

24. Multiple comparisons

In the [Chapter 23.2](#) ANOVA example, we rejected the null hypothesis that all fig wasp species have the same mean wing length. We can therefore conclude that at least one species has a different mean wing length than the rest; can we determine which one(s)? We can try to find this out using a post hoc comparison (*post hoc* is Latin for ‘after the event’). That is, after we have reject the null hypothesis in the one-way ANOVA, we can start comparing individual groups (Het1 vs Het2, Het1 vs LO1, etc.). Nevertheless, we need some way to correct for the Type I error problem explained at the beginning of [Chapter 23](#). That is, if we run a large enough number of t-tests, then we are almost guaranteed that we will find a significant difference between means ($P < 0.05$) where none really exists. A way to avoid this inflated Type I error rate is to set our significance threshold to be lower than the usual $\alpha = 0.05$. We can, for example, divide our α value by the total number of pair-wise t-tests tests that we run. This is called a Bonferonni correction ([Dytham, 2011](#)), and it is an especially cautious approach to post hoc comparisons between groups ([Narum, 2006](#)). For the fig wasp wing lengths, recall that there are 10 possible pairwise comparisons between the 5 species. This means that if we were to apply a Bonferonni correction and run 10 separate t-tests, then we would only conclude that species mean wing lengths were different when $P < 0.005$ instead of $P < 0.05$.

Another approach to correcting for multiple comparisons is a Tukey’s honestly significant difference test (Tukey’s HSD, or just a ‘Tukey’s test’). The general idea of a Tukey’s test is the same as the Bonferonni. Multiple t-tests are run in a way that controls the Type I error rate so that the probability of making a Type I error across the whole set of comparisons is fixed (e.g., at $\alpha = 0.05$). The Tukey’s test does this by using a modified t-test, with a t-distribution called the “studentised range distribution” that applies the range of mean group values (i.e., $\max(\bar{x}) - \min(\bar{x})$) and uses the sample variance across the groups with the highest and lowest sample means ([Tukey, 1949](#); [Box et al., 1978](#)).

Multiple comparisons tests can be run automatically in statistical programs such as Jamovi and R. Figure 24.1 shows a post-hoc comparisons table for all pair-wise combinations of fig wasp species wing length means.

The column ‘p’ in Figure 24.1 is the uncorrected p-value, i.e., the p-value that a t-test would produce without any correction for multiple comparisons. The columns p_{tukey} and $p_{bonferonni}$ show corrected p-values for the Tukey’s test and Bonferroni corrected t-test, respectively. We can interpret these p-values as usual, concluding that two species have different means if $P < 0.05$ (i.e., Jamovi does the correction for us; we do not need

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Comparison		Mean Difference	SE	df	t	p	Ptukey	Pbonferroni
Species	Species							
Het1	- Het2	0.17350	0.11862	15.00000	1.46270	0.164	0.600	1.000
	- LO1	0.17000	0.11862	15.00000	1.43319	0.172	0.617	1.000
	- SO1	0.36600	0.11862	15.00000	3.08557	0.008	0.050	0.075
	- SO2	0.35125	0.11862	15.00000	2.96122	0.010	0.063	0.097
Het2	- LO1	-0.00350	0.11862	15.00000	-0.02951	0.977	1.000	1.000
	- SO1	0.19250	0.11862	15.00000	1.62288	0.125	0.506	1.000
	- SO2	0.17775	0.11862	15.00000	1.49853	0.155	0.579	1.000
LO1	- SO1	0.19600	0.11862	15.00000	1.65238	0.119	0.489	1.000
	- SO2	0.18125	0.11862	15.00000	1.52803	0.147	0.561	1.000
SO1	- SO2	-0.01475	0.11862	15.00000	-0.12435	0.903	1.000	1.000

Note. Comparisons are based on estimated marginal means

Figure 24.1.: Jamovi output showing a table of 10 post hoc comparisons between species mean wing lengths for 5 different species of fig wasps. The last 3 columns show the uncorrected p-value (p), a p-value obtained from a Tukey's test, and a p-value obtained from a Bonferroni correction. Species wing length measurements were collected in 2010 near La Paz in Baja, Mexico.

to divide $\alpha = 0.05$ to figure out what the significance threshold should be given the Bonferonni correction).

Note that from Figure 24.1, it appears that both the Tukey's test and the Bonferonni correction fail to find that any pair of species have significantly different means. This does not mean that we have done the test incorrectly. The multiple comparisons tests are asking a slightly different question than the one-way ANOVA. The multiple comparisons tests are testing the null hypothesis that two *particular* species have the same mean wing lengths. The one-way ANOVA tested the null hypothesis that all species have the same mean, and our result for the ANOVA was barely below the $\alpha = 0.05$ threshold ($P = 0.042$). The ANOVA also has more statistical power because it makes use of all 20 measurements in the dataset, not just a subset of measurements between 2 of the 5 species. It is therefore not particularly surprising or concerning that we rejected H_0 for the ANOVA, but the multiple comparisons tests failed to find any significant difference between group means.