MATE PREFERENCE WITH BACKGROUND COLOR INTERFERENCE IN GUPPIES

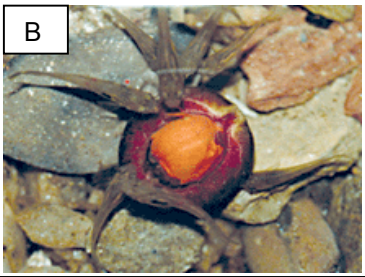
In a variety of species, females choose mates on the basis of traits such as male body size, ornamentation, courtship vigor or morphological symmetry. In general, the two sexes have very different reproductive strategies, females being more discriminating in their choice of mates than males are. For example, Bateman (1948) observed that male fruit flies try to mate with almost any female, whereas females tend to interact with a number of males before they allow one to mate. These sex differences in mating tactics are probably related to sex differences in the costs of making poor mating choices. Compared to males, females typically incur greater reproductive costs (egg production, gestation, incubation, lactation), and as a result, females run the risk of paying a greater cost for making poor mate choices (Trivers 1972). Consequently, females often choose carefully among potential mates, and males display vigorously to attract females. (For further explanation see chapter 8 in Essential Animal Behavior, or Chapter 10 in Alcock).

Guppies (*Poecilia reticulata*) have been used extensively to gain an understanding of sexual selection and the evolution of mating systems. Several competing and complementary theories can be used to explain the evolution of female choice. Careful empirical studies are important in order to determine which theories apply to which species.

The purpose of the current study is to determine the effect of the immediate environment on mate choice by female guppies.

It has been shown that females prefer "showy" males, those with a larger area of orange color (Figure 1A). The orange color on guppies is similar to, and is in part due to, the coloration of a preferred food source, the fruit from the cabrehash tree in Trinidad (Figure 1 B) (Rodd et al., 2002). This color may signal to females that the male is healthy due to efficient feeding capabilities. According to the “Good genes hypothesis” (Andersson, 1994) females benefit indirectly by choosing males based upon "honest signals" that indicate genes for good health.

Figure 1 Orange coloration of *Poecilia reticulata* (A) is similar to orange color of cabrehash fruits found locally in Trinidad.



Is the orange coloration an honest signal of healthy genes in male guppies?

An alternate theory suggests that the male's orange coloration may be a “sensory trap”. According to this theory the males orange color exploits the female's preference for orange that initially evolved for detecting the nutrient rich cabrehash fruits (Christy, 1995). Signals that contrast the environment are more readily detected. Therefore, once established, preference for a color may evolve further due to contrast with the environment.

By determining whether a female's preference varies with the degree of contrast between signal and environment, we can address the relative contribution of these two hypotheses. We will measure the strength of a female's preference for showy over drab males on orange (low contrast) and blue (high contrast) backgrounds. We will also measure the coloration of the males in the experiment to determine which body color, blue or orange, affects preference in different environments.

Before you begin lab, consider what experimental results will support the "Good genes" theory over the "Sensory Trap enhanced by Contrast" theory.

METHODS

Animals:

The guppies used for this lab are a captive reared population obtained from NatureBoysPets suppliers. They have been maintained at Reed since 2008.

Materials for each photostation (3/lab):

1 digital camera (batteries, CF card, download cable)
1 tripod
paper towels
ruler
pencils

Materials for each pair of students:

Animals: 4 female guppies (housed 4 weeks in all female tanks)
8 male guppies (housed 4 weeks in all male tanks)
(4 showy and 4 drab)
(ideally all animals would be inexperienced virgin animals)

Setup: 2 five gallon aquaria
4 clear plexiglass dividers
4 black plastic dividers
4 finger bowls (for holding males)
1 orange, 1 blue, 1 white and 1 black tank shroud
(10 inches X tank circumference)
1 small dip net
ruler
sharpie
timer

Note: This lab could possibly be scored more accurately with a computerized event recorder such as JWatcher. However, the time required to create the *.gdf, *.fmf and *.faf would preclude an introduction to ImageJ, the image analysis software.

Setup:
A 5 gallon aquarium will be divided into 3 sections as shown in Figure 2. Each end section, 6 cm, will be used for one male while the center section will contain the female. The center section is divided into three equal sections. The water depth will be only 6 centimeters and the background color shroud will extend 4 centimeters above the water. Use tape on the table under the tank to identify the center sections because the experiment will be viewed from above. **Make a record of your setup including any discrepancies that might be important.**

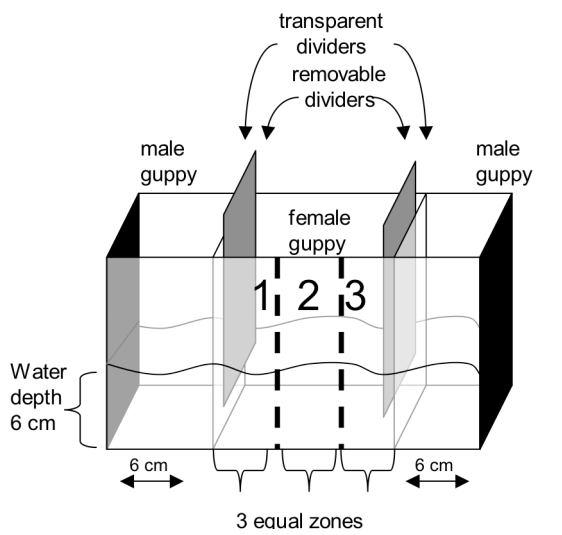


Figure 2: Female guppy preference setup in a 5 gallon glass aquarium

Experimental Trial:
Each pair of students will test 2 females in each of 4 background color conditions (orange, blue, white, black). The female will be placed in the center compartment while 1 showy male will be placed on one end compartment and one dull male will be placed in the other end compartment. The fish should be allowed to acclimate to the testing apparatus for 10 minutes. Begin the trial by lifting the two removable black dividers. The timer should be set to beep every 10 seconds for 5 minutes. A score will be recorded on each beep (see Preference Score below).

Instructions for setting a stopwatch to beep every 10 seconds:

upper left button = Reset \Select

upper right button = Start\Stop\Set

Lower right button = Chrono/Timer

Chrono/Timer switch from Chrono to Timer
Select 3 times
Set 1 time
Select 3 times

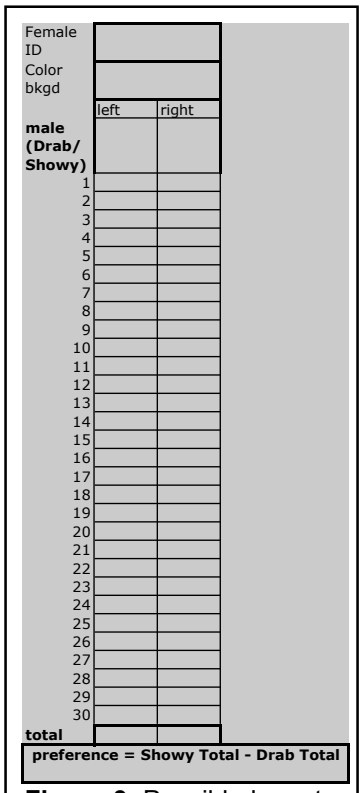
press-Start
Stopwatch will beep every 10 seconds and count the number of 10-second intervals.
press-Stop
Reset

Experimental Design:
The testing should be randomized. **(Consider why this is important).** Before you begin do the following coin flips to randomize your experimental design. Repeat this process for the 4 females that you will test and **record your experimental design in your lab notebook before you begin working.**

1. Flip a coin. If it is heads, the bright male will be placed in the compartment on the right.
2. Flip the coin again. If it is heads, the black or white background will be used before the blue and orange backgrounds.
3. Flip the coin again. If it is heads, the white will be used before black.
4. Flip the coin again. If it is heads, the orange will be used before blue.
5. All 4 backgrounds will be tested for each set of 1 female and 2 males.

Preference Score:
Female preference can be scored in many ways. Preference could be considered to be a categorical result; either the female showed a preference or she did not. Some studies use >60% time spent as the threshold for "preference". **Consider what statistical test you would use if you collected categorical for preference in the 4 different background color environments.**

Anne Houde (Lakeforest College) has developed a scoring system for preference by female guppies in the experiment set up described above (reference). This scoring system employs timed recording. Every 10 seconds both males will receive a score according to the female's behavior using the chart below. Only one male can receive a positive score and the other is given a 0 (or both males can be given a 0). At the end of 5 minutes a total score will be calculated for the showy male and a total score will be calculated for the drab male. The preference score is equal to the total showy male's score minus the total drab male's score. **You will want to create a data record table in your lab notebook similar to the example in Figure 3.** At the end of your experiment the preference scores will be recorded on the computer but the raw data should be in your notebook.



Female ID		
Color bkgd		
	left	right
male (Drab/ Showy)		
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
total		
preference = Showy Total - Drab Total		

Figure 3. Possible layout for data collection during female preference test.

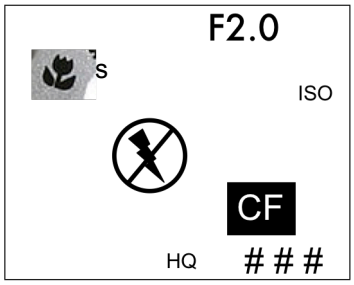
<u>Table 1. Scoring for Female guppies behavior</u>

SCORE	Female Behavior
0	- in section 2; not paying attention to either male
1	- in section 2; orientated towards one side
1	- in section 1 or 3; not paying attention to male
2	- in section 1 or 3; oriented towards the male
3	- in section 1 or 3; closely following male (within 1 body length of the plexi-glass)

Male coloration photography:

Each photograph must include a **metric ruler** as well as a piece of paper indicating the identity of each fish according to date_station(A-K)_trial(1/2/3/4)_Color(S/D) (*e.g.* **20081014_B_3_S**).

Check that the camera is set up correctly before taking the fish out of the water.



- The camera should be mounted on a tri-pod, ~ 4 inches above the photography surface.
- The camera should be set to:
- Macro (flower symbol)S
- no flash
- saving images in high quality (HQ)
- saved to the Flash Card (CF).

1. Place the fish, the ruler, and the label on a damp paper towel under the camera.
2. Take a picture of the **right** side of the fish.
3. View the image on the camera screen to be sure the focus is crisp and the full fish is in view.
4. When all fish from all stations on that lab bench have been photographed, the images will be transferred to the courses server.

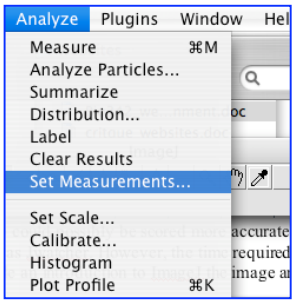
Image Analysis:

File Transfer:

1. Connect to the courses server.
2. Find your images in the folder. **2008_labs/week6_guppy_wed(thurs)/raw_images**.
3. Drag your 8 images to your desktop.
4. Rename your images according to the label in the photograph (date_station_trial_Color (*e.g.* **20081014_B_3_S**)). If you took more than one photo decide which one is best for each fish, rename that one and continue with that one.

Measuring in ImageJ:

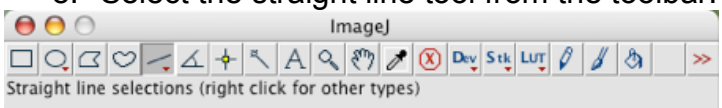
This is free image analysis software developed by NIH. Several Plugins are available which allow very sophisticated image analysis. It can be downloaded for MAC or PC from http://rsb.info.nih.gov/ij/



1. Open **ImageJ** by clicking on the microscope icon in the dock.
2. Select **Analyze > Set Measurements**....
3. A pop-up window will open showing all measurement options.
4. Check the box by **Area**
5. Click **OK**
6. Open you first image
7. The scale must be set for each image using the rultr

included in the image.

8. Select the straight line tool from the toolbar.



9. To calibrate this line tool with the ruler in the photo, draw a line across an object of known length by clicking on one end and dragging across it.
10. Select **Analyze** > **Set Scale** from the drop-down menu.
11. In the popup window, enter the length of the calibration object in **Known Distance** field and the appropriate units in the **Unit of Length** field. Ignore **Distance in Pixels** and **Pixel Aspect Ratio**. Click **OK**.

You must Set Scale every time you open a new file! but once you have set the scale it is ok to use the magnify tool to change the size of the image on the screen for a particular image.

Measurements:

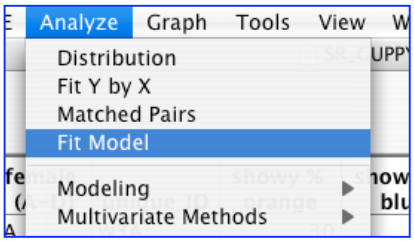
12. Select the **Freehand** selections tool.
13. Trace the outline of the fish body, excluding the tail.
14. You may start over if you are not happy with the outline you have drawn.
15. Select **Analyze > Measure** from the top menu.
16. Notice, ImageJ calculates the area inside the outline of your fish shape. This value is recorded in the **Results** window.
17. If you make a mistake you may delete an entry by selecting the erroneous measurement in the **Results** window and then selecting **Edit > Clear.**
18. Use the mouse to outline the most anterior orange patch
19. Select **Analyze > Measure**.
20. Select **Analyze > Label**. Notice that the corresponding number will appear on your image.
21. Each time you measure an object it must be labeled.
22. Measure and label each orange patch working from the head to the base of the tail.
23. In your lab notebook record how many orange patches were measured for this fish.
24. Measure and label each blue patch working from the head to the base of the tail.
25. In your lab notebook record how many blue patches were measured for this fish.
26. When you have measured all orange and blue color patches for this fish save the results file. Name it according to date_station_trial_Color (*e.g.* **20081014_B_3_S.txt**).
27. Save your labeled image with a **new name** as a .jpg file (e.g. **20081014_B_3_S_label.jpg**).
28. Open the next image file and repeat steps 2 - 27.
29. The scale should be roughly the same if the camera mount was not

changed, but it will be more accurate to reset the scale for each image.

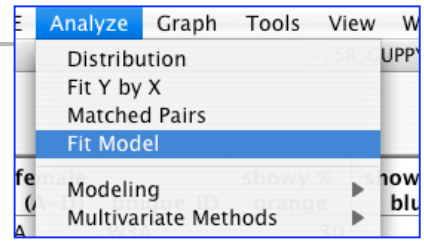
30. The name of the image file is recorded in the Results file so one Results file can be used for all 4 images. It is a good idea to save the Results file after each image to protect your data from computer crashes.
31. By opening your .txt results file in Excel (or by hand), calculate the **total orange area** and **the total blue area** for each fish.
32. Divide those values by total body area to obtain the **% orange** and **% blue**.

These measurements made in ImageJ are your raw data. Because it is very easy to loose track of computer files (i.e. hard drives crash and files become corrupted) it is a good idea to back this data up either by printing a hard copy of the results file at the end of the day, recording the measurements directly into your lab notebook, or burning the data to a second hard drive or other data storage medium. You lab notebook should provide a clear record of where this raw data can be found. Often times, a data set collected on a computer is too large to consider printing a hard copy. In these cases the data back up becomes very important!

Data Entry:

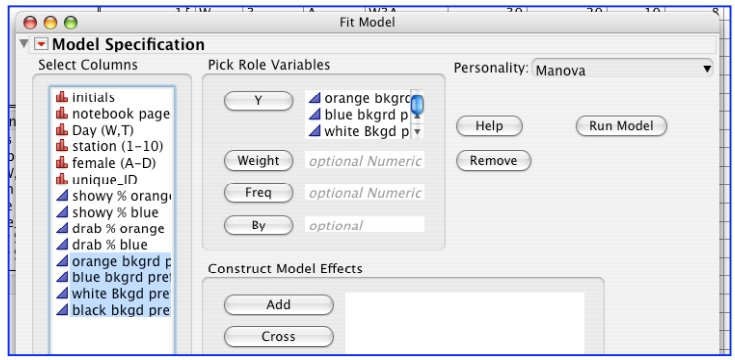


1. Drag the file guppy_data_template to your desktop from Courses/Biology/Bio342/Labs/guppy.
2. Rename this file **guppy_data_date_initials.**
3. Open this file in JMP and enter your data in the appropriate columns.
4. Save your data file.
5. Drag a copy of your data file back to Couses/Biology/Bio342/Labs/guppy so that the class dataset can be combined.
6. **By entering only "Preference Score" we have omitted some information. This data processing step will simplify our analysis, but it may cause us to miss some important behavior. Consider why you might want to analyze the raw data using the total score for drab and showy males.**

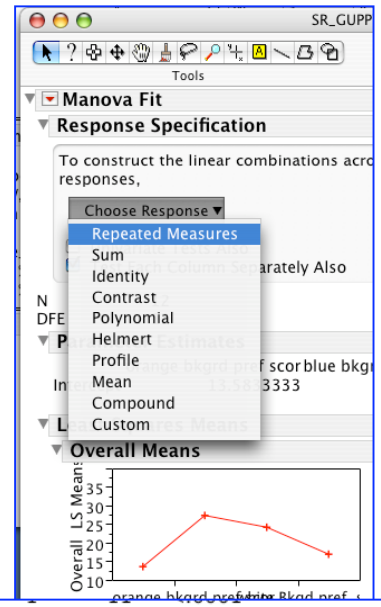


Data Analysis: (with JMP)

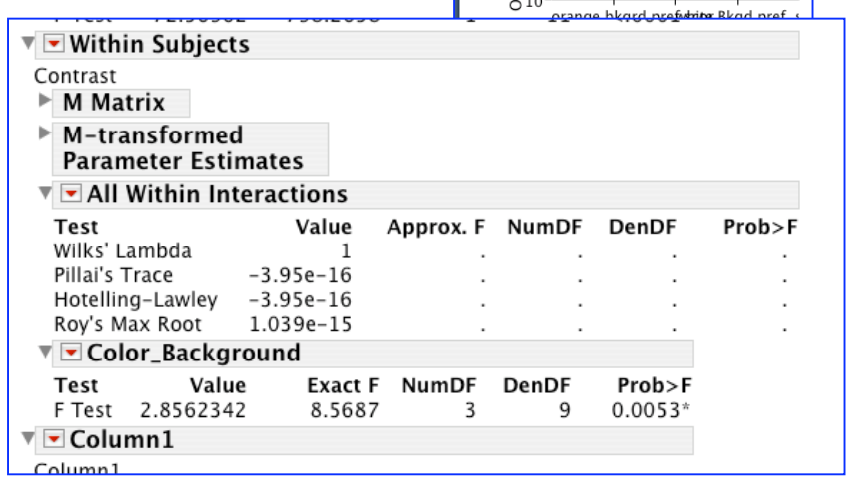
Because each female was measured 4 times, we have a repeated measures design. We want test the null hypothesis that background color did not affect the female's preference. In JMP, to form a multivariate model, choose **Fit Model** from the **Analyze** menu. In the Fit model window, assign all 4 preference scores as multiple Y's in the dialog. Then select **Manova** from the fitting personality menu, as shown below, and click **Run Model**.



The multivariate fitting platform fits the responses to the effects using least squares. You must still specify a response design by choosing Repeated Measures from the Choose Response popup menu. Also select the radio button to "Test Each Column Separately Also". A popup window will appear in which you can enter a name for the term to represent going across y-variables (e.g. Background_Color).



We are interested in the Within Subjects test for Color_Background and the Prob>F provides the p-value that we can use to reject the null hypothesis. A p-value less than 0.05 supports the Environment Contrast Hypothesis. Each of the three other color backgrounds will be compared to Orange (because it was the first in the list) so we can determine whether there is a statistically significant difference between orange (low contrast) and the other conditions. **How would you test whether the Blue and Black backgrounds had different effects on the female's preference score?**



Within Subjects

Contrast

M Matrix

M-transformed Parameter Estimates

All Within Interactions

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	1	.	.	.	.
Pillai's Trace	-3.95e-16	.	.	.	.
Hotelling-Lawley	-3.95e-16	.	.	.	.
Roy's Max Root	1.039e-15	.	.	.	.

Color_Background

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	2.8562342	8.5687	3	9	0.0053*

Column1

During this lab we collected a lot of phenotypic data from the males, such as total body size, % orange, and % blue. These factors could affect female preference in ways more subtle than the gross categorization of "Showy" and "Drab". Does the relative amount of orange in the two males affect the strength of female choice? Does the relative ratio of orange to blue on the showy male correlate with female choice? Does that correlation change on the different backgrounds? **For your own records (which we will discuss in lab next week), create a graph that depicts one potentially interesting relationship, explain why (from a behavioral or ecological perspective) this is an "interesting" relationship. Then apply the appropriate statistical tests to address a hypothesis related to your explanation.**

CONCLUSIONS:

There is no write up due for this lab. However, in addition to a clear record of what you did in lab today, your lab notebook should include:

- ☐ A simple concluding statement that evaluates your results in light of the alternate hypotheses that were proposed to explain the evolution of female preference for orange color in male guppies.
- ☐ There should be evidence in your lab notebook of statistical tests that were run, and the p-values obtained, which led you to this conclusion.
- ☐ Your additional hypothesis, as well as the graphs, and statistical tests associated with that hypothesis.
- ☐ A simple concluding statement that evaluates your results in light of this additional hypothesis.

Aspects of this experiment have been abbreviated in order to fit within the 4 hour lab period and adjustments have been made to the protocol in order to allow 18 students to simultaneously work efficiently. Consider how you would run the experiment differently if it were your own independent research.

- ☐ Record your suggestions for changes to this experiment if it was your goal to collect data for publication.
- ☐ Record any ideas that you have that would increase efficiency or improve this experiment in a lab course setting.

Acknowledgements:

This lab exercise builds upon an idea discussed with Mike Kinneson (University of Maine) at the 2008 Ecological and Evolutionary Ethology of Fishes Conference in Boston MA. The text is adapted from a preliminary report written by Emily Stevens (University of Maine).

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Independent project ideas:

1) Fluctuating asymmetry and fluctuating environments

It has been hypothesized that mate choice should favor individuals that display high symmetry because small random deviations from bilateral symmetry result from developmental "noise" in the morphological traits of individuals (Lens *et al*., 2002). This type of random developmental asymmetry is known as "Fluctuating Asymmetry" (FA). According to these hypotheses, FA is an indicator of reduced genetic quality, or lack of buffering from environmental perturbations during development. However, this theory remains controversial and few robust empirical tests have been conducted.

Despite current research that has shown behavioral lateralization for a great number of species in different contexts, many studies focus on the benefits of symmetry. Therefore, behavioral ecology has largely ignored the potential for compensatory or exploitative behaviors that capitalize upon the subtle asymmetries resulting from developmental instability.

A recent study (Gross et al., 2007) has been the first to demonstrate behavioral responses to subtle fluctuating asymmetry. Gross et al (2007) reasoned that in organisms where symmetry is not a trait targeted by females, one might anticipate that males would attempt to take advantage of their own fluctuating asymmetry and "put their best side forward". They used the guppy as their model organism and demonstrated that male guppies with more symmetric body color display both sides equally to the female during courtship, while those with high asymmetry in body color preferentially display their most colorful side.

If contrast with background color affects female preference and a male has asymmetric coloration, his "best-side" might be different in two different environments. One could test to see if the male will display different lateralization in different environments.

Consider how the male would know which side has higher contrast and would therefore be preferred?

2) Environmental Contrast, female preference and speciation.

There are several populations of guppies that show little genetic differentiation, and no evidence for post-zygotic isolation, yet the male sexually selected traits and behaviors are substantially different between populations (Alexander and Breden, 2004) . These characteristics present an opportunity to test theories concerning the role of sexual selection and mate choice behavior in driving speciation.

It was previously thought that the group of guppies called Endler's livebearer ("*Poecilia wingei*") and the common guppy (*Poecilia reticulata*) were distinct species, however breeding experiments and genetic analyses have shown that the two groups are not only genetically compatible but do not differ according to neutral genetic markers. In the wild however, the two groups are essentially reproductively isolated due to mate choice. Since reproductive isolation is one of the harbingers of speciation, these two populations provide an exciting opportunity to observe the role of behavior in shaping evolution.

Does the environment play an important role in the ability of these animals to perform con-specific mate choice? One could ask if contrast with background color affects a females ability to select her own species as the preferred mate.

3) Environmental Contrast and mate choice copying.

There have been several studies that demonstrate mate choice copying among female guppies. Younger females tend to copy older females. One could ask if this copying behavior is affected by the contrast with the environment.