Buddies or Siblings? Determining Relatedness in Anegada Iguanas

Funding for this project was provided by a grant from the Institute of Museum and Library Services.

Objective: The following lesson helps introduce students to methods for examining microsatellites to determine relatedness.

Summary: This activity will introduce students to terminologies and methodologies associated with using microsatellites as a way to assess relatedness of animals of unknown parentage.

Grade level: These activities are designed for secondary students (ages 14 to 18) with knowledge of basic genetics. Although the activities themselves are fairly basic, the subject matter on which they are based is very complex. Previous knowledge of genetic concepts, including the vocabulary, is an important prerequisite for using this lesson.

Time: Approximately 45 to 60 minutes to read the introduction, do problem sets, and discuss the results.

Subject: Science

Skills: Students will use the lesson to improve their conceptual understanding of how genetic diversity, specifically of microsatellites, can be used to understand relatedness of individuals.

Learning objectives: Students should be able to discuss the reasoning for their answers on the third exercise using the terms from the vocabulary list.

Vocabulary: microsatellites*, genome, markers, locus (plural: loci), allele**, homozygous, heterozygous, genetic diversity.

*Definition for **microsatellites**: Microsatellites are defined by the *Merriam-Webster Medical Dictionary* as “any of numerous short segments of DNA that are distributed throughout the **genome**, that consist of repeated sequences of usually two to five nucleotides, and that are often useful **markers** in studies of genetic linkage because they tend to vary from one individual to another.” The nucleotides found in DNA are adenine (A), thymine (T), guanine (G), and cytosine (C).

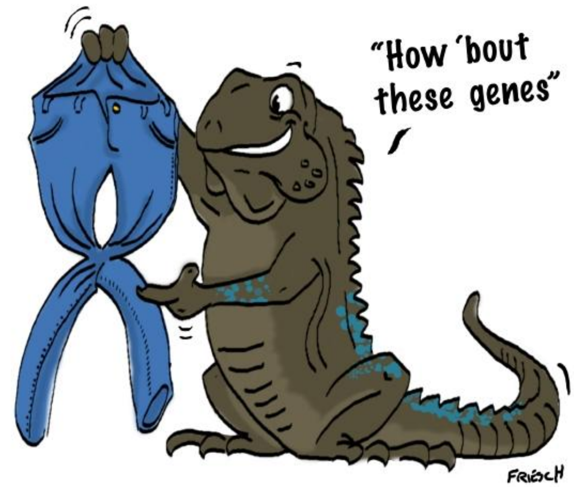
Definition for **allele: Alternative forms of a genetic locus; a single allele for each locus is inherited separately from each parent. We defined allele specifically because the typical definition describes it as alternate forms of a gene. But in the case of microsatellites, they are not genes, but molecular markers.

Teacher Background

In humans, microsatellites are often used for criminal investigations to link genetic information found in tissue, hair, blood, or other biological materials to an individual. In animals (as well as humans), microsatellites can help determine relatedness of individuals (called paternity identification when used for determining the lineage on the father’s side). The place where a microsatellite occurs is called a **locus**. To determine relatedness, scientists must look at the microsatellites at as many **loci** (plural of locus) as possible. At each locus, an individual inherits one marker, or **allele,** from his/her father and one allele from his/her mother. These two alleles can have microsatellites of the same size (termed **homozygous**) or different sizes (**heterozygous**). For example, if at a specific locus there are two types of microsatellites found, one being AGAGAGAGAG (allele 1 which has AG repeated 5 times, or $(AG)_5$) and the other being AGAGAGAGAGAG (allele 2 which has AG repeated 6 times, or $(AG)_6$), then the locus is called heterozygous. These are the most useful for studies of relatedness. For the Anegada iguana, scientists looked at about 50 loci. Only about half were found to be useful; that is, they were heterozygous. The amount of variety in the microsatellites is one measure of **genetic diversity**. Genetic diversity can be measured both within an individual and within a population. It is important to have a certain amount of genetic diversity within a population for that population to be healthy and sustainable.

Student Activity

Microsatellites help scientists determine which animals are related and which ones are not. At the San Diego Zoo, scientists used these molecular markers to find out if six confiscated Anegada iguanas were related.



Read the following introduction and complete the three exercises. These exercises will help you better understand and discuss the genetic methodology for using microsatellites to determine relatedness.

Introduction:
When six confiscated Anegada iguanas were given to the San Diego Zoo by authorities, it was a mixed blessing. On one hand, these six individuals were the only representatives of the critically endangered species outside of the British Virgin Islands. And if keepers at the Zoo could get them to breed, the iguanas would become the founders of a captive population, serving to safeguard against the loss of the few hundred individuals left on the island of Anegada. But first the Zoo had to know if these iguanas were closely related.

To get to the bottom of this mystery, scientists knew they would have to compare the genetic diversity of the six iguanas to those of the wild population. For determining relatedness, scientists can look at parts of the genome called microsatellites. Microsatellites are strings of DNA that repeat a sequence of nucleotides (the building block of DNA). For example, one microsatellite could be AGAGAGAGAGAGAG, which could be written as $(AG)_7$ (AG repeated 7 times). Like most animals, including humans, iguanas have two microsatellites at each locus (or position) on the chromosome where a microsatellite occurs: one microsatellite from each parent.

Determining relatedness takes more than just knowing where the microsatellites are; it takes genetic diversity, meaning there has to be some kind of uniqueness within those microsatellites. For example, if all the iguanas have the microsatellite $(AG)_7$ at one locus, it might mean that they are all siblings or it might not. Only by looking at many loci (the plural of locus) can scientists determine how related, if at all, animals are. Say you were trying to determine who was related in a room full of people. Using one characteristic, such as hair color, wouldn't help very much. But by using as many characteristics as possible—nose shape, eye color, type of smile, etc.—you would have a much better chance of figuring out who is related. That's why scientists look at microsatellites at as many loci as possible.

Student Exercises

#1. Microsatellites are usually 100 to 400 base pairs long! To understand how microsatellites work, we will look at partial microsatellite sequences.

Examples of partial microsatellite sequences are:
GAGAGAGAGA
ACGACGACGACGACG
CTAGCTAGCTAGCTAG

Complete the following partial microsatellite sequences.
1. AGAGAGAG_ _ _ _ _ _
2. TGATGATGA_ _ _ _ _ _
3. CTCT_T_ _ C _ _ T
4. GACGACG_CGA _ _ AC
5. CTTGCTT_ _ TT_ C_ T _
6. GCATT_ _ A _ T GC _ TT

Bonus
_ _ CAAC _ AACCAACC_

#2. Microsatellites are designated by the number of times they repeat a given sequence. For example, for the sequence AGAGAG, AG is repeated 3 times. It can be shortened to $(AG)_3$. TCGTCGTCGTCGTCGTCG is $(TCG)_6$.

Write the labels for the following sequences next to them.

1. GATGATGATGATGATGAT

2.CTCTCTCTCTCTCTCTCTCT

3. GGTGGTGGTGGTGGTGGTGGT

4. GTGTGTGTGTGTGTGTGTGT

5. CTCTCTCTCTCTCTCT

6. AGTAGTAGTAGTAGT

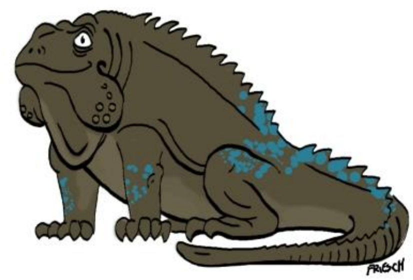
7. AGAGAGAGAGAGAGAGAG

8. CTTCCTTCCTTCCTTCCTTCCTTC

9. GATGATGATGATGAT

10. CTGGCTGGCTGGCTGG

#3. Who is related to whom? At each locus there are two microsatellites, one on each chromosome. Remember, if the microsatellites have repeats of different lengths, the locus is called heterozygous. If the repeats are the same size, the locus is called homozygous. Offspring receive one microsatellite from each parent. In the following example, there are six offspring of unknown paternity. There is only one possible female parent but each of the offspring has two possible fathers. Complete the following exercise to find out which male sired each offspring.



ADULTS

Female
Locus 1 $(AG)_{140}$ and $(AG)_{144}$
Locus 2 $(AG)_{140}$ and $(AG)_{150}$

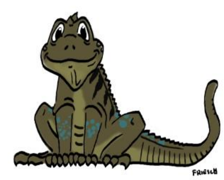
Male A
Locus 1 $(AG)_{144}$ and $(AG)_{150}$
Locus 2 $(AG)_{150}$ and $(AG)_{158}$

Male B
Locus 1 $(AG)_{150}$ and $(AG)_{158}$
Locus 2 $(AG)_{140}$ and $(AG)_{158}$

OFFSPRING

Offspring 1
Locus 1 $(AG)_{140}$ and $(AG)_{144}$
Locus 2 $(AG)_{140}$ and $(AG)_{158}$

Offspring 2
Locus 1 $(AG)_{144}$ and $(AG)_{158}$
Locus 2 $(AG)_{140}$ and $(AG)_{150}$



Offspring 3
Locus 1 $(AG)_{140}$ and $(AG)_{150}$
Locus 2 $(AG)_{150}$ and $(AG)_{150}$

Offspring 4
Locus 1 $(AG)_{144}$ and $(AG)_{150}$
Locus 2 $(AG)_{150}$ and $(AG)_{158}$

Offspring 5
Locus 1 $(AG)_{144}$ and $(AG)_{144}$
Locus 2 $(AG)_{140}$ and $(AG)_{150}$

Offspring 6
Locus 1 $(AG)_{144}$ and $(AG)_{150}$
Locus 2 $(AG)_{140}$ and $(AG)_{140}$

Answer Key

EXERCISE 1:
1. AGAGAG; 2. TGATGA; 3. C,CT,TC; 4. A,CG; 5. GC,G,T,G; 6. GC,T,A; Bonus. AC,C,A

EXERCISE 2:
1. $(GAT)_6$ 2. $(CT)_{10}$ 3. $(GGT)_7$ 4. $(GT)_{10}$ 5. $(CT)_8$ 6. $(AGT)_5$ 7. $(AG)_9$ 8. $(CTTC)_6$ 9. $(GAT)_5$ 10. $(CTGG)_4$

EXERCISE 3:
Offspring 1- Male A; Offspring 2- Male B; Offspring 3- Male A; Offspring 4- Unknown; Offspring 5- Male A; Offspring 6- Male B