**Lesson Description:**

The goal of this lesson is to answer the question: Are pikas living at low elevations genetically different than those at high elevations? Students investigate this question in two ways, both commonly used by modern population geneticists. First, students investigate general gene flow in these populations by looking at the proportion of individuals that are heterozygous in a population. Second, they will look at single nucleotide polymorphism (SNP) data to identify DNA regions (loci) that may be undergoing natural selection and will use their creativity to guess possible functions of these genes. Finally, students will develop methods for testing their gene’s function and consider the ability of low elevation pikas to survive in the future.

**Learning Goals:**

1. Reaffirm Mendelian genetics concepts and be able to test a population for inbreeding and understand the consequences of inbreeding on a population
2. Understand what a SNP is and how scientists use SNPs to understand natural selection
3. Improve math and spreadsheet skills, as well as graphing and interpreting data trends
4. Develop a scientific proposal for studying pika adaptation at different elevations

**Prerequisites**

This lesson is intended for an advanced biology audience. Students should be familiar with the structure of DNA, alleles, genes, loci, heterozygosity, inbreeding, and have a general sense for how natural selection works prior to this lesson. ScienceLIVE’s Pika Body Size and Adaptation lesson (available at [www.science-live.org/teachers](http://www.science-live.org/teachers)) is a nice adaptation introduction lesson.

**Materials**

* This is a computer-based lesson that should be conducted in a computer lab in which students can work individually or in small groups. The lesson uses Google Spreadsheets, however a Google account is not necessary to use that data. You can log in using scienceliveoutreach, password: k\*12class if needed.
* Students will receive a worksheet and an instruction sheet, available at [www.science-live.org/teachers](http://www.science-live.org/teachers).

**Standards**

**High School Next Generation Science Standards Addressed\*:**

* HS-LS3-3. Variation and distribution of expressed traits in a population.
* HS-LS4-2. Evolution.
* HS-LS4-3. Advantageous heritable traits.
* HS-LS4-4. Natural selection.
	+ LS4.C: Adaptation

**Part 1: Inbreeding on an Elevational Gradient**

1. To begin this exercise, it is helpful to start with some basic Punnett squares to demonstrate the following (these can be for any sample trait you choose):
	1. Tt x Tt = 1:2:1 ratio, with 50% of offspring heterozygous
	2. TT x tt = All heterozygous offspring
	3. TT x Tt = 50% heterozygous
	4. tt x Tt = 50% heterozygous
	5. TT x TT = no heterozygotes
	6. tt x tt = no heterozygotes
2. This demonstration should remind students that heterozygotes are made through many different parental combinations, and in a healthy, non-inbred population, heterozygosity should be relatively high. The lower the heterozygosity, the more likely the population has been subject to inbreeding.
3. Next, lead students in a discussion for what might cause inbreeding in a population. The goal here is to lead students to the idea that inbreeding occurs due to reproductive isolation of some kind. This can be because populations are small, far apart, or both (along with many other social reasons, but we’ll focus on these two).
4. Usually we talk about heterozygosity at particular loci (locations) or genes (like Mendel’s yellow and green peas). In this lesson we will focus on specific loci that are the sites of single nucleotide polymorphisms (SNPs, or snips). The University of Utah has put together a nice description and demonstration of SNPs, which you can find here: <http://learn.genetics.utah.edu/content/pharma/snips/> If your students aren’t familiar with SNPs, having them run through the “What is a SNP” chapter on this site should help. This can also be a nice chance to review mutations and the errors in cellular processes that could lead to an initial nucleotide substitution in an individual.
5. You can now introduce students to pikas via this video: (<https://vimeo.com/122452931>)
6. The first question students will be answering today is whether pika populations at all elevations in the North Cascades are healthy, or if some populations are too small and fragmented, causing inbreeding. To answer this, students will follow the instruction sheet to summarize and graph heterozygosity data in Google Spreadsheets.
7. This part of the lesson is fairly cookie-cutter, leading students step-by-step through the analysis and graphing exercise. We STRONGLY recommend that you run through this lesson on your own before class, so you are ready to troubleshoot with students.
8. Their final graph should be something like this:
9. *Question 15:* Students should notice that heterozygosity increases with elevation, meaning low elevation populations are the most inbred. This is likely due to a lack of population connectivity at low elevations, since pikas are more suited to cooler, wetter climates at higher elevations. The less suitable habitat at low elevations probably leads to less successful movement between populations, as well as smaller populations, both of which increase the likelihood of mating with a close relative.

**Part 2: Identifying adaptive loci (SNPs)**

We often teach genetics as Mendel first studied the subject – we know there is a “heritable unit” for green peas and a “heritable unit” for yellow peas, one is dominant over the other, etc. But Mendel didn’t know there were genes! Others figured out that DNA was behind the scenes of these phenotypes. Now we know there are genes at different loci, comprised of alleles that represent different nucleotide sequences at a specific locus. Genetic analyses have gotten more complicated since Mendel’s day. One reason for this is that we often have to work backwards to figure out what a gene does. Often, genetic analyses are done as follows:

* Step One: Find regions of the genome that don’t look like the rest – regions that show signs of selection because their allelic frequencies are too high or low, relative to the rest of the genome (remember ~95% of our genetic code doesn’t directly code for proteins). Because the non-coding regions of DNA are changing at a background, normal rate of mutation, any region (locus) that shows substantially more or fewer changes than this background rate gives us a sign that it may be undergoing selection.
* Step Two: Once outlier loci have been identified, scientists then experiment to figure out what those regions do through correlation of different phenotypes. The Utah SNP site has a nice description of this process as well, under the “What is a Haplotype” and “Applying SNP Profiles to Drug Choices” chapters of the SNP site.

Today we are going to see if we can pick out regions of the pika genome in North Cascades National Park that appear to be undergoing selection based on elevation. You can begin this section of the lesson by showing this overview of ecological genetics research in the North Cascades: <https://vimeo.com/122543923>

1. After the very recipe-like Part 1, Part 2 of this lesson is designed to give students more freedom to develop an analysis plan and theories about elevational adaptation. Students should read the introductory page (Page 5 of the Instruction Sheet) and develop a plan for calculating allele frequencies. You may want to have them discuss their plan before enacting it.
2. They can achieve allele frequencies fairly easily, by using the =COUNT() and =COUNTIF() functions in Google Spreadsheets. The most efficient way of doing this is to use this equation:

 =COUNTIF(B2:C9,”1”)/COUNT(B2:C9)

 and

 =COUNTIF(B2:C9,”2”)/COUNT(B2:C9)

This will calculate the proportion of alleles present in low elevation populations that are Allele 1 and Allele 2, respectively. Note that the sum of the frequency of Allele 1 and Allele 2 should equal 1.

1. Once students have calculated all their frequencies, they should get the following:

|  |  |  |  |
| --- | --- | --- | --- |
|  | SNP 1306 | SNP 3453 | SNP 3606 |
|  | Allele 1 | Allele 2 | Allele 1 | Allele 2 | Allele 1 | Allele 2 |
| Low | 0 | 1 | 0.5 | 0.5 | 1 | 0 |
| Mid-Lo | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 0 |
| Mid-Hi | 0.4 | 0.6 | 0.5 | 0.5 | 1 | 0 |
| High | 0.95 | 0.05 | 0.5 | 0.5 | 0.95 | 0.05 |

1. These three SNP loci show very different patterns. SNP 1306 shows clear elevational selection, with Allele 1 becoming more prevalent as you move up the mountain, and Allele 2 showing the opposite trend. Students should note this on their worksheets, indicating that Allele 1 appears to be a high-altitude adaptation of some sort, while Allele 2 is a low-altitude adaptation (though again, these are two sides of the same coin).
2. For *Question 3* on the students’ worksheet, they can come up with any number of gene functions. These can be creative and fun, but we would encourage you to steer students toward what a gene actually does: coding for protein. So if students think the gene impacts fur thickness, perhaps there is a stronger keratin protein created by this gene. If students think that Allele 1 of the gene aids pikas in acquiring oxygen, perhaps it codes for one of hemoglobin’s subunits. Again, there are myriad possibilities here!
3. For *Question 4* on the worksheet, students should think about how to figure out what a gene of interest does. Most scientists begin by collecting lots of data – in this case you would need to get lots of pika genetic samples and, if we go with the hair thickness hypothesis, they would need hair samples from each of them as well. Scientists would then analyze the keratin content of all the hair samples and run statistics to determine if the presence of Allele 1 on SNP 1306 correlates to higher keratin levels in hair. There are many ways to achieve this, but in all cases the animals’ phenotypes (e.g. hair thickness) will need to be tested based on genotype.
4. What about the other two SNPs? In *Question 5*, students should note that neither of the other two loci appear to be adaptive for elevation. In the case of SNP 3453, nearly every individual is heterozygous at the locus, while for SNP 3603, only one individual has Allele 2. This could mean that Allele 2 is either a very new mutation or has been strongly selected against throughout these pika populations.
5. For *Question 6*, there is no one right answer. Our inbreeding analyses indicate that low elevation populations aren’t doing as well as high elevations, and yet finding an adaptive locus like SNP 1306 is a sign of hope that there may be adaptive potential in these populations. It really comes down to what the genes at this adaptive locus and others like it do. If they represent minor changes in pikas’ phenotypes, it may not be enough to save these populations, but if the adaptation represents a large improvement in survival or reproductive success at low elevations, they may be able to sustain further environmental change.