except that all four variables are listed in the `data` and `proc corr` steps. We see there is a highly significant correlation between sepal length and width ($r = 0.743, P < 0.0001$), and petal length and width are also significantly correlated ($r = 0.332, P = 0.0186$). All the remaining correlations are nonsignificant. It appears that measurements of the same structure (petal or sepal) are correlated, but the correlation is weaker between structures. The scatterplot matrix (Fig. 18.10 reflects these patterns, with sepal length and width showing a strong positive association, with a weaker one for petal length and width. The remaining pairs show no obvious relationships.

SAS Program

```
* Iris_all.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Correlation for Iris data";
data iris;
        input seplen sepwid petlen petwid;
        datalines;
5.1 3.5 1.4 0.2
4.9 3.0 1.4 0.2
4.7 3.2 1.3 0.2
4.6 3.1 1.5 0.2
5.0 3.6 1.4 0.2

etc.

4.8 3.0 1.4 0.3
5.1 3.8 1.6 0.2
4.6 3.2 1.4 0.2
5.3 3.7 1.5 0.2
5.0 3.3 1.4 0.2
;
run;
* Print data set;
proc print data=iris;
run;
* Correlation analysis and scatterplots;
ods graphics on;
proc corr data=iris plots=(scatter matrix);
        var seplen sepwid petlen petwid;
run;
ods graphics off;
quit;
```

SAS Output

```
                          Correlation for Iris data                          1
                                        16:36 Wednesday, June 11, 2014

                  Obs    seplen    sepwid    petlen    petwid

                    1      5.1       3.5       1.4       0.2
                    2      4.9       3.0       1.4       0.2
                    3      4.7       3.2       1.3       0.2
```

```
                  4      4.6       3.1       1.5       0.2
                  5      5.0       3.6       1.4       0.2
etc.

                 46      4.8       3.0       1.4       0.3
                 47      5.1       3.8       1.6       0.2
                 48      4.6       3.2       1.4       0.2
                 49      5.3       3.7       1.5       0.2
                 50      5.0       3.3       1.4       0.2


                         Correlation for Iris data                          2
                                                 16:36 Wednesday, June 11, 2014

                             The CORR Procedure

              4  Variables:    seplen   sepwid   petlen   petwid


                             Simple Statistics

 Variable         N        Mean     Std Dev         Sum     Minimum     Maximum

 seplen          50     5.00600     0.35249   250.30000     4.30000     5.80000
 sepwid          50     3.42800     0.37906   171.40000     2.30000     4.40000
 petlen          50     1.46200     0.17366    73.10000     1.00000     1.90000
 petwid          50     0.24600     0.10539    12.30000     0.10000     0.60000


                   Pearson Correlation Coefficients, N = 50
                           Prob > |r| under H0: Rho=0

                        seplen        sepwid        petlen        petwid

         seplen        1.00000       0.74255       0.26718       0.27810
                                      <.0001        0.0607        0.0505

         sepwid        0.74255       1.00000       0.17770       0.23275
                       <.0001                      0.2170        0.1038

         petlen        0.26718       0.17770       1.00000       0.33163
                       0.0607        0.2170                      0.0186

         petwid        0.27810       0.23275       0.33163       1.00000
                       0.0505        0.1038        0.0186
```

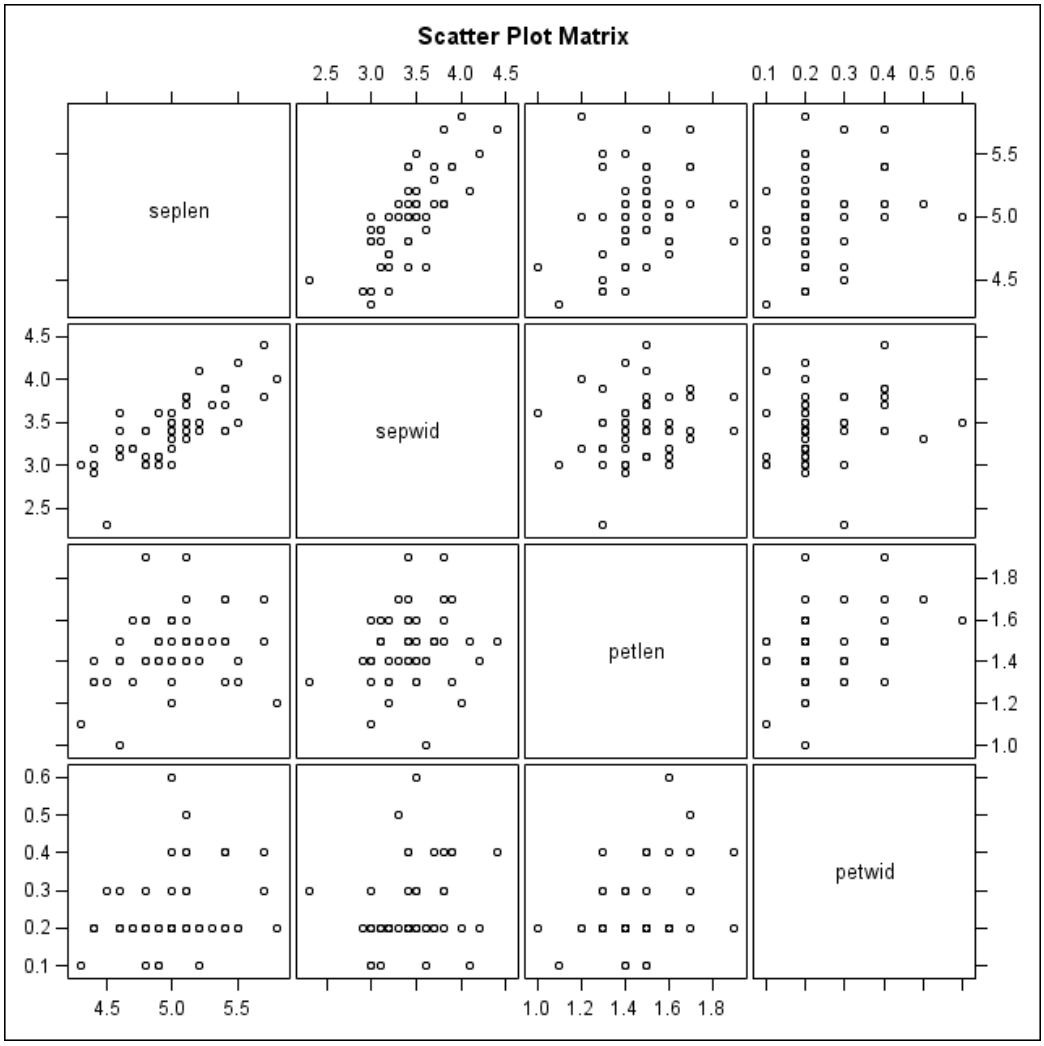

Figure 18.10: Scatterplot matrix for *I. setosa* sepal length and width, and petal length and width.

18.3 Correlation assumptions

The main assumption of correlation is that the data have a bivariate normal distribution. If the data do not appear to be bivariate normal, it may be useful to transform one or both variables. The same transformations used in linear regression may be helpful (see Chapter 17). For example, suppose that the relationship between Y_1 and Y_2 appears to be curved (Fig. 18.11). A log transformation of Y_2 makes the overall distribution more similar to the bivariate normal (Fig. 18.12). Once the distribution appears correct, we would calculate the correlation coefficient r and conduct our tests.

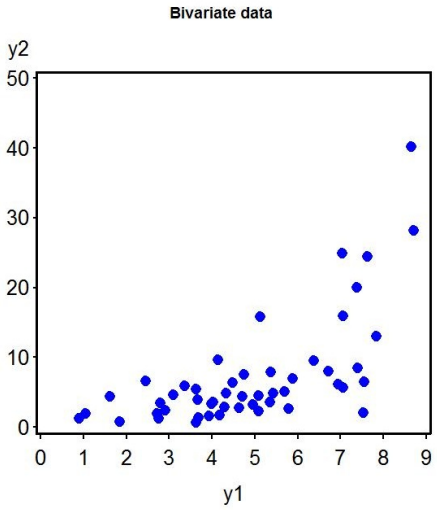

Figure 18.11: Simulated data showing a curved relationship between Y_1 and Y_2.

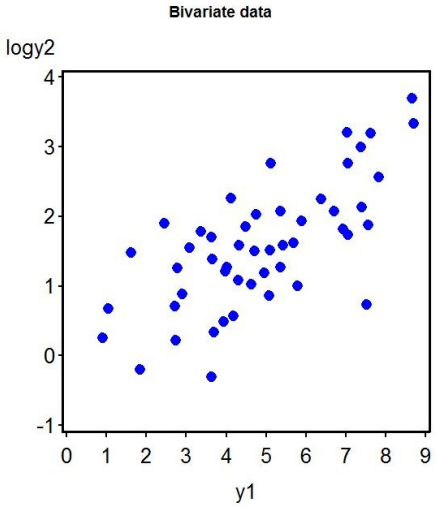

Figure 18.12: Simulated data showing a bivariate normal distribution for Y_1 and $\ln(Y_2)$.

18.4 Nonparametric correlation

There are nonparametric correlation methods useful when the observations are not bivariate normal. One common method is the Spearman rank correlation test (Hollander et al. 2014). This procedure simply substitutes the rank values of Y_1 and Y_2 in the formula for r, then proceeds as before. We are still interested in testing whether Y_1 and Y_2 are independent, but no distribution is specified.

We will illustrate the Spearman rank correlation procedure using the Example 1 data set. The initial calculations are shown in Table 18.2. We next calculate the Spearman rank correlation r_s using the results from this table. We have

$$r_s = \frac{62}{\sqrt{81 \times 81.5}} = 0.763. \tag{18.17}$$

If we want to test whether Y_1 and Y_2 are independent, we can use the same test procedure as before, but substituting r_s for r. For the Table 18.2 data, we have

$$T_s = r_s \sqrt{\frac{n-2}{1-r_s^2}} = 0.763 \sqrt{\frac{10-2}{1-0.763^2}} = 3.339. \tag{18.18}$$

Using Table T with $10-2 = 8$ degrees of freedom, we see that $P < 0.02$. This result suggests the two variables are not independent ($r_s = 0.763, P < 0.02$).

Table 18.2: Preliminary calculations for Spearman rank correlation using the Example 1 data. Here R_{1i} and R_{2i} are the rank values of sepal length and width. Tied values were assigned the average of their ranks.

i	Y_{1i} = Sepal length	Y_{2i} = Sepal width	R_{1i}	R_{2i}	$(R_{1i} - \bar{R}_1)(R_{2i} - \bar{R}_2)$	$(R_{1i} - \bar{R}_1)^2$	$(R_{2i} - \bar{R}_2)^2$
1	5.1	3.5	9	8	8.75	12.25	6.25
2	4.9	3.0	5.5	2	0.00	0.00	12.25
3	4.7	3.2	4	5	0.75	2.25	0.25
4	4.6	3.1	2.5	3.5	6.00	9.00	4.00
5	5.0	3.6	7.5	9	7.00	4.00	12.25
6	5.4	3.9	10	10	20.25	20.25	20.25
7	4.6	3.4	2.5	6.5	-3.00	9.00	1.00
8	5.0	3.4	7.5	6.5	2.00	4.00	1.00
9	4.4	2.9	1	1	20.25	20.25	20.25
10	4.9	3.1	5.5	3.5	0.00	0.00	4.00
$\sum$	-	-	-	-	62.00	81.00	81.50

18.4.1 Spearman rank correlation for Example 1 - SAS demo

The Spearman rank correlation and tests can be conducted in SAS by adding the `spearman` option to the `proc corr` statement. For the Table 18.2 data, we obtain $r_s = 0.763, P = 0.0102$. See SAS output below showing the Spearman section.

SAS Output

```
Spearman Correlation Coefficients, N = 10
       Prob > |r| under H0: Rho=0

                    seplen        sepwid

seplen           1.00000       0.76308
                               0.0102

sepwid           0.76308       1.00000
                 0.0102
```

18.5 Problems

1. An entomologist is interested in variation in eye and head size for leaf-cutting ants (Moser et al. 2004). A microscope is used to measure the width of the head (mm), and the surface area of the eyes and ocelli (mm). The following data were obtained for the females of one species (*Atta sexdens*).

Head	$\sqrt{\text{Eye}}$	$\sqrt{\text{Ocelli}}$	Head	$\sqrt{\text{Eye}}$	$\sqrt{\text{Ocelli}}$
4.1	0.660	0.311	3.8	0.633	0.290
4.1	0.651	0.301	3.9	0.659	0.293
4.1	0.614	0.287	4.0	0.633	0.287
4.1	0.668	0.301	4.1	0.614	0.295
4.0	0.659	0.298	4.2	0.678	0.295
4.1	0.659	0.306	4.2	0.668	0.292
4.1	0.678	0.311	4.1	0.668	0.304
4.0	0.668	0.311	4.2	0.678	0.298
4.0	0.601	0.285	4.2	0.678	0.286
3.9	0.651	0.288	3.9	0.646	0.295
4.1	0.678	0.303	4.0	0.633	0.295
4.1	0.665	0.298	4.1	0.659	0.295
4.2	0.668	0.306	4.0	0.646	0.296
4.0	0.668	0.306	4.1	0.655	0.298
4.1	0.678	0.306	4.0	0.659	0.290
4.0	0.659	0.301	4.1	0.678	0.298
3.9	0.659	0.298	4.1	0.678	0.301
4.1	0.678	0.304	4.1	0.668	0.298
4.2	0.668	0.299	4.1	0.659	0.295
4.1	0.659	0.304	4.2	0.678	0.301
4.1	0.665	0.301	3.9	0.687	0.296
4.2	0.665	0.307	4.0	0.614	0.293
4.1	0.651	0.306	4.1	0.668	0.298
4.2	0.659	0.293	4.3	0.678	0.304
4.1	0.659	0.301	4.1	0.646	0.297
4.0	0.659	0.301	4.2	0.655	0.301

(a) Calculate all pairwise correlations among these variables using SAS. Interpret the results of this analysis, providing a P value and discussing the significance of the test. Provide a biological explanation for the positive correlations among these variables.

(b) Test whether each of the pairwise correlations is significantly different from 0.2.

(c) Calculate all pairwise Spearman rank correlations using SAS. Interpret the results of this analysis.

18.6 References

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Moser, J. C., Reeve, J. D., Bento, J. M. S., Della Lucia, T. M. C., Cameron, R. S. & Heck, N. M. (2004) Eye size and behaviour of day- and night-flying leafcutting ant alates. *Journal of Zoology* 264: 69-75.

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Chapter 19

More Complex ANOVA Designs

This chapter examines three designs that incorporate more factors and introduce some new elements of experimental design. They are three-way ANOVA, one-way nested ANOVA, and analysis of covariance (ANCOVA). These are common designs whose elements can be combined to generate even more elaborate ones. A useful guide to complex ANOVA designs is Winer et al. (1991), who provide a description and statistical model for each design. Once a particular design is identified, the statistical model can be used to program the analysis in SAS or other software.

19.1 Three-way ANOVA

We will first discuss three-way ANOVA, an analysis which examines how three different factors influence the means of the different groups. The three factors may be any combination of fixed or random effects and are typically referred to a Factors A, B, and C. In this design, there are one or more replicate observations for each combination of the three factors. The statistical analysis for three-way ANOVA designs may include F tests for the main effects of the factors as well as the interactions among them. For example, if the design has replication and all three factors are fixed, there are F tests for the main effects (Factor A, B, C), each pairwise interaction (A $\times$ B, A $\times$ C, B $\times$ C), and a three-way interaction (A $\times$ B $\times$ C). The additional complexity of this design with its many interactions can make interpretation

of the results quite challenging.

As an example of three-way ANOVA, we will analyze data from an experiment by Maestre & Reynolds (2007). This study examined how overall nutrient and water availability, and nutrient heterogeneity, affected grassland biomass production (Table 19.1). Nutrient heterogeneity was manipulated by placing the nitrogen at a particular location within the container vs. an even distribution. See Chapter 14 for further description of this experiment. We will use the notation Y_{ijkl} to reference the observations in three-way ANOVA designs. The i subscript refers to the group or treatment within Factor A (in this case nitrogen heterogeneity), j the treatment within Factor B (nitrogen levels), k the treatment within Factor C (water levels), while l refers to the observation within the treatment. For example, Y_{1134} refers to the fourth observation in the no nutrient heterogeneity, 40 mg N, 375 ml water treatment, which is 7.901.

Table 19.1: Example 1 - Effect of nitrogen heterogeneity, nitrogen availability, and water availability on the total biomass of grassland plants grown in microcosms (Maestre & Reynolds 2007). The table illustrates how the subscripts for Y_{ijkl} vary across treatments for a portion of the data set (see Chapter 21 for the full version).

N het. (Y/N)	N (mg)	Water (ml/week)	Y_{ijkl} = Biomass	i	j	k	l
N	40	125	4.372	1	1	1	1
N	40	125	4.482	1	1	1	2
N	40	125	4.221	1	1	1	3
N	40	125	3.977	1	1	1	4
N	40	250	7.400	1	1	2	1
N	40	250	8.027	1	1	2	2
N	40	250	7.883	1	1	2	3
N	40	250	7.769	1	1	2	4
N	40	375	7.226	1	1	3	1
N	40	375	8.126	1	1	3	2
N	40	375	6.840	1	1	3	3
N	40	375	7.901	1	1	3	4
etc.							
Y	120	250	10.731	2	3	2	1
Y	120	250	12.640	2	3	2	2
Y	120	250	10.350	2	3	2	3
Y	120	250	11.550	2	3	2	4
Y	120	375	14.697	2	3	3	1
Y	120	375	17.826	2	3	3	2
Y	120	375	14.711	2	3	3	3
Y	120	375	13.614	2	3	3	4

19.1.1 Three-way fixed effects model

Suppose that we want to model the observations in a study like Example 1, where there are Factors A, B, and C. Assume the design is factorial with every possible combination of the three factors, with $n > 1$ observations of each one. This design is often called three-way ANOVA with replication. A common model for the observations Y_{ijkl} in such designs (Winer et al. 1991) is

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + + \gamma_k + (\alpha\beta)_{ij} + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkl}. \quad (19.1)$$

Here μ is the grand mean of the observations, while α_i is the deviation from μ caused by the ith level or treatment of Factor A, β_j the deviation caused by the jth level of Factor B, and γ_k is the deviation caused by the kth level of Factor C. These terms are the **main effects** in the model. The terms $(\alpha\beta)_{ij}$, $(\beta\gamma)_{jk}$, and $(\alpha\gamma)_{ik}$ are pairwise or first-order interactions among Factors A and B, B and C, and A and C. These interactions are also symbolized as A $\times$ B, B $\times$ C, and A $\times$ C. They are similar to the interaction term in two-way ANOVA, but with three factors in the design there are more possibilities for interaction among them. The term $(\alpha\beta\gamma)_{ijk}$ models a second-order interaction (symbolized as A $\times$ B $\times$ C) among all three factors. It can be thought of as an interaction of interactions, i.e., the interaction between Factors A and B could change across levels of C. The ϵ_{ijkl} term represents the usual random departures from the mean value predicted by the main effects and interactions due to natural variability.

The objective in three-way ANOVA is to test whether Factor A, B, and C have an effect on the group means, and whether there are interactions among these factors. For Factor A this amounts to testing H_0 : all $\alpha_i = 0$, and similarly H_0 : all $\beta_j = 0$ for Factor B and H_0 : all $\gamma_k = 0$ for Factor C. For the A $\times$ B interaction, we would test $H_0 : (\alpha\beta)_{ij} = 0$, and similarly $H_0 : (\alpha\gamma)_{ik} = 0$ for the A $\times$ C and $H_0 : (\beta\gamma)_{jk} = 0$ for the B $\times$ C interactions. For the second-order interaction A $\times$ B $\times$ C, we are interested in testing H_0 : all $(\alpha\beta\gamma)_{ijk} = 0$. The F tests for these hypotheses can be constructed using various sums of squares and mean squares, similar to two-way ANOVA, and are also examples of likelihood ratio tests. We will not consider this process in detail but instead proceed directly to the analysis and interpretation of the Example 1 data set.

19.1.2 Three-way ANOVA for Example 1 - SAS demo

The first step in the program (see below) is to read in the observations using a `data` step, with the first variable (`nitrohet`) denoting the nitrogen heterogeneity treatment, while `nitrogen` and `water` represent the nitrogen and water levels. The variable `biomass` is then log-transformed before analysis, yielding the dependent variable `y = log10(biomass)`. Three separate plots then requested using `proc gplot` (SAS Institute Inc. 2014a), one for every pairwise combination of `nitrohet`, `nitrogen`, and `water`. These plots will allow us to examine the main effects and all first order (pairwise) interactions among the treatments. The choice as to whether a particular treatment is plotted on the x-axis or appears as separate groups (lines) on the graph is arbitrary. Like two-way ANOVA, if the lines are not parallel in a plot this suggests there is an interaction between the factors.

The second set of plots is intended to illustrate the second-order interaction among the three factors. Each plot illustrates the interaction between nitrogen and water at one level of nitrogen heterogeneity. If there is a second-order interaction, then the plots will appear different from one another.

The next section of the program conducts the three-way ANOVA using `proc glm` (SAS Institute Inc. 2014b). The `class` statement tells SAS that `nitrohet`, `nitrogen`, and `water` are used to classify the observations into the 18 different treatment groups. The `model` statement tells SAS the form of the ANOVA model. Recall that the model for fixed effects three-way ANOVA (Eq. 19.1). The statement `nitrohet|nitrogen|water` is SAS shorthand for this model, and will automatically generate all the possible main effects and interactions of the three factors.

The `lsmeans` statement causes `proc glm` to calculate quantities called least squares means for each level of `nitrohet`, `nitrogen`, and `water`. When the data are balanced these are equivalent to the means for each treatment group, but least squares means have some advantages for unbalanced data and other statistical models. The option `adjust=tukey` requests multiple comparisons among treatments using the Tukey method. This is useful for comparing the different levels of the main effects. However, tests for the main effects as well as multiple comparisons should be treated with caution in the presence of strong interaction (see Chapter 14 for discussion of this issue).

We now examine the results of the tests generated by SAS, examining the interactions first (see SAS output below). We are primarily interested in the results for Type III sums of squares. We see that the second-order

nitrogen heterogeneity × nitrogen × water interaction was nonsignificant ($F_{4,54} = 1.39, P = 0.2492$). The two graphs that illustrate this interaction appear similar, further indicating this interaction is weak or absent (Fig. 19.1, 19.2). Turning to the first order interactions, we see that the nitrogen heterogeneity × nitrogen interaction was nonsignificant ($F_{2,54} = 0.93, P = 0.4017$). In agreement with this result, the corresponding graph for this interaction (Fig. 19.3) suggests these two treatments are additive. The nitrogen × water interaction ($F_{4,54} = 12.90, P < 0.0001$) was highly significant. Examining Fig. 19.4, we see that the source of this interaction was a reduced effect of watering at lower nitrogen levels. The nitrogen heterogeneity × water interaction was also highly significant ($F_{2,54} = 13.10, P < 0.0001$). This interaction was apparently generated by a stronger effect of nitrogen heterogeneity at the lowest water level (Fig. 19.5). Overall, the significant interactions suggest that effects of these factors on biomass are not additive (Maestre & Reynolds 2007).

The SAS analysis also found highly significant main effects of nitrogen heterogeneity ($F_{1,54} = 144.14, P < 0.0001$), nitrogen ($F_{2,27} = 129.71, P < 0.0001$) and water ($F_{2,27} = 657.00, P < 0.0001$) on biomass. We can judge the strength of these effects through the interaction plots as well as the sum of squares values. Watering appears to have the largest effect on biomass, followed by nitrogen and nitrogen heterogeneity. The heterogeneity result is particularly intriguing, because more biomass was generated when this nutrient was heterogeneously distributed in space. Maestre & Reynolds (2007) suggest this occurred because nutrient patches encourage root proliferation, leading to increased nutrient uptake and overall growth. Even though there were significant interactions in this analysis, the main effects were larger and explained most of the variation in these data.

SAS program

```
* Maestre_biomass_3way.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Three-way ANOVA for biomass";
title2 "Data from Maestre and Reynolds (2007)";
data maestre;
        input nitrohet $ nitrogen water biomass;
        * Apply transformations here;
        y = log10(biomass);
        datalines;
N   40  125   4.372
N   40  125   4.482
N   40  125   4.221
N   40  125   3.977
N   40  250   7.400
N   40  250   8.027
N   40  250   7.883
N   40  250   7.769

etc.

Y  120  375  14.697
Y  120  375  17.826
Y  120  375  14.711
Y  120  375  13.614
;
run;
* Print data set;
proc print data=maestre;
run;
proc gplot data=maestre;
        plot y*nitrohet=nitrogen y*nitrogen=water y*nitrohet=water / vaxis=axis1
        haxis=axis1 legend=legend1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
        legend1 label=(height=2) value=(height=2);
run;
* Sort data by nitrohet levels;
proc sort data=maestre;
        by nitrohet;
run;
* Plots to show three-way interaction;
proc gplot data=maestre;
```

```
        by nitrohet;
        plot y*nitrogen=water / vaxis=axis1 haxis=axis1 legend=legend1;
        symbol1 i=std1mjt v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
        legend1 label=(height=2) value=(height=2);
run;
* Three-way ANOVA with all fixed effects;
proc glm data=maestre;
        class nitrohet nitrogen water;
        model y = nitrohet|nitrogen|water;
        lsmeans nitrohet nitrogen water / adjust=tukey cl lines;
        output out=resids p=pred r=resid;
run;
goptions reset=all;
title "Diagnostic plots to check anova assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
        plot resid*pred=1 / vaxis=axis1 haxis=axis1;
        symbol1 v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
        qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS Output

```
                    Three-way ANOVA for biomass                        1
                 Data from Maestre and Reynolds (2007)
                                        09:49 Friday, June 7, 2013

        Obs    nitrohet    nitrogen    water    biomass       y

          1       N           40       125       4.372    0.64068
          2       N           40       125       4.482    0.65147
          3       N           40       125       4.221    0.62542
          4       N           40       125       3.977    0.59956
          5       N           40       250       7.400    0.86923
          6       N           40       250       8.027    0.90455
          7       N           40       250       7.883    0.89669
          8       N           40       250       7.769    0.89037
```

etc.

```
                    Three-way ANOVA for biomass                        3
                 Data from Maestre and Reynolds (2007)
                                        09:49 Friday, June 7, 2013

                          The GLM Procedure

                       Class Level Information

                 Class         Levels    Values

                 nitrohet           2    N Y

                 nitrogen           3    40 80 120

                 water              3    125 250 375


                Number of Observations Read          72
                Number of Observations Used          72
```

Three-way ANOVA for biomass 4
Data from Maestre and Reynolds (2007)
09:49 Friday, June 7, 2013

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	1.86010971	0.10941822	106.05	<.0001
Error	54	0.05571723	0.00103180		
Corrected Total	71	1.91582694			

R-Square	Coeff Var	Root MSE	y Mean
0.970917	3.492176	0.032122	0.919818

Source	DF	Type I SS	Mean Square	F Value	Pr > F
nitrohet	1	0.14872636	0.14872636	144.14	<.0001
nitrogen	2	0.26766625	0.13383312	129.71	<.0001
nitrohet*nitrogen	2	0.00191433	0.00095717	0.93	0.4017
water	2	1.35577897	0.67788949	657.00	<.0001
nitrohet*water	2	0.02702407	0.01351204	13.10	<.0001
nitrogen*water	4	0.05325694	0.01331423	12.90	<.0001
nitroh*nitroge*water	4	0.00574279	0.00143570	1.39	0.2492

Source	DF	Type III SS	Mean Square	F Value	Pr > F
nitrohet	1	0.14872636	0.14872636	144.14	<.0001
nitrogen	2	0.26766625	0.13383312	129.71	<.0001
nitrohet*nitrogen	2	0.00191433	0.00095717	0.93	0.4017
water	2	1.35577897	0.67788949	657.00	<.0001
nitrohet*water	2	0.02702407	0.01351204	13.10	<.0001
nitrogen*water	4	0.05325694	0.01331423	12.90	<.0001
nitroh*nitroge*water	4	0.00574279	0.00143570	1.39	0.2492

Three-way ANOVA for biomass 5
Data from Maestre and Reynolds (2007)
09:49 Friday, June 7, 2013

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

nitrohet	y LSMEAN	H0:LSMean1=LSMean2 Pr > |t|
N	0.87436837	<.0001
Y	0.96526708	

nitrohet	y LSMEAN	95% Confidence Limits	
N	0.874368	0.863635	0.885102
Y	0.965267	0.954534	0.976000

Least Squares Means for Effect nitrohet

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	-0.090899	-0.106077	-0.075720

Tukey Comparison Lines for Least Squares Means of nitrohet

LS-means with the same letter are not significantly different.

	y LSMEAN	nitrohet	LSMEAN Number
A	0.96526708	Y	2
B	0.87436837	N	1

etc.

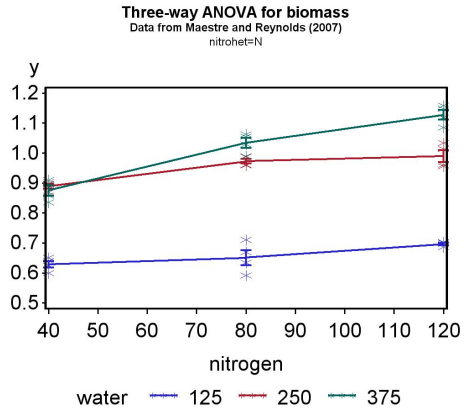

Figure 19.1: Means ± standard errors and data for the Example 1 experiment, where $Y = \log_{10}(\text{Biomass})$. This figure is the first of two figures illustrating any second order interaction, i.e., nitrogen heterogeneity × nitrogen × water.

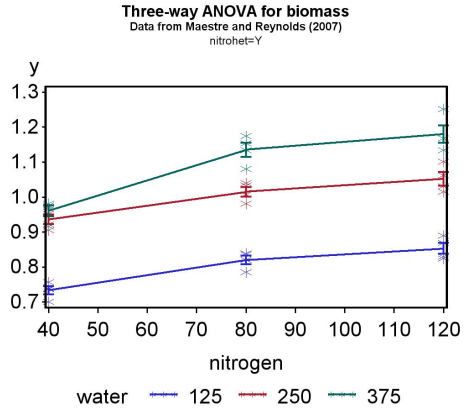

Figure 19.2: Means ± standard errors and data for the Example 1 experiment, where $Y = \log_{10}(\text{Biomass})$. This figure is the second of two figures illustrating any second-order interaction, i.e., nitrogen heterogeneity × nitrogen × water.

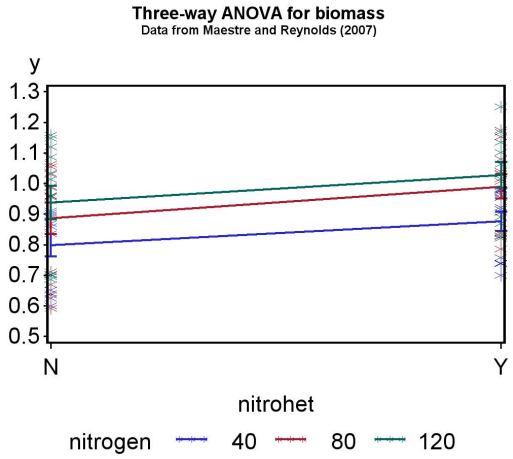

Figure 19.3: Means ± standard errors and data for the Example 1 experiment, where $Y = \log_{10}(\text{Biomass})$. This figure illustrates any nitrogen heterogeneity × nitrogen interaction, and the main effects of nitrogen heterogeneity and nitrogen.

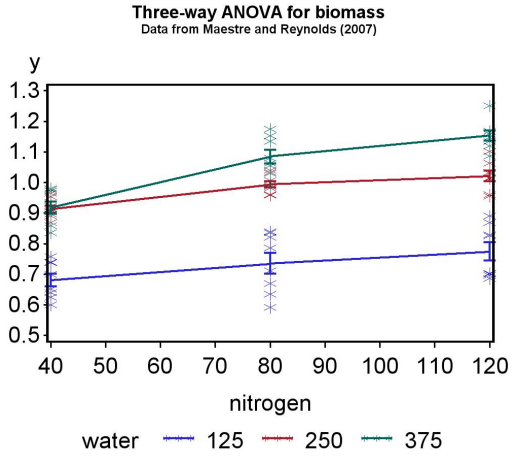

Figure 19.4: Means ± standard errors and data for the Example 1 experiment, where $Y = \log_{10}(\text{Biomass})$. This figure illustrates any nitrogen × water interaction, and the main effects of nitrogen and water.

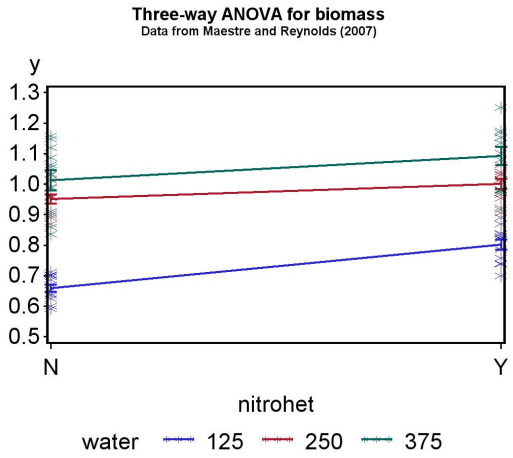

Figure 19.5: Means ± standard errors and data for the Example 1 experiment, where $Y = \log_{10}(\text{Biomass})$. This figure illustrates any nitrogen heterogeneity × water interaction, and the main effects of nitrogen heterogeneity and water.

19.1.3 Tests for main effects with interaction

As discussed in Chapter 14, there are questions as to whether tests of main effects are appropriate when interaction is significant, and these extend to three-way designs. As an alternative, we can use the `slice` option for `lsmeans` to avoid tests of the main effects. The modified SAS code is listed below along with the output. We first fit the full model including all the interactions, and observe that the nitrogen heterogeneity × nitrogen × water interaction is nonsignificant ($F_{4,54} = 1.39, P = 0.2492$), as is the nitrogen heterogeneity × nitrogen interaction ($F_{2,54} = 0.93, P = 0.4017$). We then drop these interactions and refit the model. The remaining two interactions are both highly significant in this reduced model (nitrogen heterogeneity × water, $F_{2,60} = 12.79, P < 0.0001$; nitrogen × water, $F_{4,60} = 12.61, P < 0.0001$). We skip the tests of the main effects because of these highly significant interactions, and instead use the `slice` option to test for a nitrogen heterogeneity effect at each water level, and vice versa. These tests were all highly significant, suggesting that nitrogen heterogeneity affects biomass at every water level, and water affects biomass at every nitrogen heterogeneity level. Similar tests could be conducted to examine the effects of nitrogen and water.

SAS Program

```
* Three-way ANOVA with interaction;
title3 "MODEL WITH ALL FOUR INTERACTIONS";
proc glm data=maestre;
        class nitrohet nitrogen water;
        model y = nitrohet|nitrogen|water / ss2;
        output out=resids p=pred r=resid;
run;
* Three-way ANOVA dropping ns interactions;
title3 "MODEL WITH ONLY SIGNIFICANT INTERACTIONS";
proc glm data=maestre;
        class nitrohet nitrogen water;
        model y = nitrohet nitrogen water nitrohet*water nitrogen*water / ss2;
        lsmeans nitrohet*water / slice=water slice=nitrohet;
run;
```

SAS Output

Three-way ANOVA for biomass 4
Data from Maestre and Reynolds (2007)
MODEL WITH ALL FOUR INTERACTIONS
15:27 Friday, November 8, 2013

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	1.86010971	0.10941822	106.05	<.0001
Error	54	0.05571723	0.00103180		
Corrected Total	71	1.91582694			

R-Square	Coeff Var	Root MSE	y Mean
0.970917	3.492176	0.032122	0.919818

Source	DF	Type II SS	Mean Square	F Value	Pr > F
nitrohet	1	0.14872636	0.14872636	144.14	<.0001
nitrogen	2	0.26766625	0.13383312	129.71	<.0001
nitrohet*nitrogen	2	0.00191433	0.00095717	0.93	0.4017
water	2	1.35577897	0.67788949	657.00	<.0001
nitrohet*water	2	0.02702407	0.01351204	13.10	<.0001
nitrogen*water	4	0.05325694	0.01331423	12.90	<.0001
nitroh*nitroge*water	4	0.00574279	0.00143570	1.39	0.2492

Three-way ANOVA for biomass 6
Data from Maestre and Reynolds (2007)
MODEL WITH ONLY SIGNIFICANT INTERACTIONS
15:27 Friday, November 8, 2013

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1.85245259	0.16840478	159.44	<.0001
Error	60	0.06337435	0.00105624		
Corrected Total	71	1.91582694			

R-Square	Coeff Var	Root MSE	y Mean
0.966921	3.533291	0.032500	0.919818

Source	DF	Type II SS	Mean Square	F Value	Pr > F
nitrohet	1	0.14872636	0.14872636	140.81	<.0001
nitrogen	2	0.26766625	0.13383312	126.71	<.0001
water	2	1.35577897	0.67788949	641.80	<.0001
nitrohet*water	2	0.02702407	0.01351204	12.79	<.0001
nitrogen*water	4	0.05325694	0.01331423	12.61	<.0001

11:20 Monday, November 25, 2013

The GLM Procedure
Least Squares Means

nitrohet	water	y LSMEAN
N	125	0.65929804
N	250	0.95137559
N	375	1.01243148
Y	125	0.80223888
Y	250	1.00139663
Y	375	1.09216574

Three-way ANOVA for biomass 8
Data from Maestre and Reynolds (2007)
MODEL WITH ONLY SIGNIFICANT INTERACTIONS
11:20 Monday, November 25, 2013

The GLM Procedure
Least Squares Means

nitrohet*water Effect Sliced by water for y

water	DF	Sum of Squares	Mean Square	F Value	Pr > F
125	1	0.122592	0.122592	116.07	<.0001
250	1	0.015013	0.015013	14.21	0.0004
375	1	0.038145	0.038145	36.11	<.0001

Three-way ANOVA for biomass 9
Data from Maestre and Reynolds (2007)
MODEL WITH ONLY SIGNIFICANT INTERACTIONS
11:20 Monday, November 25, 2013

The GLM Procedure
Least Squares Means

nitrohet*water Effect Sliced by nitrohet for y

nitrohet	DF	Sum of Squares	Mean Square	F Value	Pr > F
N	2	0.854961	0.427481	404.72	<.0001
Y	2	0.527842	0.263921	249.87	<.0001

19.1.4 Other three-way designs

The Maestre & Reynolds (2007) experiment had four replicate containers for each treatment combination ($n = 4$), and so it was possible to fit a model with a second order interaction, namely nitrogen heterogeneity $\times$ nitrogen $\times$ water. Suppose now there was only observation for each treatment combination ($n = 1$). It is still possible to analyze these data using three-way ANOVA, but the data are not sufficient to fit a model with a second-order interaction. We would therefore use the model

$$Y_{ijk} = \mu + \alpha_i + \beta_j + +\gamma_k + (\alpha\beta)_{ij} + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + \epsilon_{ijk}. \quad (19.2)$$

The equivalent model statement for `proc glm` would be

```
model y = nitrohet nitrogen water nitrohet*nitrogen nitrohet*water
nitrogen*water;
```

There is no shorthand method of specifying this model. The SAS output would be interpreted in the same way as the model with replication, except there would be no test for a second-order interaction.

Another common three-way design could have one or more factors that are random effects. For example, suppose that one manipulated nitrogen and water levels similar to Maestre & Reynolds (2007) but conducted the experiment in three different blocks, either different locations in the greenhouse or points in time. Block could be a random effect in this design, and the corresponding model would be

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + C_k + (\alpha\beta)_{ij} + (\beta C)_{jk} + (\alpha C)_{ik} + (\alpha\beta C)_{ijk} + \epsilon_{ijkl}. \quad (19.3)$$

Here C stands for a random block effect, with $C \sim N(0, \sigma^2_C)$. Note that every interaction term involving C is also considered a random effect. This model could be analyzed with `proc mixed` (SAS Institute Inc. 2014b) using the following SAS statements:

```
proc mixed cl;
        class nitrogen water block;
        model y = nitrogen water nitrogen*water / ddfm=kr outp=resids;
        random block block*nitrogen block*water block*nitrogen*water;
run;
```

19.2 One-way nested ANOVA

The second design we will examine are called one-way nested designs. There are two factors in this design, a Factor A that may be a fixed or random effect, and a random nested Factor B. Nested means that for each level of Factor A, there are several levels of Factor B that are unique to that level of A. There are several replicate observations for each combination of Factor A and B.

As an example of this design, we will examine a genetic study of a minute parasitic wasp, *Anagrus delicatus* (Hymenoptera: Mymaridae). This wasp attacks eggs of the planthopper *Prokelisia marginata* (Homoptera: Delphacidae), a salt marsh insect that feeds on *Spartina* plants. Cronin & Strong (1996) were interested in the genetics of various wasp traits, including the number of eggs carried by the wasps themselves, ovipositor length, and various behavioral traits. They collected female wasps from three separate sites in San Franciso Bay and established genetically identical isolines from individual wasps collected from each site. They then measured the traits for a number of individuals from each isoline. Isolines are the nested factor in this design, because each isoline was established from a single site. Sites were classified as a fixed effect because there were essentially only three sites available for sampling, and so the sites were not randomly selected from a population of sites. Example 2 below shows a simulated data set based on this study, with three sites, 14 isolines per site, and eight individuals per isoline.

Table 19.2: Example 2 - Fecundity for *Anagrus delicatus* collected from three different sites, with 14 isolines per site and eight wasps per isoline. The data were simulated from results presented in Cronin and Strong (1996). Note that the values in the site, isoline, and wasp columns also correspond to the subscripts for Y_{ijk}. See Chapter 21 for the full version of this data set.

Site	Isoline	Wasp	Y_{ijk} = eggs
1	1	1	37
1	1	2	41
1	1	3	46
1	1	4	44
1	1	5	43
1	1	6	41
1	1	7	38
1	1	8	37
1	2	1	37
1	2	2	28
1	2	3	34
1	2	4	37
1	2	5	35
1	2	6	39
1	2	7	36
etc.			
3	13	1	36
3	13	2	39
3	13	3	36
3	13	4	30
3	13	5	37
3	13	6	32
3	13	7	38
3	13	8	39
3	14	1	32
3	14	2	34
3	14	3	41
3	14	4	33
3	14	5	35
3	14	6	35
3	14	7	34
3	14	8	31

19.2.1 Nested ANOVA models

Suppose that we want to model the observations in a study like Example 2, where there is a fixed Factor A and a nested Factor B. A common model for the observations Y_{ijk} in such designs (Winer et al. 1991) is

$$Y_{ijk} = \mu + \alpha_i + B_{j(i)} + \epsilon_{ijk}. \tag{19.4}$$

Here μ is the grand mean of the observations, α_i the deviation from μ caused by the ith level or treatment of Factor A, and $B_{j(i)}$ the random deviation caused by the jth level of Factor B within the ith level of Factor A. $B_{j(i)}$ is assumed to be normally distributed with mean zero and variance $\sigma^2_{B(A)}$, or $B_{j(i)} \sim N(0, \sigma^2_{B(A)})$, while $\epsilon_{ijk} \sim N(0, \sigma^2)$ as usual. $B_{j(i)}$ and ϵ_{ijk} are assumed to be independent. This model has two variance components, namely $\sigma^2_{B(A)}$ and σ^2.

The behavior of this model is illustrated in Fig. 19.6, for $a = 3$ levels of Factor A and $b = 4$ levels of Factor B nested within A. The figure illustrates how the value of α_i shifts the mean of the observations away from μ, similar to other ANOVA models. The $B_{j(i)}$ values, which are random variables, shift the observations for each nested level away from the values set by $\mu + \alpha_i$. Because they are random variables, the values of $B_{j(i)}$ are different for each level of Factor A.

The usual objectives for this nested ANOVA design are to test for Factor A effects, and estimate the variance components $\sigma^2_{B(A)}$ and σ^2. For Factor A this amounts to testing H_0 : all $\alpha_i = 0$. We will not consider this process in detail but proceed to the analysis and interpretation of the Example 2 data set. We will use `proc mixed` for the analysis because this design involves a mixed model.

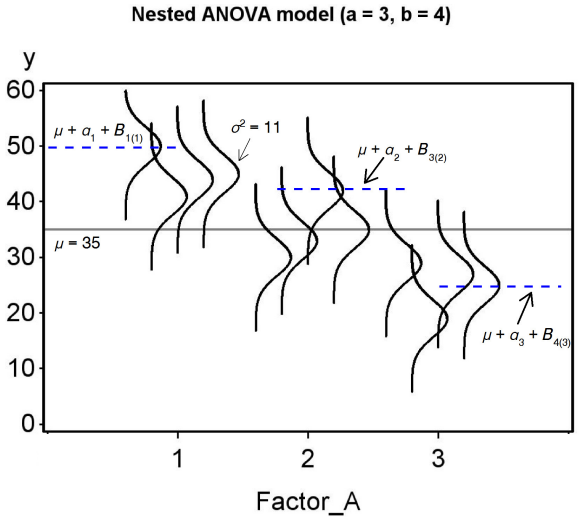

Figure 19.6: Mixed model for nested ANOVA showing the Factor A and B effects.

19.2.2 Nested ANOVA for Example 2 - SAS demo

The first step in analyzing the Example 2 data is to read the observations using a `data` step, with the variables `site` and `isoline` denoting the collection site and *Anagrus* isoline (see below). Although the isolines are numbered similarly across the three sites, note they are actually unique to each site and so are nested within sites. The variable `wasp` refers to a particular wasp within each isoline, but is not used in the analyses. Two plots are then requested using `proc gplot` (SAS Institute Inc. 2014a), one showing the mean for each site and so illustrating the site effect. The second plot shows the individual wasps color-coded by isoline, allowing for a visual comparison of variation among and within isolines. The x-axis position of each wasp is jittered to keep the points from overlapping. This involves adding a small random quantity to the `site` value, generating a new variable called `site_jit` that differs for each wasp.

The next section of the program conducts the nested ANOVA using `proc mixed` (SAS Institute Inc. 2014b). The `class` statement tells SAS that `site` and `isoline` are used to classify the observations. Next, the fixed effect site is listed in the `model` statement, while the random, nested effect of isoline

is incorporated in the `random` statement. SAS uses the syntax `isoline(site)` to indicate that isoline is nested within site. An `lsmeans` statement is used to compared the different sites using the Tukey method.

The analysis found no significant effect of site ($F_{2,39} = 2.3, P = 0.1323$) on the number of eggs per wasp (Fig. 19.7. The estimated variance among isolines within sites ($\hat{\sigma}^2_{B(A)} = \hat{\sigma}^2_{\text{site(isoline)}} = 10.17$) was substantial relative to the variance among wasps within isolines ($\hat{\sigma}^2 = 11.02$). This pattern can be observed in Fig. 19.8, with the observations for each isoline falling into discernable groups. This suggests that variation in egg number has a significant genetic component.

We can use the two variance components to estimate the heritability of egg number, which is the proportion of the variance due to genotypic vs. phenotypic differences among individuals (Falconer & Mackay 1996). The genotypic variance, V_G, is estimated by the variance among isolines within sites, because each isoline represents a different genetic group. For the wasp example, we have $V_G = \hat{\sigma}^2_{\text{site(isoline)}} = 10.17$. The environmental variance, V_E, is estimated by the variance among individuals within isolines, and represents variation among individuals not due to genotype. It is estimated by the variance among wasps within isolines, or $V_E = \hat{\sigma}^2 = 11.02$. The phenotypic variance is defined as the sum of the genotypic and environmental variance, or $V_P = V_G + V_E$. Heritability is then defined $h^2 = V_G/V_P = V_G/(V_G + V_E)$. It follows that $h^2 = 10.17/(10.17 + 11.02) = 0.48$ for the number of eggs in the wasps. This is relatively large value, suggesting that egg number could readily evolve in response to selection pressure.

SAS program

```
* Nested_ANOVA_Anagrus.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Nested ANOVA for fecundity";
title2 "Data simulated from Cronin and Strong (1996)";
data anagrus;
        * Read large data set from external text file;
        * Set SAS current folder to the location of the text file;
        infile "Nested_ANOVA_Anagrus.txt";
        input site isoline wasp eggs;
        * Apply transformations here;
        y = eggs;
        * Make jittered data for plots;
        site_jit = site + 0.1*rannor(0);
run;
* Print data set;
proc print data=anagrus;
run;
* Plot means and standard errors for each site;
proc gplot data=anagrus;
        plot y*site=1 / vaxis=axis1 haxis=axis1;
        symbol1 i=std1jmt v=none height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Plot observations for each site and isoline;
proc gplot data=anagrus;
        plot y*site_jit=isoline / vaxis=axis1 haxis=axis1;
        symbol1 i=none v=dot height=0.5;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Nested ANOVA mixed model;
proc mixed cl data=anagrus;
        class site isoline;
        model y = site / ddfm=kr outp=resids;
        random isoline(site);
        * Compare levels of fixed effect using Tukey's HSD;
        lsmeans site / diff=all adjust=tukey cl adjdfe=row;
run;
goptions reset=all;
title "Diagnostic plots to check ANOVA assumptions";
* Plot residuals vs. predicted values;
proc gplot data=resids;
        plot resid*pred=1 / vaxis=axis1 haxis=axis1;
```

```
        symbol1 v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
        qqplot resid / normal waxis=3 height=4;
run;
quit;
```

SAS output

Nested ANOVA for fecundity 1
Data simulated from Cronin and Strong (1996)
10:55 Tuesday, June 11, 2013

Obs	site	isoline	wasp	eggs	y	site_jit
1	1	1	1	37	37	1.10326
2	1	1	2	41	41	0.90939
3	1	1	3	46	46	1.18465
4	1	1	4	44	44	1.12283
5	1	1	5	43	43	1.09742
6	1	1	6	41	41	0.95798
7	1	1	7	38	38	1.11470
8	1	1	8	37	37	0.98907

etc.

329	3	14	1	32	32	2.89845
330	3	14	2	34	34	2.96535
331	3	14	3	41	41	3.02094
332	3	14	4	33	33	2.92618
333	3	14	5	35	35	2.93152
334	3	14	6	35	35	3.01175
335	3	14	7	34	34	3.06111
336	3	14	8	31	31	2.93977

Nested ANOVA for fecundity 8
Data simulated from Cronin and Strong (1996)
10:55 Tuesday, June 11, 2013

The Mixed Procedure

Model Information

Data Set	WORK.ANAGRUS
Dependent Variable	y
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
site	3	1 2 3
isoline	14	1 2 3 4 5 6 7 8 9 10 11 12 13 14

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	42
Subjects	1
Max Obs Per Subject	336

Number of Observations

Number of Observations Read	336
Number of Observations Used	336
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	1965.68443676	
1	1	1841.14730382	0.00000000

Convergence criteria met.

Nested ANOVA for fecundity 9
Data simulated from Cronin and Strong (1996)
10:55 Tuesday, June 11, 2013

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate	Alpha	Lower	Upper
isoline(site)	10.1664	0.05	6.5003	18.1260
Residual	11.0187	0.05	9.4338	13.0417

Fit Statistics

-2 Res Log Likelihood	1841.1
AIC (smaller is better)	1845.1
AICC (smaller is better)	1845.2
BIC (smaller is better)	1848.6

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
site	2	39	2.13	0.1323

Least Squares Means

Effect	site	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha
site	1	34.4821	0.9081	39	37.97	<.0001	0.05
site	2	34.2946	0.9081	39	37.77	<.0001	0.05
site	3	32.0982	0.9081	39	35.35	<.0001	0.05

Least Squares Means

Effect	site	Lower	Upper
site	1	32.6454	36.3188
site	2	32.4579	36.1313
site	3	30.2615	33.9349

Differences of Least Squares Means

Effect	site	_site	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment

site	1	2	0.1875	1.2842	39	0.15	0.8847	Tukey
site	1	3	2.3839	1.2842	39	1.86	0.0710	Tukey

Nested ANOVA for fecundity 10
Data simulated from Cronin and Strong (1996)
08:29 Monday, November 11, 2013

The Mixed Procedure

Differences of Least Squares Means

Effect	site	_site	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
site	2	3	2.1964	1.2842	39	1.71	0.0951	Tukey

Differences of Least Squares Means

Effect	site	_site	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
site	1	2	0.9883	0.05	-2.4100	2.7850	-2.9411	3.3161
site	1	3	0.1651	0.05	-0.2136	4.9814	-0.7447	5.5126
site	2	3	0.2142	0.05	-0.4011	4.7939	-0.9322	5.3251

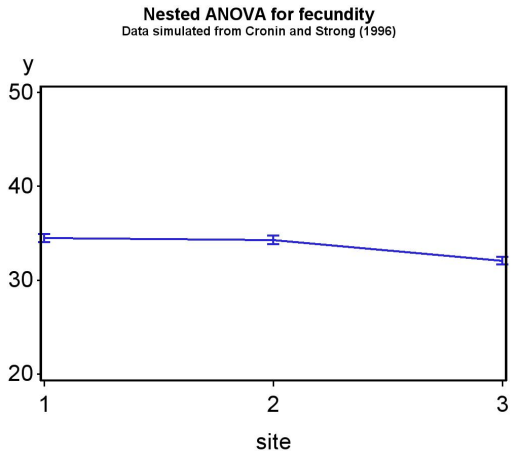

Figure 19.7: Means ± standard errors for each site in the Example 2 study, where Y = eggs.

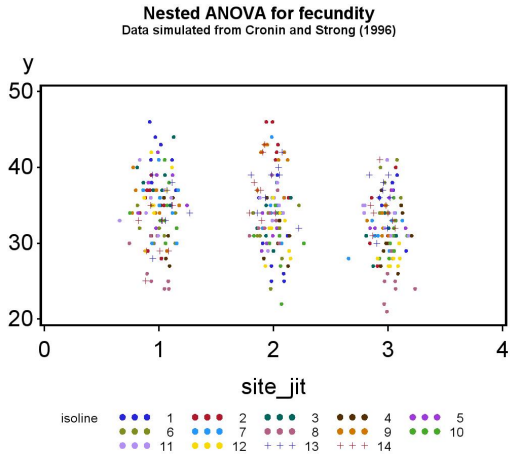

Figure 19.8: Observations for each site and isoline in the Example 2 study, where Y = eggs.

19.3 Analysis of covariance

Analysis of covariance, or ANCOVA, is a design that combines elements of ANOVA and regression. The simplest ANCOVA design is a combination of one-way ANOVA and linear regression. Factor A in the design is typically a fixed effect. For each observation in the design, a covariate X is measured and along with the dependent variable Y. The covariate X is thought to explain some level of variation in Y, and by including it in the design this may increase the power to detect treatment effects. Y is often assumed to be linearly related to X, although nonlinear relationships can be accomodated. More generally, a study might involve a mixture of factors and covariates, and the covariate effects may be of equal or greater interest than the factors.

As an example of ANCOVA, we will analyze a study of the fitness of adult *Thanasimus dubius*, a bark beetle predator, reared on an artificial diet vs. individuals collected from the wild (Reeve et al. 2003). The fitness variables measured were the total number of eggs laid (fecundity) and elytral length (Table 19.3). Body size and fecundity are often related in insects, so elytral length was used as a covariate in the analysis. This helps control for natural variation in body size to better see the treatment effect. The three treatments in the study were (1) artificial diet as larvae and *Ips grandicollis* as adults (`DietIG`), (2) artificial diet and cowpea weevils (`DietCPW`), and (3) wild adults fed cowpea weevils (`WildCPW`). The wild adults were collected from the field and so reared on natural prey as larvae. We will use the notation Y_{ij} to reference the observations in ANCOVA designs, with the i subscript refering to the Factor A or treatment group, while j is the observation within the treatment.

Table 19.3: Example 3 - Fitness of the predator *T. dubius*, reared on an artificial diet as larvae vs. wild individuals collected from the field (Reeve et al. 2003). See Chapter 21 for the full data set.

Y_{ij} = Eggs	X_{ij} = Length (mm)	Treatment	i	j
290	5.7	DietIG	1	1
99	5.2	DietIG	1	2
340	5.5	DietIG	1	3
271	4.8	DietIG	1	4
200	5.2	DietIG	1	5
etc.				
66	4.6	DietCPW	2	1
93	5.0	DietCPW	2	2
9	5.4	DietCPW	2	3
404	5.4	DietCPW	2	4
244	5.1	DietCPW	2	5
etc.				
62	4.7	WildCPW	3	1
290	5.0	WildCPW	3	2
488	5.8	WildCPW	3	3
336	5.2	WildCPW	3	4
337	5.8	WildCPW	3	5
etc.				

19.3.1 ANCOVA model

The following model is commonly used for simple ANCOVA designs (Winer et al. 1991). We have

$$Y_{ij} = \mu + \alpha_i + \beta(X_{ij} - \bar{X}) + \epsilon_{ij}, \tag{19.5}$$

where μ is the grand mean and α_i is the deviation from μ caused by the ith level of Factor A. The term X_{ij} is the value of the covariate for observation Y_{ij}, while $\bar{X}$ is the average of all the covariate values. The parameter β is the slope of the relationship between Y_{ij} and X_{ij}. This slope is assumed to be the same across all levels of Factor A. We will later see how to test this assumption. As usual, the model assumes $\epsilon_{ij} \sim N(0, \sigma^2)$.

The model can also be written in the form

$$Y'_{ij} = Y_{ij} - \beta(X_{ij} - \bar{X}) = \mu + \alpha_i + \epsilon_{ij}. \tag{19.6}$$

Displayed this way, we can see that ANCOVA is equivalent to carrying out a one-way ANOVA on values of Y_{ij} that have been adjusted for the covariate X, namely the values of Y'_{ij}.

Another adjustment of the model is needed by SAS and other statistical software. Combining some elements, the model can be written as

$$Y_{ij} = \mu' + \alpha_i + \beta X_{ij} + \epsilon_{ij}, \tag{19.7}$$

where $\mu' = \mu - \beta\bar{X}$. The quantity μ' represents a grand mean adjusted for the effect of the covariate. The objective in ANCOVA is to test whether Factor A and the covariate have an effect, and so test H_0 : all $\alpha_i = 0$ and $H_0 : \beta = 0$. However, before conducting these F tests we will first test whether the slopes across Factor A groups are identical by including an interaction term in the SAS model. If the slopes are significantly different, we have a scenario similar to ANOVA when interaction is present (see Chapter 14). Like ANOVA, when the interaction is significant tests of the main effects in ANCOVA, namely Factor A and the covariate X, may not make sense.

19.3.2 ANCOVA for Example 3 - SAS demo

The first step in the analysis (see program below) is to plot the number of eggs (`y`) for each treatment (`treat`) against elytral length, the covariate (`x`), using `proc gplot` (SAS Institute Inc. 2014a). This gives some idea whether

each treatment group has the same slope, a key assumption of ANCOVA. The slopes do appear to be similar (Fig. 19.9).

We then fit the ANCOVA model using `proc glm`, because all the effects in the model are fixed effects (SAS Institute Inc. 2014b). The first step is to fit a model with an interaction between the treatment and covariate, and examine the test for the interaction (see first SAS output below). We see that it is non-significant ($F_{2,35} = 0.02, P = 0.9781$), and so can assume the slopes are the same across treatments. We then rerun the program using the model without interaction. We see a highly significant effect of the covariate ($F_{1,37} = 9.99, P = 0.0031$), illustrating the typical strong relationship between body size and fecundity in insects. The treatment effect was nonsigificant ($F_{2,37} = 0.52, P = 0.5976$), implying the treatments themselves had no effect on egg numbers. Predators reared on the artificial diet are apparently similar to wild predators on this measure of fitness, controlling for elytral length and so body size.

The program also includes an `lsmeans` statement to calculate the least squares means for each treatment group, and test for differences among them using the Tukey method. Least squares means are means adjusted for the effect of other variables in the model, and in the case of ANCOVA are the treatment means adjusted for the covariate. In particular, they have the form

$$\bar{Y}_i(adj) = \bar{Y}_i - \hat{\beta}(\bar{X}_i - \bar{\bar{X}}). \tag{19.8}$$

We can see they are composed of two terms, the treatment means and the adjustment for the covariate. Treatment groups that have covariate means ($\bar{X}_i$ values) far from the overall covariate mean ($\bar{\bar{X}}$) receive a larger adjustment. No significant differences were found among the treatment groups, which is not surprising given the overall treatment effect was nonsignificant.

SAS Program

```
* ANCOVA_fitness.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'ANCOVA for T. dubius fitness';
data fitness;
        input eggs length treat $;
        * Choose y and x variables;
        y = eggs;
        x = length;
        datalines;
290    5.7   DietIG
 99    5.2   DietIG
340    5.5   DietIG
271    4.8   DietIG
200    5.2   DietIG

etc.

;
run;
* Print data set;
proc print data=fitness;
run;
* Plot data and regression line;
proc gplot data=fitness;
        plot y*x=treat / vaxis=axis1 haxis=axis1 legend=legend1;
        symbol1 i=rl v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
        legend1 label=(height=2) value=(height=2);
run;
* ANCOVA;
proc glm data=fitness;
        class treat;
        * Model with interaction;
        *model y = treat x treat*x;
        * Model without interaction;
        model y = treat x;
        lsmeans treat / pdiff=all adjust=tukey cl lines;
        output out=resids p=pred r=resid;
run;
goptions reset=all;
title "Diagnostic plots to check ANCOVA assumptions";
* Plot residuals vs. predicted values;
```

```
proc gplot data=resids;
        plot resid*pred=1 / vaxis=axis1 haxis=axis1;
        symbol1 v=star height=2 width=3;
        axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
run;
* Normal quantile plot of residuals;
proc univariate noprint data=resids;
        qqplot resid / normal waxis=3 height=4;
run;
quit;
```

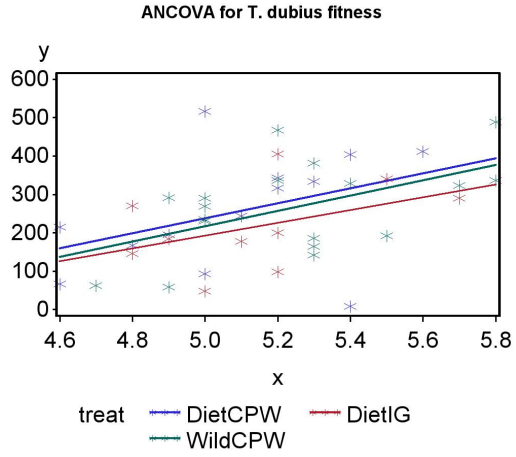

Figure 19.9: Eggs laid by adult *T. dubius* in three treatments vs. elytra length.

SAS Output - Model with Interaction

ANCOVA for T. dubius fitness 3
13:26 Thursday, September 26, 2013

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	149241.7740	29848.3548	2.13	0.0845
Error	35	489963.3479	13998.9528		
Corrected Total	40	639205.1220			

R-Square	Coeff Var	Root MSE	y Mean
0.233480	47.29918	118.3172	250.1463

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	2	16193.0211	8096.5105	0.58	0.5661
x	1	132427.1693	132427.1693	9.46	0.0041
x*treat	2	621.5837	310.7918	0.02	0.9781

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	2	396.6464	198.3232	0.01	0.9859
x	1	114086.8726	114086.8726	8.15	0.0072
x*treat	2	621.5837	310.7918	0.02	0.9781

SAS Output - Model without Interaction

ANCOVA for T. dubius fitness 1
08:29 Monday, November 11, 2013

Obs	eggs	length	treat	y	x
1	290	5.7	DietIG	290	5.7
2	99	5.2	DietIG	99	5.2
3	340	5.5	DietIG	340	5.5
4	271	4.8	DietIG	271	4.8
5	200	5.2	DietIG	200	5.2

etc.

ANCOVA for T. dubius fitness 2
08:29 Monday, November 11, 2013

The GLM Procedure

Class Level Information

Class	Levels	Values
treat	3	DietCPW DietIG WildCPW

Number of Observations Read	41
Number of Observations Used	41

ANCOVA for T. dubius fitness 3
08:29 Monday, November 11, 2013

The GLM Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	148620.1904	49540.0635	3.74	0.0193
Error	37	490584.9316	13259.0522		

Corrected Total	40	639205.1220

R-Square	Coeff Var	Root MSE	y Mean
0.232508	46.03224	115.1480	250.1463

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	2	16193.0211	8096.5105	0.61	0.5484
x	1	132427.1693	132427.1693	9.99	0.0031

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	2	13846.2749	6923.1375	0.52	0.5976
x	1	132427.1693	132427.1693	9.99	0.0031

ANCOVA for T. dubius fitness 4
08:29 Monday, November 11, 2013

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

treat	y LSMEAN	LSMEAN Number
DietCPW	270.496170	1
DietIG	221.056056	2
WildCPW	251.610513	3

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: y

i/j	1	2	3
1		0.5702	0.8992
2	0.5702		0.7839

```
         3        0.8992        0.7839


treat           y LSMEAN      95% Confidence Limits

DietCPW       270.496170      205.021331   335.971009
DietIG        221.056056      147.207956   294.904156
WildCPW       251.610513      195.890594   307.330433


          Least Squares Means for Effect treat

                Difference          Simultaneous 95%
                   Between       Confidence Limits for
i    j               Means        LSMean(i)-LSMean(j)

1    2          49.440114       -69.094011   167.974239
1    3          18.885656       -85.958935   123.730248
2    3         -30.554457      -142.397015    81.288100


Tukey-Kramer Comparison Lines for Least Squares Means of treat

LS-means with the same letter are not significantly different.

                                          LSMEAN
                     y LSMEAN    treat    Number

                A     270.496    DietCPW       1
                A
                A     251.611    WildCPW       3
                A
                A     221.056    DietIG        2
```

19.4 References

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Reeve, J. D., Rojas, M. G. & Morales-Ramos, J. A. (2003) Artificial diet and rearing methods for *Thanasimus dubius* (Coleoptera: Cleridae), a predator of bark beetles (Coleoptera: Scolytidae). *Biological Control* 27: 315-322.

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19.5 Problems

1. A limnologist wants to examine the length of a zooplankton species reared using four different algal growth media (1, 2, 3, and 4). She is also interested in whether there is variation among the containers used to rear the organisms. An experiment is conducted where three containers are used for each rearing medium, for a total of 12 different containers. The containers were randomly selected from a box of containers. The length of four animals was determined for each container, yielding the following data:

Medium	Container	Lengths 1-4 (mm)
1	1	3.1, 3.0, 3.2, 3.0
1	2	3.3, 3.6, 2.8, 2.5
1	3	3.7, 3.4, 3.4, 3.6
2	1	2.7, 2.9, 3.2, 3.0
2	2	2.9, 3.4, 3.5, 2.9
2	3	3.5, 3.5, 3.7, 4.0
3	1	2.8, 2.7, 1.8, 2.5
3	2	2.6, 2.5, 3.2, 2.4
3	3	2.6, 2.9, 1.8, 2.4
4	1	4.1, 4.6, 3.3, 4.5
4	2	3.7, 3.9, 4.0, 3.9
4	3	4.4, 4.4, 3.9, 4.6

   (a) Write an appropriate ANOVA model for this design, stating which factors are fixed, random, and possibly nested.

   (b) Use SAS to analyze these data using your ANOVA model, transforming the observations only if necessary. Is there a significant difference among the four media in zooplankton length?

   (c) Use the Tukey method to compare the media treatments. Interpret your results.

   (d) Compare the magnitude of your variance components. Does there appear to be much variation among containers?

2. An ecologist is interested in the effect of three management treatments (labeled 1, 2, and 3) on the abundance of an endangered snail. Treatment 2 is a control treatment. Twenty-four plots are established and

the three treatments assigned at random to the plots. The density of snails is then measured at a later time, as well as a covariate in the form of a habitat index. Larger values of the habitat index are thought to indicate better snail habitat. See data set below.

Treatment	Index	Snails
1	9.3	23.0
1	9.8	24.9
1	9.9	24.7
1	10.1	24.6
1	8.9	23.4
1	10.8	27.1
1	9.6	25.4
1	10.7	25.4
2	11.9	21.8
2	9.6	18.8
2	10.3	21.0
2	10.8	21.5
2	9.9	20.9
2	10.9	22.6
2	8.9	19.8
2	10.2	22.4
3	11.2	23.4
3	10.3	18.5
3	11.1	22.3
3	9.8	20.5
3	11.2	20.5
3	8.7	18.4
3	8.4	18.7
3	10.5	19.2

(a) Test for equality of slopes among the different treatment groups using SAS. Is this key assumption of ANCOVA satisfied?

(b) Use ANCOVA and SAS to test for overall treatment and covariate effects in this experiment, and the Tukey method to compare the different treatments. Interpret and discuss your results. Is there a significant treatment and covariate effect? How do the different treatments compare?

3. A scientist interested in aquaculture raises fish using three kinds of treatments in a factorial design. There were two fish diets (A and B), two strains of fish (1 and 2), and three temperatures (22^o, 24^o, and 26^oC). Two fish were reared for each combination of the treatments. The following data were obtained:

Diet	Strain	Temp	Weight (lb)
A	1	22	5.5
A	1	22	5.8
A	1	24	5.9
A	1	24	5.7
A	1	26	6.2
A	1	26	5.9
A	2	22	5.2
A	2	22	5.0
A	2	24	5.4
A	2	24	5.6
A	2	26	5.0
A	2	26	4.9
B	1	22	5.4
B	1	22	4.8
B	1	24	5.4
B	1	24	5.4
B	1	26	5.7
B	1	26	5.5
B	2	22	5.2
B	2	22	4.8
B	2	24	5.1
B	2	24	5.1
B	2	26	4.8
B	2	26	4.5

   (a) Write an appropriate ANOVA model for this design, stating which factors are fixed or random.

   (b) Use SAS to analyze these data using your ANOVA model, transforming the observations only if necessary. Interpret the results of your analysis.

Chapter 20

Methods for Categorical Data

Categorical data are observations that fall into two or more discrete categories, such as female vs. male organisms, age or size classes, or different phenotypes in genetic studies (Chapter 1). This requires a different type of statistical model than in previous chapters, where the observations were assumed to have a normal distribution. We will instead use the binomial and multinomial distributions to model categorical data, and derive likelihood ratio and chi-square tests of various hypotheses. Recall that the binomial distribution can be used to model data with two categories (see Chapter 5). **The multinomial distribution is a generalization of the binomial to data with more than two categories.**

One class of test we will examine are called **goodness-of-fit tests**. These tests compare the observed frequencies of different categories of observations with those expected under some null hypothesis. For example, recall the laboratory rearing study of *Thanasimus dubius* described in Chapter 3. We might be interested in whether the sex ratio for these predatory beetles is close to 1:1 (50% females, 50% males), as occurs in many diploid sexual organisms. This is our null hypothesis and it implies that the probability p a sampled individual is female is 0.5, or $H_0 : p = 0.5$. Suppose we have a sample of $n = 130$ beetles as in this data set. What are the expected frequencies of females and males in this sample? Under H_0, we would expect $E_1 = np = 130(0.5) = 65$ females and $E_2 = n(1-p) = 130(0.5) = 65$ males. These are also the values one would expect to see if the observations have a binomial distribution (see Chapter 5). The observed frequencies are $O_1 = 60$ females and $O_2 = 70$ males for this data set. It is common to organize these results into following form (Table 20.1):

Table 20.1: Observed and expected frequencies of female and male *T. dubius* from a laboratory rearing study (Reeve et al. 2003).

	Females	Males	$\sum$
i	1	2	
O_i	60	70	130
E_i	65	65	130

A goodness-of-fit test for $H_0 : p = 0.5$ provides a way of comparing these observed and expected frequencies, generating a test statistic and P value for the test. Based on these results we may accept or reject this null hypothesis, and in this case the result is non-significant ($P = 0.3805$). We will later see how goodness-of-fit tests may be applied to data with more categories and cases where certain model parameters are estimated from the data.

Tests of independence are a second class of tests for categorical data. Suppose that the observations in a data set can be classified in two different ways. For example, a sample of amphibians could be classified into different species and whether individuals of a given species are infected with a pathogen. Using a test of independence, we can test whether species and infection status are independent events (see Chapter 4). Equivalently, we can test whether the probability of being infected is the same across species. To make things more concrete, suppose that four amphibian species (A, B, C, and D) are randomly sampled and scored for infection, yielding Table 20.2. The null hypothesis of independence, or an equal probability of being infected across all species, can be expressed as follows. Let p_A be the overall probability an individual of species A is sampled (infected or not), while p_I is the probability it is infected (across all four species). If species and infection status are independent, we would expect by definition that the probability of sampling an infected individual of species A would be $p_A p_I$. A similar relationship would hold for the other possible outcomes, and the null hypothesis of independence can be expressed in this form.

Tests of independence also make use of observed and expected frequencies, with the expected frequencies calculated under the null hypothesis of independence (see Table 20.2). Subscripts are commonly used to indicate the observed and expected frequencies in particular cells of the table, with the first subscript indicating the row and the second the column in the table. For example, in Table 20.2 we have $O_{11} = 7, O_{21} = 18, O_{12} = 12, O_{22} = 38$,

and so forth. We will later see how to calculate the expected frequencies under the null hypothesis of independence. There appear to be substantial differences between the observed and expected frequencies in this table, and in fact the test of independence is highly significant ($P = 0.0002$), suggesting that amphibian species and infection status are **not** independent. We will focus on two-way tables like the one below, but it is also possible to conduct tests of independence for three-way or higher tables. However, these problems are more commonly addressed using **loglinear models**, which have an ANOVA-like structure and feel but focus on testing the interactions between factors, which are equivalent to tests of independence (Agresti 1990).

Table 20.2: Observed frequencies of infected and non-infected individuals in four amphibian species. Below each observed frequency is the expected frequency under the null hypothesis of independence.

	Species				
Infected	A	B	C	D	$\sum$
Yes	7	12	15	27	61
	10.167	20.333	14.233	16.267	
No	18	38	20	13	89
	14.833	29.667	20.767	23.733	
$\sum$	25	50	35	40	150

20.1 Goodness-of-fit tests

As a simple example of a goodness-of-fit test, consider the data set involving male and female *T. dubius*. Suppose we want to test the hypothesis that the sex ratio is 1:1 (50% female, 50% male) in this species. The population falls into two categories, female or male, which suggests using the binomial distribution to model the observations. Suppose that we have a sample of size n from this population and let Y be the number of females in the sample, a binomial random variable. If p is the probability that a *T. dubius* adult is female, then the probability the sample will have y females is given by the formula

$$P[Y = y] = \binom{n}{y} p^y (1-p)^{n-y}. \tag{20.1}$$

The null hypothesis that the sex ratio is 1:1 implies that $p = 0.5$, which can be written as $H_0 : p = 0.5$. The alternative is that the sex ratio differs from 1:1, or $H_1 : p \neq 0.5$. More generally, we will be interested in testing $H_0 : p = p_0$ vs. $H_1 : p \neq p_0$ where p_0 is some probability.

We now develop a likelihood ratio test for $H_0 : p = p_0$ vs. $H_1 : p \neq p_0$, assuming the observations have a binomial distribution. It is a goodness-of-fit test because we will be comparing the observed frequencies of females and males with that expected under H_0, and if observed and expected frequencies are substantially different we will likely reject H_0. The likelihood ratio test uses the ratio of the likelihoods under H_0 and H_1 as the test statistic (see Chapter 10).

Recall that the likelihood function for discrete distributions is just the probability of the observed data (see Chapter 8). The data are fixed quantities in this function, while the parameters of the distribution are free to vary. In this case, the value of y (the number of females in the sample) is the data while p is the parameter that is free to vary, and so the likelihood function for binomial data would be

$$L(p) = \binom{n}{y} p^y (1-p)^{n-y}. \tag{20.2}$$

We first need to find the maximum value of the likelihood under H_0. Under the null hypothesis the parameter p is set equal to p_0, and so we have

$$L_{H_0} = \binom{n}{y} p_0^y (1-p_0)^{n-y}. \tag{20.3}$$

This is the only value that can be taken by L_{H_0}, because all the other quantities are fixed, and so this is also its maximum. Under H_1, the parameter p is free to vary in $L(p)$. The maximum value of the likelihood function occurs at $\hat{p} = y/n$, the maximum likelihood estimate of p. This is simply the proportion of females in the sample. Thus,

$$L_{H_1} = \binom{n}{y} \hat{p}^y (1-\hat{p})^{n-y} = \binom{n}{y} (y/n)^y (1-y/n)^{n-y}. \tag{20.4}$$

The test statistic is the ratio of these two likelihoods:

$$\lambda = \frac{L_{H_0}}{L_{H_1}} \tag{20.5}$$

$$= \frac{\binom{n}{y} p_0^y (1-p_0)^{n-y}}{\binom{n}{y} (y/n)^y (1-y/n)^{n-y}} \tag{20.6}$$

$$= \frac{p_0^y (1-p_0)^{n-y}}{(y/n)^y (1-y/n)^{n-y}} \tag{20.7}$$

$$= \left(\frac{p_0}{y/n}\right)^y \left(\frac{1-p_0}{1-y/n}\right)^{n-y} \tag{20.8}$$

$$= \left(\frac{np_0}{y}\right)^y \left(\frac{n(1-p_0)}{n-y}\right)^{n-y} \tag{20.9}$$

$$= \left(\frac{E_1}{O_1}\right)^{O_1} \left(\frac{E_2}{O_2}\right)^{O_2}. \tag{20.10}$$

Here O_1 and O_2 would be the observed frequencies of females and males, while $E_1 = np_0$ and $E_2 = n(1-p_0)$ are the corresponding expected frequencies (see Table 20.1). Under H_0, the quantity

$$G^2 = -2 \ln \lambda \tag{20.11}$$

has approximately a χ^2 distribution with one degree of freedom, with the approximation improving as n increases (Agresti 1990). In terms of the observed and expected frequencies, we have

$$G^2 = -2 \ln \lambda \tag{20.12}$$

$$= -2 \ln \left[\left(\frac{E_1}{O_1}\right)^{O_1} \left(\frac{E_2}{O_2}\right)^{O_2} \right] \tag{20.13}$$

$$= -2[O_1 \ln(E_1/O_1) + O_2 \ln(E_2/O_2)] \tag{20.14}$$

$$= 2[O_1 \ln(O_1/E_1) + O_2 \ln(O_2/E_2)]. \tag{20.15}$$

Similar to other likelihood ratio tests that utilize the χ^2 distribution, the degrees of freedom are equal to the difference in the number of parameters free between the H_1 and H_0 models (see Chapter 14). There is one free parameter under H_1, namely p, but under H_0 we have $p = p_0$, a fixed quantity. Thus, there is a difference of one parameter between the two models, implying one

degree of freedom. G^2 values will become large if the observed and expected frequencies are different.

Another commonly used statistic for this goodness-of-fit test is the quantity

$$X^2 = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2} \tag{20.16}$$

(Agresti 1990). Under H_0, X^2 has approximately a χ^2 distribution with one degree of freedom. Although the two test statistics G^2 and X^2 are different in form, they usually yield similar values and test results. X^2 values also become large as the observed and expected frequencies diverge. This test is often called a 'chi-square' or 'χ^2' test, although the likelihood ratio test also uses the χ^2 distribution.

Sample calculation

We now conduct a goodness-of-fit test for the Table 20.1 data, testing $H_0 : p = 0.5$. We have

$$\begin{aligned} G^2 &= 2[O_1 \ln(O_1/E_1) + O_2 \ln(O_2/E_2)] && (20.17)\\ &= 2[60 \ln(60/65) + 70 \ln(70/65)] && (20.18)\\ &= 2[-4.803 + 5.188] && (20.19)\\ &= 0.770. && (20.20) \end{aligned}$$

We next find the P value from Table C and obtain a non-significant result ($G^2 = 0.770, df = 1, P < 0.5$). Thus, there is no evidence against a 1:1 sex ratio in this study.

We next calculate the equivalent X^2 statistic for these data. We have

$$\begin{aligned} X^2 &= \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2} && (20.21)\\ &= \frac{(60 - 65)^2}{65} + \frac{(70 - 65)^2}{65} && (20.22)\\ &= 0.385 + 0.385 && (20.23)\\ &= 0.770. && (20.24) \end{aligned}$$

The result is identical to G^2 and so the P value is the same ($X^2 = 0.770, df = 1, P < 0.5$). The test results are often similar for these two statistics, although seldom identical as in this case.

Goodness-of-fit test - SAS demo

We can use `proc freq` in SAS to conduct a goodness-of-fit test for the Table 20.1 data using the X^2 statistic (SAS Institute Inc. 2014a). This procedure does not provide the likelihood ratio test involving G^2, but there is another option that is actually better than both. SAS can conduct an exact chi-square (X^2) test where the distribution of the test statistic under H_0 is determined exactly, instead of approximating it with a χ^2 distribution. This approach is computationally intensive and may be impractical for large sample sizes, but in this case the chi-square (X^2) test would be valid and the exact test unnecessary.

The first step in the analysis is to make a SAS data set using the observed frequencies in Table 20.1. The variable `obsfreq` contains this information for each value of `sex` (see SAS program below). The data could also have been entered as individual observations with a single data line for each observation, as in the original data set (see Chapter 3). We would then use `proc freq` to tabulate the data.

Now examine the `proc freq` portion of the program. The `order=data` option asks SAS to use the order of the categories (values of `sex`) given by the data, rather than alphabetically. The `tables` line requests a frequency table for `sex`. The next step is to tell SAS the probabilities under H_0 for each sex, which are $p = 0.5$ for females and $1 - p = 0.5$ for males. This is accomplished using the option `testp = (0.5 0.5)`. The order of the probabilities in the `testp` statement should match the order of the categories in the data. The `weight` command tells `proc freq` that the data are in the form of frequencies, and the name of the variable containing these frequencies (`obsfreq`). An exact chi-square (X^2) test is requested by the command `exact chisq`.

Examining the SAS output, we find that the exact chi-square (X^2) test is non-significant ($X^2 = 0.769, df = 1, P = 0.4300$). There is no evidence that the sex ratio differs from 1:1 in this organism.

SAS Program

```
* gof_clerids.sas;
options pageno=1 linesize=80;
title 'Goodness-of-fit test for T. dubius data';
data elytra;
    input sex \$ obsfreq;
    datalines;
F  60
M  70
;
run;
* Print data set;
proc print data=elytra;
run;
* Goodness-of-fit test (Chi-square only);
proc freq data=elytra order=data;
    tables sex / testp=(0.5 0.5) chisq cellchi2 expected;
    weight obsfreq;
    * Compute exact test if frequencies low, takes too long for large data sets;
    exact chisq;
run;
quit;
```

SAS Output

```
                    Goodness-of-fit test for T. dubius data                    1
                                                  12:40 Monday, November 29, 2010

                              Obs    sex    obsfreq

                               1      F        60
                               2      M        70

                    Goodness-of-fit test for T. dubius data                    2
                                                  12:40 Monday, November 29, 2010

                                The FREQ Procedure

                                                    Test     Cumulative    Cumulative
      sex    Frequency     Percent     Percent     Frequency      Percent

      ------------------------------------------------------------------------
      F            60       46.15       50.00            60        46.15
      M            70       53.85       50.00           130       100.00
```

```
            Chi-Square Test
        for Specified Proportions
---------------------------------------
Chi-Square                       0.7692
DF                                    1
Asymptotic Pr >  ChiSq           0.3805
Exact      Pr >= ChiSq           0.4300

           Sample Size = 130
```

20.1.1 Goodness-of-fit tests for a categories

We now examine goodness-of-fit tests for data with more than two categories. A common type occurs in genetic studies where different genotypes are crossed, such as Mendel's classic experiments involving pea plants (Mendel 1865). One of his experiments created hybrids for two genes governing the shape (round or wrinkled) and color (yellow or green) of the peas, which were then crossed and the phenotypes of the offspring scored. A total of $n = 556$ peas were observed (Table 20.3).

Table 20.3: Observed and expected frequencies for a dihybrid cross (Mendel 1865).

	Round yellow	Round green	Wrinkled yellow	Wrinkled green	$\sum$
i	1	2	3	4	
O_i	315	101	108	32	556
E_i	312.75	104.25	104.25	34.75	556

If we assume Mendelian genetics, with the round allele dominant over the wrinkled one and yellow color dominant over green, we would expect to see these four phenotypes in a 9:3:3:1 ratio. This forms the null hypothesis for this problem. We can express it in the form $H_0 : p_1 = 9/16 = 0.5625, p_2 = 3/16 = 0.1875, p_3 = 3/16 = 0.1875$, and $p_4 = 1/16 = 0.0625$. The alternative H_1 is that the probabilities differ from these values. More generally, we will

be interested in testing $H_0 : p_1 = p_{10}, p_2 = p_{20}, p_3 = p_{30}$, and $p_4 = p_{40}$ vs. some alternative hypothesis H_1 where the probabilities differ from these values.

Also shown in Table 20.3 are the expected frequencies under H_0, calculated using the formula $E_i = np_i$. We have $E_1 = 556(0.5625) = 312.75$, $E_2 = 556(0.1875) = 104.25 = E_3$, and $E_4 = 556(0.0625) = 34.75$. These are the expected numbers of peas for each phenotype assuming that H_0 is true.

We need a different distribution to model these observations, a generalization of the binomial distribution called the **multinomial distribution**. Suppose that n total peas are sampled, and let Y_1, Y_2, Y_3 and Y_4 be random variables corresponding to the four phenotypes, with y_1 the observed number of round and yellow peas, y_2 the number of round and green, y_3 the number of wrinkled and yellow, while y_4 is wrinkled and green. Because $n = Y_1 + Y_2 + Y_3 + Y_4$ there is some dependence among the four variables (if we know three, the fourth is determined by this relationship). Let p_1 be the probability that a pea is round and yellow, with p_2, p_3, and p_4 similarly defined. The four probabilities sum to one ($p_1 + p_2 + p_3 + p_4 = 1$), which implies the distribution really has only three parameters. Then, the probability of observing y_1, y_2, y_3, and y_4 peas of each type is given by the multinomial distribution, which has the form

$$P[Y_1 = y_1, Y_2 = y_2, Y_3 = y_3, Y_4 = y_4] = \frac{n!}{y_1!y_2!y_3!y_4!} p_1^{y_1} p_2^{y_2} p_3^{y_3} p_4^{y_4}. \quad (20.25)$$

This distribution can be readily generalized to any number of categories.

Using the multinomial distribution as a model for the observations, it can be shown that the G^2 statistic can be used for tables with a cells ($a = 2, 3, 4, \ldots$) by adding more terms of the form $O_i \ln(O_i/E_i)$. For a table with a cells, we have

$$G^2 = 2 \sum_{i=1}^{a} O_i \ln(O_i/E_i). \quad (20.26)$$

Under H_0, G^2 has a χ^2 distribution with $a - 1$ degrees of freedom. They are equal to $a - 1$ because there are $a - 1$ free parameters (p_1, p_2, etc.) under H_1 but none free under H_0. Similarly, the X^2 statistic can be generalized as

$$X^2 = \sum_{i=1}^{a} \frac{(O_i - E_i)^2}{E_i}. \quad (20.27)$$

This statistic also has $a - 1$ degrees of freedom under H_0.

Sample calculation

We illustrate a goodness-of-fit test for $a = 4$ categories using the pea data, testing $H_0 : p_1 = 0.5625, p_2 = 0.1875, p_3 = 0.1875$, and $p_4 = 0.0625$. Table 20.3 presents the observed and expected frequencies, from which we can calculate G^2. We have

$$G^2 = 2\sum_{i=1}^{a} O_i \ln(O_i/E_i) \tag{20.28}$$

$$= 2[315\ln(315/312.75) + 101\ln(101/104.25) \tag{20.29}$$

$$+ 108\ln(108/104.25) + 32\ln(32/34.75)] \tag{20.30}$$

$$= 2[2.258 - 3.199 + 3.817 - 2.638] \tag{20.31}$$

$$= 0.476. \tag{20.32}$$

The degrees of freedom for the test are $a - 1 = 4 - 1 = 3$. We next find the P value from Table C and obtain a non-significant result ($G^2 = 0.476, df = 3, P < 0.95$). The observed frequencies apparently agree with the Mendelian ratio of 9:3:3:1.

We next conduct a chi-square (X^2) test for these data. We have

$$X^2 = \sum_{i=1}^{a} \frac{(O_i - E_i)^2}{E_i} \tag{20.33}$$

$$= \frac{(315 - 312.75)^2}{312.75} + \frac{(101 - 104.25)^2}{104.25} \tag{20.34}$$

$$+ \frac{(108 - 104.25)^2}{104.25} + \frac{(32 - 34.75)^2}{34.75} \tag{20.35}$$

$$= 0.016 + 0.101 + 0.135 + 0.218 \tag{20.36}$$

$$= 0.470 \tag{20.37}$$

We also obtain a non-significant result with this test ($X^2 = 0.470, df = 3, P < 0.95$).

Goodness-of-fit test - SAS demo 2

The chi-square (X^2) test for the Table 20.3 data can also be conducted in SAS. A data set is first made using the observed frequencies, with `proc freq` then used to carry out the test. The `testp` statement lists the probabilities under $H_0 : p_1 = 0.5625, p_2 = 0.1875, p_3 = 0.1875$, and $p_4 = 0.0625$. The

order of the probabilities matches the order of the phenotypes in the data set. See SAS program and output below. An exact chi-square test is also requested which may take SAS some period of time to calculate.

Examining the SAS output, we find that the exact chi-square (X^2) test is non-significant ($X^2 = 0.470, df = 3, P = 0.9272$). There is no evidence that the ratios of the phenotypes differ from the Mendelian 9:3:3:1 ratio.

SAS Program

```
* gof_peas.sas;
options pageno=1 linesize=80;
title 'Goodness-of-fit test for Mendel data';
data peas;
    input phenotype :\$12. obsfreq;
    datalines;
round_yellow  315
round_green   101
wrink_yellow  108
wrink_green    32
;
run;
* Print data set;
proc print data=peas;
run;
* Goodness-of-fit test (Chi-square only);
proc freq data=peas order=data;
    tables phenotype / testp=(0.5625 0.1875 0.1875 0.0625) chisq cellchi2 expected;
    weight obsfreq;
    * Compute exact test if frequencies low, takes too long for large data sets;
    exact chisq;
run;
quit;
```

SAS Output

Goodness-of-fit test for Mendel data 1
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Obs	phenotype	obsfreq
1	round_yellow	315
2	round_green	101
3	wrink_yellow	108
4	wrink_green	32

Goodness-of-fit test for Mendel data 2
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The FREQ Procedure

phenotype	Frequency	Percent	Test Percent	Cumulative Frequency	Cumulative Percent
round_yellow	315	56.65	56.25	315	56.65
round_green	101	18.17	18.75	416	74.82
wrink_yellow	108	19.42	18.75	524	94.24
wrink_green	32	5.76	6.25	556	100.00

Chi-Square Test
for Specified Proportions

Chi-Square		0.4700
DF		3
Asymptotic	Pr > ChiSq	0.9254
Exact	Pr >= ChiSq	0.9272

Sample Size = 556

20.1.2 Goodness-of-fit tests with estimated parameters

Another common type of goodness-of-fit test compares the observed frequencies with that expected for some theoretical distribution, such as the Poisson. We previously fitted a Poisson distribution to count data and compared graphically the observed and expected frequencies (5). We now compare these frequencies using a goodness-of-fit test similar to previous examples. The null hypothesis in this case is that the observations are Poisson in distribution, while the alternative is that some other distribution describes them.

There are two additional considerations with these goodness-of-fit tests. One is that the Poisson parameter λ must be estimated from the observations, using the estimator $\hat{\lambda} = \bar{Y}$. This requires an adjustment to the degrees of freedom for the test (Agresti 1990). **In particular, one degree of freedom is subtracted from the total for every parameter estimated.** For the Poisson distribution we have to estimate λ, and so the degrees of freedom are $a - 1 - 1 = a - 2$. A second consideration involves the expected frequencies in the tests. The distributions of both G^2 and X^2 are approximately χ^2 under H_0, but this approximation works better if the expected frequencies are not too small, although there is no universal rule on what constitutes small (Agresti 1990). **One commonly used but overly conservative rule is $E_i \geq 5$ - the expected frequencies must equal or exceed five for all cells.** We have not encountered this problem in previous examples but it does occur with goodness-of-fit tests for the Poisson and other discrete distributions. **The solution is to combine adjacent cells in the table until the expected frequencies equal or exceed five. The observed frequencies are also combined to match the expected ones.**

We will use a SAS program to automate most of the calculations for these tests. The tests cannot be completely automated because the expected frequencies need to be manually combined at some point. Recall the corn borers data and SAS program from Chapter 5. The program listed below is similar, except that some additional quantities needed for the tests are calculated in the second `data` step. In particular, the program calculates the individual terms for the X^2 and G^2 tests, defined as the SAS variables `cellchi2` and `olnoe`, and keeps a running total of these values in the variables `sumchi2` and `sumlike`.

As before, define E_1 to be the expected frequency for the first cell ($y = 0$), E_2 the expected frequency for the second cell ($y = 1$), and so forth. We see

that the expected frequency $E_8 = 3.2041 < 5$, as are the remaining values. We therefore add them together so that the combined expected frequency is greater than five. We have

$$\begin{aligned} E_{\text{combined}} &= 3.204 + 1.268 + 0.446 && (20.38)\\ &\quad + 0.141 + 0.041 + 0.011 && (20.39)\\ &= 5.111. && (20.40) \end{aligned}$$

We must also combine the observed frequencies for these cells, to obtain

$$\begin{aligned} O_{\text{combined}} &= 5 + 3 + 4 + 3 + 0 + 1 && (20.41)\\ &= 16. && (20.42) \end{aligned}$$

We then calculate an overall G^2 statistic as follows. First, we calculate the component of this test statistic for the combined cells, obtaining

$$O_{\text{combined}} \ln(O_{\text{combined}}/E_{\text{combined}}) = 16 \ln(16/5.111) = 18.259. \quad (20.43)$$

The program calculates a running total of these components using the variable `sumlike`, and we find that the total prior to the combined cells is 13.078. The overall test statistic value is therefore equal to

$$G^2 = 2[13.078 + 18.259] = 62.674. \quad (20.44)$$

There are $a = 8$ categories in the test, so the degrees of freedom are $a - 2 = 8 - 2 = 6$. Using Table C, we find that the test is highly significant ($G^2 = 62.674, df = 6, P < 0.001$). This result strongly suggests the observations do not have a Poisson distribution. Instead, they appear to have an overdispersed pattern that is better described by the negative binomial distribution (Chapter 5).

We now calculate a chi-square (X^2) goodness-of-fit test for these observations. We first calculate the component of this statistic for the combined cells, obtaining

$$\frac{(O_{\text{combined}} - E_{\text{combined}})^2}{E_{\text{combined}}} = \frac{(16 - 5.111)^2}{5.111} = 23.199. \quad (20.45)$$

We then find the running total of these components (`sumchi2`) prior to the combined cells from the SAS output, which is 80.705. The overall test statistic is equal to

$$X^2 = 80.705 + 23.199 = 103.904. \quad (20.46)$$

The degrees of freedom are $a - 2 = 7 - 2 = 6$, the same as above. The test is again highly significant ($X^2 = 103.904, df = 6, P < 0.001$).

SAS Program

```
* Poisson_fit2_gof.sas;
options pageno=1 linesize=80;
goptions reset=all;
title 'Fitting the Poisson to frequency data';
data poisson;
    input y obsfreq;
    * Generate offset y values for plot;
    yexp = y - 0.1; yobs = y + 0.1;
    datalines;
0  24
1  16
2  16
3  18
4  15
5   9
6   6
7   5
8   3
9   4
10  3
11  0
12  1
;
run;
* Print data set;
proc print data=poisson;
run;
* Descriptive statistics, save ybar, n, and var to data file;
proc univariate data=poisson;
    var y;
    histogram y / vscale=count;
    freq obsfreq;
    output out=stats mean=ybar n=n var=var;
run;
* Print output data file;
proc print data=stats;
run;
* Calculate expected frequencies using ybar;
data poisfit;
    if _n_ = 1 then set stats;
    set poisson;
```

```
    poisprob = pdf('poisson',y,ybar);
    expfreq = n*poisprob;
    * Calculate test values for each cell;
    cellchi2 = ((obsfreq - expfreq)**2)/expfreq;
    sumchi2 + cellchi2;
    olnoe = obsfreq*log(obsfreq/expfreq);
    sumlike + olnoe;
run;
* Print observed and expected frequencies;
proc print data=poisfit;
run;
* Plot observed and expected frequencies;
proc gplot data=poisfit;
    plot expfreq*yexp=1 obsfreq*yobs=2 / overlay legend=legend1 vref=0 wvref=3
    vaxis=axis1 haxis=axis1;
    symbol1 i=needle v=circle c=red width=3 height=2;
    symbol2 i=needle v=square c=blue width=3 height=2;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
    legend1 label=(height=2) value=(height=2);
run;
quit;
```

SAS Output

Fitting the Poisson to frequency data 1
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Obs	y	obsfreq	yexp	yobs
1	0	24	-0.1	0.1
2	1	16	0.9	1.1
3	2	16	1.9	2.1
4	3	18	2.9	3.1
5	4	15	3.9	4.1
6	5	9	4.9	5.1
7	6	6	5.9	6.1
8	7	5	6.9	7.1
9	8	3	7.9	8.1
10	9	4	8.9	9.1
11	10	3	9.9	10.1
12	11	0	10.9	11.1
13	12	1	11.9	12.1

etc.

Fitting the Poisson to frequency data 5
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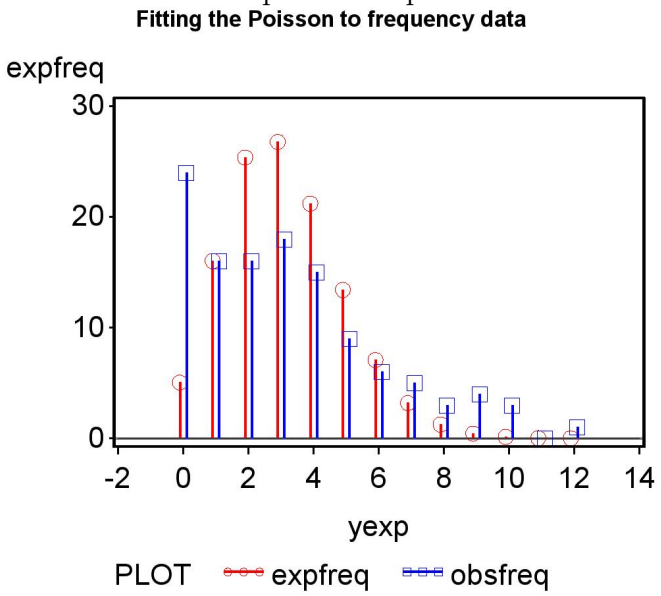
Obs	n	ybar	var	y	obsfreq	yexp	yobs	poisprob
1	120	3.16667	7.77031	0	24	-0.1	0.1	0.04214
2	120	3.16667	7.77031	1	16	0.9	1.1	0.13346
3	120	3.16667	7.77031	2	16	1.9	2.1	0.21130
4	120	3.16667	7.77031	3	18	2.9	3.1	0.22304
5	120	3.16667	7.77031	4	15	3.9	4.1	0.17658
6	120	3.16667	7.77031	5	9	4.9	5.1	0.11183
7	120	3.16667	7.77031	6	6	5.9	6.1	0.05902
8	120	3.16667	7.77031	7	5	6.9	7.1	0.02670
9	120	3.16667	7.77031	8	3	7.9	8.1	0.01057
10	120	3.16667	7.77031	9	4	8.9	9.1	0.00372
11	120	3.16667	7.77031	10	3	9.9	10.1	0.00118
12	120	3.16667	7.77031	11	0	10.9	11.1	0.00034
13	120	3.16667	7.77031	12	1	11.9	12.1	0.00009

Obs	expfreq	cellchi2	sumchi2	olnoe	sumlike
1	5.0573	70.9529	70.953	37.3735	37.3735
2	16.0147	0.0000	70.953	-0.0147	37.3588
3	25.3565	3.4526	74.405	-7.3672	29.9917
4	26.7652	2.8705	77.276	-7.1412	22.8505
5	21.1892	1.8078	79.084	-5.1816	17.6689
6	13.4198	1.4557	80.539	-3.5956	14.0733
7	7.0827	0.1655	80.705	-0.9953	13.0780
8	3.2041	1.0067	81.712	2.2251	15.3031
9	1.2683	2.3645	84.076	2.5829	17.8859
10	0.4462	28.3010	112.377	8.7727	26.6587
11	0.1413	57.8306	170.208	9.1662	35.8249
12	0.0407	0.0407	170.248	.	35.8249
13	0.0107	91.1630	261.411	4.5342	40.3591

Figure 20.1: Observed and expected frequencies - Poisson distribution



20.2 Tests of independence

We now develop tests of independence for tables in which the observations are classified in two different ways, known as two-way tables. The test statistics are similar to previous likelihood ratio (G^2) and chi-square (X^2) goodness-of-fit tests. Because the null hypothesis is different for tests of independence, however, the expected frequencies are calculated differently as are the degrees of freedom. Further details are provided in Agresti (1990).

We first examine how the expected frequencies are constructed for tests of independence, but these calculations will require estimates of the probabilities for certain events. Recall the Table 20.2 example where amphibians were sampled and classified by species and infection status. What is the overall probability of sampling species A, regardless of infection status? Let the quantity p_{+1} stand for this probability, where the + symbol indicates the overall probability combining infected and uninfected individuals while '1' stands for the first column in Table 20.2, which is species A. We can estimate this probability by summing the number of infected and uninfected individuals for species A and dividing by the sample size n. If we let O_{+1} stand for this sum, we have

$$\hat{p}_{+1} = \frac{O_{+1}}{n} = \frac{25}{150} = 0.167. \tag{20.47}$$

This is just the column total for species A divided by the sample size n. We can similarly calculate the probability of sampling species B, obtaining

$$\hat{p}_{+2} = \frac{O_{+2}}{n} = \frac{50}{150} = 0.333. \tag{20.48}$$

For species C, we obtain $\hat{p}_{+3} = 0.233$, while for species D we have $\hat{p}_{+4} = 0.267$.

What about the overall probability of being infected, across all species? Let the quantity p_{1+} stand for this probability, where '1' stands for the first row in Table 20.2, while + indicates the overall probability combining species A through D. We can estimate this probability by summing the infected individuals across all four species and dividing by the sample size n. If we let O_{1+} stand for this sum, we obtain

$$\hat{p}_{1+} = \frac{O_{1+}}{n} = \frac{61}{150} = 0.407. \tag{20.49}$$

This is just the row total of the infected amphibians divided by n. The overall probability of not being infected, p_{2+}, is estimated using the formula

$$\hat{p}_{2+} = \frac{O_{2+}}{n} = \frac{89}{150} = 0.593. \tag{20.50}$$

We are now in a position to calculate the expected frequencies under the null hypothesis of independence. Recall that events A and B are independent then $P[A \cap B] = P[A]P[B]$. Thus, the probability of both A and B occurring is the product of their individual probabilities, provided they are independent (see Chapter 4). Thus, if p_{11} is the probability of sampling an individual of species A that is infected, then if species and infection status are independent we can estimate this probability using

$$\hat{p}_{11} = \hat{p}_{1+}\hat{p}_{+1}. \tag{20.51}$$

The expected frequency for this cell, E_{11}, would be n times this probability, or

$$\begin{aligned} E_{11} &= n\hat{p}_{11} && (20.52)\\ &= n\hat{p}_{1+}\hat{p}_{+1} && (20.53)\\ &= n\frac{O_{1+}}{n}\frac{O_{+1}}{n} && (20.54)\\ &= \frac{O_{1+}O_{+1}}{n}. && (20.55) \end{aligned}$$

Thus, the expected frequency for this cell is the product of its column and row totals divided by the sample size. Using the Table 20.2 data, we find that

$$E_{11} = \frac{61(25)}{150} = 10.167. \tag{20.56}$$

All other cells are calculated in a similar manner. For example, we have

$$E_{13} = \frac{O_{1+}O_{+3}}{n} = \frac{61(35)}{150} = 14.233. \tag{20.57}$$

The remaining expected values are given in Table 20.2. The general formula for any cell would be

$$E_{ij} = \frac{O_{i+}O_{+j}}{n}. \tag{20.58}$$

This formula says that the expected value for any cell is the product of the row and column totals for that cell, divided by the sample size n.

Now suppose a particular two-way table has r rows and c columns. The likelihood ratio test statistic (G^2) for a test of independence is given by the general formula

$$G^2 = 2\sum_{i=1}^{r}\sum_{j=1}^{c} O_{ij}\ln(O_{ij}/E_{ij}). \tag{20.59}$$

G^2 has a χ^2 distribution under H_0 with $(r-1)(c-1)$ degrees of freedom. The explanation for the degrees of freedom is as follows (Agresti 1990). Under H_1, where the observations are not independent, the probability of an observation falling into a particular cell could be anything. Thus, there are rc values of p_{ij} that are free to vary except that they must sum to one, so there are $rc-1$ free parameters under H_1. Under H_0 there are r values of p_{i+} but only $r-1$ free to vary because these probabilities also sum to one. Similarly, there are $c-1$ values of p_{+j} free to vary. The difference in the number of free parameters under H_1 vs. H_0 is the degrees of freedom for the test, similar to goodness-of-fit tests. We therefore have

$$df = rc - 1 - (r-1) - (c-1) = rc - r - c + 1 = (r-1)(c-1). \tag{20.60}$$

The chi-square (X^2) statistic for a test of independence is given by the general formula

$$X^2 = \sum_{i=1}^{r}\sum_{j=1}^{c} \frac{(O_{ij} - E_{ij})^2}{E_{ij}}. \tag{20.61}$$

Under H_0, X^2 also has a χ^2 distribution with $(r-1)(c-1)$ degrees of freedom.

20.2.1 Sample calculation

We illustrate these tests of independence using the Table 20.2 data, for which the expected frequencies have already been calculated. For the likelihood

ratio test, we have

$$G^2 = 2\sum_{i=1}^{r}\sum_{j=1}^{c} O_{ij}\ln(O_{ij}/E_{ij}) \tag{20.62}$$
$$= 2[7\ln(7/10.167) + 12\ln(12/20.333) + 15\ln(15/14.233) \tag{20.63}$$
$$+ 27\ln(27/16.267) + 18\ln(18/14.833) + 38\ln(38/29.667) \tag{20.64}$$
$$+ 20\ln(20/20.767) + 13\ln(13/23.733)] \tag{20.65}$$
$$= 2[-2.613 - 6.328 + 0.787 + 13.681 \tag{20.66}$$
$$+ 3.483 + 9.407 - 0.753 - 7.825] \tag{20.67}$$
$$= 2[9.839] \tag{20.68}$$
$$= 19.678. \tag{20.69}$$

There are $r = 2$ rows and $c = 4$ columns in the table, so the degrees of freedom are $(r-1)(c-1) = (2-1)(4-1) = 3$. From Table C, we see that the test is highly significant ($G^2 = 19.678, df = 3, P < 0.001$). This provides some evidence that species and infection status are not independent.

For the chi-square (X^2) version of this test, we have

$$X^2 = \sum_{i=1}^{r}\sum_{j=1}^{c} \frac{(O_{ij} - E_{ij})^2}{E_{ij}} \tag{20.70}$$
$$= \frac{(7-10.167)^2}{10.167} + \frac{(12-20.333)^2}{20.333} + \frac{(15-14.233)^2}{14.233} \tag{20.71}$$
$$+ \frac{(27-16.267)^2}{16.267} + \frac{(18-14.833)^2}{14.833} + \frac{(38-29.667)^2}{29.667} \tag{20.72}$$
$$+ \frac{(20-20.767)^2}{20.767} + \frac{(13-23.733)^2}{23.733} \tag{20.73}$$
$$= 0.987 + 3.415 + 0.041 + 7.082 + 0.676 + 2.341 \tag{20.74}$$
$$+ 0.028 + 4.854 \tag{20.75}$$
$$= 19.424. \tag{20.76}$$

The test is highly significant ($X^2 = 19.424, df = 3, P < 0.001$), similar to the likelihood ratio test.

20.2.2 Test of independence - SAS demo

We can carry out the same calculations using SAS and `proc freq` (SAS Institute Inc. 2014a). See program and output below. A two-way table of infec-

tion status and species is requested using the command `tables infected*species`. Likelihood ratio (G^2) and chi-square (X^2) tests are then requested using the `chisq` option. Because sample sizes are relatively small in this example, we can also request an exact version of both tests using the `exact chisq` option. See program and output below

The option `out=percents outpct` requests an output data file called `percents` that contains various percentages, including the column percents from the two-way table. This file is used by `proc gchart` to generate a vertical bar chart with `species` on the x-axis (SAS Institute Inc. 2014b). The percentage of infected and uninfected amphibians shown within each bar are generated using the option `subgroup=infected`.

Examining the SAS output, we see that both tests are highly significant ($G^2 = 19.618, df = 3, P = 0.0002; X^2 = 19.425, df = 3, P = 0.0002$). The exact tests give similar results in this case. For the exact likelihood ratio test (G^2) we have $P = 2.42 \times 10^{-4}$, while for the chi-square (X^2) the result is $P = 1.73 \times 10^{-4}$. The graph generated by `proc gchart` suggests that the infection rate is low for species A and B, intermediate for species C, and highest for species D (Fig. 20.2).

SAS Program

```
* chytrid.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Tests of independence - species vs. infection";
data chytrid;
    input species $ infected $ obsfreq;
    datalines;
A  yes   7
A  no   18
B  yes  12
B  no   38
C  yes  15
C  no   20
D  yes  27
D  no   13
;
run;
* Print data set;
proc print data=chytrid;
run;
* Tests of independence;
proc freq data=chytrid order=data;
    tables infected*species / chisq cellchi2 expected out=percents outpct;
    weight obsfreq;
    * Can compute an exact test if frequencies are low;
    * Not recommended for large data sets;
    exact chisq;
run;
* Print output data file containing percents;
proc print data=percents;
run;
* Generate bar chart showing percentages;
proc gchart data=percents;
    vbar species / sumvar=pct_col subgroup=infected width=10 woutline=3
    raxis=axis1 maxis=axis2 legend=legend1;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
    axis2 label=(height=2) value=(height=2) width=3;
    legend1 label=(height=2) value=(height=2);
run;
quit;
```

SAS Output

```
          Tests of independence - species vs. infection                1
                                        14:06 Thursday, December 2, 2010

              Obs    species    infected    obsfreq

               1        A         yes          7
               2        A         no          18
               3        B         yes         12
               4        B         no          38
               5        C         yes         15
               6        C         no          20
               7        D         yes         27
               8        D         no          13


          Tests of independence - species vs. infection                2
                                        14:06 Thursday, December 2, 2010

                        The FREQ Procedure

                    Table of infected by species

infected        species

Frequency       |
Expected        |
Cell Chi-Square|
Percent         |
Row Pct         |
Col Pct         |A       |B       |C       |D       |  Total
----------------------------------------------------------
yes             |      7 |     12 |     15 |     27 |     61
                | 10.167 | 20.333 | 14.233 | 16.267 |
                | 0.9863 | 3.4153 | 0.0413 | 7.0822 |
                |   4.67 |   8.00 |  10.00 |  18.00 |  40.67
                |  11.48 |  19.67 |  24.59 |  44.26 |
                |  28.00 |  24.00 |  42.86 |  67.50 |
----------------|--------|--------|--------|--------|
no              |     18 |     38 |     20 |     13 |     89
                | 14.833 | 29.667 | 20.767 | 23.733 |
                |  0.676 | 2.3408 | 0.0283 | 4.8541 |
                |  12.00 |  25.33 |  13.33 |   8.67 |  59.33
                |  20.22 |  42.70 |  22.47 |  14.61 |
```

	72.00	76.00	57.14	32.50	
Total	25	50	35	40	150
	16.67	33.33	23.33	26.67	100.00

Statistics for Table of infected by species

Statistic	DF	Value	Prob
Chi-Square	3	19.4245	0.0002
Likelihood Ratio Chi-Square	3	19.6810	0.0002
Mantel-Haenszel Chi-Square	1	15.9999	<.0001
Phi Coefficient		0.3599	
Contingency Coefficient		0.3386	
Cramer's V		0.3599	

Pearson Chi-Square Test

Chi-Square	19.4245
DF	3
Asymptotic Pr > ChiSq	0.0002
Exact Pr >= ChiSq	1.730E-04

Tests of independence - species vs. infection 3
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The FREQ Procedure

Statistics for Table of infected by species

Likelihood Ratio Chi-Square Test

Chi-Square	19.6810
DF	3
Asymptotic Pr > ChiSq	0.0002
Exact Pr >= ChiSq	2.417E-04

Mantel-Haenszel Chi-Square Test

Chi-Square	15.9999
DF	1

```
Asymptotic Pr >  ChiSq      <.0001
Exact      Pr >= ChiSq   6.462E-05

Sample Size = 150
```

```
Tests of independence - species vs. infection                4
                                    14:06 Thursday, December 2, 2010
```

Obs	infected	species	COUNT	PERCENT	PCT_ROW	PCT_COL
1	yes	A	7	4.6667	11.4754	28.0000
2	yes	B	12	8.0000	19.6721	24.0000
3	yes	C	15	10.0000	24.5902	42.8571
4	yes	D	27	18.0000	44.2623	67.5000
5	no	A	18	12.0000	20.2247	72.0000
6	no	B	38	25.3333	42.6966	76.0000
7	no	C	20	13.3333	22.4719	57.1429
8	no	D	13	8.6667	14.6067	32.5000

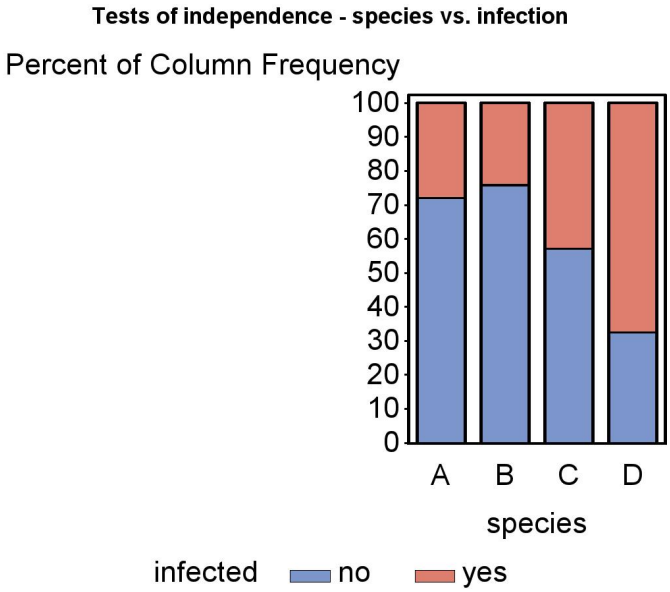

Figure 20.2: Stacked bar chart showing the percent infected vs. not infected in four different amphibian species.

20.2.3 Test of independence - SAS demo 2

The age structure of a population can provide clues about birth and death rates in the population, and the growth rate of the population may be reflected in its age structure. For example, a population with a higher proportion of young individuals could indicate the population is increasing through higher birth rates. An ecologist samples 100 individuals from three different populations and classifies them by age. There are five age classes, including newborns (age 0) and individuals 1, 2, 3, or 4 years old. See Table 20.4 for the results.

These data were obtained using a sampling scheme that selected 100 individuals for each population, so that the column totals are fixed at 100 while the row totals are free to vary. This differs from the previous example (Table 20.2), where amphibians in general were sampled and the number of each species was a random quantity. It turns out the multinomial distribution can be used to describe both sampling methods, and the tests for independence are the same (Agresti 1990).

Table 20.4: Observed frequencies of age 0, 1, 2, 3, and 4 year old individuals for three different populations.

	Population			
Age class	1	2	3	$\sum$
0	36	48	60	144
1	22	24	21	67
2	18	14	12	44
3	13	10	12	28
4	11	4	2	17
$\sum$	100	100	100	300

We will conduct tests of independence for these data using SAS and `proc freq` (see program and output below). As before, we will conduct both the likelihood ratio (G^2) and chi-square (X^2) tests. We have not calculated expected frequencies for this problem as in previous examples, but these can be found using the `expected` option of the `tables` command. One difference in this program is that the option for exact tests is turned off, because they are quite time consuming (and unnecessary) for large data sets. An output file is used by `proc gchart` to generate a vertical bar chart with `pop` on the x-axis, with the divisions within each bar the percentages of each age group. These were generated using the option `subgroup=age`.

The likelihood ratio test of independence was significant ($G^2 = 18.920, df = 8, P = 0.0153$) as was the chi-square test ($X^2 = 18.864, df = 8, P = 0.0156$). Examining the bar chart, we see that the percentage of younger individuals is lowest for population 1 and highest for population 3 (Fig. 20.3). One possible explanation is that population 3 has the highest birth rate while population 1 has the lowest.

SAS Program

```
* age_structure.sas;
options pageno=1 linesize=80;
goptions reset=all;
title "Tests of independence - age structure";
data age;
    input pop $ age $ obsfreq;
    datalines;
1  0  36
1  1  22
1  2  18
1  3  13
1  4  11
2  0  48
2  1  24
2  2  14
2  3  10
2  4   4
3  0  60
3  1  21
3  2  12
3  3   5
3  4   2
;
run;
* Print data set;
proc print data=age;
run;
* Tests of independence;
proc freq data=age order=data;
    tables age*pop / chisq cellchi2 expected out=percents outpct;
    weight obsfreq;
    * Can compute an exact test if frequencies are low;
    * Not recommended for large data sets;
    *exact chisq;
run;
* Print output data file containing percents;
proc print data=percents;
run;
* Generate bar chart showing percentages;
proc gchart data=percents;
    vbar pop / sumvar=pct_col subgroup=age width=10 woutline=3
    raxis=axis1 maxis=axis2 legend=legend1;
    axis1 label=(height=2) value=(height=2) width=3 major=(width=2) minor=none;
```

```
    axis2 label=(height=2) value=(height=2) width=3;
    legend1 label=(height=2) value=(height=2);
run;
quit;
```

SAS Output

Tests of independence - age structure 1
14:06 Thursday, December 2, 2010

Obs	pop	age	obsfreq
1	1	0	36
2	1	1	22
3	1	2	18
4	1	3	13
5	1	4	11
6	2	0	48
7	2	1	24
8	2	2	14
9	2	3	10
10	2	4	4
11	3	0	60
12	3	1	21
13	3	2	12
14	3	3	5
15	3	4	2

Tests of independence - age structure 2
14:06 Thursday, December 2, 2010

The FREQ Procedure

Table of age by pop

age	pop			
Frequency				
Expected				
Cell Chi-Square				
Percent				
Row Pct				
Col Pct	1	2	3	Total
0	36	48	60	144
	48	48	48	
	3	0	3	
	12.00	16.00	20.00	48.00
	25.00	33.33	41.67	
	36.00	48.00	60.00	
1	22	24	21	67
	22.333	22.333	22.333	
	0.005	0.1244	0.0796	
	7.33	8.00	7.00	22.33
	32.84	35.82	31.34	
	22.00	24.00	21.00	
2	18	14	12	44
	14.667	14.667	14.667	
	0.7576	0.0303	0.4848	
	6.00	4.67	4.00	14.67
	40.91	31.82	27.27	
	18.00	14.00	12.00	
3	13	10	5	28
	9.3333	9.3333	9.3333	
	1.4405	0.0476	2.0119	
	4.33	3.33	1.67	9.33
	46.43	35.71	17.86	
	13.00	10.00	5.00	

```
4              |     11 |      4 |      2 |     17
               | 5.6667 | 5.6667 | 5.6667 |
               | 5.0196 | 0.4902 | 2.3725 |
               |   3.67 |   1.33 |   0.67 |   5.67
               |  64.71 |  23.53 |  11.76 |
               |  11.00 |   4.00 |   2.00 |
---------------|--------|--------|--------|
Total              100      100      100      300
                 33.33    33.33    33.33   100.00
```

```
      Tests of independence - age structure                    3
                                   14:06 Thursday, December 2, 2010

                The FREQ Procedure

          Statistics for Table of age by pop

Statistic                     DF       Value      Prob
------------------------------------------------------
Chi-Square                     8     18.8640    0.0156
Likelihood Ratio Chi-Square    8     18.9195    0.0153
Mantel-Haenszel Chi-Square     1     17.5932    <.0001
Phi Coefficient                      0.2508
Contingency Coefficient              0.2432
Cramer's V                           0.1773

                Sample Size = 300
```

```
      Tests of independence - age structure                    4
                                   14:06 Thursday, December 2, 2010

 Obs    age    pop    COUNT    PERCENT    PCT_ROW    PCT_COL

   1      0      1       36    12.0000    25.0000         36
   2      0      2       48    16.0000    33.3333         48
   3      0      3       60    20.0000    41.6667         60
   4      1      1       22     7.3333    32.8358         22
   5      1      2       24     8.0000    35.8209         24
```

etc.

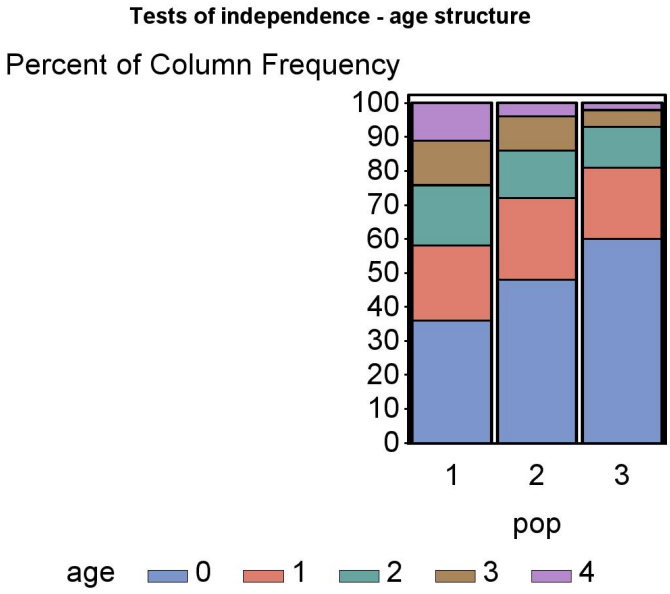

Figure 20.3: Stacked bar chart showing the percent of age 0, 1, 2, 3, 4, and 5 year old individuals for three populations.

20.3 Problems

1. An ecologist wants to characterize the spatial distribution of an uncommon plant species in the forest. One hundred quadrats are established and the number of plants counted in each quadrat. The following data were obtained:

Plants	Frequency
0	42
1	23
2	12
3	8
4	4
5	3
6	3
7	2
8	1
9	1
10	0
11	0
12	0

   Test whether these data have a Poisson distribution, using both likelihood ratio (G^2) and X^2 (χ^2) tests. Discuss your results. Do the data appear to be Poisson, overdispersed, or underdispersed?

2. Some species of snakes can imitate a rattlesnake and thereby avoid being eaten by predators, a phenomenon known as Batesian mimicry. Individuals of one such species were randomly selected from locations where rattlesnakes were absent, at moderate density, and at high density. Each snake was then scored for whether or not it imitated a rattlesnake when disturbed. The following results were obtained.

	Rattlesnake density		
Imitated a rattlesnake?	Absent	Moderate	High
Yes	65	76	82
No	35	24	18

   (a) Test if imitation of a rattlesnake is independent of rattlesnake density using a manual likelihood ratio (G^2) test. Show your calculations.

   (b) Test if imitation of a rattlesnake is independent of rattlesnake density using a manual $X^2(\chi^2)$ test. Show your calculations.

   (c) Check your above answers by having SAS carry out the same two tests.

   (d) Interpret the results of your tests. Does the frequency of rattlesnake imitation vary significantly with the density of rattlesnakes, and if so what is the pattern?

20.4 References

Agresti, A. (1990). *Categorical Data Analysis.* John Wiley & Sons, New York, NY.

Mendel, G. (1865) Experiments in plant hybridization. http://www.mendelweb.org/Mendel.html

Reeve, J. D., Rojas, M. G. & Morales-Ramos, J. A. (2003) Artificial diet and rearing methods for *Thanasimus dubius* (Coleoptera: Cleridae), a predator of bark beetles (Coleoptera: Scolytidae). *Biological Control* 27: 315-322.

SAS Institute Inc. (2014a) *Base SAS 9.4 Procedures Guide: Statistical Procedures, Third Edition.* SAS Institute Inc., Cary, NC, USA

SAS Institute Inc. (2014b) *SAS/GRAPH 9.4: Reference, Third Edition.* SAS Institute Inc., Cary, NC.

Chapter 21

Data Sets

21.1 Elytra Length

Elytra length of male and female clerid beetles (*Thanasimus dubius*) including a sample SAS `data` step. Data drawn from Reeve et al. (2003).

```
data elytra;
    input sex $ length;
    datalines;
M   4.9
F   5.2
M   4.9
F   4.2
F   5.7
M   4.6
M   3.8
F   5.4
F   4.0
F   4.5
M   4.9
F   5.2
M   4.9
F   4.2
F   5.7
M   4.6
M   3.8
F   5.4
F   4.0
F   4.5
F   5.2
F   4.9
M   5.0
M   4.4
M   5.0
M   5.0
M   4.9
F   4.5
F   4.5
M   5.1
F   5.5
M   4.8
F   4.9
M   4.8
M   4.5
```

```
M   4.5
M   4.4
M   5.2
M   4.1
F   5.0
M   4.4
F   4.9
M   4.7
M   4.4
F   4.8
F   4.5
M   4.0
M   3.4
F   5.5
M   4.7
M   4.8
F   4.8
F   3.7
M   5.3
M   4.6
F   4.8
M   4.5
M   5.0
M   4.4
F   4.6
M   4.4
M   4.9
F   5.3
F   5.0
F   4.7
F   5.2
M   5.0
M   5.0
M   4.8
M   5.8
F   5.7
F   5.2
M   4.9
F   5.1
F   5.3
F   5.3
F   5.9
F   5.3
M   4.5
F   5.2
```

```
M   5.1
F   4.6
M   4.8
M   3.5
F   4.6
F   5.3
M   5.2
F   4.8
M   5.1
M   5.2
M   4.9
M   5.3
M   5.2
F   4.9
F   5.6
M   5.0
M   5.0
F   5.1
M   5.1
F   5.5
M   5.1
F   4.8
F   4.9
F   5.0
M   4.9
M   5.0
F   5.0
M   4.9
M   4.8
F   5.2
F   4.8
M   4.7
F   5.1
M   4.5
M   5.0
F   5.4
F   4.6
M   4.0
M   4.2
F   5.2
F   4.6
M   5.0
M   3.7
M   4.6
M   4.0
```

```
M   5.1
F   4.4
M   4.8
M   4.6
F   3.7
;
run;
```

21.2 Development Time

Development times for the clerid beetle *Thanasimus dubius* The variables `time_pp` and `time_adult` are the development time from the larval to the prepupal stage, and the prepupal to the adult stage, respectively (Reeve et al. 2003).

```
data devel_time;
    input time_pp time_adult;
    datalines;
34  65
31  48
29   .
30  55
32  62
32  47
37  44
34  53
31   .
37  53
32   .
31  42
29   .
35   .
39   .
34  43
32   .
34   .
34 113
32  47
32 100
41   .
32  49
29   .
32  53
39   .
39  84
35   .
32   .
35  74
36  43
31  50
34   .
```

```
35  44
35 116
34   .
34   .
37  58
36 101
32  67
34  68
34  61
28  66
31  84
30  68
28 106
28  42
31  58
31  42
28  68
32  55
32   .
30 101
30  99
39  43
30  80
28  52
27  50
28 110
28  42
30   .
28  66
28 147
27   .
37 135
30 119
29 113
30 103
30  95
27  87
29  89
33   .
27  76
27   .
30   .
30  49
30  81
29  85
```

```
27   .
31 104
27  73
27 110
27   .
31  99
31  55
31  59
27   .
30  93
27   .
28  84
28  93
29   .
29 108
31 103
33   .
29  92
;
run;
```

21.3 Plant Biomass

Effect of nitrogen heterogeneity, nitrogen availability, and water availability on the total biomass of grassland plants grown in microcosms (Maestre & Reynolds 2007).

```
data maestre;
    input nitrohet $ nitrogen water biomass;
    datalines;
N   40  125   4.372
N   40  125   4.482
N   40  125   4.221
N   40  125   3.977
N   40  250   7.400
N   40  250   8.027
N   40  250   7.883
N   40  250   7.769
N   40  375   7.226
N   40  375   8.126
N   40  375   6.840
N   40  375   7.901
N   80  125   5.140
N   80  125   3.913
N   80  125   4.669
N   80  125   4.306
N   80  250   9.099
N   80  250   9.711
N   80  250   9.123
N   80  250   9.709
N   80  375  10.701
N   80  375  11.552
N   80  375  11.356
N   80  375   9.759
N  120  125   5.021
N  120  125   4.970
N  120  125   5.055
N  120  125   4.862
N  120  250   9.029
N  120  250  10.791
N  120  250   9.115
N  120  250  10.319
N  120  375  12.189
N  120  375  14.381
```

```
N  120  375  13.153
N  120  375  14.066
Y   40  125   5.458
Y   40  125   5.017
Y   40  125   5.479
Y   40  125   5.714
Y   40  250   8.972
Y   40  250   9.234
Y   40  250   8.032
Y   40  250   8.372
Y   40  375   9.464
Y   40  375   9.563
Y   40  375   9.385
Y   40  375   8.226
Y   80  125   6.616
Y   80  125   6.909
Y   80  125   6.851
Y   80  125   6.098
Y   80  250  10.792
Y   80  250  10.164
Y   80  250  10.947
Y   80  250   9.582
Y   80  375  14.936
Y   80  375  13.607
Y   80  375  14.231
Y   80  375  12.038
Y  120  125   7.389
Y  120  125   6.683
Y  120  125   7.759
Y  120  125   6.752
Y  120  250  10.731
Y  120  250  12.640
Y  120  250  10.350
Y  120  250  11.550
Y  120  375  14.697
Y  120  375  17.826
Y  120  375  14.711
Y  120  375  13.614
;
run;
```

21.4 *Anagrus* fecundity

Fecundity for the parasitoid *Anagrus delicatus* collected from different sites, with 14 isolines per site and eight individual wasps per isoline. The data were simulated from the results presented in Cronin and Strong (1996).

```
data anagrus;
        input site isoline wasp eggs;
        datalines;
1   1  1  37
1   1  2  41
1   1  3  46
1   1  4  44
1   1  5  43
1   1  6  41
1   1  7  38
1   1  8  37
1   2  1  37
1   2  2  28
1   2  3  34
1   2  4  37
1   2  5  35
1   2  6  39
1   2  7  36
1   2  8  29
1   3  1  35
1   3  2  37
1   3  3  40
1   3  4  39
1   3  5  37
1   3  6  44
1   3  7  35
1   3  8  38
1   4  1  28
1   4  2  36
1   4  3  31
1   4  4  27
1   4  5  36
1   4  6  33
1   4  7  31
1   4  8  35
1   5  1  34
1   5  2  35
```

```
1   5  3  30
1   5  4  39
1   5  5  42
1   5  6  39
1   5  7  38
1   5  8  32
1   6  1  30
1   6  2  32
1   6  3  35
1   6  4  35
1   6  5  32
1   6  6  31
1   6  7  34
1   6  8  30
1   7  1  30
1   7  2  36
1   7  3  37
1   7  4  30
1   7  5  41
1   7  6  35
1   7  7  34
1   7  8  37
1   8  1  25
1   8  2  31
1   8  3  24
1   8  4  26
1   8  5  30
1   8  6  31
1   8  7  25
1   8  8  24
1   9  1  34
1   9  2  35
1   9  3  29
1   9  4  34
1   9  5  34
1   9  6  40
1   9  7  37
1   9  8  37
1  10  1  38
1  10  2  30
1  10  3  33
1  10  4  32
1  10  5  33
1  10  6  34
1  10  7  35
```

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1  10  8  41
1  11  1  36
1  11  2  33
1  11  3  36
1  11  4  34
1  11  5  37
1  11  6  41
1  11  7  37
1  11  8  31
1  12  1  35
1  12  2  36
1  12  3  35
1  12  4  37
1  12  5  40
1  12  6  34
1  12  7  29
1  12  8  42
1  13  1  33
1  13  2  39
1  13  3  33
1  13  4  37
1  13  5  28
1  13  6  35
1  13  7  34
1  13  8  38
1  14  1  35
1  14  2  33
1  14  3  25
1  14  4  29
1  14  5  29
1  14  6  35
1  14  7  33
1  14  8  29
2   1  1  26
2   1  2  39
2   1  3  36
2   1  4  27
2   1  5  25
2   1  6  31
2   1  7  30
2   1  8  25
2   2  1  42
2   2  2  46
2   2  3  46
2   2  4  42
```

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2   2   5   43
2   2   6   36
2   2   7   36
2   2   8   41
2   3   1   38
2   3   2   36
2   3   3   35
2   3   4   31
2   3   5   36
2   3   6   32
2   3   7   29
2   3   8   34
2   4   1   28
2   4   2   36
2   4   3   33
2   4   4   32
2   4   5   27
2   4   6   31
2   4   7   30
2   4   8   32
2   5   1   30
2   5   2   35
2   5   3   32
2   5   4   31
2   5   5   36
2   5   6   34
2   5   7   29
2   5   8   36
2   6   1   28
2   6   2   34
2   6   3   34
2   6   4   35
2   6   5   32
2   6   6   31
2   6   7   24
2   6   8   31
2   7   1   35
2   7   2   34
2   7   3   44
2   7   4   34
2   7   5   35
2   7   6   36
2   7   7   32
2   7   8   30
2   8   1   37
```

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2   8  2  32
2   8  3  33
2   8  4  39
2   8  5  30
2   8  6  31
2   8  7  32
2   8  8  34
2   9  1  41
2   9  2  41
2   9  3  43
2   9  4  36
2   9  5  43
2   9  6  42
2   9  7  42
2   9  8  37
2  10  1  34
2  10  2  30
2  10  3  35
2  10  4  27
2  10  5  30
2  10  6  22
2  10  7  31
2  10  8  31
2  11  1  34
2  11  2  36
2  11  3  38
2  11  4  36
2  11  5  34
2  11  6  33
2  11  7  35
2  11  8  29
2  12  1  28
2  12  2  29
2  12  3  27
2  12  4  36
2  12  5  33
2  12  6  32
2  12  7  34
2  12  8  32
2  13  1  40
2  13  2  39
2  13  3  39
2  13  4  34
2  13  5  32
2  13  6  42
```

```
2  13  7  36
2  13  8  39
2  14  1  38
2  14  2  42
2  14  3  37
2  14  4  37
2  14  5  34
2  14  6  33
2  14  7  43
2  14  8  34
3   1  1  30
3   1  2  35
3   1  3  36
3   1  4  37
3   1  5  29
3   1  6  27
3   1  7  39
3   1  8  38
3   2  1  30
3   2  2  37
3   2  3  30
3   2  4  31
3   2  5  27
3   2  6  31
3   2  7  36
3   2  8  40
3   3  1  27
3   3  2  33
3   3  3  31
3   3  4  32
3   3  5  34
3   3  6  31
3   3  7  31
3   3  8  31
3   4  1  26
3   4  2  27
3   4  3  37
3   4  4  30
3   4  5  29
3   4  6  35
3   4  7  34
3   4  8  31
3   5  1  36
3   5  2  32
3   5  3  34
```

```
3   5  4  37
3   5  5  32
3   5  6  34
3   5  7  33
3   5  8  32
3   6  1  33
3   6  2  40
3   6  3  34
3   6  4  38
3   6  5  36
3   6  6  35
3   6  7  41
3   6  8  34
3   7  1  31
3   7  2  33
3   7  3  31
3   7  4  34
3   7  5  29
3   7  6  33
3   7  7  28
3   7  8  33
3   8  1  22
3   8  2  25
3   8  3  29
3   8  4  24
3   8  5  24
3   8  6  26
3   8  7  25
3   8  8  21
3   9  1  32
3   9  2  31
3   9  3  28
3   9  4  28
3   9  5  35
3   9  6  34
3   9  7  33
3   9  8  31
3  10  1  31
3  10  2  32
3  10  3  29
3  10  4  30
3  10  5  28
3  10  6  31
3  10  7  28
3  10  8  36
```

```
3  11  1  32
3  11  2  31
3  11  3  34
3  11  4  35
3  11  5  35
3  11  6  31
3  11  7  41
3  11  8  34
3  12  1  28
3  12  2  27
3  12  3  27
3  12  4  27
3  12  5  27
3  12  6  30
3  12  7  28
3  12  8  28
3  13  1  36
3  13  2  39
3  13  3  36
3  13  4  30
3  13  5  37
3  13  6  32
3  13  7  38
3  13  8  39
3  14  1  32
3  14  2  34
3  14  3  41
3  14  4  33
3  14  5  35
3  14  6  35
3  14  7  34
3  14  8  31
;
run;
```

21.5 Fitness of *T. dubius*

Fitness of adult *T. dubius*, a bark beetle predator, reared on an artificial diet as larvae vs. wild individuals collected from the field (Reeve et al. 2003). The adults were fed either *Ips grandicollis*) or cowpea weevils.

```
data fitness;
        input eggs longevity length treat $;
        datalines;
290    78   5.7   DietIG
 99    40   5.2   DietIG
340    70   5.5   DietIG
271    67   4.8   DietIG
200    84   5.2   DietIG
405    80   5.2   DietIG
178    80   5.1   DietIG
 48    23   5.0   DietIG
146    62   4.8   DietIG
184    82   4.9   DietIG
 66    67   4.6   DietCPW
 93    45   5.0   DietCPW
  9    49   5.4   DietCPW
404   121   5.4   DietCPW
244   114   5.1   DietCPW
195    72   4.9   DietCPW
343   126   5.2   DietCPW
516   138   5.0   DietCPW
215   108   4.6   DietCPW
412   156   5.6   DietCPW
167    79   4.8   DietCPW
316   117   5.2   DietCPW
334   127   5.3   DietCPW
 62   221   4.7   WildCPW
290   180   5.0   WildCPW
488   175   5.8   WildCPW
336   177   5.2   WildCPW
337   164   5.8   WildCPW
230    93   5.0   WildCPW
381   155   5.3   WildCPW
192   152   5.5   WildCPW
186   143   5.3   WildCPW
467   140   5.2   WildCPW
 59    42   4.9   WildCPW
```

```
323   138   5.7   WildCPW
291   117   4.9   WildCPW
164   112   5.3   WildCPW
142   112   5.3   WildCPW
269   110   5.0   WildCPW
329    91   5.4   WildCPW
235    84   5.0   WildCPW
;
run;
```

21.6 *Iris* flower measurements

Sepal and petal measurements for *I. setosa* (Fisher 1936).

```
data iris;
        input seplen sepwid petlen petwid;
        datalines;
5.1 3.5 1.4 0.2
4.9 3.0 1.4 0.2
4.7 3.2 1.3 0.2
4.6 3.1 1.5 0.2
5.0 3.6 1.4 0.2
5.4 3.9 1.7 0.4
4.6 3.4 1.4 0.3
5.0 3.4 1.5 0.2
4.4 2.9 1.4 0.2
4.9 3.1 1.5 0.1
5.4 3.7 1.5 0.2
4.8 3.4 1.6 0.2
4.8 3.0 1.4 0.1
4.3 3.0 1.1 0.1
5.8 4.0 1.2 0.2
5.7 4.4 1.5 0.4
5.4 3.9 1.3 0.4
5.1 3.5 1.4 0.3
5.7 3.8 1.7 0.3
5.1 3.8 1.5 0.3
5.4 3.4 1.7 0.2
5.1 3.7 1.5 0.4
4.6 3.6 1.0 0.2
5.1 3.3 1.7 0.5
4.8 3.4 1.9 0.2
5.0 3.0 1.6 0.2
5.0 3.4 1.6 0.4
5.2 3.5 1.5 0.2
5.2 3.4 1.4 0.2
4.7 3.2 1.6 0.2
4.8 3.1 1.6 0.2
5.4 3.4 1.5 0.4
5.2 4.1 1.5 0.1
5.5 4.2 1.4 0.2
4.9 3.1 1.5 0.2
5.0 3.2 1.2 0.2
5.5 3.5 1.3 0.2
```

```
4.9 3.6 1.4 0.1
4.4 3.0 1.3 0.2
5.1 3.4 1.5 0.2
5.0 3.5 1.3 0.3
4.5 2.3 1.3 0.3
4.4 3.2 1.3 0.2
5.0 3.5 1.6 0.6
5.1 3.8 1.9 0.4
4.8 3.0 1.4 0.3
5.1 3.8 1.6 0.2
4.6 3.2 1.4 0.2
5.3 3.7 1.5 0.2
5.0 3.3 1.4 0.2
;
run;
```

References

Cronin, J. T. & Strong, D. R. (1996) Genetics of oviposition success of a thelytokous fairyfly parasitoid, *Anagrus delicatus*. *Heredity* 76: 43-54.

Fisher, R. A. (1936) The use of multiple measurements in taxonomic problems. *Annals of Eugenics* 7: 179188.

Maestre, F. T. & Reynolds, J. F. (2007) Amount or pattern? Grassland responses to the heterogeneity and availability of two key resources. *Ecology* 88: 501-511.

Reeve, J. D., Rojas, M. G. & Morales-Ramos, J. A. (2003) Artificial diet and rearing methods for *Thanasimus dubius* (Coleoptera: Cleridae), a predator of bark beetles (Coleoptera: Scolytidae). *Biological Control* 27: 315-322.

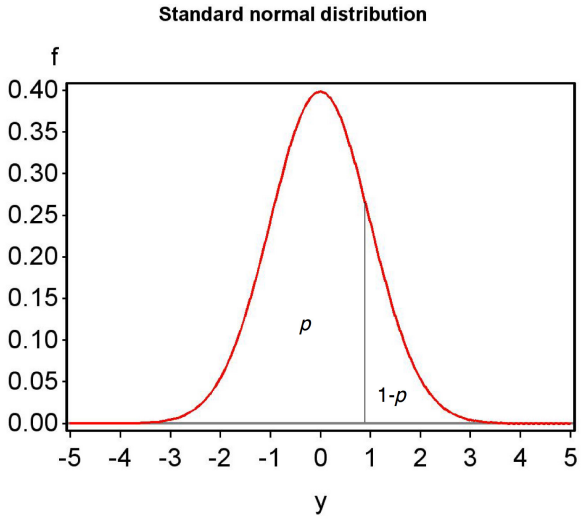
Chapter 22

Statistical Tables

22.1 Table Z: Probabilities for the standard normal distribution.

Suppose a random variable Z has a standard normal distribution ($Z \sim N(0,1)$). This table gives $P[Z < z] = p$ where the first two digits of z are given on the left, while the last digit is given in the top row. The values in the table were generated using the SAS function `probnorm` (SAS Institute Inc. 2014).

Figure 22.1: Plot of the standard normal distribution illustrating the probability shown in the table below.



References

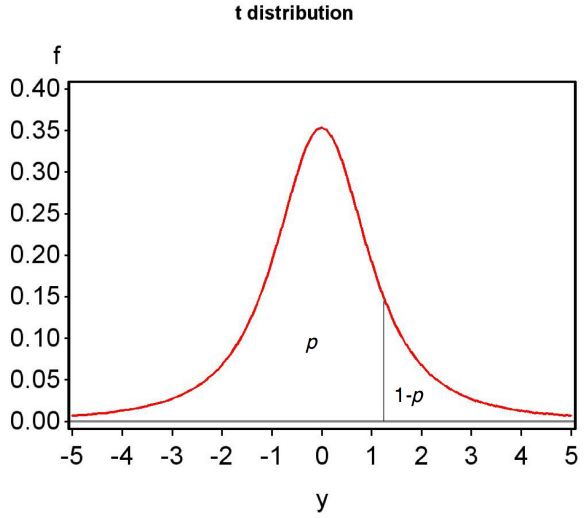
SAS Institute Inc. (2014) *SAS 9.4 Functions and CALL Routines, Fourth Edition*. SAS Institute Inc., Cary, NC.

z	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	0.5000	0.5040	0.5080	0.5120	0.5160	0.5199	0.5239	0.5279	0.5319	0.5359
0.1	0.5398	0.5438	0.5478	0.5517	0.5557	0.5596	0.5636	0.5675	0.5714	0.5753
0.2	0.5793	0.5832	0.5871	0.5910	0.5948	0.5987	0.6026	0.6064	0.6103	0.6141
0.3	0.6179	0.6217	0.6255	0.6293	0.6331	0.6368	0.6406	0.6443	0.6480	0.6517
0.4	0.6554	0.6591	0.6628	0.6664	0.6700	0.6736	0.6772	0.6808	0.6844	0.6879
0.5	0.6915	0.6950	0.6985	0.7019	0.7054	0.7088	0.7123	0.7157	0.7190	0.7224
0.6	0.7257	0.7291	0.7324	0.7357	0.7389	0.7422	0.7454	0.7486	0.7517	0.7549
0.7	0.7580	0.7611	0.7642	0.7673	0.7704	0.7734	0.7764	0.7794	0.7823	0.7852
0.8	0.7881	0.7910	0.7939	0.7967	0.7995	0.8023	0.8051	0.8078	0.8106	0.8133
0.9	0.8159	0.8186	0.8212	0.8238	0.8264	0.8289	0.8315	0.8340	0.8365	0.8389
1.0	0.8413	0.8438	0.8461	0.8485	0.8508	0.8531	0.8554	0.8577	0.8599	0.8621
1.1	0.8643	0.8665	0.8686	0.8708	0.8729	0.8749	0.8770	0.8790	0.8810	0.8830
1.2	0.8849	0.8869	0.8888	0.8907	0.8925	0.8944	0.8962	0.8980	0.8997	0.9015
1.3	0.9032	0.9049	0.9066	0.9082	0.9099	0.9115	0.9131	0.9147	0.9162	0.9177
1.4	0.9192	0.9207	0.9222	0.9236	0.9251	0.9265	0.9279	0.9292	0.9306	0.9319
1.5	0.9332	0.9345	0.9357	0.9370	0.9382	0.9394	0.9406	0.9418	0.9429	0.9441
1.6	0.9452	0.9463	0.9474	0.9484	0.9495	0.9505	0.9515	0.9525	0.9535	0.9545
1.7	0.9554	0.9564	0.9573	0.9582	0.9591	0.9599	0.9608	0.9616	0.9625	0.9633
1.8	0.9641	0.9649	0.9656	0.9664	0.9671	0.9678	0.9686	0.9693	0.9699	0.9706
1.9	0.9713	0.9719	0.9726	0.9732	0.9738	0.9744	0.9750	0.9756	0.9761	0.9767
2.0	0.9772	0.9778	0.9783	0.9788	0.9793	0.9798	0.9803	0.9808	0.9812	0.9817
2.1	0.9821	0.9826	0.9830	0.9834	0.9838	0.9842	0.9846	0.9850	0.9854	0.9857
2.2	0.9861	0.9864	0.9868	0.9871	0.9875	0.9878	0.9881	0.9884	0.9887	0.9890
2.3	0.9893	0.9896	0.9898	0.9901	0.9904	0.9906	0.9909	0.9911	0.9913	0.9916
2.4	0.9918	0.9920	0.9922	0.9925	0.9927	0.9929	0.9931	0.9932	0.9934	0.9936
2.5	0.9938	0.9940	0.9941	0.9943	0.9945	0.9946	0.9948	0.9949	0.9951	0.9952
2.6	0.9953	0.9955	0.9956	0.9957	0.9959	0.9960	0.9961	0.9962	0.9963	0.9964
2.7	0.9965	0.9966	0.9967	0.9968	0.9969	0.9970	0.9971	0.9972	0.9973	0.9974
2.8	0.9974	0.9975	0.9976	0.9977	0.9977	0.9978	0.9979	0.9979	0.9980	0.9981
2.9	0.9981	0.9982	0.9982	0.9983	0.9984	0.9984	0.9985	0.9985	0.9986	0.9986
3.0	0.9987	0.9987	0.9987	0.9988	0.9988	0.9989	0.9989	0.9989	0.9990	0.9990
3.1	0.9990	0.9991	0.9991	0.9991	0.9992	0.9992	0.9992	0.9992	0.9993	0.9993
3.2	0.9993	0.9993	0.9994	0.9994	0.9994	0.9994	0.9994	0.9995	0.9995	0.9995
3.3	0.9995	0.9995	0.9995	0.9996	0.9996	0.9996	0.9996	0.9996	0.9996	0.9997
3.4	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9998
3.5	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998
3.6	0.9998	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999

22.2 Table T: Quantiles of the t distribution

Suppose a random variable T has a t distribution. This table gives values of the quantile q such that $P[T < q] = p$, where $p = 0.75, 0.9, ..., 0.9995$. Degrees of freedom are given on the left. The values in the table were generated using the SAS function `tinv` (SAS Institute Inc. 2014).

Figure 22.2: Plot of the t distribution illustrating p and $1 - p$ in the table below.



References

SAS Institute Inc. (2014) *SAS 9.4 Functions and CALL Routines, Fourth Edition*. SAS Institute Inc., Cary, NC.

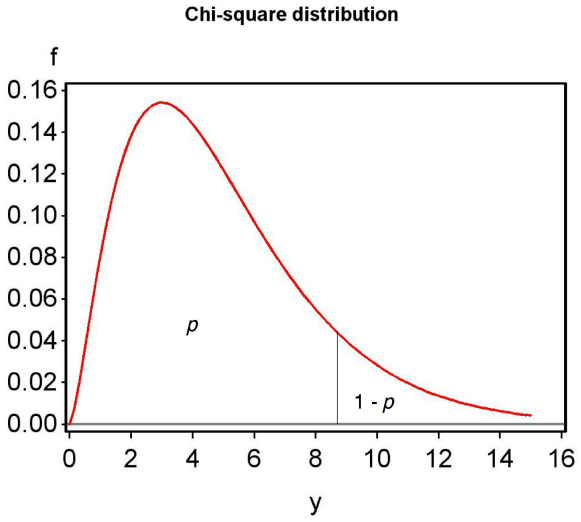
	p	0.75	0.90	0.95	0.975	0.990	0.995	0.9995
	$1-p$	0.25	0.10	0.05	0.025	0.010	0.005	0.0005
	$2(1-p)$	0.50	0.20	0.10	0.050	0.020	0.010	0.0010
	1	1.000	3.078	6.314	12.706	31.821	63.657	636.619
	2	0.816	1.886	2.920	4.303	6.965	9.925	31.599
	3	0.765	1.638	2.353	3.182	4.541	5.841	12.924
	4	0.741	1.533	2.132	2.776	3.747	4.604	8.610
	5	0.727	1.476	2.015	2.571	3.365	4.032	6.869
	6	0.718	1.440	1.943	2.447	3.143	3.707	5.959
	7	0.711	1.415	1.895	2.365	2.998	3.499	5.408
	8	0.706	1.397	1.860	2.306	2.896	3.355	5.041
	9	0.703	1.383	1.833	2.262	2.821	3.250	4.781
	10	0.700	1.372	1.812	2.228	2.764	3.169	4.587
	11	0.697	1.363	1.796	2.201	2.718	3.106	4.437
	12	0.695	1.356	1.782	2.179	2.681	3.055	4.318
	13	0.694	1.350	1.771	2.160	2.650	3.012	4.221
	14	0.692	1.345	1.761	2.145	2.624	2.977	4.140
	15	0.691	1.341	1.753	2.131	2.602	2.947	4.073
	16	0.690	1.337	1.746	2.120	2.583	2.921	4.015
	17	0.689	1.333	1.740	2.110	2.567	2.898	3.965
df	18	0.688	1.330	1.734	2.101	2.552	2.878	3.922
	19	0.688	1.328	1.729	2.093	2.539	2.861	3.883
	20	0.687	1.325	1.725	2.086	2.528	2.845	3.850
	21	0.686	1.323	1.721	2.080	2.518	2.831	3.819
	22	0.686	1.321	1.717	2.074	2.508	2.819	3.792
	23	0.685	1.319	1.714	2.069	2.500	2.807	3.768
	24	0.685	1.318	1.711	2.064	2.492	2.797	3.745
	25	0.684	1.316	1.708	2.060	2.485	2.787	3.725
	26	0.684	1.315	1.706	2.056	2.479	2.779	3.707
	27	0.684	1.314	1.703	2.052	2.473	2.771	3.690
	28	0.683	1.313	1.701	2.048	2.467	2.763	3.674
	29	0.683	1.311	1.699	2.045	2.462	2.756	3.659
	30	0.683	1.310	1.697	2.042	2.457	2.750	3.646
	31	0.682	1.309	1.696	2.040	2.453	2.744	3.633
	32	0.682	1.309	1.694	2.037	2.449	2.738	3.622
	33	0.682	1.308	1.692	2.035	2.445	2.733	3.611
	34	0.682	1.307	1.691	2.032	2.441	2.728	3.601
	35	0.682	1.306	1.690	2.030	2.438	2.724	3.591

	p	0.75	0.90	0.95	0.975	0.990	0.995	0.9995
	$1-p$	0.25	0.10	0.05	0.025	0.010	0.005	0.0005
	$2(1-p)$	0.50	0.20	0.10	0.050	0.020	0.010	0.0010
df	36	0.681	1.306	1.688	2.028	2.434	2.719	3.582
	37	0.681	1.305	1.687	2.026	2.431	2.715	3.574
	38	0.681	1.304	1.686	2.024	2.429	2.712	3.566
	39	0.681	1.304	1.685	2.023	2.426	2.708	3.558
	40	0.681	1.303	1.684	2.021	2.423	2.704	3.551
	50	0.679	1.299	1.676	2.009	2.403	2.678	3.496
	60	0.679	1.296	1.671	2.000	2.390	2.660	3.460
	70	0.678	1.294	1.667	1.994	2.381	2.648	3.435
	80	0.678	1.292	1.664	1.990	2.374	2.639	3.416
	90	0.677	1.291	1.662	1.987	2.368	2.632	3.402
	100	0.677	1.290	1.660	1.984	2.364	2.626	3.390
	∞	0.674	1.282	1.645	1.960	2.326	2.576	3.291

22.3 Table C: Quantiles of the χ^2 distribution

Suppose a random variable X has a χ^2 distribution with df degrees of freedom. This table gives values of the quantile q such that $P[X < q] = p$, where $p = 0.005, ..., 0.999$. The values in the table were generated using the SAS function `cinv` (SAS Institute Inc. 2014).

Figure 22.3: Plot of the χ^2 distribution ($df = 5$) illustrating p and $1 - p$ in the table below.



References

SAS Institute Inc. (2014) *SAS 9.4 Functions and CALL Routines, Fourth Edition*. SAS Institute Inc., Cary, NC.

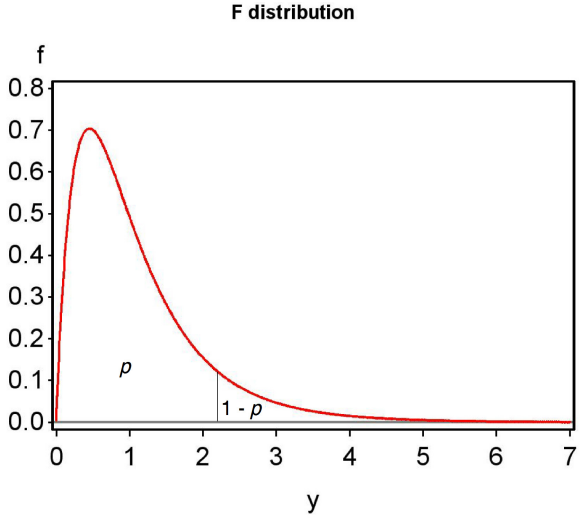
	p	0.005	0.010	0.025	0.050	0.100	0.250	0.500	0.750	0.900	0.950	0.975	0.990	0.995	0.999
	$1-p$	0.995	0.990	0.975	0.950	0.900	0.750	0.500	0.250	0.100	0.050	0.025	0.010	0.005	0.001
df	1	$3.93e^{-5}$	$1.57e^{-4}$	$9.82e^{-4}$	$3.93e^{-3}$	0.016	0.102	0.455	1.323	2.706	3.841	5.024	6.635	7.879	10.828
	2	0.010	0.020	0.051	0.103	0.211	0.575	1.386	2.773	4.605	5.991	7.378	9.210	10.597	13.816
	3	0.072	0.115	0.216	0.352	0.584	1.213	2.366	4.108	6.251	7.815	9.348	11.345	12.838	16.266
	4	0.207	0.297	0.484	0.711	1.064	1.923	3.357	5.385	7.779	9.488	11.143	13.277	14.860	18.467
	5	0.412	0.554	0.831	1.145	1.610	2.675	4.351	6.626	9.236	11.07	12.833	15.086	16.750	20.515
	6	0.676	0.872	1.237	1.635	2.204	3.455	5.348	7.841	10.645	12.592	14.449	16.812	18.548	22.458
	7	0.989	1.239	1.690	2.167	2.833	4.255	6.346	9.037	12.017	14.067	16.013	18.475	20.278	24.322
	8	1.344	1.646	2.180	2.733	3.490	5.071	7.344	10.219	13.362	15.507	17.535	20.09	21.955	26.124
	9	1.735	2.088	2.700	3.325	4.168	5.899	8.343	11.389	14.684	16.919	19.023	21.666	23.589	27.877
	10	2.156	2.558	3.247	3.940	4.865	6.737	9.342	12.549	15.987	18.307	20.483	23.209	25.188	29.588
	11	2.603	3.053	3.816	4.575	5.578	7.584	10.341	13.701	17.275	19.675	21.920	24.725	26.757	31.264
	12	3.074	3.571	4.404	5.226	6.304	8.438	11.34	14.845	18.549	21.026	23.337	26.217	28.300	32.909
	13	3.565	4.107	5.009	5.892	7.042	9.299	12.34	15.984	19.812	22.362	24.736	27.688	29.819	34.528
	14	4.075	4.660	5.629	6.571	7.790	10.165	13.339	17.117	21.064	23.685	26.119	29.141	31.319	36.123
	15	4.601	5.229	6.262	7.261	8.547	11.037	14.339	18.245	22.307	24.996	27.488	30.578	32.801	37.697
	16	5.142	5.812	6.908	7.962	9.312	11.912	15.338	19.369	23.542	26.296	28.845	32.000	34.267	39.252
	17	5.697	6.408	7.564	8.672	10.085	12.792	16.338	20.489	24.769	27.587	30.191	33.409	35.718	40.790
	18	6.265	7.015	8.231	9.390	10.865	13.675	17.338	21.605	25.989	28.869	31.526	34.805	37.156	42.312
	19	6.844	7.633	8.907	10.117	11.651	14.562	18.338	22.718	27.204	30.144	32.852	36.191	38.582	43.820
	20	7.434	8.260	9.591	10.851	12.443	15.452	19.337	23.828	28.412	31.410	34.170	37.566	39.997	45.315
	21	8.034	8.897	10.283	11.591	13.240	16.344	20.337	24.935	29.615	32.671	35.479	38.932	41.401	46.797
	22	8.643	9.542	10.982	12.338	14.041	17.24	21.337	26.039	30.813	33.924	36.781	40.289	42.796	48.268
	23	9.260	10.196	11.689	13.091	14.848	18.137	22.337	27.141	32.007	35.172	38.076	41.638	44.181	49.728
	24	9.886	10.856	12.401	13.848	15.659	19.037	23.337	28.241	33.196	36.415	39.364	42.980	45.559	51.179
	25	10.520	11.524	13.120	14.611	16.473	19.939	24.337	29.339	34.382	37.652	40.646	44.314	46.928	52.620
	26	11.160	12.198	13.844	15.379	17.292	20.843	25.336	30.435	35.563	38.885	41.923	45.642	48.29	54.052
	27	11.808	12.879	14.573	16.151	18.114	21.749	26.336	31.528	36.741	40.113	43.195	46.963	49.645	55.476
	28	12.461	13.565	15.308	16.928	18.939	22.657	27.336	32.620	37.916	41.337	44.461	48.278	50.993	56.892
	29	13.121	14.256	16.047	17.708	19.768	23.567	28.336	33.711	39.087	42.557	45.722	49.588	52.336	58.301
	30	13.787	14.953	16.791	18.493	20.599	24.478	29.336	34.800	40.256	43.773	46.979	50.892	53.672	59.703
	31	14.458	15.655	17.539	19.281	21.434	25.390	30.336	35.887	41.422	44.985	48.232	52.191	55.003	61.098
	32	15.134	16.362	18.291	20.072	22.271	26.304	31.336	36.973	42.585	46.194	49.480	53.486	56.328	62.487
	33	15.815	17.074	19.047	20.867	23.110	27.219	32.336	38.058	43.745	47.400	50.725	54.776	57.648	63.870
	34	16.501	17.789	19.806	21.664	23.952	28.136	33.336	39.141	44.903	48.602	51.966	56.061	58.964	65.247
	35	17.192	18.509	20.569	22.465	24.797	29.054	34.336	40.223	46.059	49.802	53.203	57.342	60.275	66.619
	36	17.887	19.233	21.336	23.269	25.643	29.973	35.336	41.304	47.212	50.998	54.437	58.619	61.581	67.985
	37	18.586	19.960	22.106	24.075	26.492	30.893	36.336	42.383	48.363	52.192	55.668	59.893	62.883	69.346
	38	19.289	20.691	22.878	24.884	27.343	31.815	37.335	43.462	49.513	53.384	56.896	61.162	64.181	70.703
	39	19.996	21.426	23.654	25.695	28.196	32.737	38.335	44.539	50.660	54.572	58.120	62.428	65.476	72.055
	40	20.707	22.164	24.433	26.509	29.051	33.660	39.335	45.616	51.805	55.758	59.342	63.691	66.766	73.402
	41	21.421	22.906	25.215	27.326	29.907	34.585	40.335	46.692	52.949	56.942	60.561	64.950	68.053	74.745
	42	22.138	23.650	25.999	28.144	30.765	35.510	41.335	47.766	54.090	58.124	61.777	66.206	69.336	76.084
	43	22.859	24.398	26.785	28.965	31.625	36.436	42.335	48.840	55.230	59.304	62.990	67.459	70.616	77.419
	44	23.584	25.148	27.575	29.787	32.487	37.363	43.335	49.913	56.369	60.481	64.201	68.710	71.893	78.750
	45	24.311	25.901	28.366	30.612	33.350	38.291	44.335	50.985	57.505	61.656	65.410	69.957	73.166	80.077
	46	25.041	26.657	29.160	31.439	34.215	39.220	45.335	52.056	58.641	62.830	66.617	71.201	74.437	81.400
	47	25.775	27.416	29.956	32.268	35.081	40.149	46.335	53.127	59.774	64.001	67.821	72.443	75.704	82.720
	48	26.511	28.177	30.755	33.098	35.949	41.079	47.335	54.196	60.907	65.171	69.023	73.683	76.969	84.037
	49	27.249	28.941	31.555	33.930	36.818	42.010	48.335	55.265	62.038	66.339	70.222	74.919	78.231	85.351
	50	27.991	29.707	32.357	34.764	37.689	42.942	49.335	56.334	63.167	67.505	71.420	76.154	79.490	86.661

	p	0.005	0.010	0.025	0.050	0.100	0.250	0.500	0.750	0.900	0.950	0.975	0.990	0.995	0.999
	$1-p$	0.995	0.990	0.975	0.950	0.900	0.750	0.500	0.250	0.100	0.050	0.025	0.010	0.005	0.001
	51	28.735	30.475	33.162	35.600	38.56	43.874	50.335	57.401	64.295	68.669	72.616	77.386	80.747	87.968
	52	29.481	31.246	33.968	36.437	39.433	44.808	51.335	58.468	65.422	69.832	73.810	78.616	82.001	89.272
	53	30.230	32.018	34.776	37.276	40.308	45.741	52.335	59.534	66.548	70.993	75.002	79.843	83.253	90.573
	54	30.981	32.793	35.586	38.116	41.183	46.676	53.335	60.600	67.673	72.153	76.192	81.069	84.502	91.872
	55	31.735	33.570	36.398	38.958	42.060	47.610	54.335	61.665	68.796	73.311	77.380	82.292	85.749	93.168
	56	32.490	34.350	37.212	39.801	42.937	48.546	55.335	62.729	69.919	74.468	78.567	83.513	86.994	94.461
	57	33.248	35.131	38.027	40.646	43.816	49.482	56.335	63.793	71.040	75.624	79.752	84.733	88.236	95.751
	58	34.008	35.913	38.844	41.492	44.696	50.419	57.335	64.857	72.160	76.778	80.936	85.950	89.477	97.039
	59	34.770	36.698	39.662	42.339	45.577	51.356	58.335	65.919	73.279	77.931	82.117	87.166	90.715	98.324
	60	35.534	37.485	40.482	43.188	46.459	52.294	59.335	66.981	74.397	79.082	83.298	88.379	91.952	99.607
	61	36.301	38.273	41.303	44.038	47.342	53.232	60.335	68.043	75.514	80.232	84.476	89.591	93.186	100.888
	62	37.068	39.063	42.126	44.889	48.226	54.171	61.335	69.104	76.630	81.381	85.654	90.802	94.419	102.166
	63	37.838	39.855	42.950	45.741	49.111	55.110	62.335	70.165	77.745	82.529	86.830	92.010	95.649	103.442
	64	38.610	40.649	43.776	46.595	49.996	56.050	63.335	71.225	78.860	83.675	88.004	93.217	96.878	104.716
	65	39.383	41.444	44.603	47.450	50.883	56.990	64.335	72.285	79.973	84.821	89.177	94.422	98.105	105.988
	66	40.158	42.240	45.431	48.305	51.770	57.931	65.335	73.344	81.085	85.965	90.349	95.626	99.330	107.258
	67	40.935	43.038	46.261	49.162	52.659	58.872	66.335	74.403	82.197	87.108	91.519	96.828	100.554	108.526
	68	41.713	43.838	47.092	50.020	53.548	59.814	67.335	75.461	83.308	88.250	92.689	98.028	101.776	109.791
	69	42.494	44.639	47.924	50.879	54.438	60.756	68.334	76.519	84.418	89.391	93.856	99.228	102.996	111.055
	70	43.275	45.442	48.758	51.739	55.329	61.698	69.334	77.577	85.527	90.531	95.023	100.425	104.215	112.317
	71	44.058	46.246	49.592	52.600	56.221	62.641	70.334	78.634	86.635	91.670	96.189	101.621	105.432	113.577
	72	44.843	47.051	50.428	53.462	57.113	63.585	71.334	79.690	87.743	92.808	97.353	102.816	106.648	114.835
	73	45.629	47.858	51.265	54.325	58.006	64.528	72.334	80.747	88.850	93.945	98.516	104.010	107.862	116.092
	74	46.417	48.666	52.103	55.189	58.900	65.472	73.334	81.803	89.956	95.081	99.678	105.202	109.074	117.346
	75	47.206	49.475	52.942	56.054	59.795	66.417	74.334	82.858	91.061	96.217	100.839	106.393	110.286	118.599
df	76	47.997	50.286	53.782	56.920	60.690	67.362	75.334	83.913	92.166	97.351	101.999	107.583	111.495	119.850
	77	48.788	51.097	54.623	57.786	61.586	68.307	76.334	84.968	93.270	98.484	103.158	108.771	112.704	121.100
	78	49.582	51.910	55.466	58.654	62.483	69.252	77.334	86.022	94.374	99.617	104.316	109.958	113.911	122.348
	79	50.376	52.725	56.309	59.522	63.380	70.198	78.334	87.077	95.476	100.749	105.473	111.144	115.117	123.594
	80	51.172	53.540	57.153	60.391	64.278	71.145	79.334	88.130	96.578	101.879	106.629	112.329	116.321	124.839
	81	51.969	54.357	57.998	61.261	65.176	72.091	80.334	89.184	97.680	103.010	107.783	113.512	117.524	126.083
	82	52.767	55.174	58.845	62.132	66.076	73.038	81.334	90.237	98.780	104.139	108.937	114.695	118.726	127.324
	83	53.567	55.993	59.692	63.004	66.976	73.985	82.334	91.289	99.880	105.267	110.090	115.876	119.927	128.565
	84	54.368	56.813	60.540	63.876	67.876	74.933	83.334	92.342	100.980	106.395	111.242	117.057	121.126	129.804
	85	55.170	57.634	61.389	64.749	68.777	75.881	84.334	93.394	102.079	107.522	112.393	118.236	122.325	131.041
	86	55.973	58.456	62.239	65.623	69.679	76.829	85.334	94.446	103.177	108.648	113.544	119.414	123.522	132.277
	87	56.777	59.279	63.089	66.498	70.581	77.777	86.334	95.497	104.275	109.773	114.693	120.591	124.718	133.512
	88	57.582	60.103	63.941	67.373	71.484	78.726	87.334	96.548	105.372	110.898	115.841	121.767	125.913	134.745
	89	58.389	60.928	64.793	68.249	72.387	79.675	88.334	97.599	106.469	112.022	116.989	122.942	127.106	135.978
	90	59.196	61.754	65.647	69.126	73.291	80.625	89.334	98.650	107.565	113.145	118.136	124.116	128.299	137.208
	91	60.005	62.581	66.501	70.003	74.196	81.574	90.334	99.700	108.661	114.268	119.282	125.289	129.491	138.438
	92	60.815	63.409	67.356	70.882	75.100	82.524	91.334	100.750	109.756	115.390	120.427	126.462	130.681	139.666
	93	61.625	64.238	68.211	71.760	76.006	83.474	92.334	101.800	110.850	116.511	121.571	127.633	131.871	140.893
	94	62.437	65.068	69.068	72.640	76.912	84.425	93.334	102.850	111.944	117.632	122.715	128.803	133.059	142.119
	95	63.250	65.898	69.925	73.520	77.818	85.376	94.334	103.899	113.038	118.752	123.858	129.973	134.247	143.344
	96	64.063	66.730	70.783	74.401	78.725	86.327	95.334	104.948	114.131	119.871	125.000	131.141	135.433	144.567
	97	64.878	67.562	71.642	75.282	79.633	87.278	96.334	105.997	115.223	120.990	126.141	132.309	136.619	145.789
	98	65.694	68.396	72.501	76.164	80.541	88.229	97.334	107.045	116.315	122.108	127.282	133.476	137.803	147.010
	99	66.510	69.230	73.361	77.046	81.449	89.181	98.334	108.093	117.407	123.225	128.422	134.642	138.987	148.230
	100	67.328	70.065	74.222	77.929	82.358	90.133	99.334	109.141	118.498	124.342	129.561	135.807	140.169	149.449

22.4 Table F: Quantiles of the F distribution

Suppose a random variable Y has an F distribution, with df_1 and df_2 the numerator and denominator degrees of freedom. This table gives values of the quantile q such that $P[Y < q] = p$, where $p = 0.005, ..., 0.999$. The values in the table were generated using the SAS function `finv` (SAS Institute Inc. 2014).

Figure 22.4: Plot of the F distribution ($df_1 = 4$, $df_2 = 20$) illustrating p and $1 - p$ in the table below.



References

SAS Institute Inc. (2014) *SAS 9.4 Functions and CALL Routines, Fourth Edition*. SAS Institute Inc., Cary, NC.

		0.900	0.950	0.975	0.990	0.995	0.999	p
		0.100	0.050	0.025	0.010	0.005	0.001	$1-p$
df_1	df_2							
1	4	4.545	7.709	12.218	21.198	31.333	74.137	
2	4	4.325	6.944	10.649	18.000	26.284	61.246	
3	4	4.191	6.591	9.979	16.694	24.259	56.177	
4	4	4.107	6.388	9.605	15.977	23.155	53.436	
5	4	4.051	6.256	9.364	15.522	22.456	51.712	
6	4	4.010	6.163	9.197	15.207	21.975	50.525	
1	5	4.060	6.608	10.007	16.258	22.785	47.181	
2	5	3.780	5.786	8.434	13.274	18.314	37.122	
3	5	3.619	5.409	7.764	12.060	16.530	33.202	
4	5	3.520	5.192	7.388	11.392	15.556	31.085	
5	5	3.453	5.050	7.146	10.967	14.940	29.752	
6	5	3.405	4.950	6.978	10.672	14.513	28.834	
1	6	3.776	5.987	8.813	13.745	18.635	35.507	
2	6	3.463	5.143	7.260	10.925	14.544	27.000	
3	6	3.289	4.757	6.599	9.780	12.917	23.703	
4	6	3.181	4.534	6.227	9.148	12.028	21.924	
5	6	3.108	4.387	5.988	8.746	11.464	20.803	
6	6	3.055	4.284	5.820	8.466	11.073	20.030	
1	7	3.589	5.591	8.073	12.246	16.236	29.245	
2	7	3.257	4.737	6.542	9.547	12.404	21.689	
3	7	3.074	4.347	5.890	8.451	10.882	18.772	
4	7	2.961	4.120	5.523	7.847	10.050	17.198	
5	7	2.883	3.972	5.285	7.460	9.522	16.206	
6	7	2.827	3.866	5.119	7.191	9.155	15.521	
1	8	3.458	5.318	7.571	11.259	14.688	25.415	
2	8	3.113	4.459	6.059	8.649	11.042	18.494	
3	8	2.924	4.066	5.416	7.591	9.596	15.829	
4	8	2.806	3.838	5.053	7.006	8.805	14.392	
5	8	2.726	3.687	4.817	6.632	8.302	13.485	
6	8	2.668	3.581	4.652	6.371	7.952	12.858	
1	9	3.360	5.117	7.209	10.561	13.614	22.857	
2	9	3.006	4.256	5.715	8.022	10.107	16.387	
3	9	2.813	3.863	5.078	6.992	8.717	13.902	
4	9	2.693	3.633	4.718	6.422	7.956	12.560	
5	9	2.611	3.482	4.484	6.057	7.471	11.714	
6	9	2.551	3.374	4.320	5.802	7.134	11.128	
1	10	3.285	4.965	6.937	10.044	12.826	21.040	
2	10	2.924	4.103	5.456	7.559	9.427	14.905	
3	10	2.728	3.708	4.826	6.552	8.081	12.553	
4	10	2.605	3.478	4.468	5.994	7.343	11.283	
5	10	2.522	3.326	4.236	5.636	6.872	10.481	
6	10	2.461	3.217	4.072	5.386	6.545	9.926	

		0.900	0.950	0.975	0.990	0.995	0.999	p
		0.100	0.050	0.025	0.010	0.005	0.001	$1-p$
df_1	df_2							
1	11	3.225	4.844	6.724	9.646	12.226	19.687	
2	11	2.860	3.982	5.256	7.206	8.912	13.812	
3	11	2.660	3.587	4.630	6.217	7.600	11.561	
4	11	2.536	3.357	4.275	5.668	6.881	10.346	
5	11	2.451	3.204	4.044	5.316	6.422	9.578	
6	11	2.389	3.095	3.881	5.069	6.102	9.047	
1	12	3.177	4.747	6.554	9.330	11.754	18.643	
2	12	2.807	3.885	5.096	6.927	8.510	12.974	
3	12	2.606	3.490	4.474	5.953	7.226	10.804	
4	12	2.480	3.259	4.121	5.412	6.521	9.633	
5	12	2.394	3.106	3.891	5.064	6.071	8.892	
6	12	2.331	2.996	3.728	4.821	5.757	8.379	
1	13	3.136	4.667	6.414	9.074	11.374	17.815	
2	13	2.763	3.806	4.965	6.701	8.186	12.313	
3	13	2.560	3.411	4.347	5.739	6.926	10.209	
4	13	2.434	3.179	3.996	5.205	6.233	9.073	
5	13	2.347	3.025	3.767	4.862	5.791	8.354	
6	13	2.283	2.915	3.604	4.620	5.482	7.856	
1	14	3.102	4.600	6.298	8.862	11.060	17.143	
2	14	2.726	3.739	4.857	6.515	7.922	11.779	
3	14	2.522	3.344	4.242	5.564	6.680	9.729	
4	14	2.395	3.112	3.892	5.035	5.998	8.622	
5	14	2.307	2.958	3.663	4.695	5.562	7.922	
6	14	2.243	2.848	3.501	4.456	5.257	7.436	
1	15	3.073	4.543	6.200	8.683	10.798	16.587	
2	15	2.695	3.682	4.765	6.359	7.701	11.339	
3	15	2.490	3.287	4.153	5.417	6.476	9.335	
4	15	2.361	3.056	3.804	4.893	5.803	8.253	
5	15	2.273	2.901	3.576	4.556	5.372	7.567	
6	15	2.208	2.790	3.415	4.318	5.071	7.092	
1	16	3.048	4.494	6.115	8.531	10.575	16.120	
2	16	2.668	3.634	4.687	6.226	7.514	10.971	
3	16	2.462	3.239	4.077	5.292	6.303	9.006	
4	16	2.333	3.007	3.729	4.773	5.638	7.944	
5	16	2.244	2.852	3.502	4.437	5.212	7.272	
6	16	2.178	2.741	3.341	4.202	4.913	6.805	
1	17	3.026	4.451	6.042	8.400	10.384	15.722	
2	17	2.645	3.592	4.619	6.112	7.354	10.658	
3	17	2.437	3.197	4.011	5.185	6.156	8.727	
4	17	2.308	2.965	3.665	4.669	5.497	7.683	
5	17	2.218	2.810	3.438	4.336	5.075	7.022	
6	17	2.152	2.699	3.277	4.102	4.779	6.562	

		0.900	0.950	0.975	0.990	0.995	0.999	p
		0.100	0.050	0.025	0.010	0.005	0.001	$1-p$
df_1	df_2							
1	18	3.007	4.414	5.978	8.285	10.218	15.379	
2	18	2.624	3.555	4.560	6.013	7.215	10.390	
3	18	2.416	3.160	3.954	5.092	6.028	8.487	
4	18	2.286	2.928	3.608	4.579	5.375	7.459	
5	18	2.196	2.773	3.382	4.248	4.956	6.808	
6	18	2.130	2.661	3.221	4.015	4.663	6.355	
1	19	2.990	4.381	5.922	8.185	10.073	15.081	
2	19	2.606	3.522	4.508	5.926	7.093	10.157	
3	19	2.397	3.127	3.903	5.010	5.916	8.280	
4	19	2.266	2.895	3.559	4.500	5.268	7.265	
5	19	2.176	2.740	3.333	4.171	4.853	6.622	
6	19	2.109	2.628	3.172	3.939	4.561	6.175	
1	20	2.975	4.351	5.871	8.096	9.944	14.819	
2	20	2.589	3.493	4.461	5.849	6.986	9.953	
3	20	2.380	3.098	3.859	4.938	5.818	8.098	
4	20	2.249	2.866	3.515	4.431	5.174	7.096	
5	20	2.158	2.711	3.289	4.103	4.762	6.461	
6	20	2.091	2.599	3.128	3.871	4.472	6.019	
1	21	2.961	4.325	5.827	8.017	9.830	14.587	
2	21	2.575	3.467	4.420	5.780	6.891	9.772	
3	21	2.365	3.072	3.819	4.874	5.730	7.938	
4	21	2.233	2.840	3.475	4.369	5.091	6.947	
5	21	2.142	2.685	3.250	4.042	4.681	6.318	
6	21	2.075	2.573	3.090	3.812	4.393	5.881	
1	22	2.949	4.301	5.786	7.945	9.727	14.380	
2	22	2.561	3.443	4.383	5.719	6.806	9.612	
3	22	2.351	3.049	3.783	4.817	5.652	7.796	
4	22	2.219	2.817	3.440	4.313	5.017	6.814	
5	22	2.128	2.661	3.215	3.988	4.609	6.191	
6	22	2.060	2.549	3.055	3.758	4.322	5.758	
1	23	2.937	4.279	5.750	7.881	9.635	14.195	
2	23	2.549	3.422	4.349	5.664	6.730	9.469	
3	23	2.339	3.028	3.750	4.765	5.582	7.669	
4	23	2.207	2.796	3.408	4.264	4.950	6.696	
5	23	2.115	2.640	3.183	3.939	4.544	6.078	
6	23	2.047	2.528	3.023	3.710	4.259	5.649	
1	24	2.927	4.260	5.717	7.823	9.551	14.028	
2	24	2.538	3.403	4.319	5.614	6.661	9.339	
3	24	2.327	3.009	3.721	4.718	5.519	7.554	
4	24	2.195	2.776	3.379	4.218	4.890	6.589	
5	24	2.103	2.621	3.155	3.895	4.486	5.977	
6	24	2.035	2.508	2.995	3.667	4.202	5.550	

		0.900	0.950	0.975	0.990	0.995	0.999	p
		0.100	0.050	0.025	0.010	0.005	0.001	$1-p$
df_1	df_2							
1	25	2.918	4.242	5.686	7.770	9.475	13.877	
2	25	2.528	3.385	4.291	5.568	6.598	9.223	
3	25	2.317	2.991	3.694	4.675	5.462	7.451	
4	25	2.184	2.759	3.353	4.177	4.835	6.493	
5	25	2.092	2.603	3.129	3.855	4.433	5.885	
6	25	2.024	2.490	2.969	3.627	4.150	5.462	
1	26	2.909	4.225	5.659	7.721	9.406	13.739	
2	26	2.519	3.369	4.265	5.526	6.541	9.116	
3	26	2.307	2.975	3.670	4.637	5.409	7.357	
4	26	2.174	2.743	3.329	4.140	4.785	6.406	
5	26	2.082	2.587	3.105	3.818	4.384	5.802	
6	26	2.014	2.474	2.945	3.591	4.103	5.381	
1	27	2.901	4.210	5.633	7.677	9.342	13.613	
2	27	2.511	3.354	4.242	5.488	6.489	9.019	
3	27	2.299	2.960	3.647	4.601	5.361	7.272	
4	27	2.165	2.728	3.307	4.106	4.740	6.326	
5	27	2.073	2.572	3.083	3.785	4.340	5.726	
6	27	2.005	2.459	2.923	3.558	4.059	5.308	
1	28	2.894	4.196	5.610	7.636	9.284	13.498	
2	28	2.503	3.340	4.221	5.453	6.440	8.931	
3	28	2.291	2.947	3.626	4.568	5.317	7.193	
4	28	2.157	2.714	3.286	4.074	4.698	6.253	
5	28	2.064	2.558	3.063	3.754	4.300	5.656	
6	28	1.996	2.445	2.903	3.528	4.020	5.241	
1	29	2.887	4.183	5.588	7.598	9.230	13.391	
2	29	2.495	3.328	4.201	5.420	6.396	8.849	
3	29	2.283	2.934	3.607	4.538	5.276	7.121	
4	29	2.149	2.701	3.267	4.045	4.659	6.186	
5	29	2.057	2.545	3.044	3.725	4.262	5.593	
6	29	1.988	2.432	2.884	3.499	3.983	5.179	
1	30	2.881	4.171	5.568	7.562	9.180	13.293	
2	30	2.489	3.316	4.182	5.390	6.355	8.773	
3	30	2.276	2.922	3.589	4.510	5.239	7.054	
4	30	2.142	2.690	3.250	4.018	4.623	6.125	
5	30	2.049	2.534	3.026	3.699	4.228	5.534	
6	30	1.980	2.421	2.867	3.473	3.949	5.122	
1	31	2.875	4.160	5.549	7.530	9.133	13.202	
2	31	2.482	3.305	4.165	5.362	6.317	8.704	
3	31	2.270	2.911	3.573	4.484	5.204	6.993	
4	31	2.136	2.679	3.234	3.993	4.590	6.067	
5	31	2.042	2.523	3.010	3.675	4.196	5.480	
6	31	1.973	2.409	2.851	3.449	3.918	5.070	

		0.900	0.950	0.975	0.990	0.995	0.999	p
		0.100	0.050	0.025	0.010	0.005	0.001	$1-p$
df_1	df_2							
1	32	2.869	4.149	5.531	7.499	9.090	13.117	
2	32	2.477	3.295	4.149	5.336	6.281	8.639	
3	32	2.263	2.901	3.557	4.459	5.171	6.936	
4	32	2.129	2.668	3.218	3.969	4.559	6.014	
5	32	2.036	2.512	2.995	3.652	4.166	5.429	
6	32	1.967	2.399	2.836	3.427	3.889	5.021	
1	33	2.864	4.139	5.515	7.471	9.050	13.039	
2	33	2.471	3.285	4.134	5.312	6.248	8.579	
3	33	2.258	2.892	3.543	4.437	5.141	6.883	
4	33	2.123	2.659	3.204	3.948	4.531	5.965	
5	33	2.030	2.503	2.981	3.630	4.138	5.382	
6	33	1.961	2.389	2.822	3.406	3.861	4.976	
1	34	2.859	4.130	5.499	7.444	9.012	12.965	
2	34	2.466	3.276	4.120	5.289	6.217	8.522	
3	34	2.252	2.883	3.529	4.416	5.113	6.833	
4	34	2.118	2.650	3.191	3.927	4.504	5.919	
5	34	2.024	2.494	2.968	3.611	4.112	5.339	
6	34	1.955	2.380	2.808	3.386	3.836	4.934	
1	35	2.855	4.121	5.485	7.419	8.976	12.896	
2	35	2.461	3.267	4.106	5.268	6.188	8.470	
3	35	2.247	2.874	3.517	4.396	5.086	6.787	
4	35	2.113	2.641	3.179	3.908	4.479	5.876	
5	35	2.019	2.485	2.956	3.592	4.088	5.298	
6	35	1.950	2.372	2.796	3.368	3.812	4.894	
1	36	2.850	4.113	5.471	7.396	8.943	12.832	
2	36	2.456	3.259	4.094	5.248	6.161	8.420	
3	36	2.243	2.866	3.505	4.377	5.062	6.744	
4	36	2.108	2.634	3.167	3.890	4.455	5.836	
5	36	2.014	2.477	2.944	3.574	4.065	5.260	
6	36	1.945	2.364	2.785	3.351	3.790	4.857	
1	37	2.846	4.105	5.458	7.373	8.912	12.771	
2	37	2.452	3.252	4.082	5.229	6.135	8.374	
3	37	2.238	2.859	3.493	4.360	5.038	6.703	
4	37	2.103	2.626	3.156	3.873	4.433	5.799	
5	37	2.009	2.470	2.933	3.558	4.043	5.224	
6	37	1.940	2.356	2.774	3.334	3.769	4.823	
1	38	2.842	4.098	5.446	7.353	8.882	12.714	
2	38	2.448	3.245	4.071	5.211	6.111	8.331	
3	38	2.234	2.852	3.483	4.343	5.016	6.665	
4	38	2.099	2.619	3.145	3.858	4.412	5.763	
5	38	2.005	2.463	2.923	3.542	4.023	5.190	
6	38	1.935	2.349	2.763	3.319	3.749	4.790	

		0.900	0.950	0.975	0.990	0.995	0.999	p
		0.100	0.050	0.025	0.010	0.005	0.001	$1-p$
df_1	df_2							
1	39	2.839	4.091	5.435	7.333	8.854	12.660	
2	39	2.444	3.238	4.061	5.194	6.088	8.290	
3	39	2.230	2.845	3.473	4.327	4.995	6.629	
4	39	2.095	2.612	3.135	3.843	4.392	5.730	
5	39	2.001	2.456	2.913	3.528	4.004	5.158	
6	39	1.931	2.342	2.754	3.305	3.731	4.759	
1	40	2.835	4.085	5.424	7.314	8.828	12.609	
2	40	2.440	3.232	4.051	5.179	6.066	8.251	
3	40	2.226	2.839	3.463	4.313	4.976	6.595	
4	40	2.091	2.606	3.126	3.828	4.374	5.698	
5	40	1.997	2.449	2.904	3.514	3.986	5.128	
6	40	1.927	2.336	2.744	3.291	3.713	4.731	
1	41	2.832	4.079	5.414	7.296	8.803	12.561	
2	41	2.437	3.226	4.042	5.163	6.046	8.214	
3	41	2.222	2.833	3.454	4.299	4.957	6.562	
4	41	2.087	2.600	3.117	3.815	4.356	5.668	
5	41	1.993	2.443	2.895	3.501	3.969	5.100	
6	41	1.923	2.330	2.736	3.278	3.696	4.703	
1	42	2.829	4.073	5.404	7.280	8.779	12.516	
2	42	2.434	3.220	4.033	5.149	6.027	8.179	
3	42	2.219	2.827	3.446	4.285	4.940	6.532	
4	42	2.084	2.594	3.109	3.802	4.339	5.640	
5	42	1.989	2.438	2.887	3.488	3.953	5.073	
6	42	1.919	2.324	2.727	3.266	3.680	4.677	
1	43	2.826	4.067	5.395	7.264	8.757	12.472	
2	43	2.430	3.214	4.024	5.136	6.008	8.146	
3	43	2.216	2.822	3.438	4.273	4.923	6.503	
4	43	2.080	2.589	3.101	3.790	4.324	5.613	
5	43	1.986	2.432	2.879	3.476	3.937	5.048	
6	43	1.916	2.318	2.719	3.254	3.665	4.653	
1	44	2.823	4.062	5.386	7.248	8.735	12.431	
2	44	2.427	3.209	4.016	5.123	5.991	8.115	
3	44	2.213	2.816	3.430	4.261	4.907	6.476	
4	44	2.077	2.584	3.093	3.778	4.308	5.588	
5	44	1.983	2.427	2.871	3.465	3.923	5.024	
6	44	1.913	2.313	2.712	3.243	3.651	4.630	
1	45	2.820	4.057	5.377	7.234	8.715	12.392	
2	45	2.425	3.204	4.009	5.110	5.974	8.086	
3	45	2.210	2.812	3.422	4.249	4.892	6.450	
4	45	2.074	2.579	3.086	3.767	4.294	5.564	
5	45	1.980	2.422	2.864	3.454	3.909	5.001	
6	45	1.909	2.308	2.705	3.232	3.638	4.608	

		0.900	0.950	0.975	0.990	0.995	0.999	p
		0.100	0.050	0.025	0.010	0.005	0.001	$1-p$
df_1	df_2							
1	46	2.818	4.052	5.369	7.220	8.695	12.355	
2	46	2.422	3.200	4.001	5.099	5.958	8.057	
3	46	2.207	2.807	3.415	4.238	4.877	6.425	
4	46	2.071	2.574	3.079	3.757	4.280	5.541	
5	46	1.977	2.417	2.857	3.444	3.896	4.979	
6	46	1.906	2.304	2.698	3.222	3.625	4.587	
1	47	2.815	4.047	5.361	7.207	8.677	12.319	
2	47	2.419	3.195	3.994	5.087	5.943	8.030	
3	47	2.204	2.802	3.409	4.228	4.864	6.401	
4	47	2.068	2.570	3.073	3.747	4.267	5.519	
5	47	1.974	2.413	2.851	3.434	3.883	4.958	
6	47	1.903	2.299	2.691	3.213	3.612	4.566	
1	48	2.813	4.043	5.354	7.194	8.659	12.286	
2	48	2.417	3.191	3.987	5.077	5.929	8.005	
3	48	2.202	2.798	3.402	4.218	4.850	6.379	
4	48	2.066	2.565	3.066	3.737	4.255	5.498	
5	48	1.971	2.409	2.844	3.425	3.871	4.938	
6	48	1.901	2.295	2.685	3.204	3.601	4.547	
1	49	2.811	4.038	5.347	7.182	8.642	12.253	
2	49	2.414	3.187	3.981	5.066	5.915	7.980	
3	49	2.199	2.794	3.396	4.208	4.838	6.357	
4	49	2.063	2.561	3.060	3.728	4.243	5.478	
5	49	1.968	2.404	2.838	3.416	3.860	4.919	
6	49	1.898	2.290	2.679	3.195	3.589	4.529	
1	50	2.809	4.034	5.340	7.171	8.626	12.222	
2	50	2.412	3.183	3.975	5.057	5.902	7.956	
3	50	2.197	2.790	3.390	4.199	4.826	6.336	
4	50	2.061	2.557	3.054	3.720	4.232	5.459	
5	50	1.966	2.400	2.833	3.408	3.849	4.901	
6	50	1.895	2.286	2.674	3.186	3.579	4.512	
1	51	2.807	4.030	5.334	7.159	8.610	12.192	
2	51	2.410	3.179	3.969	5.047	5.889	7.934	
3	51	2.194	2.786	3.385	4.191	4.814	6.317	
4	51	2.058	2.553	3.049	3.711	4.221	5.441	
5	51	1.964	2.397	2.827	3.400	3.838	4.884	
6	51	1.893	2.283	2.668	3.178	3.568	4.495	
1	52	2.805	4.027	5.328	7.149	8.595	12.164	
2	52	2.408	3.175	3.963	5.038	5.877	7.912	
3	52	2.192	2.783	3.379	4.182	4.803	6.298	
4	52	2.056	2.550	3.044	3.703	4.210	5.424	
5	52	1.961	2.393	2.822	3.392	3.828	4.867	
6	52	1.891	2.279	2.663	3.171	3.558	4.479	

		0.900	0.950	0.975	0.990	0.995	0.999	p
		0.100	0.050	0.025	0.010	0.005	0.001	$1-p$
df_1	df_2							
1	53	2.803	4.023	5.322	7.139	8.581	12.137	
2	53	2.406	3.172	3.958	5.030	5.865	7.892	
3	53	2.190	2.779	3.374	4.174	4.793	6.280	
4	53	2.054	2.546	3.038	3.695	4.200	5.407	
5	53	1.959	2.389	2.817	3.384	3.818	4.852	
6	53	1.888	2.275	2.658	3.163	3.549	4.464	
1	54	2.801	4.020	5.316	7.129	8.567	12.111	
2	54	2.404	3.168	3.953	5.021	5.854	7.872	
3	54	2.188	2.776	3.369	4.167	4.783	6.262	
4	54	2.052	2.543	3.034	3.688	4.191	5.391	
5	54	1.957	2.386	2.812	3.377	3.809	4.836	
6	54	1.886	2.272	2.653	3.156	3.540	4.449	
1	55	2.799	4.016	5.310	7.119	8.554	12.085	
2	55	2.402	3.165	3.948	5.013	5.843	7.853	
3	55	2.186	2.773	3.364	4.159	4.773	6.246	
4	55	2.050	2.540	3.029	3.681	4.181	5.375	
5	55	1.955	2.383	2.807	3.370	3.800	4.822	
6	55	1.884	2.269	2.648	3.149	3.531	4.435	
1	56	2.797	4.013	5.305	7.110	8.541	12.061	
2	56	2.400	3.162	3.943	5.006	5.833	7.834	
3	56	2.184	2.769	3.359	4.152	4.763	6.230	
4	56	2.048	2.537	3.024	3.674	4.172	5.361	
5	56	1.953	2.380	2.803	3.363	3.791	4.808	
6	56	1.882	2.266	2.644	3.143	3.523	4.421	
1	57	2.796	4.010	5.300	7.102	8.529	12.038	
2	57	2.398	3.159	3.938	4.998	5.823	7.817	
3	57	2.182	2.766	3.355	4.145	4.754	6.214	
4	57	2.046	2.534	3.020	3.667	4.164	5.346	
5	57	1.951	2.377	2.798	3.357	3.783	4.794	
6	57	1.880	2.263	2.639	3.136	3.514	4.408	
1	58	2.794	4.007	5.295	7.093	8.517	12.015	
2	58	2.396	3.156	3.934	4.991	5.813	7.800	
3	58	2.181	2.764	3.351	4.138	4.746	6.199	
4	58	2.044	2.531	3.016	3.661	4.156	5.333	
5	58	1.949	2.374	2.794	3.351	3.775	4.781	
6	58	1.878	2.260	2.635	3.130	3.507	4.396	
1	59	2.793	4.004	5.290	7.085	8.506	11.994	
2	59	2.395	3.153	3.929	4.984	5.804	7.784	
3	59	2.179	2.761	3.347	4.132	4.737	6.185	
4	59	2.043	2.528	3.012	3.655	4.148	5.319	
5	59	1.947	2.371	2.790	3.345	3.767	4.769	
6	59	1.876	2.257	2.631	3.124	3.499	4.384	

		0.900	0.950	0.975	0.990	0.995	0.999	p
		0.100	0.050	0.025	0.010	0.005	0.001	$1-p$
df_1	df_2							
1	60	2.791	4.001	5.286	7.077	8.495	11.973	
2	60	2.393	3.150	3.925	4.977	5.795	7.768	
3	60	2.177	2.758	3.343	4.126	4.729	6.171	
4	60	2.041	2.525	3.008	3.649	4.140	5.307	
5	60	1.946	2.368	2.786	3.339	3.760	4.757	
6	60	1.875	2.254	2.627	3.119	3.492	4.372	

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