

# 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 where 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 frontalinal + 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 ( $\bar{Y}_{ij\cdot}$ ) and preliminary calculations to find  $SS_{within}$

Bait	Color	<i>T. dubius</i>	$Y_{ijk} = \log_{10}(T.dubius + 1)$	<i>i</i>	<i>j</i>	<i>k</i>	$\bar{Y}_{ij\cdot}$	$(Y_{ijk} - \bar{Y}_{ij\cdot})^2$
FRT	B	18	1.279	1	1	1	1.150	$1.664 \times 10^{-2}$
FRT	B	12	1.114	1	1	2		$1.296 \times 10^{-3}$
FRT	B	22	1.362	1	1	3		$4.494 \times 10^{-2}$
FRT	B	6	0.845	1	1	4		$9.303 \times 10^{-2}$
FRT	W	12	1.114	1	2	1	0.980	$1.796 \times 10^{-2}$
FRT	W	15	1.204	1	2	2		$5.018 \times 10^{-2}$
FRT	W	7	0.903	1	2	3		$5.929 \times 10^{-3}$
FRT	W	4	0.699	1	2	4		$7.896 \times 10^{-2}$
IDT	B	0	0.000	2	1	1	0.369	$1.363 \times 10^{-1}$
IDT	B	2	0.477	2	1	2		$1.161 \times 10^{-2}$
IDT	B	1	0.301	2	1	3		$4.658 \times 10^{-3}$
IDT	B	4	0.699	2	1	4		$1.087 \times 10^{-1}$
IDT	W	2	0.477	2	2	1	0.314	$2.665 \times 10^{-2}$
IDT	W	1	0.301	2	2	2		$1.626 \times 10^{-4}$
IDT	W	2	0.477	2	2	3		$2.665 \times 10^{-2}$
IDT	W	0	0.000	2	2	4		$9.844 \times 10^{-2}$

Bait	Color	<i>T. dubius</i>	$Y_{ijk} = \log_{10}(T.dubius + 1)$	<i>i</i>	<i>j</i>	<i>k</i>	$\bar{Y}_{ij.}$	$(Y_{ijk} - \bar{Y}_{ij.})^2$
IST	B	2	0.477	3	1	1	0.725	$6.126 \times 10^{-2}$
IST	B	2	0.477	3	1	2		$6.126 \times 10^{-2}$
IST	B	10	1.041	3	1	3		$1.002 \times 10^{-1}$
IST	B	7	0.903	3	1	4		$3.186 \times 10^{-2}$
IST	W	1	0.301	3	2	1	0.719	$1.745 \times 10^{-1}$
IST	W	4	0.699	3	2	2		$3.901 \times 10^{-4}$
IST	W	14	1.176	3	2	3		$2.091 \times 10^{-1}$
IST	W	4	0.699	3	2	4		$3.901 \times 10^{-4}$

Table 14.2: Example 2 - Effect of nutrient and water availability on the total biomass of grassland plants grown in microcosms (Maestre &amp; Reynolds 2007).

N (mg)	Water (ml/week)	$Y_{ijk} = \text{Biomass}$	$i$	$j$	$k$
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)	$Y_{ijk} = \text{Biomass}$	$i$	$j$	$k$
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.

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SAS Program

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```
* 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

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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.

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## 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  $k$ th observation ( $k = 1, 2, \dots, n$ ) in the  $i$ th Factor A treatment and  $j$ th 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

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}. \quad (14.1)$$

Here  $\mu$  is the grand mean of the observations, while  $\alpha_i$  is the deviation from  $\mu$  caused by the  $i$ th treatment in Factor A, while  $\beta_j$  is the deviation caused by the  $j$ th 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  $\epsilon_{ijk}$  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  $i$ th level of Factor A and  $j$ th 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  $i$ th and  $j$ th 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  $\mu$  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  $\mu$ , 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  $H_0 : \text{all } \alpha_i = 0$ , while for Factor B we would test  $H_0 : \text{all } \beta_j = 0$ . For interaction between the two factors, we would test  $H_0 : \text{all } (\alpha\beta)_{ij} = 0$ . The corresponding alternative hypotheses are  $H_1 : \text{some } \alpha_i \neq 0$ ,  $H_1 : \text{some } \beta_j \neq 0$ , and  $H_1 : \text{some } (\alpha\beta)_{ij} \neq 0$ . We will discuss how these null hypotheses are tested in the next section.

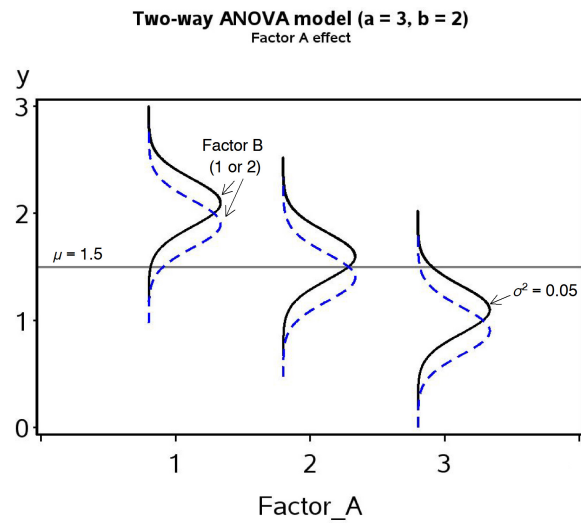


Figure 14.1: Fixed effects model for two-way ANOVA showing a Factor A effect.

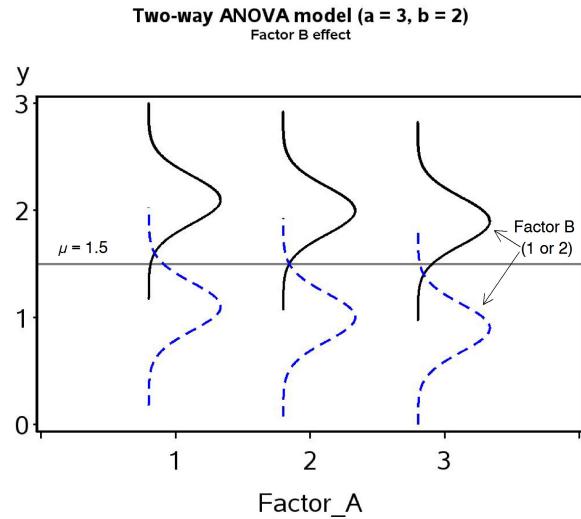


Figure 14.2: Fixed effects model for two-way ANOVA showing a Factor B effect.

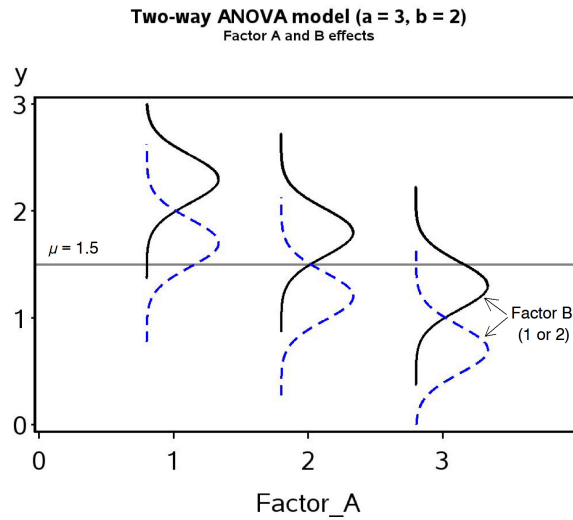


Figure 14.3: Fixed effects model for two-way ANOVA showing both Factor A and B effects.

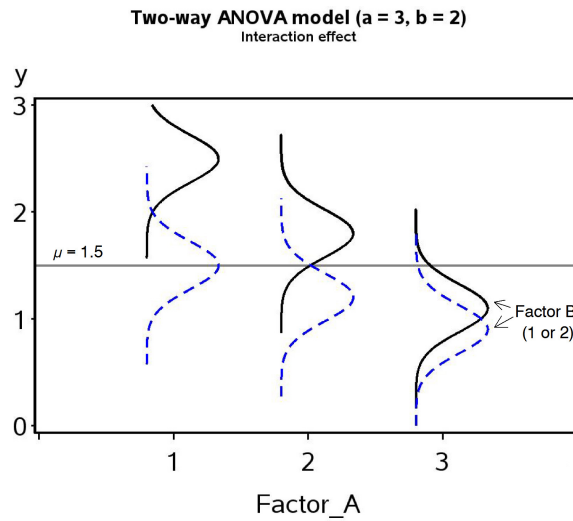


Figure 14.4: Fixed effects model for two-way ANOVA showing an  $A \times B$  interaction between the two factors.

## 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 ( $k = 1, 2, \dots, n$ ). 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

$$\bar{\bar{Y}} = \frac{\sum_{i=1}^a \sum_{j=1}^b \bar{Y}_{ij\cdot}}{ab}. \quad (14.2)$$

For the Example 1 data set, we have

$$\bar{\bar{Y}} = \frac{1.150 + 0.980 + 0.369 + 0.314 + 0.725 + 0.719}{6} = 0.709. \quad (14.3)$$

We next calculate a mean corresponding to each level of Factor A by averaging across the levels of Factor B, which we denote as  $\bar{\bar{Y}}_{i\cdot}$ . It can be calculated using the formula

$$\bar{\bar{Y}}_{i\cdot} = \frac{\sum_{j=1}^b \bar{Y}_{ij\cdot}}{b}. \quad (14.4)$$

For the Example 1 data set, we have

$$\bar{\bar{Y}}_{1\cdot} = \frac{1.150 + 0.980}{2} = 1.065, \quad (14.5)$$

$$\bar{Y}_{2..} = \frac{0.369 + 0.314}{2} = 0.342, \quad (14.6)$$

and

$$\bar{Y}_{3..} = \frac{0.725 + 0.719}{2} = 0.722. \quad (14.7)$$

The difference  $\bar{Y}_{i..} - \bar{\bar{Y}}$  is a measure of the shift generated by Factor A in the observations, as well as an estimate of  $\alpha_i$  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

$$SS_A = nb \sum_{i=1}^a (\bar{Y}_{i..} - \bar{\bar{Y}})^2. \quad (14.8)$$

$SS_A$  has  $a - 1$  degrees of freedom. We can calculate a mean square for Factor A using the formula

$$MS_A = \frac{SS_A}{a - 1}. \quad (14.9)$$

Note the factor  $nb$  in the expression for  $SS_A$ , which scales  $MS_A$  so that it estimates  $\sigma^2$  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

$$SS_A = 4(2) [(1.065 - 0.709)^2 + (0.342 - 0.709)^2 + (0.722 - 0.709)^2] \quad (14.10)$$

$$= 8 [1.265 \times 10^{-1} + 1.353 \times 10^{-1} + 1.501 \times 10^{-4}] = 2.096. \quad (14.11)$$

and

$$MS_A = \frac{2.096}{3 - 1} = 1.048. \quad (14.12)$$

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

$$\bar{\bar{Y}}_{.j} = \frac{\sum_{i=1}^a \bar{Y}_{ij.}}{a}. \quad (14.13)$$

For the Example 1 data set, we have

$$\bar{\bar{Y}}_{.1} = \frac{1.150 + 0.369 + 0.725}{3} = 0.748 \quad (14.14)$$



and

$$\bar{\bar{Y}}_{.2} = \frac{0.980 + 0.314 + 0.719}{3} = 0.671. \quad (14.15)$$

The difference  $\bar{\bar{Y}}_{.j} - \bar{\bar{Y}}$  is a measure of the shift generated by Factor B in the observations, as well as an estimate of  $\beta_j$  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

$$SS_B = na \sum_{j=1}^b (\bar{\bar{Y}}_{.j} - \bar{\bar{Y}})^2. \quad (14.16)$$

$SS_B$  has  $b - 1$  degrees of freedom. We can then calculate a mean square for Factor B using the formula

$$MS_B = \frac{SS_B}{b - 1}. \quad (14.17)$$

For the Example 1 data, we have

$$SS_B = 4(3) [(0.748 - 0.709)^2 + (0.671 - 0.709)^2] \quad (14.18)$$

$$= 12 [1.485 \times 10^{-3} + 1.485 \times 10^{-3}] = 3.565 \times 10^{-2} \quad (14.19)$$

and

$$MS_B = \frac{3.565 \times 10^{-2}}{2 - 1} = 3.565 \times 10^{-2}. \quad (14.20)$$

We can also calculate a sum of squares and mean square to test for the  $A \times B$  interaction. The sum of squares for interaction,  $SS_{AB}$ , is calculated in general using the formula

$$SS_{AB} = n \sum_{i=1}^a \sum_{j=1}^b (\bar{Y}_{ij} - \bar{\bar{Y}}_{i.} - \bar{\bar{Y}}_{.j} + \bar{\bar{Y}})^2. \quad (14.21)$$

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

$$MS_{AB} = \frac{SS_{AB}}{(a - 1)(b - 1)}. \quad (14.22)$$

For the Example 1 data, we have

$$SS_{AB} = 4[(1.150 - 1.065 - 0.748 + 0.709)^2] \quad (14.23)$$

$$+ (0.980 - 1.065 - 0.671 + 0.709)^2 \quad (14.24)$$

$$+ (0.369 - 0.342 - 0.748 + 0.709)^2 \quad (14.25)$$

$$+ (0.314 - 0.342 - 0.671 + 0.709)^2 \quad (14.26)$$

$$+ (0.725 - 0.722 - 0.748 + 0.709)^2 \quad (14.27)$$

$$+ (0.719 - 0.722 - 0.671 + 0.709)^2 \quad (14.28)$$

$$= 5[2.111 \times 10^{-3} + \cdots + 2.836 \times 10^{-2}] = 2.836 \times 10^{-2}. \quad (14.29)$$

and

$$MS_{AB} = \frac{2.836 \times 10^{-2}}{(3-1)(2-1)} = 1.418 \times 10^{-2}. \quad (14.30)$$

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

$$SS_{within} = \sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n (Y_{ijk} - \bar{Y}_{ij\cdot})^2 \quad (14.31)$$

which has  $ab(n-1)$  degrees of freedom. The associated mean square is calculated as

$$MS_{within} = \frac{SS_{within}}{ab(n-1)}. \quad (14.32)$$

The last column of Table 14.1 shows the preliminary calculations for  $SS_{within}$ . Adding this column across all the treatment groups yields

$$SS_{within} = 1.644 \times 10^{-2} + \cdots + 3.901 \times 10^{-4} = 1.361 \quad (14.33)$$

and

$$MS_{within} = \frac{1.361}{(3)(2)(4-1)} = 7.561 \times 10^{-2}. \quad (14.34)$$

There is one more sum of squares that is often calculated in two-way ANOVA, the total sum of squares. It is defined as

$$SS_{total} = \sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n (Y_{ijk} - \bar{\bar{Y}})^2. \quad (14.35)$$

It measures the variability of the observations around the grand mean of the data ( $\bar{\bar{Y}}$ ) 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

$$SS_A + SS_B + SS_{AB} + SS_{within} = SS_{total} \quad (14.36)$$

and

$$(a - 1) + (b - 1) + (a - 1)(b - 1) + ab(n - 1) = abn - 1. \quad (14.37)$$

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

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}. \quad (14.38)$$

where  $\mu$  is the grand mean,  $\alpha_i$  is the effect of the  $i$ th level of Factor A,  $\beta_j$  is the effect of the  $j$ th 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

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \epsilon_{ijk}. \quad (14.39)$$

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

$$\lambda = \frac{L_{H_0}}{L_{H_1}}. \quad (14.40)$$

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  $\times$  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	$3.565 \times 10^{-2}$	$3.565 \times 10^{-2}$	0.47	$> 0.100$
Bait $\times$ Color	2	$2.836 \times 10^{-2}$	$1.418 \times 10^{-2}$	0.19	$> 0.100$
Within	18	1.361	$7.561 \times 10^{-2}$		
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  $y = \log_{10}(Tdubius+1)$ . 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

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk} \quad (14.41)$$

The  $\alpha_i$ ,  $\beta_j$ , 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 × 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

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for 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.

	y	LSMEAN	bait	LSMEAN 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

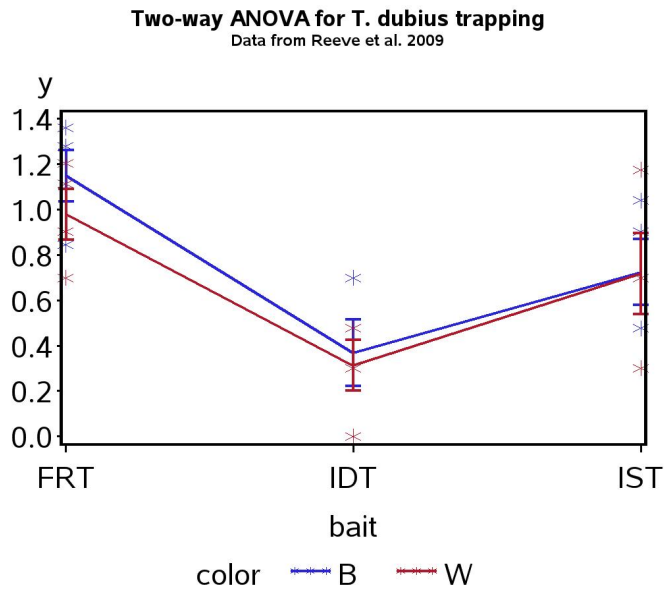


Figure 14.5: Means  $\pm$  standard errors and individual data points for the Example 1 experiment, where  $Y = \log_{10}(T.dubius + 1)$ .

### 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.

```
* 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=stdimjt 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

Source	DF	Sum of 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 0.975756  
 Coeff Var 3.499961  
 Root MSE 0.030603  
 y Mean 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

Two-way ANOVA for total biomass  
Data from Maestre and Reynolds 2007

7

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 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

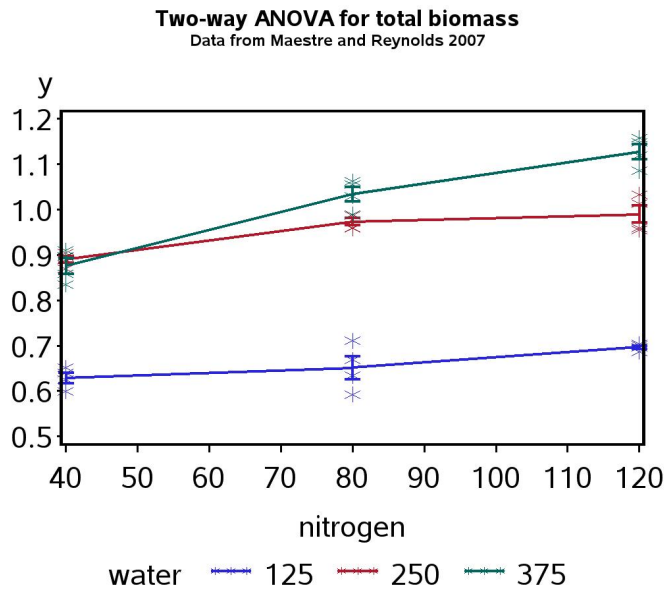


Figure 14.6: Means  $\pm$  standard errors and individual data points for the Example 2 experiment, where  $Y = \log_{10}(\text{Biomass})$ .

### 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

Source	DF	Sum of 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, possibly 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	<i>T. dubius</i>
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  
Data from Reeve et al. (2009)

3

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

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}. \quad (14.42)$$

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  $H_0 : \text{all } \alpha_i = 0$ , while for Factor B we would test  $H_0 : \text{all } \beta_j = 0$ . 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  $\bar{Y}_{i.}$ . It can be calculated using the formula

$$\bar{Y}_{i.} = \frac{\sum_{j=1}^b Y_{ij}}{b}. \quad (14.43)$$

For example, we have

$$\bar{Y}_{1.} = \frac{0.379 + 0.325 + 0.615}{3} = 0.440 \quad (14.44)$$

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

$$\bar{Y}_{.j} = \frac{\sum_{i=1}^a Y_{ij}}{a}. \quad (14.45)$$

For the first humidity treatment in Example 3, we have

$$\bar{Y}_{.1} = \frac{0.379 + 0.439 + 0.358 + 0.466 + 0.970 + 1.571}{6} = 0.697. \quad (14.46)$$

The means for the other humidity treatments are  $\bar{Y}_{.2} = 0.731$  and  $\bar{Y}_{.3} = 0.719$ . A grand mean  $\bar{\bar{Y}}$  can then be calculated by averaging across the values of  $\bar{Y}_{i.}$  or equivalently by summing all the observations and dividing by their total number. It can be generally calculated using the formula

$$\bar{\bar{Y}} = \frac{\sum_{i=1}^a \bar{Y}_{i.}}{a}. \quad (14.47)$$

For the Example 3 data set, we have

$$\bar{\bar{Y}} = \frac{0.440 + 0.502 + 0.454 + 0.521 + 0.806 + 1.571}{6} = 0.716. \quad (14.48)$$

We next develop sums of squares and means squares for this design. The difference  $\bar{Y}_i - \bar{Y}$  is a measure of the shift generated by Factor A in the observations, and also estimates  $\alpha_i$ . Squaring and summing them across all the levels of Factor A, we obtain  $SS_A$ . It is calculated using the general formula

$$SS_A = b \sum_{i=1}^a (\bar{Y}_i - \bar{Y})^2. \quad (14.49)$$

$SS_A$  has  $a - 1$  degrees of freedom. Its mean square is calculated using the formula

$$MS_A = \frac{SS_A}{a - 1}. \quad (14.50)$$

Note the factor  $b$  in the expression for  $SS_A$ , which as usual scales  $MS_A$  so that it estimates  $\sigma^2$  under  $H_0$ . For the Example 3 data, we have

$$SS_A = 3 [(0.440 - 0.716)^2 + (0.502 - 0.716)^2 + \cdots + (1.571 - 0.716)^2] \quad (14.51)$$

$$= 3 [0.076176 + 0.045796 + 0.068644 + 0.038025 + 0.008100 + 0.731025] \quad (14.52)$$

$$= 2.903298 \quad (14.53)$$

$$(14.54)$$

and

$$MS_A = \frac{2.903298}{6 - 1} = 0.580660. \quad (14.55)$$

We similarly define  $SS_B$  using the general formula

$$SS_B = a \sum_{j=1}^b (\bar{Y}_j - \bar{Y})^2. \quad (14.56)$$

$SS_B$  has  $b - 1$  degrees of freedom. We can then calculate a mean square for Factor B using the formula

$$MS_B = \frac{SS_B}{b - 1}. \quad (14.57)$$

For the Example 3 data, we have

$$SS_B = 6 [(0.697 - 0.716)^2 + (0.731 - 0.716)^2 + (0.719 - 0.716)^2] \quad (14.58)$$

$$= 6 [0.000361 + 0.000225 + 0.000009] \quad (14.59)$$

$$= 0.003570. \quad (14.60)$$

and

$$MS_B = \frac{0.003570}{3 - 1} = 0.001785. \quad (14.61)$$

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

$$SS_{within} = \sum_{i=1}^a \sum_{j=1}^b (Y_{ij} - \bar{Y}_{i\cdot} - \bar{Y}_{\cdot j} + \bar{\bar{Y}})^2. \quad (14.62)$$

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

$$MS_{within} = \frac{SS_{within}}{(a - 1)(b - 1)}. \quad (14.63)$$

The last column of Table 14.6 shows the preliminary calculations for  $SS_{within}$ . Adding this column across all the treatment groups yields

$$SS_{within} = 0.131334 \quad (14.64)$$

and

$$MS_{within} = \frac{0.131334}{(6 - 1)(3 - 1)} = 0.013133. \quad (14.65)$$

The total sum of squares is given by the formula

$$SS_{total} = \sum_{i=1}^a \sum_{j=1}^b (Y_{ij} - \bar{\bar{Y}})^2 \quad (14.66)$$

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  $H_0 : \text{all } \alpha_i = 0$  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  $H_0 : \text{all } \beta_j = 0$  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 ( $\bar{Y}_i$ ) and preliminary calculations to find  $SS_{within}$

Temp. (°C)	Humidity (%)	Mortality	$Y_{ij} = \sin^{-1}(\sqrt{\text{Mortality}})$	$i$	$j$	$\bar{Y}_i$	$(Y_{ij} - \bar{Y}_i - \bar{Y}_j + \bar{\bar{Y}})^2$
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 non-significant ( $F_{2,10} = 0.13, P = 0.8777$ ). 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

14.5. TWO-WAY ANOVA WITHOUT REPLICATION

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

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for 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.

	y LSMEAN	temp	LSMEAN Number
A	1.57079633	37.5	6
B	0.80600259	35	5
B			
C	0.52129702	30	4
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.

	y LSMEAN	rh	LSMEAN 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.

	y LSMEAN	rh	LSMEAN Number
A			
A	0.69723464	55	1

---

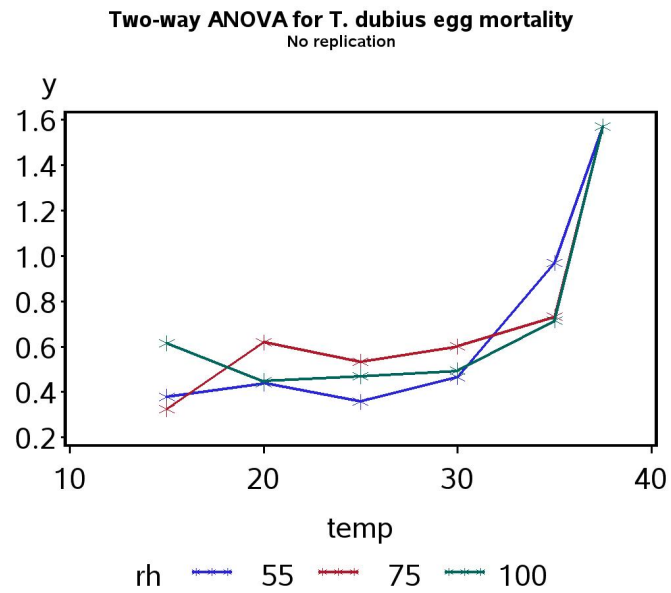


Figure 14.7: Mortality of *T. dubius* eggs at different temperatures and humidities (Table 14.6). Mortality rates were arcsine-square root transformed.

## 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.

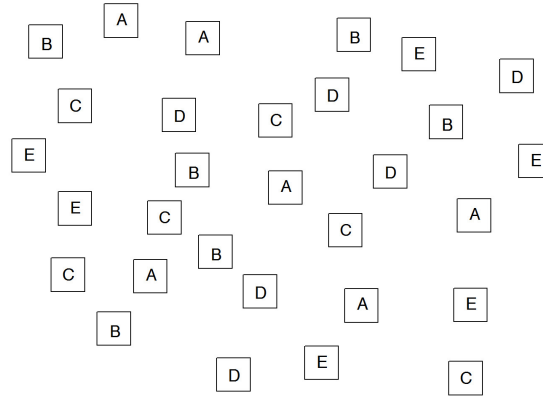


Figure 14.8: Completely randomized design with five treatments (A, B, C, D, and E) and six replicates per treatment.

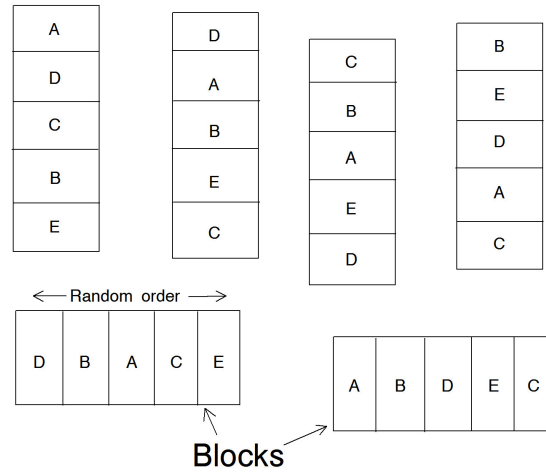


Figure 14.9: Randomized block design with five treatments (A, B, C, D, and E) and six blocks.

### 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

$$Y_{ij} = \mu + \alpha_i + B_j + \epsilon_{ij}. \quad (14.67)$$

Here  $\mu$ ,  $\alpha_i$ , and  $\epsilon_{ij}$  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 ( $\sigma_B^2$ ) and the variance of  $\epsilon_{ij}$  ( $\sigma^2$ ).

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

$$Y_{ij} = \mu + \alpha_i + B_j + (\alpha B)_{ij} + \epsilon_{ij}. \quad (14.68)$$

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 ( $\sigma_{AB}^2$ ), the block variance ( $\sigma_B^2$ ), and the variance of  $\epsilon_{ij}$  ( $\sigma^2$ ).

### 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  $H_0 : \text{all } \alpha_i = 0$ . 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`),  $\alpha$ -pinene (`AP`), frontalin +  $\alpha$ -pinene (`FRAP`), ipsdienol +  $\alpha$ -pinene (`IDAP`), and ipsenol +  $\alpha$ -pinene (`ISAP`). As mentioned earlier, frontalin, ipsdienol, and ipsenol are bark beetle pheromones while  $\alpha$ -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

$$Y_{ij} = \mu + \alpha_i + B_j + \epsilon_{ij}. \quad (14.69)$$

Here, the SAS variable `treat` corresponds to  $\alpha_i$ , 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 `ddf=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 ( $\sigma_B^2$ ) and the variance of  $\epsilon_{ij}$  ( $\sigma^2$ ). 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.

Randomized block anova for trapping experiment data      3  
 09:28 Tuesday, October 26, 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

## 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

---

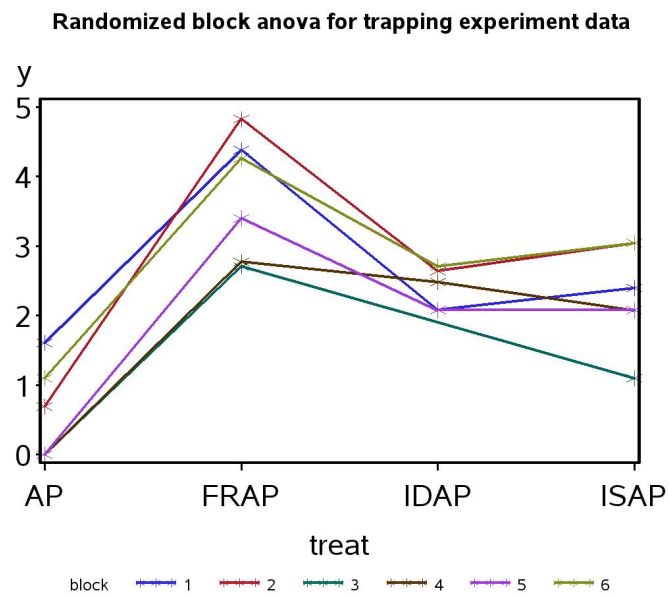


Figure 14.10: Log-transformed trap catches of *T. dubius* for four different bait treatments. Different line colors denote different blocks.



### 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  $\epsilon_{ij}$ . 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

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \quad (14.70)$$

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:

$$Y_{ij} = \mu + \alpha_i + B_j + \epsilon_{ij} \quad (14.71)$$

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

$$-2 \ln(\lambda) = 2 \ln(L_{H_1}) - 2 \ln(L_{H_0}). \quad (14.72)$$

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

$$-2 \ln(\lambda) = -39.0 - (-47.4) = -39.0 + 47.4 = 8.4 \quad (14.73)$$

How do we obtain a  $P$  value for this test statistic? For any likelihood ratio test, the quantity  $-2 \ln(\lambda)$  has approximately a  $\chi^2$  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  $\sigma_B^2$  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  
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

Obs	m2ln LH1	m2ln 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.