Repeatability exercise for morphometric measurements

CURE created by: Paula A. Trillo, Gettysburg University; Elissa Sorojsrisom, Columbia University; Carly Jordan, The George Washington University; Janice Krumm, Widener University

INTRODUCTION

In this activity, you will learn how to measure repeatability for the morphological measures you will collect with ImageJ. Repeatability analyses allow scientists to measure how consistent something is, whether this is a specific behavior for a specific animal (preference for red color by female guppies), or a specific measurement by an individual scientist (the pigmented area of a specific butterfly's eyespot). Repeatability is usually a measure of how consistent the individual is, whether it is the female guppy or the individual researcher.

When scientists measure morphology, coloration patterns, or behavior, we need to be very careful to be consistent with our measurements. One way to do this is to assess our measurement error with a repeatability index or estimate. To calculate repeatability, we conduct the same measurement multiple times and perform an analysis on the results. In some cases, it is possible to use this information to train ourselves and decrease our measurement error or *increase* our repeatability estimates. We can calculate repeatability using a one-way analysis of variance (ANOVA), or we can estimate repeatability by measuring standard error. Both methods will be explained on the pages that follow.

After this activity, you will be able to:

- Explain the function of repeatability measures.
- Gather data in image J to determine repeatability for a given set of measurements.
- Analyze data to determine repeatability using one of several methods.

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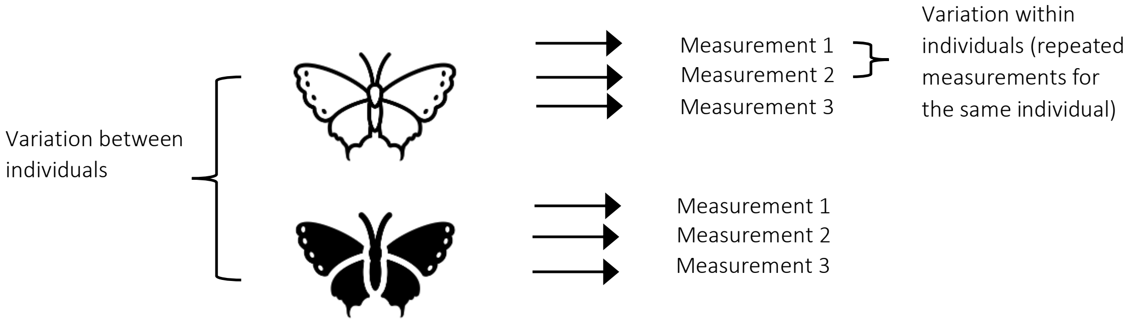
MEASURING REPEATABILITY USING ANOVA

Overview


Variation within individuals (repeated measurements for the same individual)
Measurement 1
Measurement 2
Measurement 3
Variation between individuals
Measurement 1
Measurement 2
Measurement 3

The repeatability coefficient or estimate **r** expresses the proportion of the total variation in a given trait (in this case wing spot area) that is due to differences between individuals instead of differences in repeated measurements for the same individual butterfly. If the measurements are highly consistent for a trait measurement, then the "within individual" repeated measurement variation should be low. This will make repeatability (the ratio of the variance between individuals to the total variance) be close to 1. Thus, repeatability (r) ranges from zero to 1.

Repeatability (r) = variance in measurements between butterflies/ [variance in measurements within individual butterflies (repeated measurements) + variance in measurements between butterflies]

Values of **r** can be interpreted as follows: 0-0.2 indicates low repeatability: 0.3-0.4 indicates fair repeatability; 0.5-0.6 indicates moderate repeatability; 0.7-0.8 indicates high repeatability; and > 0.8 indicates very high repeatability.

To determine your repeatability for wing pattern measurements, you will need to randomly identify **four** of your assigned images, chose the wing and perform the same measurements (wing length, wing width, anterior patch and wing apex) on each one of them **four** different times.

Repeatability measures variation that occurs when conditions are constant, and the measures are collected by the same person within a short amount of time. Therefore, all measures used for this exercise will need to be **done within a short period of time**.

Calculating repeatability

To calculate repeatability (r), you will need to first calculate Mean Square Between ($MS_{between}$) and the Mean Square Within (also called Mean Square Error or Mean Square Residuals) (MS_{within} or MS_{error} or $MS_{residuals}$) for your measurements. Both of these values can be found in a one-way ANOVA table. It will look similar to the table below. Your instructor will provide directions on how to complete an ANOVA on your data to create your ANOVA table. The two values you will need are bolded and highlighted.

ANOVA Table

	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F	Significance (Sig.) or P-value
Between Groups	32.5541	3	10.8514	190.2985	2.21275E-10
Within Groups (Residuals or Error)	0.6843	12	0.0570		
Total	33.2384	15			

Using the values from your ANOVA table, follow the example below to complete the following calculations to find your repeatability (r) value and enter the information in the calculations table below.

1. Find the variance within individual butterflies.

$$\boldsymbol{variance\ within\ individual\ butterflies = MS_{within} = 0.0570}$$

2. Calculate the variance between butterflies.

$$\boldsymbol{variance\ between\ butterflies = \frac{(MS_{between} - MS_{within})}{N_0} = \frac{(10.8514 - 0.0570)}{4} = 2.6986}$$

$$\boldsymbol{N_0 = number\ of\ repeated\ measures = 4}$$

3. Calculate the repeatability (r).

$$\boldsymbol{r = \frac{variance\ between}{(variance\ within + variance\ between)} = \frac{2.6986}{0.0570 + 2.6986} = 0.9793}$$

Calculations Table (complete using your data)

Morphological characteristic	**Mean Square (MS) between**	**Variance within butterflies (MS within)**	**Variance between butterflies**	**Repeatability (r)**
Wing length				
Wing width				
Wing anterior spot area				
Wing Apex				

Interpreting r scores:

Compare your r scores to those given as criteria for low-high repeatability above. Are your scores showing high, medium or low repeatability? Discuss these scores with your instructor.

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Performing a one-way ANOVA in R

1. Complete the repeatability spreadsheet by including all 4 measurements per butterfly image for a total of 4 specimens.
2. Open the file “RepeatabilityScript.R” on R studio- you will find instructions of how to calculate a one-way ANOVA in R.
3. Open your repeatability datasheet (dataframe) in R studio. Here you will find the columns for “butterfly_image_number”, “repeated_measure_number" and the different measurements (e.g. “RWingLength”, “RWingWidth”, “RAnteriorSpot_Area”, “RWingApex_Area”)
4. Follow instructions on the script to calculate a one-way ANOVA for each measurement.
5. From the **Analysis of Variance Table** that you get at the end of each analysis, record the Mean Square value for “Residuals” and the Mean Square value for “butterfly_image_number” to calculate the **variance within individual butterflies (repeated measures)** and the **variance between butterflies.** You can use this to complete the data table and calculate repeatability.

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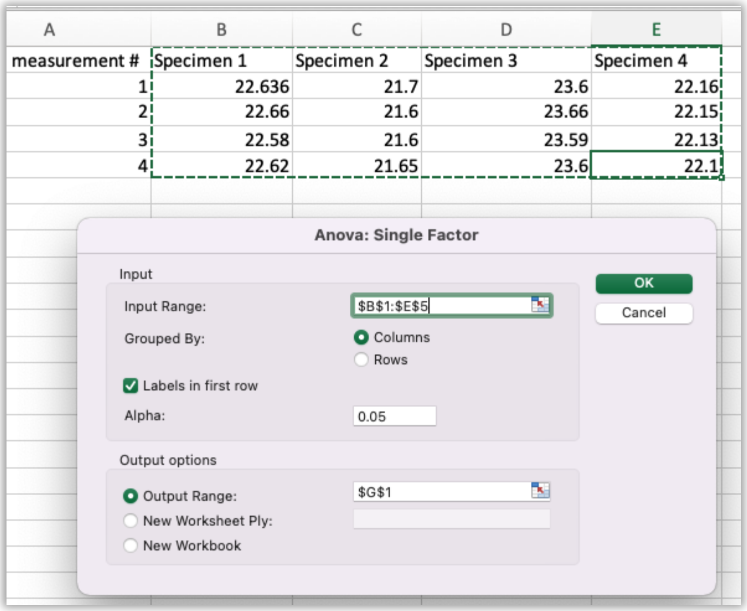
Performing a one-way ANOVA in Excel

1. Complete the repeatability spreadsheet by including all 4 measurements per butterfly image for a total of 4 specimens. Note that there are separate sheets in the file for each measurement type- right wing length, right wing width, right anterior spot, and right apex area.
2. Select the **Data** tab and click **Analysis Tools**. If “Analysis ToolPak” is checked, great! If not, check it and click OK.
3. Still on the Data tab, click **Data Analysis** and select **Anova: Single Factor**. This will bring up the dialog box shown below.



A	B	C	D	E
measurement #	Specimen 1	Specimen 2	Specimen 3	Specimen 4
1	22.636	21.7	23.6	22.16
2	22.66	21.6	23.66	22.15
3	22.58	21.6	23.59	22.13
4	22.62	21.65	23.6	22.1

Anova: Single Factor

Input

Input Range: $B$1:$E$5

Grouped By: ● Columns ○ Rows

☑ Labels in first row

Alpha: 0.05

Output options

● Output Range: $G$1

○ New Worksheet Ply:

○ New Workbook

4. Click in the **Input Range** and select all of the data for a single measurement type (ie, wing length). Be sure to select all 4 measurements for all 4 butterflies. If you include the header row, be sure to check “Labels in first row”.
5. Click in the **Output Range** and click in an empty cell on the sheet. This is where your ANOVA table will appear. Click OK.
6. Repeat steps 3-5 for each measurement type to complete the data table and calculate repeatability.

MEASURING REPEATABILITY USING STANDARD ERROR

Repeatability in measurements can be estimated by calculating the standard deviation or the standard error for a series of repeated measurements that were collected from a given individual image and using this as a proxy.

To determine your repeatability for wing pattern measurements, you will need to randomly identify 1 specimen for each measurement (right wing length, right wing width, right wing anterior spot area, right wing apex area) and perform repeated measurements on it 8 times. From this collection of 8 measurements, you will first calculate the standard deviation using the equation:

$$\text{SD} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

n = number of measurements you made

x_i = value of each individual measurement

$\bar{x}$ = the mean of all measurements

You can also calculate standard deviation in Excel using the function =STDEV.S(cell:cell)

After you have calculated the standard deviation, calculate the standard error of the mean. This is the standard deviation divided by the square root of the number of results:

$$\text{SE} = \frac{\text{SD}}{\sqrt{\text{n}}}$$

In this case, the smaller the number, the lower the variation between measurements and the higher the repeatability and the reliability of the results. A low standard error (close to 0) means no variation among the measurements. Discuss these scores with your instructor.