Behavioral Classroom Activity: Female Mate Choice

Background

Many animals use vocalizations or acoustic signals to communicate. Frogs are one group that has specialized in acoustic communication for reproductive purposes. Males of many species gather at ponds on warm nights and advertise for mates (Fig. 1). This aggregation of calling males is called a ***chorus***. Females invest substantially more energy in reproduction than do males, primarily because eggs with rich yolks are much more physiologically costly to produce than sperm. This asymmetrical investment between the sexes means that on any given night, there are far fewer females available to reproduce than males. This skews the ***sex ratio***, resulting in strong vocal competition among the males for the few available females at the pond.

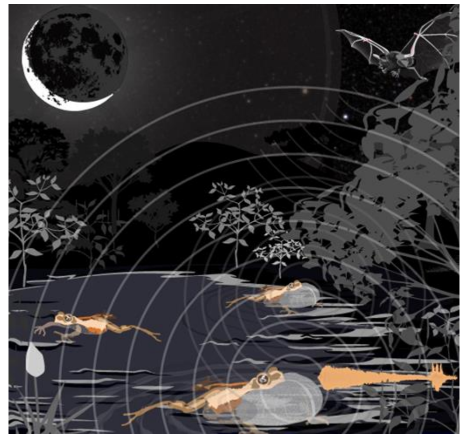


Figure 1. When Panamanian túngara frogs call they simultaneously produce sound, vibrations of the vocal sac, and ripples on the water's surface. All three components are perceived by intended receivers (frogs) and an unintended receivers (frog-eating bats). Illustration by Damond Kyllo.

Males stake out calling sites and vocalize, while females evaluate the males and are selective about the male with whom she chooses to mate. In particular, females are quite selective about certain properties of the males' vocal signal. They prefer to mate with males who make calls that best match her auditory sensitivity. When a female selects a male to mate with, she approaches and touches him. The male then clasps her, holding on to her back in a position called ***amplexus***. The pair then moves down to the pond where the female deposits her eggs (called ***oviposition***) and the male fertilizes them externally. The highly skewed sex ratio in the mating system means that most females in the population will mate. Some males will get multiple mates but many males will never mate during their lifetime. Thus the choosiness of females, called ***female mate choice***, determines which males, and hence which vocal traits (determined by a male's genes) get passed along into the next generation.

The process of female mate choice determining which males' genes go into the next generation is part of an evolutionary process called ***sexual selection***. Like ***natural selection***, sexual selection can determine which genes get passed into the next generation and this then can influence the evolution of animal signals, such as how calls sound. For example, next time you are outside, listen for any birds, insects, or frogs that you may hear calling. If more than one species is calling, you will notice that the calls sound distinctly different. The process of sexual selection, over evolutionary time, has shaped how these calls (acoustic signals) sound.

So how do biologists know this? There are lots of ways to study evolutionary processes, but one of the easiest ways to study sexual selection is to conduct female mate choice experiments. Because female frogs search out males based on how their calls sound, they are especially good candidates for studying sexual selection. We can digitally manipulate male calls, play them back to females, and then record which call she chooses. This is representative of the call she would have chosen in the wild. By testing multiple females, we can get an understanding of what most females in a population prefer and make estimates then of how the female choice can skew the odds of a male's genes getting into the next generation.

Basics of Female Mate Choice Experiments

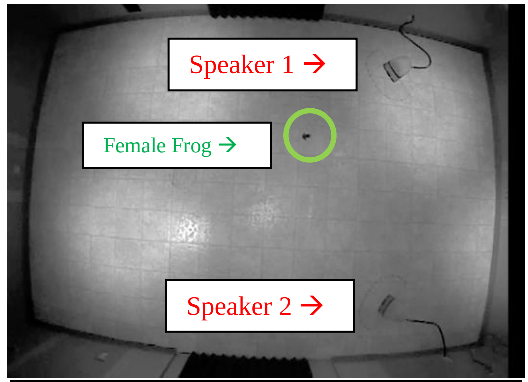


Figure 2. The experimental set-up in the sound chamber. Each female choice experiment is recorded.

To measure female mate choice, we are going to conduct a ***phonotaxis*** experiment (**phonotaxis = sound orientation**). While the logistics of conducting such an experiment can be rather labor intensive, the experiment itself is quite simple. We catch amplexed pairs in the wild and since the female made a choice we know she is reproductively active. Then, we will present female frogs with two different speakers, each playing a different call in ***semi-anechoic chamber***. This chamber is small room that limits echoes and outside noise. A female will be restrained under a funnel and then released while the speakers are playing their calls. The females perceive the calls as coming from a male and approach the speaker that broadcasts the call she prefers. When the female approaches to within *5 cm of the speaker* and stays within that vicinity for *at least 3 seconds*, we record this as her mate choice.

In nature, all individual organisms exhibit variation. For mate choice, this means that not all females will necessarily choose the same male. If the majority of females in a population prefer a particular call type, however, those genes will be selected for and most males in the population will begin to produce this type of call over evolutionary time. To gain an understanding of what most females in a population prefer, we have to test our hypothesis by testing multiple females and then evaluate the results statistically.

Class Procedure

1. Study Organisms
   We will use two frog species to make a general test of sexual selection through female mate choice, the ***green treefrog (Hyla cinerea)*** and the ***túngara frog (Physalaemus pustulosus)***. The green treefrog is found North America and the túngara frog in Central America. Each species makes a different kind of call and we will test hypotheses specific to each species. For background information on these frogs and to hear their calls, see the species profiles on the Taylor/Hunter website.

Green treefrog

Picture	oscillogram of Attractive Call	Call (sound)
	Oscillogram of Unattractive Call	Call (sound

Túngara frog

Picture	oscillogram of Complex Call (Attactive)	Call (sound)
	Oscillogram of simple call (unattractive)	Call (sound)

2. <u>Hypothesis Testing</u>
   Hypothesis 1: **Female green treefrogs** prefer to mate with males that produce low frequency (pitch) calls over high frequency calls.

   Hypothesis 2: **Female túngara frogs** prefer to mate with males the produce complex calls over simple calls.

3. <u>Phonotaxis Procedure</u>
   a. **Videos**: The Taylor/Hunter lab has compiled a series of videos from our actual experiments in Maryland (green treefrogs) and Panama (túngara frogs). In the videos you will see a series of trials (each trial is labeled at the beginning and represents a unique female frog). **Once the funnel is lifted from the female, watch to see which speaker she chooses.**

   b. **Experimental Details**: Between trials we alternate the speaker from which each call is played. This ensures that we are not biasing our experiments by some quirk of experimental design. For example, if one speaker happened to accidentally play a little louder, then all females might prefer Speaker 1, irrespective of the actual call playing. So by switching sides, we can eliminate any side bias.

   c. **Conduct Experiment**
      a. Simply watch all the trials for each species and record the number of females that choose each speaker.

      b. **Record the frog species, the trial number, the speaker choice.**

      c. Record a choice when the females enters the choice zone, marked on the floor around the speaker. Speaker 1 is in the upper right hand corner of the video screen and Speaker 2 is in the lower right hand corner (Fig. 2).

      d. Helpful hint: If you are a little unsure if a female actually chose a particular speaker, her choice is the last speaker she was at when that particular video trial ends. There is no audio on the video feed. <u>**Since we switch the position of the calls between trials, it is important that you keep careful track of which speaker the female chooses in each trial.**</u>

      e. Once you are finished recording the trials, ask your teacher for the key so that you will know which call type the female chose in each trial. Scoring the videos without first knowing what the females are choosing is called a "***blind study***" and it is one method scientists use to help ensure that their data are not biased.

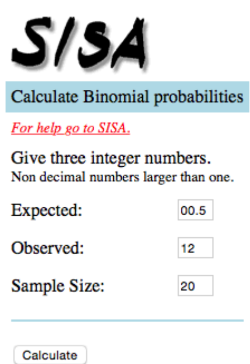
   d. **Statistical Analysis (**We will use the same statistical analysis for both experiments.)

      a. ***Binomial Exact Test***: This statistical test allows us to analyze whether two possible outcomes occur from random chance. The easiest way to think about this a coin toss. If you toss a fair coin a large number of times, statistically you will get equal numbers of heads and tails. Because either outcomes is equally likely, the probability of getting heads on any given flip is 0.5. If you make a relatively small number of coin flips, however, it is likely that you will not get exactly equal numbers of heads or tails.

b. ***Expectations for the Female Choice:*** For the frogs, we are also giving females two choices. If the females exhibit no mate preference, their choice of each call type should occur with roughly equal probability. That is, roughly half the females in our experiment should choose each call type. But since we are only testing a relatively small number of females, it is possible that we could get unequal choices even if they are choosing randomly. On the other hand, if females really do prefer one call, we should see a substantially higher proportion of females in our experiment choosing that particular call type. The results of our binomial exact test will tell us if the proportion of females choosing a particular call occurs by random chance or if our results are statistically unlikely to occur from random choice.

c. ***Running the Statistical Analysis***: Once you've collected the behavioral choice data from the videos, we'll use the follow site for our statistical analysis: http://www.quantitativeskills.com/sisa/distributions/binomial.htm

At the site you'll see a dialogue box that looks like this:



The expected value should be 00.5, indicating that the null hypothesis is random choice. So the test will evaluate whether the frog choices were statistically different from random (e.g. that females generally have a preference for one call type over the other). **You'll analyze the results of your green treefrog and túngara frog experiments separately**.

For Green treefrogs, put the number of choices you recorded for low frequency calls in the "observed" box. In the "Sample Size" box, put the total number of females that were tested (= 24). For túngara frogs, put the number of choices you recorded for complex calls in the "observed" box. The sample size for túngara frogs will be 20.

Once you've entered your data for a particular experiment, click "calculate." You will get an output box that looks like this:

S/SA

Calculate Binomial probabilities

Expected: 0.5

Observed: 12

Sample Size: 20

If 00.5 is a significance:

Binomial mean= 0.6229
as a fraction of n: = 12.46
(14 iterations)

Expected: 10 (=0.5*20)

BINOMIAL PROBABILITIES
single; cumulative

p(=8): 0.120134; p(>8): 0.748278
p(=9): 0.160179; p(>9): 0.588099
p(=10): 0.176197; p(>10): 0.411901
p(=11): 0.160179; p(>11): 0.251722
p(=12): 0.120134; p(>12): 0.131588

*** summary ***

Point probability= 0.120134
p(Obs>=12): 0.2517; (<12): 0.7483
p(Obs>12): 0.1316; (<=12): 0.8684
Mid-p: 0.192; 1-p: 0.808; 2*p: 0.383

Two sided probabilities:

abs(Exp-Obs)=abs(10-12)=2
Pointpr(Exp-Obs=2): 0.120134
p(Exp-Obs>=2)= 0.50344
p(Exp-Obs>2)= 0.38331
Mid-p= 0.44338

The value that you are interested in is your ***p value***. In your results box, this is listed as "Mid-p =   ", under "two-sided probabilities". In our hypothetical example here, we see that "Mid-p = 0.443). In the binomial test, a result that is statistically unlikely to occur from random chance, is indicated by a p value of less than 0.05. In our hypothetical example, you can see that we are way above 0.05. This indicates that if 12 out of 20 females choose a particular call this ouctcome is likely due to females choosing between the speakers randomly.

So in this case, if we tested the hypothesis that female túngara frogs prefer complex calls, **our hypothesis would not be supported**. Instead, our results are highly likely to have occurred as a result of females just randomly choosing between speakers – therefore NOT preferring a particular call type. On the other hand, if our data had been different, and our p value was less than 0.05, then our hypothesis that females prefer complex calls would have been supported.