Exploration 2.1: Female zebra finches' responses to songs

Learning Goals:
- Use pairing to potentially reduce unexplained variation and increase the power of a study
- Explain how to analyze paired data appropriately

Background: Among songbirds, male birds use songs to attract female birds. How do the female birds respond to these songs? Does it matter whether the male is their mate or an unfamiliar male? Does it matter whether the song is directed towards the female, or an undirected song (that is, a song the male would sing when in isolation)? Researchers Woolley and Doupe (2008, "Social Context–Induced Song Variation Affects Female Behavior and Gene Expression," *PLoS Biology,* Volume 6, Issue 3) investigated some of these questions with female zebra finches. (*Image from National Audubon Society.)*

One experiment investigated how a mated female zebra finch (that is, one who already is partnered up with a male zebra finch) responds to songs directed at her – either from her own mate or an unfamiliar male zebra finch. In the experiment each female zebra finch was placed in a central chamber, between two other chambers, one on each end. A male zebra finch song could be played from a speaker next to either one of the end chambers, and the female could move into either chamber See Figure 1, as presented in the original research paper.

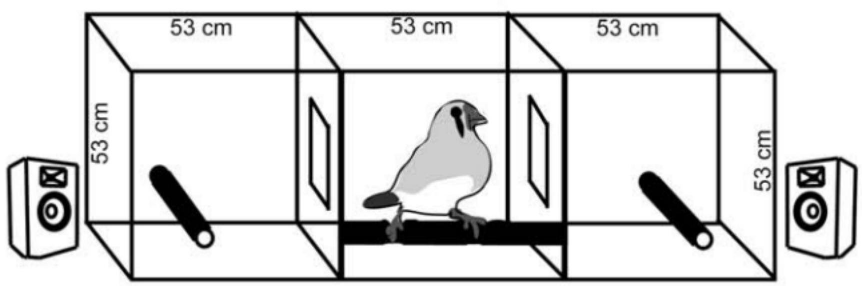

Figure 1: Setup of the experiment (from Woolley and Doupe, 2008)

For this experiment, either her mate's directed song or an unfamiliar male zebra finch's directed song would be played on one of the speakers. For their response variable, the researchers recorded what percentage of song-playing time the female zebra finch spent inside the chamber with the speaker playing the song rather than the central chamber or the chamber on the other end. So for example, if the song stimulus was played for 30 minutes in the leftmost chamber, and the female zebra finch spent 15 of those minutes in the the leftmost chamber, the response will be recorded as 50%.

Each female zebra finch was subjected to both conditions: listening to songs from her mate, as well as listening to songs from an unfamiliar male zebra finch. Let us assume that the order of mate and unfamiliar male was randomized for each female zebra finch.

1. Suppose that you were designing this study. Brainstorm a Sources of Variation diagram, including discussion of any "controls" you will use in the study to minimize unexplained variation.

Observed Variation in:	**Sources of explained variation**	**Sources of unexplained variation**
Inclusion criteria *Design*	•	•

Definition: A ***matched pairs design*** pairs experimental units that are similar to each other and randomly assigns the members within a pair each to one of the two treatment groups. A special type of matched pairs design is ***repeated measures*** where the same individual receives both treatments in a randomly assigned order.

2. Is the study design used by Woolley and Doupe a repeated measures type of matched pairs design? Explain how you are deciding. Also, explain why a repeated measures type of design might be a good choice in this context.

Key idea: By having the same experimental unit (e.g., bird) complete both treatments, the variability from experimental unit to experimental unit (e.g., bird to bird) is held constant and is no longer "unexplained variation."

3. Does random assignment still play an important role in a matched pairs design? Explain why it matters and what could happen if you didn't use random assignment.

4. Would a two-sample *t*-test (or a separate means model) be an appropriate analysis to compare the percentages for the two types of male songs for this matched pairs study design? Explain.

Key Idea: In a paired design, it is not reasonable to consider the multiple observations on the same individual as *independent*, so we cannot carry out a procedure (e.g., a two-sample *t*-test) that assumes we have two independent samples.

Key Idea: To analyze a paired design you can use, for example, a *one-sample t-test* (and confidence interval) on the *differences* (also known as a *paired t-test*) or a simulation that maintains the paired nature of the study design (e.g. randomizing the order of the two responses on the same individual). The validity condition of the one-sample *t*-test is that the distribution of the differences is reasonably symmetric or the sample size is large with no severe skewness or outliers (e.g., larger than 30).

The paired *t*-test approach only works with two treatments, so let's develop a more general method that uses the idea of partitioning the variability in the responses among the birds, the two treatments, and the unexplained variation.

Open the data file **zebrafinches** containing the results for 17 females. (Note: The data were not provided with the paper. The data here were recreated from the provided graph of the data.)

5. Use technology to find each of the following:

Overall mean % time spent in the "song chamber":

Overall standard deviation of the % time spent in the song chamber:

Sum of Squares Total:

Mean % time when listening to mate:

Mean %time when listening to unfamiliar male:

Effect of mate's song: Effect of unfamiliar male's song:

To represent the variability in the data that is due to who is singing the song (familiar mate or unfamiliar male), calculate *SSwho*, the sum of squares for the groups:

SSwho = $(\sum_{all\ groups}(group\ size) \times (effect)^2)$:

To account for the bird-to-bird variation, we want to similarly determine the *SSbird.*

6. Calculate the "effect" of bird one (bird mean – overall mean):

(99 + 5)/ 2 – 42.53 =

Was this bird generally above or below average in percentage of time spent in the song chamber?

7. Find the effect of each bird.

Note: You may want to use a spreadsheet program to answer this question.

8. Explain what it means for a female zebra finch to have a larger positive effect than another female zebra finch. Explain what it means for a female zebra finch to have a larger negative effect.

9. (Using a spreadsheet) In the next column, square each bird effect, multiply each by 2 (because the effect came from two responses) and then find the sum of all of the squared effects. This is the *sum of squares* for the "female zebra finch effect," *SSbird*.

10. Fill in the following ANOVA table, remembering that Mean Square = Sum of Squares / df

Source	DF	Sum Sq	Mean Sq
Bird			
Who			
Error			
Total			

11. What proportion of the total variation is explained by whether the male is the mate or unfamiliar? What proportion is explained by the female finch herself?

Notice that if we had ignored the variability explained by the birds, all of that variation would be "unexplained" and *SSbird* would be added to *SSError*.

12. What is the main advantage of removing the bird-to-bird variation from the unexplained variation?

In the table above, we call the combination of the *SSbird* and the *SSwho* the *SSModel*, where the model is accounting for both "bird" and "who is singing" effects, representing the total variation explained by the statistical model.

Definition: In general, ***SSModel*** refers to all of the variation explained by having the entire collection of variables in the model. Numerically, *SSModel = SSTotal – SSError.*

13. Now compute an *F* statistic for comparing the average song chamber times for the two types of males by dividing the Mean Square for who is singing by the Mean Square for Error. Is this bird-adjusted *F*-statistic large? Statistically significant? [Recall that *F*-statistics greater than 4 are usually considered strong evidence against the null (just like *t*-statistics greater than 2).]

Note: The prediction equation for this matched pairs design is of the form:

$$predicted\ song\ chamber\ time = overall\ mean + effect\ of\ bird + effect\ of\ male\ type$$

Because each female zebra finch has a different effect, the predicted song chamber times depend on both the female zebra finch and the type of male doing the singing.

14. Use the prediction equation above to find the predicted song chamber time (percentage) for the mate song condition and the predicted song chamber time (percentage) for the unfamiliar male condition for the bird you used in #6.

prediction for mate:

prediction for unfamiliar male:

15. Now, determine the residuals for the two predictions in the previous question. How do you interpret these residuals?

If we found every bird's residuals, squared them and summed them up, these would give us a direct computation of the *SSError* shown in the ANOVA table above.

Extension: What if we had more than two conditions?

16. Describe the primary modifications you would need to make to the ANOVA calculations if we had tested four different song sources rather than just two.

Summary

In this section we saw that pairing was one way to explain what, in some studies, can be a major source of unexplained variation – ***experimental unit-to-experimental unit variation***. So, why don't we always do paired studies? There are a number of reasons. Sometimes, there is no obvious way to pair the data, sometimes pairing doesn't help explain variation in the response, and sometimes pairing isn't possible. For example, a particular test or measurement might "use up" or destroy the experimental unit. That said, a reliable statistical mantra says that you should pair if you can because, even if it doesn't help (much), it usually doesn't hurt and you may not know how much it will help before the study starts.