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Population Genetics and Behavioral Ecology: Orange, Blue, & Yellow Male *Uta stansburiana*

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Variation in throat color among male side-blotched lizards, *Uta stansburiana*, is associated with variation in male size and mating strategies. Success of alternative mating strategies is dependent on the frequencies of types of males in the population. In this research-based case study, students use population genetics (Hardy-Weinberg Equilibrium), descriptions of mating strategies, and the results of a behavioral ecology field experiment to explore the evolutionary processes that maintain multiple male throat colors in populations. The activity illustrates the maintenance of variation in a population through balancing frequency-dependent selection.

Keywords: Population genetics, behavioral ecology

Introduction

The purpose of this activity is to engage students in the process of investigating evolutionary biology. The case study integrates quantitative approaches of population genetics with observational and experimental studies of behavioral ecology. The behavioral ecology approach describes a set of alternative male throat colors of side-blotched lizards; each throat color is tied to a different overall morphology and a different mating strategy. The success of each morphology and associated set of behaviors is dependent on the social context, specifically on the frequency of alternative mating strategies in the population. The frequency-dependent sexual selection results in balancing selection that maintains a high frequency of three different phenotypes in populations. This case study could be used in a "dry laboratory" that requires no preparation of supplies and equipment besides worksheet illustrations and materials. Such exercises are useful to have on-hand in case a wet laboratory experiment runs into a last-minute problem, such as organisms failing to arrive or equipment failures. It can also be used in a workshop setting. It could be adapted for use in break-out sessions in flipped lecture courses. It is written for a freshman university science majors' introductory biology course. It could be adapted for sophomore through senior levels. Adaptation of the exercise for higher levels could include the addition of inferential statistics (both data sets could be analyzed with Chi^2 Contingency Tables).

Student Outline

As you can see in the images on the screen or in the photos in the plastic sleeves at your tables, male side-blotched lizards (*Uta stansburiana*) vary in throat color. Their throats are orange, blue, or yellow. You will conduct a series of investigations using data from a study of the population genetics of *U. stansburiana* and data from a study of the behavioral ecology of these lizards to determine the biological significance, if any, of the variation in male throat color.

Before we begin working our way through the studies of the orange, blue, or yellow throat color of male *U. stansburiana*, let's step back and consider why this has attracted the interest of biologists who view the populations of lizards through the lens of evolution in response to natural selection. Work with your group to develop some initial speculations about this trait.

What might be the adaptive value of a colorful male neck patch?

If your speculation about the adaptive value of this trait is true, how might this affect the frequencies of the different phenotypes?

Part I: Population Genetics of *Uta stansburiana*

Variation in throat color of the males is influenced by a gene. This gene is present but does not have the same phenotypic effect in females. Although there are likely more than two alleles of this gene that determines male throat color, we are going to simplify the problem by associating variation in male throat color with the following three genotypes: *BB* = orange, *Bb* = blue, *bb* = yellow.

You will conduct a Hardy-Weinberg analysis to determine if any evolutionary processes —natural selection, genetic drift, assortative mating, gene flow, or mutation — are affecting the frequencies of these color morphs in male lizards.

Initial Observations

A survey of male side-blotched lizards in Arizona and California revealed the following counts of the three throat-color phenotypes: Orange = 254, Blue = 236, Yellow = 247. Count the number and record the frequency of each genotype, and each allele, from this sample data and record it in Table 1.

Table 1. Observed genotypes and allelic frequencies.

Counts of genotypes			
BB =	*Bb* =	*bb* =	Total =
Observed genotypes frequencies			
BB =	*Bb* =	*bb* =	Total =
Counts of alleles			
B =	*b* =	Total =	
Observed allele frequencies			
$p = f(B) =$	$q = f(b) =$	Total =	

Predictions of Hardy-Weinberg Equilibrium

The Hardy-Weinberg Equilibrium (HWE) equations predict the relationships between genotypic and allele frequencies if the gene is not being influenced by natural selection, genetic drift, assortative mating, gene flow, or mutation. Comparing the predictions of HWE to the counted frequencies will allow you to determine if the male throat colors are associated with any of these important processes.

In the Hardy-Weinberg equations, the frequency of the dominant allele is symbolized with p; the frequency of the recessive allele is symbolized with q. If the gene is not being influenced by any evolutionary processes, these allele frequencies p & q can be used to predict the frequencies of the three genotypes. The frequency of the homozygous dominant genotype is p^2; the frequency of the heterozygous genotype is $2pq$; and the frequency of the recessive genotype is q^2. Since there are only three possible genotypes of a gene with two alleles, these three genotypic frequencies should add up to one.

Use the Hardy-Weinberg relationships to estimate the expected genotypic frequencies of the three color morphs of lizards if throat color is not influenced by any of the evolutionary processes. Start by transferring the observed allele frequencies from Table 1 into Table 2. Use these observed allele frequencies and the HWE relationships between allele and genotype frequencies to calculate the expected genotypic frequencies if the population is in Hardy-Weinberg Equilibrium.

Table 2. Hardy-Weinberg predicted frequencies

Observed allele frequencies (from last page)			
p (frequency of the *B* allele) =		q (frequency of the *b* allele) =	
p^2 (freq. of the *BB* genotype)	$2pq$ (freq. of the *Bb* genotype)	q^2 (freq. of the *bb* genotype)	Total

Work with your group to design and sketch a bar graph that compares the observed and HWE expected frequencies of the three throat colors.

Describe the general pattern, or uniformity, in the observed allele frequencies and in the observed genotypic frequencies.

What does this comparison indicate about this gene and its associated phenotypes in this population of lizards?

How do these results compare to your original speculation about how evolutionary processes might influence male throat color?

Part II: Behavioral Ecology of *Uta stansburiana*

While the population genetics study showed that male throat color in side-blotched lizards is tied to an evolutionary process, it did not reveal the nature of the evolutionary process. We will use a study of the lizards' ecology and behavior to explore potential selection pressures associated with variation in male throat color. Bleay *et al.* (2007) investigated the effects of the frequencies of the three male throat-color types on the abilities of males of each type to find a mate and produce offspring. After considering this investigation of how throat color influences behaviors, we'll return to the question of what evolutionary processes are acting on this trait.

Behavioral Observation 1

Behavioral observations of side-blotched lizards have shown that orange-throated males are very adept at dominating territories and taking territories from blue-throated males, in spite of the ability of blue-throats to work together trying to defend their territories and mates. Orange-throated males are larger, more aggressive, and their territories can encompass the territories of many females. Successful orange-throated males are polygynous (one male mates with many females).

Behavioral Observation 2

Although yellow-throated males are weaker than the large orange throats, the yellow-throated males sneak into orange throat's territories and when approached by an orange-throated male they move their head up and down imitating a signal that females use to indicate that they are not in a receptive state for mating. The large orange throats confuse the yellow-throated males for unreceptive females, they leave them alone, and then the yellow throats mate with orange-throated males' females.

Behavioral Observation 3

Blue-throated males are more monogamous and are cooperative (Sinervo and Clobert, 2003). Neighboring blue-throated males work cooperatively to prevent yellow-throated males from sneaking into their territories. The blue-throated males are not tricked by yellow-throated male's attempts to sneak in and act as though they are females. Blue-throated males, while not as aggressive as the large orange throats, are able to out-compete the yellow-throated males.

In light of these three behavioral observations, Bleay *et al.* (2007) were testing the following hypothesis: mating success of male side-blotched lizards depends on how their throat color compares to the overall frequencies of throat colors of males in their population. In order to evaluate this hypothesis, they manipulated the frequencies of the three male throat colors in a variety of populations. They set up their field experiment with three treatment levels of this independent variable: *Treatment 1* Populations with a high frequency of orange males; *Treatment 2* populations with high frequency of yellow males; and *Treatment 3* populations with high frequency of blue males.

During the experiment they measured the dependent variable, which was the number of hatchlings sired by orange, yellow, and blue males in each of the three types of populations.

Considering their experiment and the behavioral information provided above, which phenotype does the hypothesis predict will have the highest mating success in each treatment?

Treatment One (high frequency of Orange-throated males)

Treatment Two (high frequency of Yellow-throated males)

Treatment Three (high frequency of Blue-throated males)

The results of the experiment described in Bleay *et al.* (2007) are shown in Table 3.

Table 3. The proportion of offspring sired by each type of male in each of the three treatments.

Treatment level: high frequency of	Throat color	Proportion of offspring sired in treatment
Orange	Orange	0.35
	Yellow	0.41
	Blue	0.24
Yellow	Orange	0.31
	Yellow	0.28
	Blue	0.41
Blue	Orange	0.73
	Yellow	0.15
	Blue	0.12

What conclusions can you draw from the Bleay *et al.* (2007) study of the behavioral ecology of *Uta stansburiana?*

How does frequency-dependent natural selection maintain a balance of the two allele frequencies at close to 0.50 to 0.50?

Compare natural selection on throat color in the side-blotched lizard to selection on the hemoglobin gene in humans in the subtropics. What is causing differences in survival in each example and how does it affect the gene pools?

Materials

Each student will need a calculator.

Students will view images of the three types of side-blotched lizards. Images of the three morphs of *Uta stansburiana* can be presented by projecting a web page to a flat screen or digital projector. Alternatively, if student groups have web access in the laboratory they can search for images of the lizards on their devices.

Notes for the Instructor

We provide our peer instructors with answer keys for this exercise. However, it is important that they work through the problems themselves and discuss where students might need support. Part 1 is designed to help students understand both the utility and the calculations associated with HWE. Because we have picked a trait in which the heterozygotes have a unique phenotype, counts and frequencies of each of the three genotypes that students enter in Table 1 can be directly derived from the provided counts of the three phenotypes (Genotype counts *BB* = 254, *Bb* = 236, *bb* = 247; Genotype frequencies *BB* = 0.345, *Bb* = 0.320, *bb* = 0.335). Some students miscount the alleles. They are normally able to correct this error if they are asked how many copies of a particular allele are in the homozygotes in contrast to the heterozygotes. If they have counted alleles correctly, in the second section of Table 1 they will have entered $B = 744$ and $b = 730$, with allele frequencies of $p = 0.505$ and $q = 0.495$. Notice that these genotype and allele counts and frequencies are all simply descriptions of an observed population.

In the next step, students calculate in Table 2 the predicted frequencies of the three genotypes based on the observed allele frequencies and on HWE. In other words, if the relationship between allele and genotype frequencies is not perturbed by an evolutionary processes. These HWE predicted genotypes are as follows: p^2 (frequency of *BB*) = 0.255; $2pq$ (frequency of *Bb)* = 0.50; and q^2 (frequency of *bb*) = 0.245. Our students sometimes conclude that because the frequencies add up to 1.0 the population is in HWE. Checking to see if the frequencies sum to 1.0 is simply a way to catch miscalculations of the frequencies. The questions following Table 2 challenge students to “make sense” out of their HWE analysis. This includes realizing that the observed frequencies of genotypes is much more uniform than HWE, indicating that some evolutionary process is reducing the frequency of the heterozygotes down to that of the two homozygotes.

In the second major part of the activity, students evaluate a behavioral field experiment that sheds light on the nature of the evolutionary process affecting this gene and its associated phenotypes in these populations. This illustrates frequency-dependent balancing selection. It is frequency-dependent in that when a phenotype becomes more prevalent, a specific alternative phenotype is favored: when orange throats are more common, yellow throats are most successful; as yellow throats then become more prevalent, blue throats are more successful; and lastly as blue throats become more prevalent, orange throats are favored. This frequency-dependent selection results in similar (balanced) frequencies of the three morphs and two alleles of the associated gene.

In the last question, we ask students to compare this example to selection on the hemoglobin gene in humans in the tropics. We present the hemoglobin example of balancing selection due to heterozygotes having increased survival in lecture and it is also presented in many text books. This comparison helps students see the general importance of evolutionary processes (e.g., balancing selection) across differences in the organisms’ behavior and ecology. If you don’t cover the hemoglobin example, you may need to skip this question.

Literature Cited

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Ralph Preszler earned his B.S. at Southern Oregon State College (now Southern Oregon University) where he was given the opportunity to work as an undergraduate teaching assistant in a botany laboratory. While he earned his M.S. and Ph.D. at Northern Arizona University he taught and coordinated botany laboratories, and worked as a lecturer. In his graduate and postgraduate research, he investigated interactions between plants, the endophygous fungi that live in their leaves, and the herbivores attempting to eat the leaves. He held a sequence of four positions at New Mexico State University 1) Post-doctoral Fellow; 2) Coordinator of lower-division laboratory courses; 3) Tenure-track Faculty; and 4) Department Head of Biology. He retired in the spring of 2016.

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