Geography and Species Diversity

4. Perplexing Possums

Objective

In managed zoo populations, some animals come with unknown origins. Species identification is the same process when looking at both zoo and wild populations. Using two different restriction enzymes, determine which possum population your unknown possum comes from by observing its DNA fragments.

Background – Natural History

Mountain brushtail possums live in old-growth forests and pass their tree hollow down from one generation to another. These long-lived animals are facing habitat loss due to logging and will not be able to survive the 200 to 500 years it takes to replace these large, old trees. Since the possums do not excavate their own tree hollow, the trees must be old enough for a hollow to have formed naturally. The possums usually claim a hollow large enough for their families after smaller animals have occupied it for some time.

A restriction enzyme is a special kind of protein that breaks apart pieces of DNA. There are many different kinds of restriction enzymes, and all are named differently (e.g., EcoRI, BamHI, HaeIII, DdeI) based on the organism from which they are derived (i.e., EcoRI comes from *E. coli* bacteria).

Restriction Enzymes
Remember that one of the functions of enzymes is to break bonds. Also, remember that DNA is made up of a series of nucleotides with the bases G, C, A, and T. Each enzyme recognizes a different sequence of letters in a strand of DNA. When enzyme Mse I sees the bases TTAA in a segment of DNA, it cuts the DNA between the first and second T. This break then creates DNA fragments. When writing the sequence, we represent the break by using a ^ symbol—for example, T^TAA. The enzyme Hae III, on the other hand, cuts the DNA strand between the G and the C (GG^CC) when it sees GGCC.

Why use restriction enzymes?
Once restriction enzymes have cut up an organism's DNA into smaller fragments, geneticists analyze the fragment lengths. They can use this information much like a fingerprint because the enzyme always cuts the DNA in the same place for the same individual. This is often used at crime scenes to compare the fragments of one suspect to another. This technique is used for many other reasons, for example to distinguish one species from another and to determine paternity. We give these fragments a special name, Restriction Length Fragment Polymorphisms, or RFLPs. A nucleotide polymorphism, or difference in DNA sequence, can alter the place where a restriction enzyme would cut and, therefore, yield different-length DNA fragments.

In this activity, you will be looking at RFLP patterns of mountain brushtail possum *(Trichosurus caninus)* in eastern Australia. On page 23, you will find a map of eastern Australia. The map indicates where four possum populations live in the wet, temperate, old-growth forests on the east coast.

You will examine the DNA of one of the possum populations. Note that scientists use acronyms to name the different populations as follows: Conondale Ranges is abbreviated CD, Whian Whian State Forest is WW, Barrington Tops National Park is BTP, and Cambarville is CB.

Locate the sheet called "Key for Possum Populations Using RFLP Patterns" on page 41. Notice how each population of possums has a different RFLP pattern when its DNA is exposed to specific restriction enzymes. If a scientist found a possum in one of these areas and wanted to know which population it belonged to, or if the scientist found a possum in a contact zone—an area where two populations or species border—he or she could match the DNA of the unknown possum to the patterns listed on this key.

Excerpts from Research Field Notes: 1993 Australia Expedition

Date: *October 24th* Location: *Bellbird, Victoria, Australia*

Arrived in Bellbird today and I was eager to start. The terrain is more difficult than I imagined but the eucalypt forests are beautiful. We set out 10 live traps to catch some possums this evening, all baited with green apples which apparently are a favorite. Will check tomorrow morning for some captures. Equipment for ear clipping and blood draws and radio collars are packed and ready. All samples will be taken right at specific trap site so that all animals can be released where they were caught and find their way back to their dens.

Date: *October 25th* Location: *Bellbird, Victoria, Australia*

Ventured out at 4am to check traps before it gets too hot for the trapped possums. Only one capture last night and he was a feisty one! We took a skin sample from the ear (1mm ear punch), recorded measurements of head, feet and tail and released him. Anesthetic not necessary and animal moving fine after release.

Date: *October 26th* Location: *Bellbird, Victoria, Australia*

Three more captures found this morning. Samples taken from 2 females and another male. One of the females had a young baby in her pouch but only took a quick sample from the mother. All fine after release.

Date: *October 29th* Location: *Cambarville, Victoria, Australia*

Arrived in Cambarville and the weather is perfect. It's hard to believe it's Spring here! We put out 10 more traps and marked them on our local map for retrieval. Will check them tomorrow.

Date: *October 30th* Location: *Cambarville, Victoria, Australia*

Very happy with the captures this morning as we caught 4 last night! 3 males and a female. One of the males must have been a capture for a previous study as his ear was clipped. Took samples for comparison. All animals moving fine after release.

Date: *November 1st* Location: *Sydney, New South Wales, Australia*

We flew to Sydney today and it was nice to see some civilization! We stopped off to mail off some of our samples to the lab and will start our long trip to Barrington Tops tomorrow.

Date: *November 4th* Location: *Allyn River Forest Park (Barrington Tops), New South Wales, Australia*

Arrived in the Allyn River Forest Park today and there is quite a difference here. Can't put my finger on it but the weather is different and the forest is drier here today than in the South. We put out 10 traps and will check tomorrow.

Date: *November 5th* Location: *Allyn River Forest Park (Barrington Tops), New South Wales, Australia*

Trapping was fair today as we caught 3 individuals. All were measured and I was surprised by longer tails and shorter toes. I wonder if this is a difference that all of the possums up here have. After seeing this, we're going to wait another day and see if we can catch a few more individuals. All animals ok after release.

Date: *November 6th* Location: *Allyn River Forest Park (Barrington Tops), New South Wales, Australia*

Caught 2 more individuals, both having tail and toe phenotypes like ones we measured yesterday. Will be interesting to see if we see more of the same measurements as we move North. Animals moving fine after release.

Date: *November 8th* Location: *Whian Whian, New South Wales, Australia*

Arrived in Whian Whian this morning and started a treacherous trek through the forest. Almost fell down a ravine this morning! Set out 10 traps and baited them. Will check tomorrow morning.

Date: *November 9th* Location: *Whian Whian, New South Wales, Australia*
Caught 3 individuals, all males, and collected samples. Noticing similar phenotypic features here as in Barrington Tops. Tails especially different than the Southern possums. Will remain another day to see if we catch any others. Animals moving quickly about after release.

Date: *November 10th* Location: *Whian Whian, New South Wales, Australia*
Caught 2 more individuals last night. These were females and their tails were very obviously different than what we saw in the South. Took morphometric data and collected samples. They both were moving fine as we released them.

Date: *November 13th* Location: *Conondale Ranges, Queensland, Australia*
We quickly stopped in Brisbane for supplies (almost forgot the apples!) and to have the truck fixed before we could move on to Conondale Ranges. We set out the usual 10 traps and will trudge back to check on them tomorrow. Difficult terrain!

Date: *November 14th* Location: *Conondale Ranges, Queensland, Australia*
We caught 4 individuals last night, a great sampling! We took samples and there is no doubt that there are some significant differences between these and what we saw in Cambarville and Bellbird. Caught a female with an older offspring on her back. No sample taken from either and both were immediately released to be sure they made it back together to their den. One of the other animals was a bit slow moving after release but all made off pretty quickly.

East coast of Australia. All populations are labeled except for the Barrington Tops population, which is found within the Allyn River Forest Park.

On the map on the left draw lines using a straight edge or ruler and trace the trip of the researchers. Number each stop they made in chronological order while reading the field notes.

Procedure

You will use morphology, or physical measurements, in order to correlate any significant differences in these populations. Using both morphology and genetics, you should be able to determine to which species your sample belongs. Each student in a group of four (4) should have a different population's sheet.

Morphology

1) Read through the field notes, which will help you understand where the researchers traveled and from where they collected each sample and measurements.

2) Using the photographs provided, take the measurements in centimeters (cm) and record this data on the data tables on page 43. Remember to calculate the average by adding up the measurements for each category and dividing it by the number of samples.

3) Compare data sheets with other members of your group to distinguish measurements. If there are similarities between your measurements, they may be the same species.

Genetic Data Analysis

1) Locate DNA strand No. 1 on page 33. Imagine that this is one connected, continuous strand of DNA and that there are no breaks between the bases (letters). The numbers 10, 20, 30, etc. listed above these bases serve only as a way to more easily count the bases.

2) Imagine that you are adding restriction enzyme MseI to DNA strand No. 1. Remember that MseI recognizes the sequence TTAA and cuts the strand after the first T. To show that the strand is being cut at that point, put a ^ after the first T in each sequence of TTAA in the strand. The enzyme continues to cut TTAA every time it comes to it.

3) Once you have cut the strand, count the number of bases in each fragment. Start from the beginning and count until the DNA is cut (^). Begin counting the next fragment from the very first base after the break. Always start counting each fragment with the number one. Count the bases in this manner through the end of the strand.

4) Record your counting data in the box labeled MseI at the bottom of the page. Using the fragment size guide as a ruler, put a small horizontal mark in the box next to the corresponding number. For example, if your fragment is 20 bases long, make a mark in the box that lines up with the number 20. If you have only six bases in a fragment, estimate where that mark would fall between 0 and 10.

   You should end up with a box that has four or five marks. There are several different strands of DNA being examined here, so don't be surprised if the person next to you has a different pattern of bands.

5) Using the fragments you have just recorded, refer to your Possum RFLP Patterns Key to see if you can determine to which possum population your DNA belongs. Once each student determines the origin for each sequence, give the sheet to the student with that population.

6) It may be that one restriction enzyme is not enough to make a positive identification. One enzyme could produce similar size fragments in two populations. To be sure, scientists run the same sample in more than one enzyme for comparison. Repeat the entire process above using a different restriction enzyme called HaeIII and cut up strand No. 2. Record the fragment sizes in the other box at the bottom of the page.

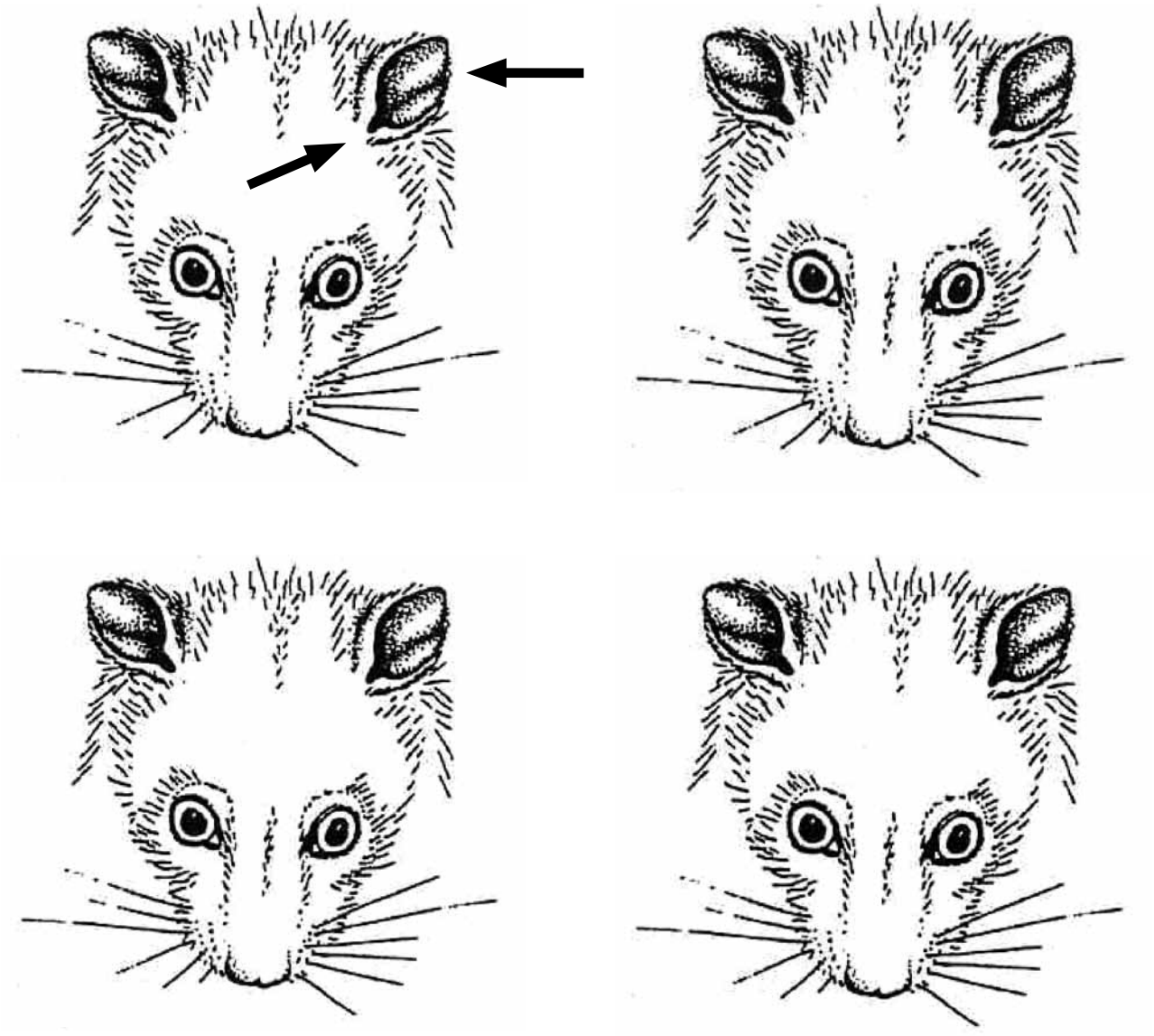
7) Again, use the key to determine the possum DNA that you have. Write your final answer in the box on the bottom of your data sheet. Once you have determined the species, obtain the corresponding morphometric sheets from your teacher and follow the steps below.

8) Using the map, note where your possum samples originated and circle the location on the map. After comparing the measurements and DNA from everyone in your group, place an X on the coast where you think the two species of pos sums are separated.

Morphometric Data Collection: Conondale Ranges Population (CD)

Using the diagrams below, take metric measurements (in centimeters) of each and log your samples into your Morphometric Data Table.

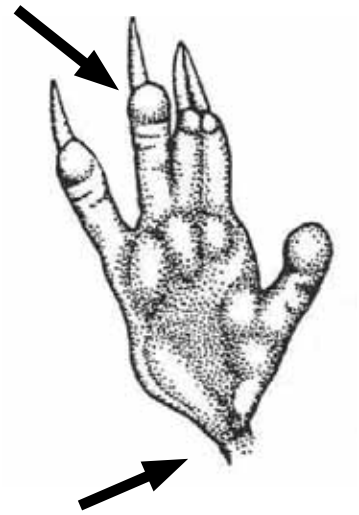
Ear morphometric data collection: Using the following possums, measure each ear, both left and right, from notch to tip of the pinna (external ear). Use arrows as a guide.



Morphometric Data Collection: Conondale Ranges Population (CD)

Using the diagrams below, take metric measurements (in centimeters) of each and log your samples into your Morphometric Data Table.

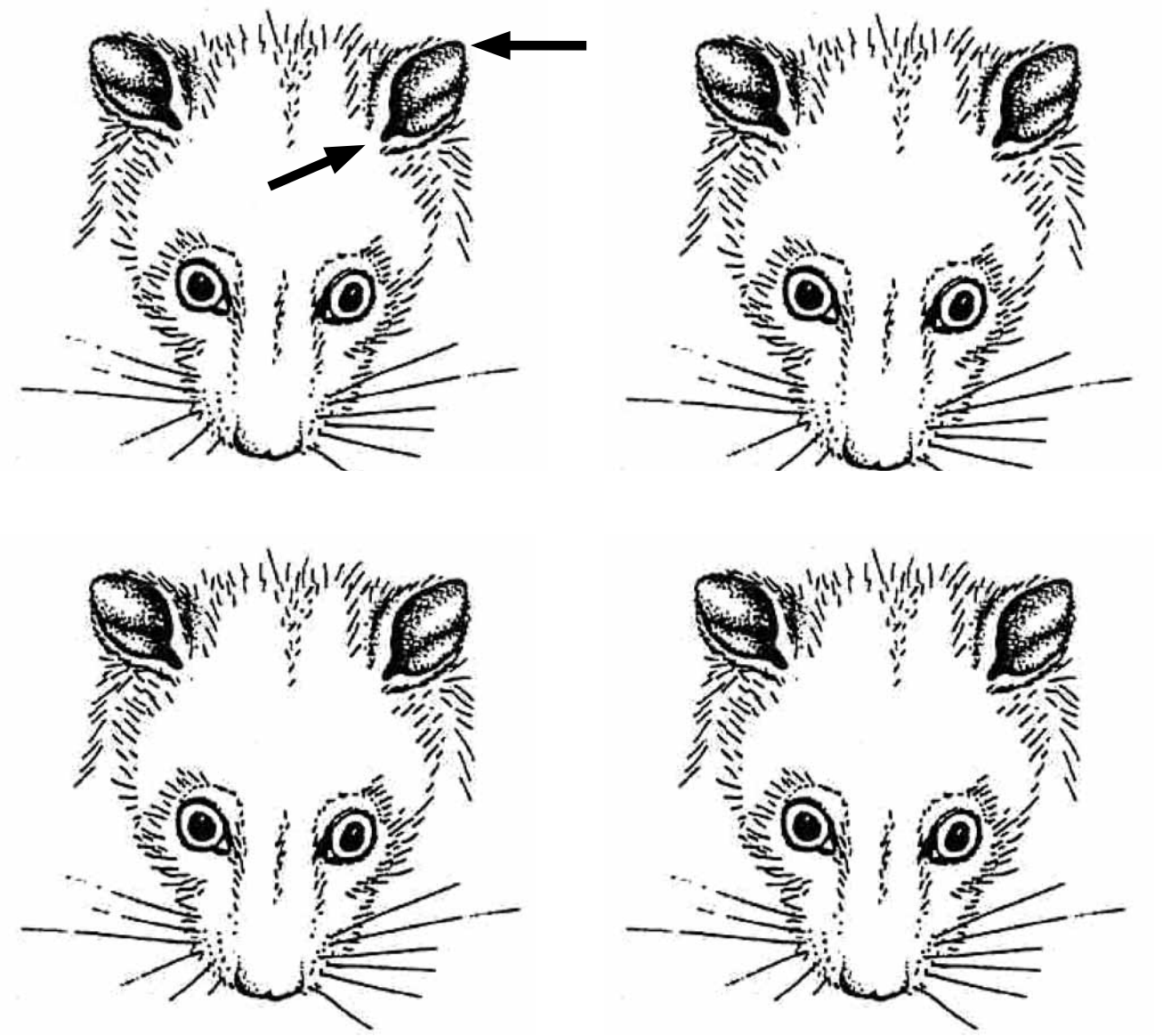
Ear morphometric data collection: Using the following diagrams, measure each foot by using the arrows as a guide. Do not include the toenail as that can be variable in each individual.



Morphometric Data Collection: Whian Whian Population (WW)

Using the diagrams below, take metric measurements (in centimeters) of each and log your samples into your Morphometric Data Table.

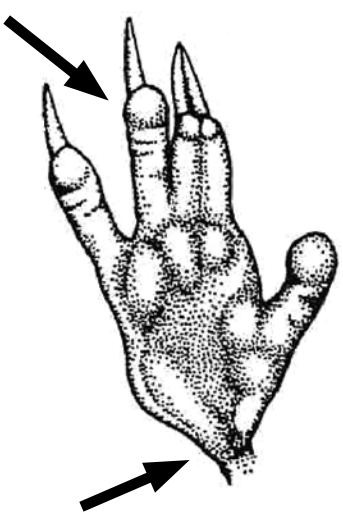
Ear morphometric data collection: Using the following possums, measure each ear, both left and right, from the notch to the tip of the pinna (external ear). Use the arrows as a guide.

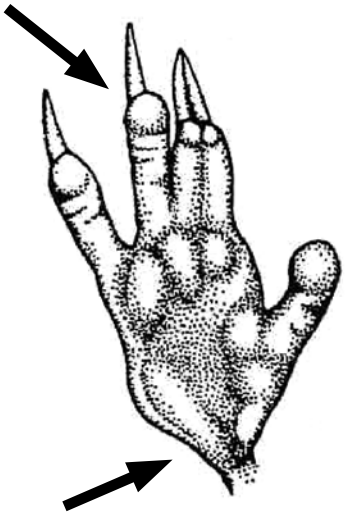


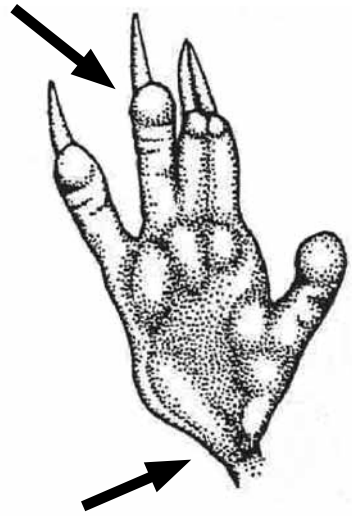
Morphometric Data Collection: Whian Whian Population (WW)

Using the diagrams below, take metric measurements (in centimeters) of each and log your samples into your Morphometric Data Table.

Ear morphometric data collection: Using the following diagrams, measure each foot by using the arrows as a guide. Do not include the toenail as that can be variable in each individual.



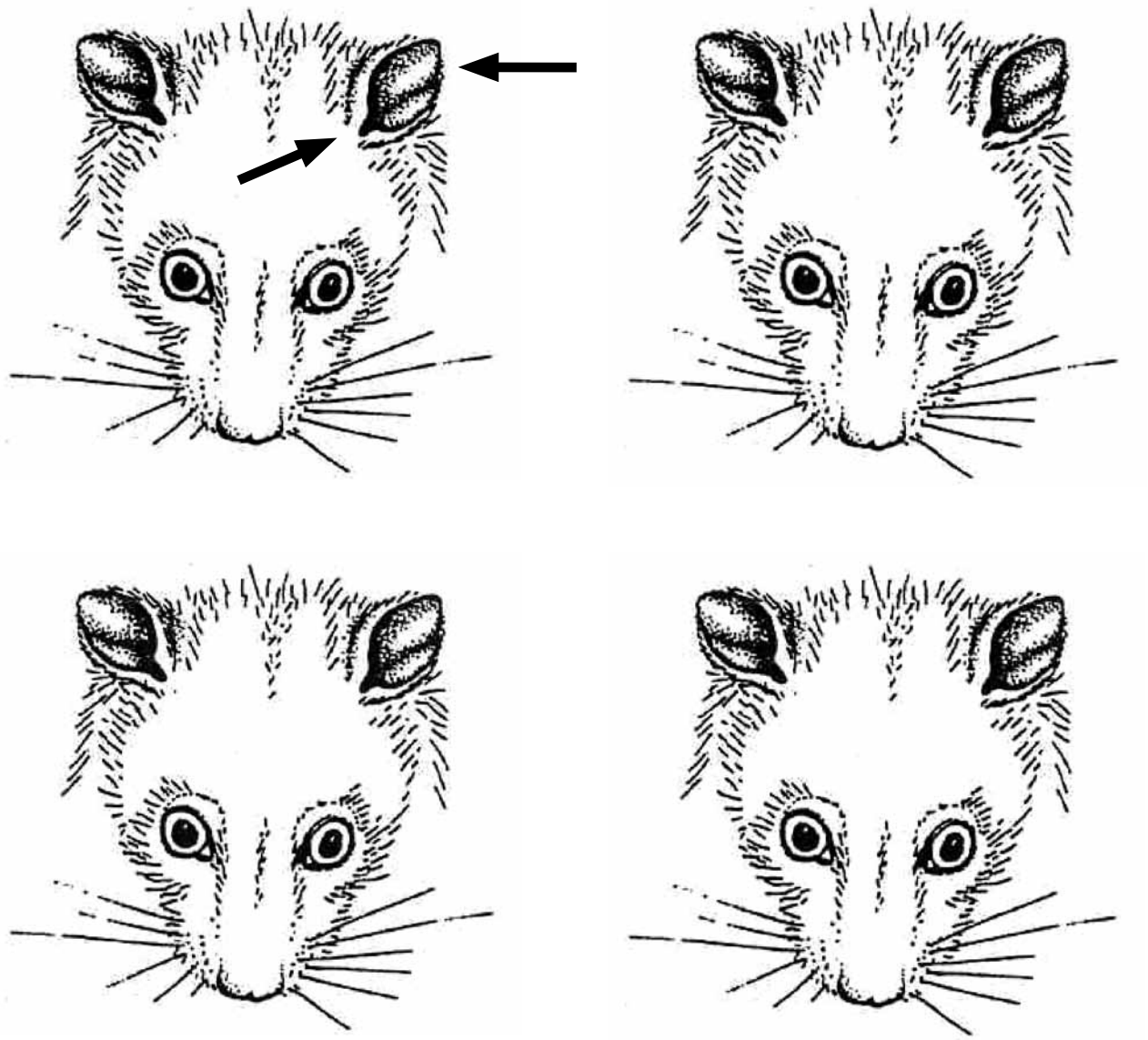




Morphometric Data Collection: Barrington Tops Population (BTP)

Using the diagrams below, take metric measurements (in centimeters) of each and log your samples into your Morphometric Data Table.

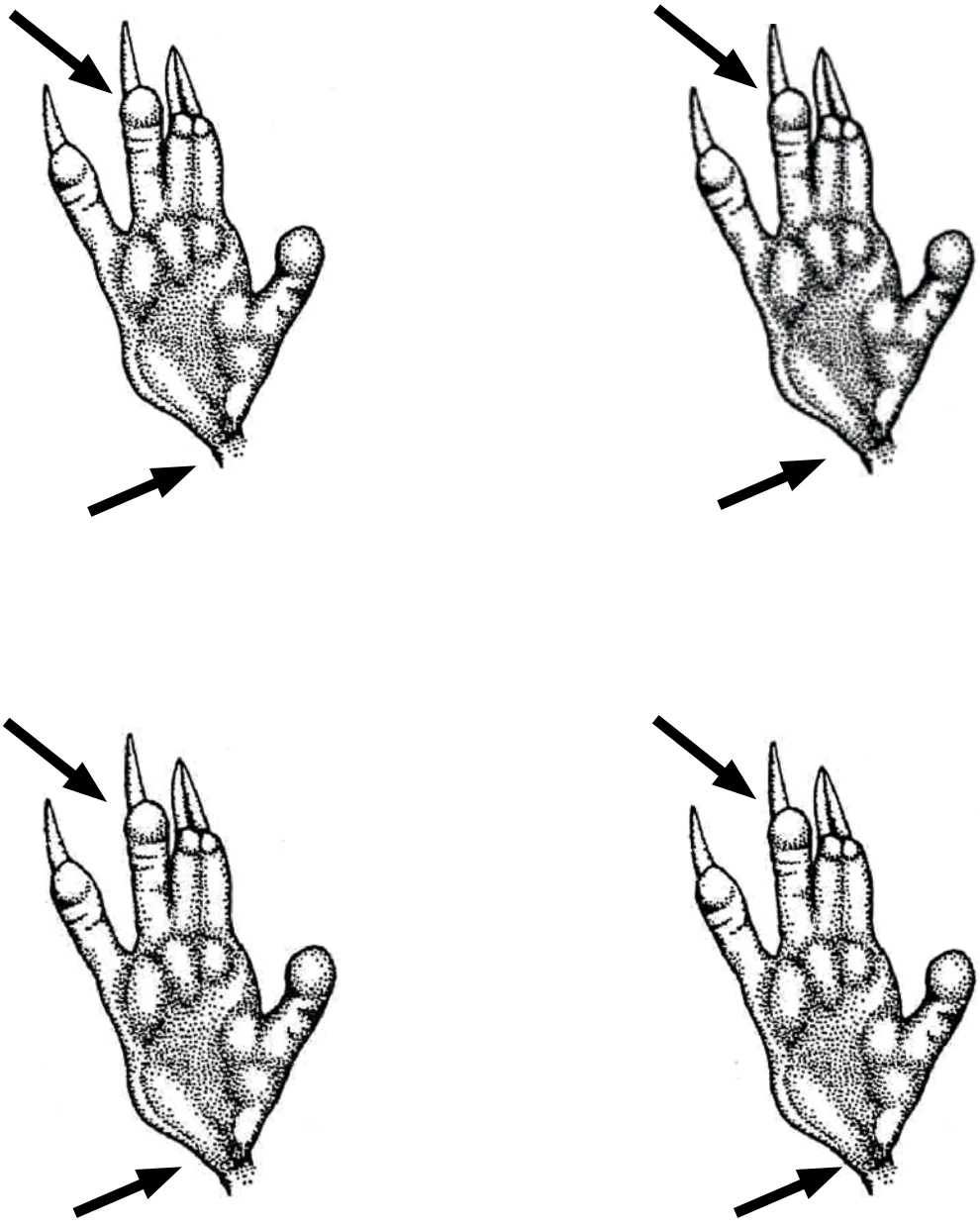
Ear morphometric data collection: Using the following possums, measure each ear, both left and right, from notch to tip of the pinna (external ear). Use arrows as a guide.



Morphometric Data Collection: Barrington Tops Population (BTP)

Using the diagrams below, take metric measurements (in centimeters) of each and log your samples into your Morphometric Data Table.

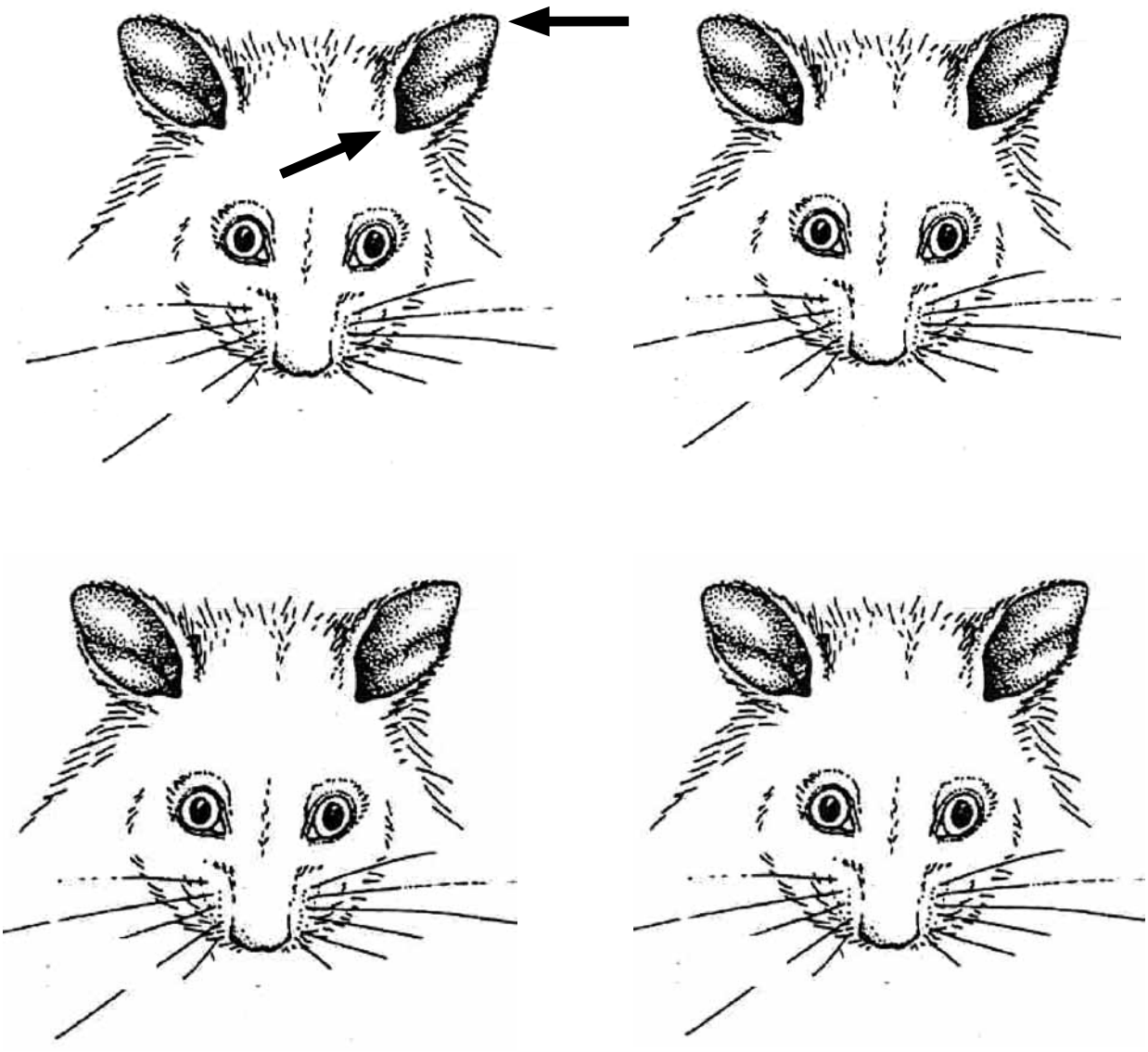
Ear morphometric data collection: Using the following diagrams, measure each foot by using the arrows as a guide. Do not include the toenail as that can be variable in each individual.



Morphometric Data Collection: Cambarville Population (CB)

Using the diagrams below, take metric measurements (in centimeters) of each and log your samples into your Morphometric Data Table.

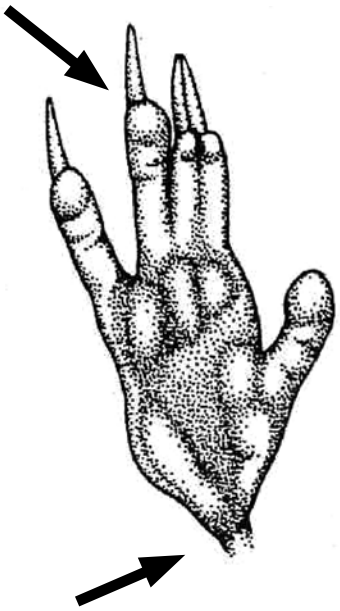
Ear morphometric data collection: Using the following possums, measure each ear, both left and right, from notch to tip of the pinna (external ear). Use arrows as a guide.

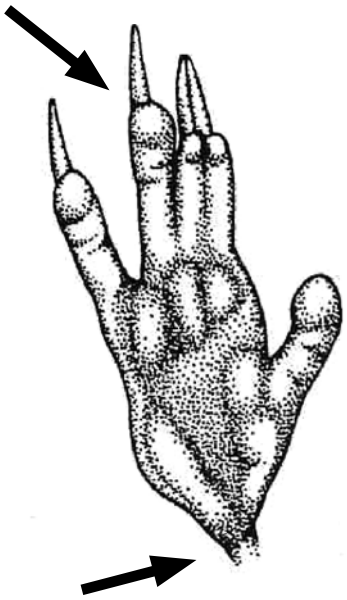


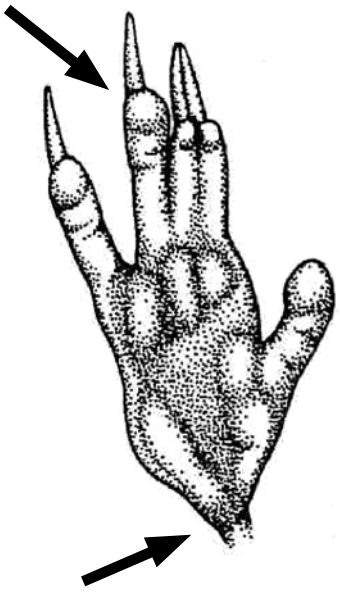
Morphometric Data Collection: Cambarville Population (CB)

Using the diagrams below, take metric measurements (in centimeters) of each and log your samples into your Morphometric Data Table.

Ear morphometric data collection: Using the following diagrams, measure each foot by using the arrows as a guide. Do not include the toenail as that can be variable in each individual.







Restriction Enzyme Data Sheet — Population #1

Strand #1

T^TAA

Mse I

```
        10         20         30         40         50         60
ATGATTAATA TTCGCAAAAC ACACTTAAGG CCTCAAATTA TCAACGACTC ATTCATTGAT

        70         80         90        100        110        120
TTACCCACAC CATGGCCTAT CCTTAAGGGA TGAAACTTCG GTTCACTCCT AGGAGTATGC

       130        140        150        160        170        180
TGCATCATTC AAAGGCCCAC AGGCTTATCT TAAGCAATAC ATTATACGTC AGACACACTA

       190        200        210        220        230        240
ACCGCATTTT CATCAGTAGC CCATATCTGC CGAGATGTGA ATTATGGAAG ..........
```

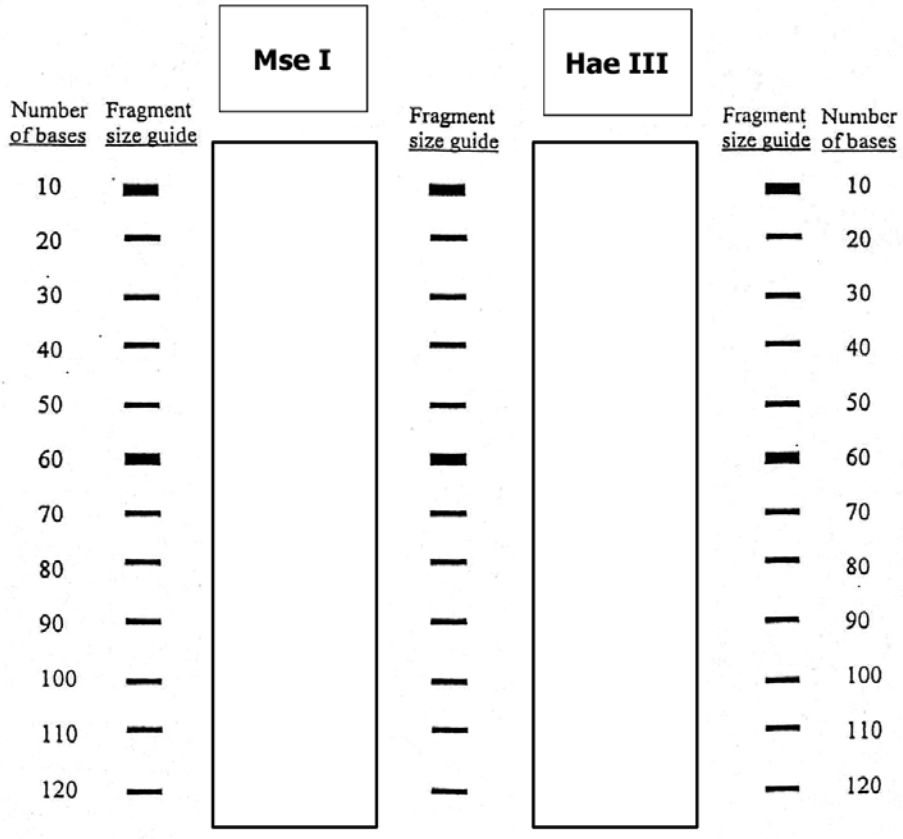
Strand #2

GG^CC

Hae III

```
        10         20         30         40         50         60
ATGATTAATA TTCGCAAAAC ACACTTAAGG CCTCAAATTA TCAACGACTC ATTCATTGAT

        70         80         90        100        110        120
TTACCCACAC CATGGCCTAT CCTTAAGGGA TGAAACTTCG GTTCACTCCT AGGAGTATGC

       130        140        150        160        170        180
TGCATCATTC AAAGGCCCAC AGGCTTATCT TAAGCAATAC ATTATACGTC AGACACACTA

       190        200        210        220        230        240
ACCGCATTTT CATCAGTAGC CCATATCTGC CGAGATGTGA ATTATGGAAG ..........
```

My DNA sample comes from the _____ possum population.



Restriction Enzyme Data Sheet — Population #2

Strand #1

T^TAA

Mse I

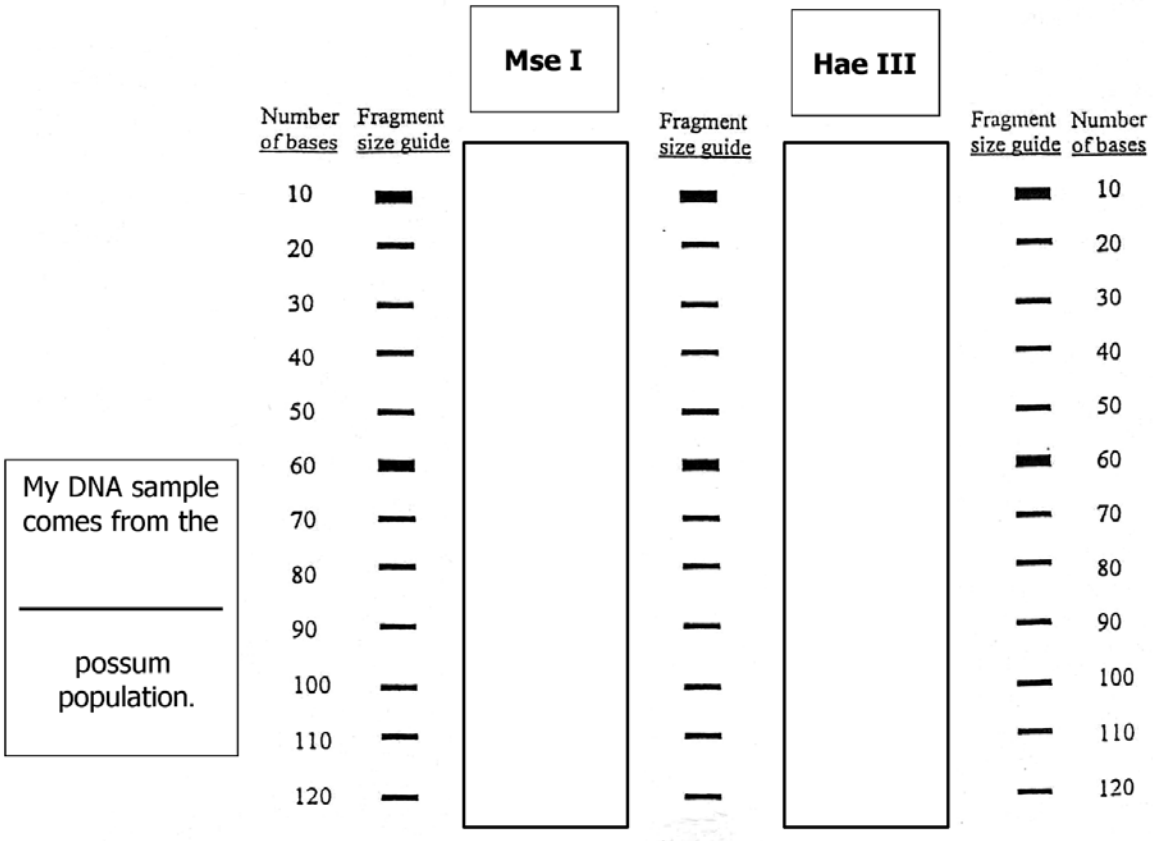
```
        10         20         30         40         50         60
ATGACCAATA TTCGCTTAAC ACACCCACTC ATAAATTAAA TCAACGACTC ATTCATTGGG

        70         80         90        100        110        120
CCACCCACAC CATCTAACAT CTCAGCTTGA TGATTAATCG GTTCACTCCT AGGAGTATGC

       130        140        150        160        170        180
TGCATCATTC AAAGGCCTAC AGGCTTATTC TTAGCAATAC ATTAAGCGTC AGACACACTA

       190        200        210        220        230        240
ACCGCATTTT CATCAGTAGC CCATATCTGC CGAGATGTGA ATTATGGAAG ..........
```

Strand #2

GG^CC

Hae III

```
        10         20         30         40         50         60
ATGACCAATA TTCGCTTAAC ACACCCACTC ATAAATTAAA TCAACGACTC ATTCATTGGG

        70         80         90        100        110        120
CCACCCACAC CATCTAACAT CTCAGCTTGA TGATTAATCG GTTCACTCCT AGGAGTATGC

       130        140        150        160        170        180
TGCATCATTC AAAGGCCTAC AGGCTTATTC TTAGCAATAC ATTAAGCGTC AGACACACTA

       190        200        210        220        230        240
ACCGCATTTT CATCAGTAGC CCATATCTGC CGAGATGTGA ATTATGGAAG ..........
```



Restriction Enzyme Data Sheet — Population #3

Strand #1

T^TAA

Mse I

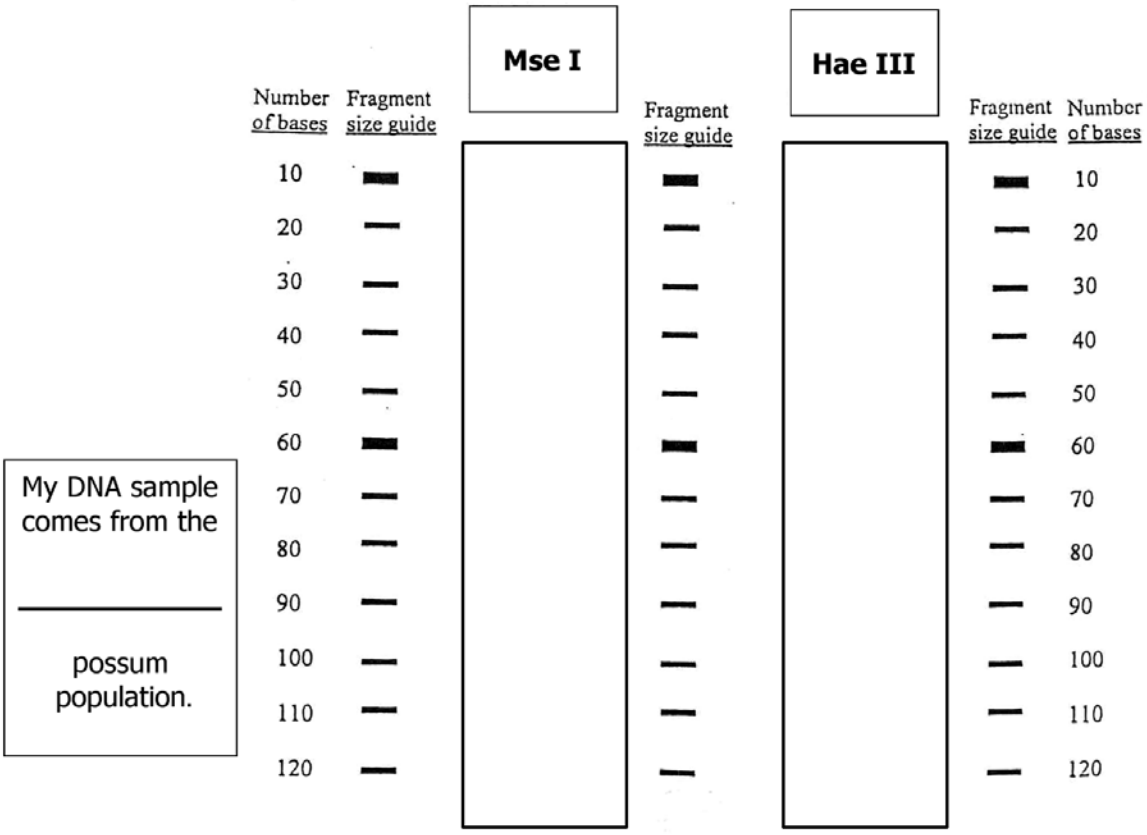
```
        10         20         30         40         50         60
ATTGGCCATA TTCGCATTTA ACACCCACGG CCAAAAATTA TCAACGACTC ATTCATTGAT

        70         80         90        100        110        120
GGTTAAACAC CATCTAACGG CCTAGCTTGA TGAAACTTCG GTTCACTCCT AGGAGTATTA

       130        140        150        160        170        180
AGCATCATTC AAATCCTCGG CCGCTTATTC TTAGCAATAC ATTATACGTC AGACACACTA

       190        200        210        220        230        240
ACCGCATTTT CATCAGTAGC CCATATCTGC CGAGATGTGA ATTATGGAAG ..........
```

Strand #2

GG^CC

Hae III

```
        10         20         30         40         50         60
ATTGGCCATA TTCGCATTTA ACACCCACGG CCAAAAATTA TCAACGACTC ATTCATTGAT

        70         80         90        100        110        120
GGTTAAACAC CATCTAACGG CCTAGCTTGA TGAAACTTCG GTTCACTCCT AGGAGTATTA

       130        140        150        160        170        180
AGCATCATTC AAATCCTCGG CCGCTTATTC TTAGCAATAC ATTATACGTC AGACACACTA

       190        200        210        220        230        240
ACCGCATTTT CATCAGTAGC CCATATCTGC CGAGATGTGA ATTATGGAAG ..........
```



Restriction Enzyme Data Sheet — Population #4

Strand #1

T^TAA

Mse I

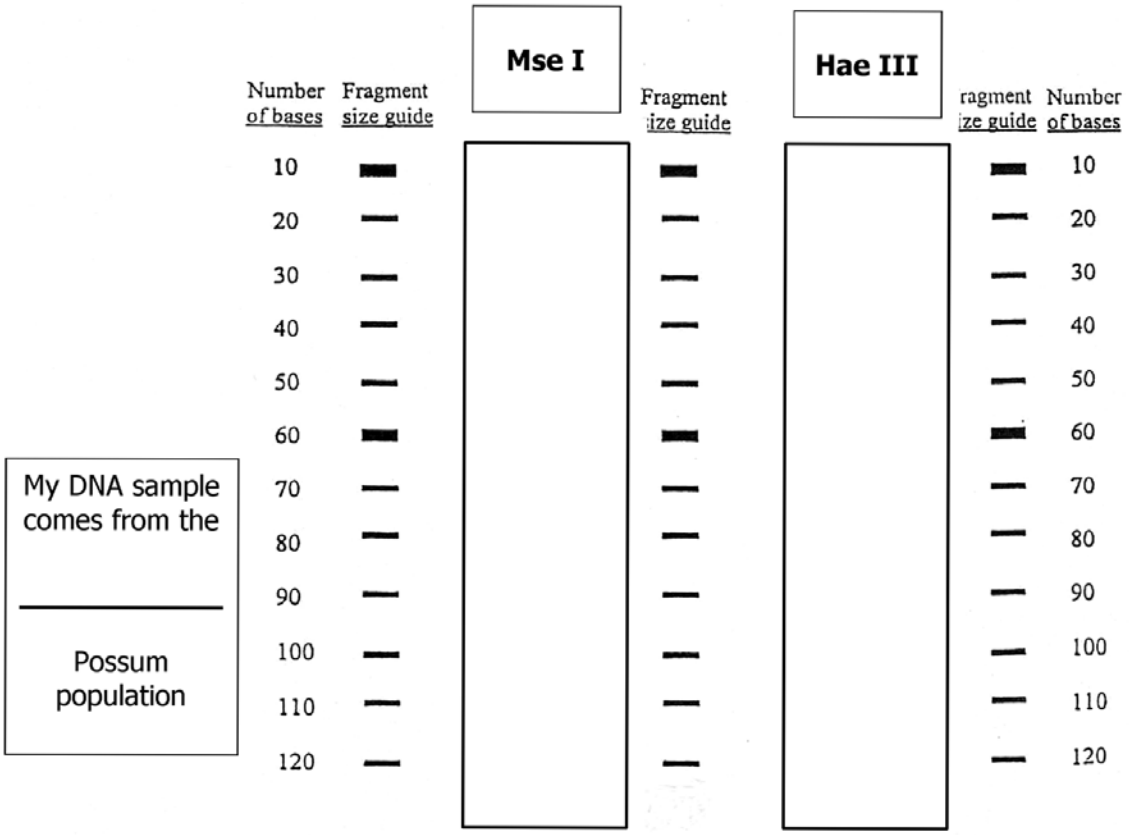
```
        10         20         30         40         50         60
ATGACCAATA TTCGCTTAAC ACACCCACTC ATAAATTAAA TCAACGACTC ATTCATTGGG

        70         80         90        100        110        120
CCACCCACAC CATCTAACAT CTCAGCTTGA TGATTAATCG GTTCACTCCT AGGAGTATGC

       130        140        150        160        170        180
TGCATCATTC AAAGGCCTAC AGGCTTATTC TTAGCAATAC ATTAAGCGTC AGACACACTA

       190        200        210        220        230        240
ACCGCATTTT CATCAGTAGC CCATATCTGC CGAGATGTGA ATTATGGAAG ..........
```

Strand #2

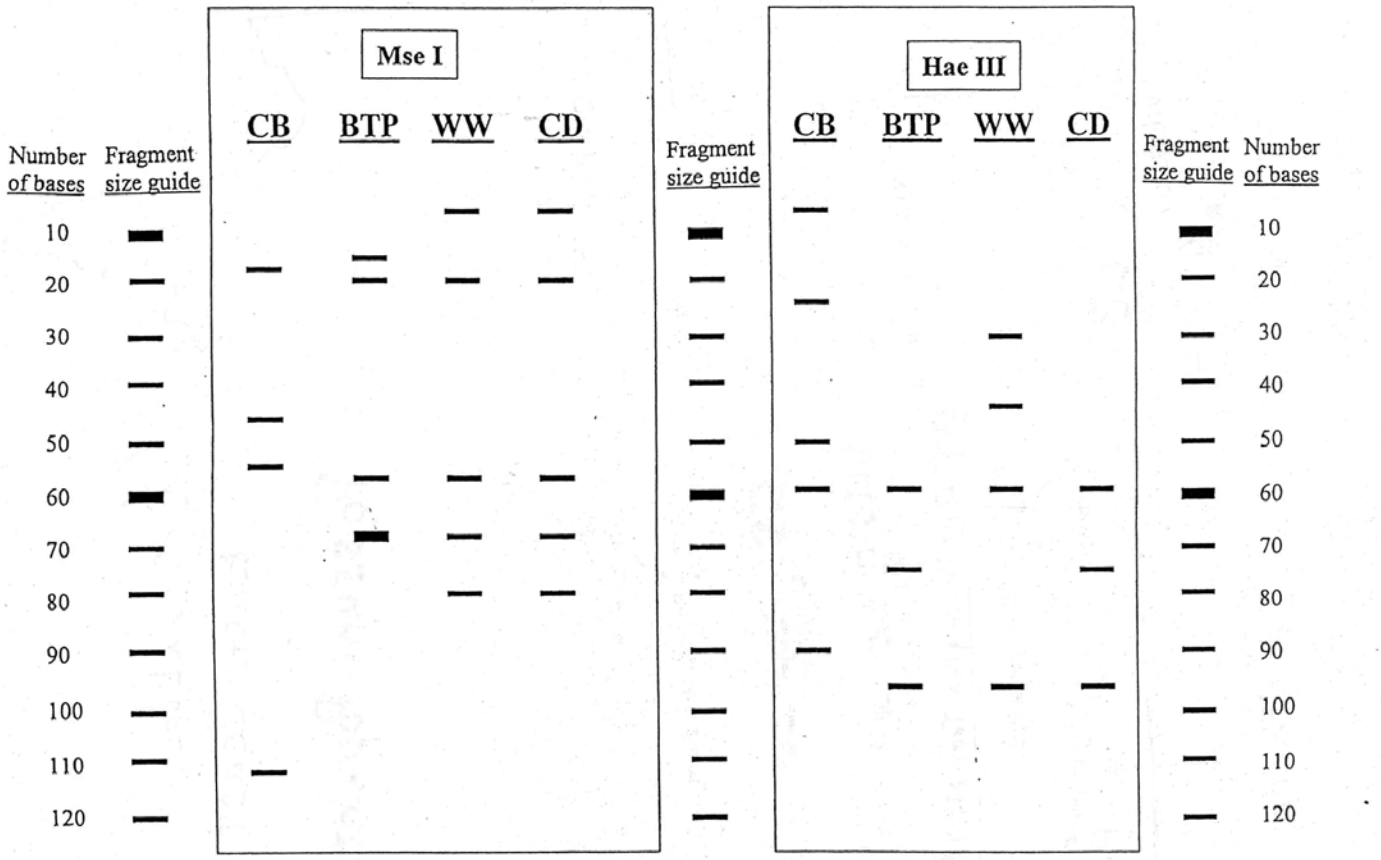
GG^CC

Hae III

```
        10         20         30         40         50         60
ATGACCAATA TTCGCTTAAC ACACCCACTC ATAAATTAAA TCAACGACTC ATTCATTGGG

        70         80         90        100        110        120
CCACCCACAC CATCTAACAT CTCAGCTTGA TGATTAATCG GTTCACTCCT AGGAGTATGC

       130        140        150        160        170        180
TGCATCATTC AAAGGCCTAC AGGCTTATTC TTAGCAATAC ATTAAGCGTC AGACACACTA

       190        200        210        220        230        240
ACCGCATTTT CATCAGTAGC CCATATCTGC CGAGATGTGA ATTATGGAAG ..........
```



Key for Possum Populations (CB, BTP, WW, and CD) Using RFLP Patterns



TEACHER SOLUTION

Population #1	WW (p. 33)
Population #2	BTP (p. 35)
Population #3	CB (p. 37)
Population #4	CD (p. 39)

Morphometric Data Tables

Collect data from every member of your research team below. Be sure to mark your population in the left column.

Cambarville Population	Ear Length	Foot Length
Individual 1		
Individual 2		
Individual 3		
Individual 4		
Average Measurement		

Barrington Tops Population	Ear Length	Foot Length
Individual 1		
Individual 2		
Individual 3		
Individual 4		
Average Measurement		

Whian Whian Population	Ear Length	Foot Length
Individual 1		
Individual 2		
Individual 3		
Individual 4		
Average Measurement		

Morphometric Data Tables *(Continued)*

Conondale Ranges Population	Ear Length	Foot Length
Individual 1		
Individual 2		
Individual 3		
Individual 4		
Average Measurement		

Analysis Questions:

1) Why do you think it was necessary for researchers to analyze both the morphology and the DNA sequence when determining if these populations were from two different species?

2) Why did the researchers collect data from the areas that they did?

3) Why was it important to categorize samples by geography?

4) Where is the most probable boundary, or contact zone, where the two species most likely meet?