26. Two-way ANOVA

The one-way ANOVA tested the null hypothesis that all group means are the same. But we might also have a dataset in which there is more than 1 type of group. For example, suppose we know that the fig wasps used in Chapters 23-25 actually came from 2 different trees (Tree A and Tree B). This would mean that there could be a confounding variable affecting wing length, violating assumption 2 from Chapter 23.3. If, for whatever reason, fig wasps on different trees have different wing lengths, then we should include tree as an explanatory variable in the ANOVA. Because we would then have 2 group types (species and tree), we would need a two-way ANOVA[1]. Here, to illustrate the key concepts as clearly as possible, we will run an example of a two-way ANOVA with just 2 of the 5 species (Het1 and SO2). Table 26.1 shows the fig wasp wing length dataset for the 2 species in a tidy format, which includes columns for 2 group types, species and tree.

Table 26.1.: Wing lengths (mm) measured for 2 unnamed species of non-pollinating fig wasps collected from 2 fig trees in 2010 near La Paz in Baja, Mexico.

Species	Tree	wing_length
Het1	A	2.122
Het1	A	1.938
Het1	B	1.765
Het1	B	1.700
SO2	A	1.635
SO2	A	1.700
SO2	B	1.407
SO2	B	1.378

We could run a one-way ANOVA (or t-test) to see if wing lengths differ between species, then run another one-way ANOVA to see if wing lengths differ between trees. But by including both group types in the same model (species and tree), we can test how one group affects wing length in the context of the other group (e.g., how tree affects wing length, while also accounting for any effects of species on wing length). We can also see if there is any synergistic effect between groups, which is called an **interaction effect**. For example, if Het1 fig wasps had longer wing lengths than SO1 on Tree A, but shorter

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[1]Yes, it is possible to have a three-way or a four-way ANOVA, but having so many group types can get very messy very quickly, especially if we want to look at interactions between group types.

wing lengths than SO1 on Tree B, then we would say that there is an interaction between species and tree. Given this kind of interaction effect, it would not make sense to say that Het1 fig wasps have longer or shorter wings than SO1 because this would depend on the tree!

This chapter will not delve into the mathematics of the two-way ANOVA[2]. Working out a two-way ANOVA by hand requires similar, albeit more laborious, calculations of sum of squares and degrees of freedom than is needed for the one-way ANOVA in Chapter 23.2. But the general concept is the same. The idea is to calculate the amount of the total variation attributable to different groups, or the interaction among group types. Note that the assumptions for the two-way ANOVA are the same as for the one-way ANOVA.

Unlikely previous statistical tests in this book, we can actually test three separate null hypotheses with a two-way ANOVA. The first test is the same as the one-way ANOVA in Chapter 23, which focuses on mean species wing lengths:

- H_0 : Both mean species wing lengths are the same
- H_A : Mean species wing lengths are not the same

The second test focuses on the other group type, Tree:

- H_0 : Wing lengths are the same in both trees
- H_A : Wing lengths are not the same in both trees

These two hypotheses address the **main effects** of the independent variables (species and tree) on the dependent variable (wing length). In other words, the mean effect of one group type (either species or tree) by itself, holding the other constant. The third hypothesis addresses the interaction effect:

- H_0 : There is no interaction between species and tree
- H_A : There is an interaction between species and tree

Interaction effects are difficult to understand at first, so we will look at two concrete examples of two-way ANOVAs. The first will use the Table 26.1 data in which no interaction effect exists. The second will use a different species pairing in which an interaction does exist.

In a two-way ANOVA, the output (Figure 26.1) appears similar to that of a one-way ANOVA (see Chapter 23.2.3), but there are 4 rows of output. Rows 1 and 2 test the null hypotheses associated with the main effects of species and tree, respectively. Row 3 tests the interaction effect. And row 4 shows us the variation in the data that cannot be attributed to either the main effects or the interaction (i.e., the residual variation). This is equivalent to the variation within groups in the one-way ANOVA.

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[2]For those interested, chapter 11 of Sokal and Rohlf (1995) works all of this out step by step.

ANOVA - wing_length

	Sum of Squares	df	Mean Square	F	p
Species	0.24675	1	0.24675	45.75115	0.002
Tree	0.16388	1	0.16388	30.38508	0.005
Species * Tree	0.00025	1	0.00025	0.04693	0.839
Residuals	0.02157	4	0.00539		

Figure 26.1.: Jamovi output table for a two-way ANOVA, which tests for the effects of species (Het1 and SO2), tree, and their interaction on fig wasp wing lengths. Species wing length measurements were collected in 2010 near La Paz in Baja, Mexico.

This is a lot more information than the one-way ANOVA, but it helps to think of each row separately as a test of a different null hypothesis. From the first row, given the high F = 45.75 and the low P = 0.002, we can reject the null hypothesis that species mean wing lengths are the same. Similarly, from the second row, we can reject the null hypothesis that wing lengths are the same in both trees (F = 30.39; P = 0.005). In contrast, from the third row, we should not reject the null hypothesis that there is no interaction between species and trees (F = 0.05, P = 0.839). Figure 26.2 shows the these results visually (The Jamovi Project, 2022).

In Figure 26.2, there is no interaction between species and tree. How can we infer this from Figure 26.2? Wing length is always significantly higher in Tree A than it is in Tree B, regardless of the species of wasp. Similarly, wing length is always higher for Het1 than it is for SO2, regardless of tree. Consequently, while wasp species is important for predicting wing length, as is the tree from which the wasp was collected, we do not need to consider one variable to know the effect of the other. This is reflected in the orange and blue lines having a similar slope, or, more technically, slopes that are not significantly different from one another. If the slopes of the two lines were significantly different, then this would be evidence for an interaction between species and tree.

What would Figure 26.2 look like if there was an interaction effect between species and tree? We can run another two-way ANOVA, this time with a different pair of species, SO1 and SO2. Table 26.2 shows the data.

Table 26.2.: Wing lengths (mm) measured for 2 unnamed species of non-pollinating fig wasps collected from 2 fig trees in 2010 near La Paz in Baja, Mexico.

Species	Tree	wing_length
SO1	A	1.557
SO1	A	1.493

Species	Tree	wing_length
SO1	B	1.470
SO1	B	1.541
SO2	A	1.635
SO2	A	1.700
SO2	B	1.407
SO2	B	1.378

If we run the two-way ANOVA in Jamovi, then we can see the ANOVA output table (Figure 26.3).

Figure 26.3 shows that the effect of species is not significant (P > 0.05), but the effect of tree is significant (P < 0.05). Consequently, in terms of the main effects, it appears that species does not affect wing length, but tree does affect wing length. Unlike the example test between Het1 and SO2, the interaction between species and tree is significant in Figure 26.3. This means that we cannot interpret the main effects in isolation because the effect of each on wing length will change in the presence of the other. In other words, the effect of tree on wing length will depend on the species. Figure 26.4 shows this interaction visually.

Note that for SO1, wing length is basically the same for Tree A and B (the 2 left points in Figure 26.4). In contrast, for SO2, tree has a very noticeable effect on wing length! Fig wasps in species SO2 clearly have higher wing lengths on Tree A than they do on Tree B. *Overall*, however, the mean wing length for SO2 appears to be about the same as it is for SO1 (i.e., the mid-way point between the blue and orange points for SO2 is at about the same wing length as it is for SO1, around 1.5 mm). Species therefore does not really have an effect on wing length, at least not by itself. But species *does* have a clear effect if you also take tree into account. In other words, to predict how tree will affect wing length (not at all for SO1, or a lot for SO2), it is necessary to know what species of fig wasp is being considered. This is how interactions work in ANOVA, and more generally in statistics.

There is one last point that is relevant to make for the two-way ANOVA. Recall that we tested 3 null hypotheses simultaneously. Should we not then apply some kind of correction for multiple comparisons, as we did in Chapter 24? This is actually not necessary. The reason is subtle. With the multiple t-tests, we wanted to know if *any* of the pair-wise differences between groups were significant. Each t-test was not really a separate question in this case. We just tried all possible combinations in search of a pair of species with significantly different wing lengths. With the two-way ANOVA, we are asking 3 separate questions, and accepting a Type I error rate of 0.05 for each of them, individually.
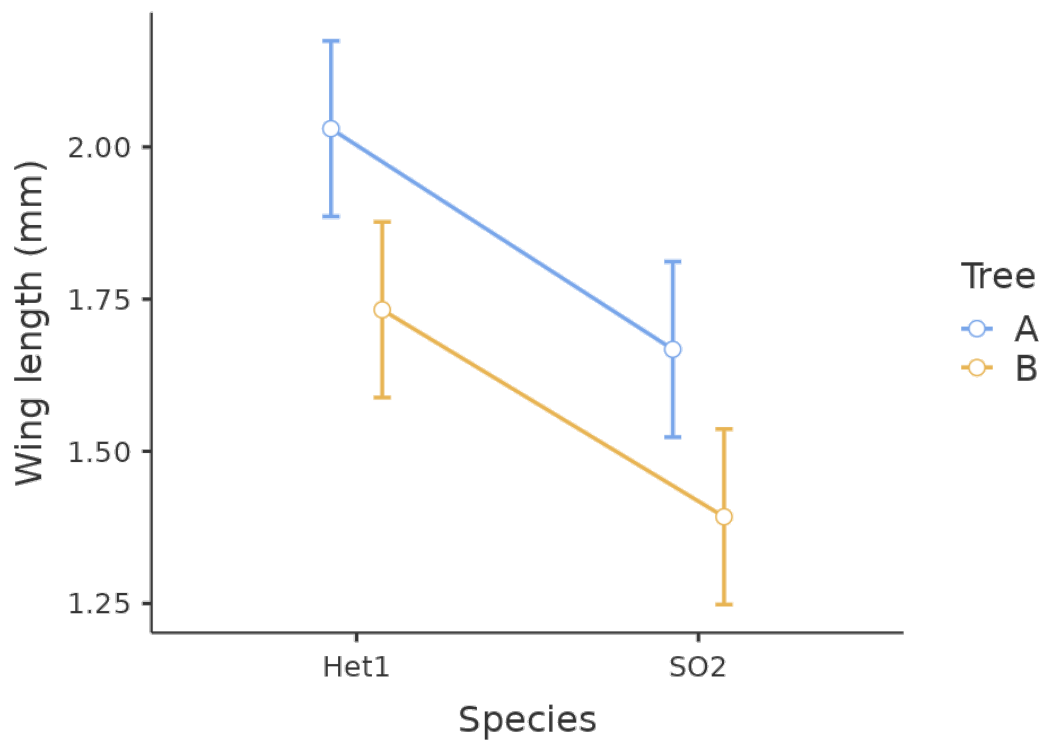
Figure 26.2.: An interaction plot for a two-way ANOVA of fig wasp wing measurements as affected by species and tree. Points show mean values for the 4 species and tree combinations (e.g., Het1 from Tree A in the upper left). Error bars show standard errors around the means. Species wing length measurements were collected in 2010 near La Paz in Baja, Mexico.

ANOVA - Wing length (mm)

	Sum of Squares	df	Mean Square	F	p
Species	0.00044	1	0.00044	0.24509	0.647
Tree	0.04337	1	0.04337	24.42590	0.008
Species ∗ Tree	0.03264	1	0.03264	18.38492	0.013
Residuals	0.00710	4	0.00178		

Figure 26.3.: Jamovi output table for a two-way ANOVA, which tests for the effects of species (SO1 and SO2), tree, and their interaction on fig wasp wing lengths. Species wing length measurements were collected in 2010 near La Paz in Baja, Mexico.
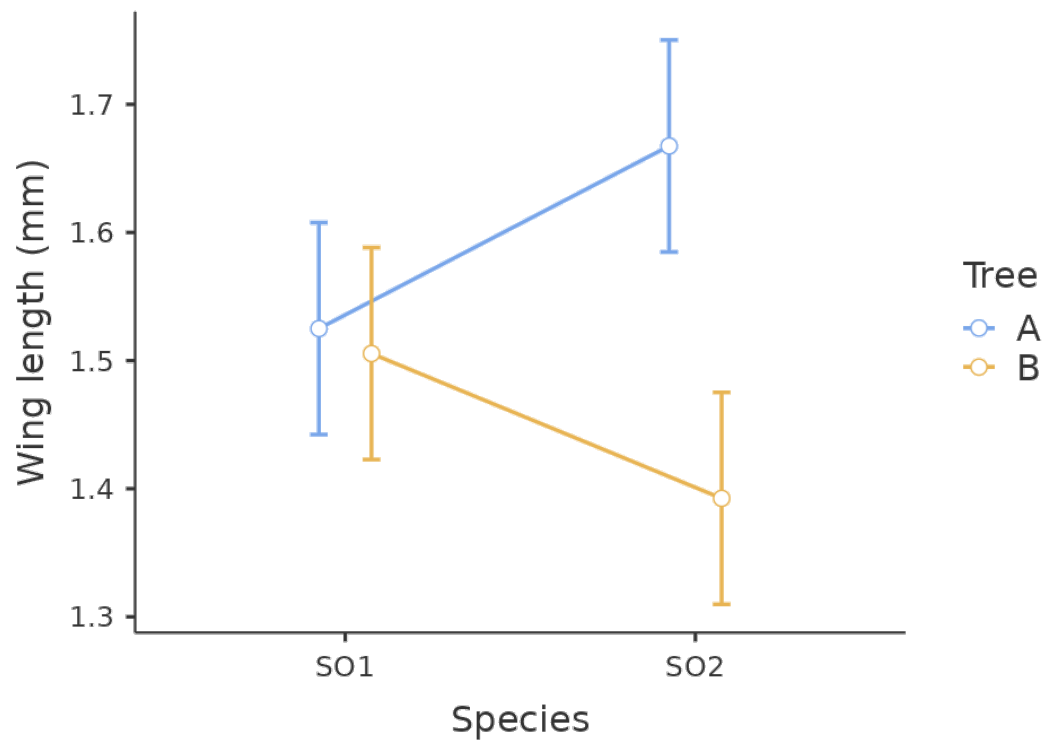
Figure 26.4.: An interaction plot for a two-way ANOVA of fig wasp wing measurements as affected by species and tree. Points show mean values for the 4 species and tree combinations (e.g., SO2 from Tree A in the upper left). Error bars show standard errors around the means. Species wing length measurements were collected in 2010 near La Paz in Baja, Mexico.