Conservation Genetics Lab

Discovering Lemur Diversity

Produced in collaboration with the Duke Lemur Center

Conservation Genetics Lab:
Discovering Lemur Diversity
KT-1505-01

Version: 1.4
Release: September 2025

Student guide

Background information

Overview

Today, many species are at risk from habitat destruction. To better understand how to protect species, conservation biologists must know how many species live in a particular area, whether they are rare or widespread, and how resilient they are in the face of ecological disruption.

The island of Madagascar holds one of the most unique assemblages of species on Earth. Unfortunately, many of the species that live there are under threat of extinction from habitat loss. To understand how to protect these organisms, it is critical to know exactly how many species there are, where they live and how they interact with the environment. There are reports that a lemur species once thought to be extinct may have been spotted in an isolated forest on Madagascar. Today, you will analyze authentic data collected by researchers to determine if this lemur species has been rediscovered.

Madagascar and lemurs

Madagascar is a large island off Africa's east coast (Figure 1) with habitats unlike any others found on Earth. Because Madagascar has been isolated from other land masses for a long time, organisms that live there have been evolving separately from species on the mainland for tens of millions of years. As a result, many organisms found on Madagascar are unique. It's estimated that roughly 90% of Madagascar's species are found nowhere else in the world.

Figure 1: Madagascar is located 250 miles off the east coast of Africa and is the world's 4th largest island.

Lemurs are perhaps the most iconic animals of Madagascar. Lemurs are small primates, meaning they are part of the same order that includes tarsiers, monkeys, apes, and humans.

Scientists think that the common ancestor of all lemurs reached Madagascar approximately 40 to 55 million years ago. Those first few animals likely floated on debris from the African coast all the way to Madagascar. The descendants of those first few animals quickly spread across the island. As different traits conferred advantages in different habitats, populations living separated from each other adapted to their local environments and eventually evolved into new species. Because Madagascar's habitats are so varied and there were so many different ecological niches that lemurs

could fill, early lemur populations evolved over the past 50 million years into the diverse array of species we see today (Figure 2).

The fact that lemurs only live on Madagascar and are so well adapted to their specific habitats also means that they are particularly vulnerable to habitat loss. In Madagascar, slash-and-burn farming practices have drastically reduced the natural forests to small fragmented patches. By some estimates, over 80% of the island's original forests are now gone. Furthermore, as the global climate changes due to human fossil fuel use, the remaining forests in which lemurs live are also changing, further threatening the lemurs' survival. According to the International Union for the Conservation of Nature (IUCN), 98% of lemur species are considered threatened and 31% are critically endangered.

Figure 2: Today there are over 100 different lemur species. They range in size from the tiny mouse lemurs (top right), where adults can be as small as 3 inches and weigh only one ounce, to the much larger indri (bottom center), which weigh about 20 pounds and are a little over two feet long, not including their tail. Some extinct lemur species may have been as large as 350 pounds, about the size of an adult male gorilla.

Dwarf lemur images courtesy Duke Lemur Center

Measuring biodiversity

Protecting threatened species is a complex problem that requires the coordinated work of many different individuals and organizations. Often a first step in this work is to identify the *biodiversity* in an area. In terms of conservation, measuring biodiversity means identifying which species live in a particular region and estimating each species' population.

Scientists can sometimes disagree on how to define a species. Here, we use the word to mean a group of animals that are able to reproduce in the wild and share a particular set of characteristics and evolutionary history. Importantly, the members of a species have been breeding exclusively with members of their group for a long time. This means that all members of a species will be more closely related to each other than to any other organism.

Of course, you can't directly see exactly how organisms are related to each other, but things that are related do tend to look more similar. So, traditionally, we have relied on visual similarities and differences to identify species because members of different species tend to look different. For example, lions and leopards look different, and intuitively, these animals belong to distinct species. *Morphological* differences, such as color patterns, head shape, or limb length are easy for us to measure and document.

The problem is that using only morphological differences to classify species can end up misrepresenting the actual number of unique species. For example, sometimes species can contain so much morphological diversity that we may think there is more than one species just by looking, but the organisms belong to a common breeding population (Figure 3). Perhaps more often, organisms may look nearly identical, but exhibit different behaviors and habits, belong to different breeding populations, and ultimately represent separate species (Figure 4).

When different species are difficult to tell apart based on morphology, we call them *cryptic species*. To figure out whether multiple species exist in a population of animals that look alike, we have to use more tools than just morphological measurements. We need to document their behavior, communication systems, and, most importantly, genetics—their DNA.

When organisms reproduce, random mutations that occur in the DNA may be passed to the offspring. Within a single species, these random mutations have the opportunity to spread through the population as different organisms reproduce together. But in other groups, different mutations will occur and eventually spread and become more common. As long as the two groups are not exchanging genes by reproducing together, the specific mutations that spread and become common will mostly be different (Figure 5). Scientists can compare these differences in the DNA to identify which groups are distinct and how different groups are related. Even if organisms look and behave similarly, their DNA can tell us if they are different species and how they are related evolutionarily.

Figure 3: These wolves are a single pack and therefore closely related members of one species, even though some look very different from each other.

Figure 4: African forest elephant (top) and African bush elephants (bottom) are difficult to distinguish based on morphology, but scientists consider them different species because they have been separate breeding populations for almost two million years.

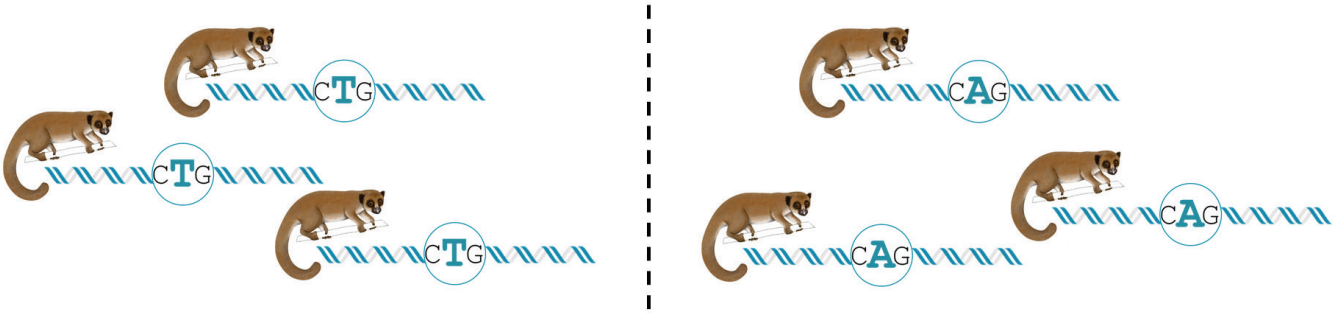


Figure 5: When a population splits into two or more groups, individuals from the different populations may not interbreed for many, many generations. When that happens, different mutations will spread and become more common in each group. By comparing DNA sequences from many different individuals, scientists can find all the different places where groups differ genetically. These genetic differences can help scientists identify different species.

In the end, DNA will provide evidence of how genetically distinct different groups are. Whether different populations are distinct enough to be considered different species is a judgment call made by experts.

What does it mean to be related?

When a person says they are related to someone biologically, they usually mean that they share ancestors. A sibling is very closely related because you share ancestors just one generation prior (your parents). A cousin is less closely related because your shared ancestors were two generations ago (your grandparents). A second cousin is more distantly related still because of how far back you need to go to find a common ancestor (your great-grandparents).

The same is true for species. When we say that species are closely related we are saying that the individuals of those two species share common ancestors in the not too distant past. When species are less closely related, it is because you need to go farther back in time to find their common ancestors. The main differences is the amount of time. Species relationships are usually measured in millions of years; family relationships are measured in a few generations.

Today's lab

Today, you will be joining our research team on an important expedition. We have reports of lemurs living in the forest that resemble a species long thought to be extinct. Help us determine if a lemur species has been rediscovered!

Figure 6: Tsinjoarivo is located on the East side of Madagascar near the edge of the central highlands, highlighted in yellow.

Today, we are trying to determine how many species of lemur live in a particular forest of the central highlands of Madagascar. The forest we are studying is a protected reserve near the town of Tsinjoarivo (*tsin-ju-a-reev-oo*) (Figure 6). We know that one species, *Cheirogaleus crossleyi (Care-o-gal-ee-us cross-lee-eye)*, sometimes called the furry-eared dwarf lemur, lives at Tsinjoarivo. *C. crossleyi* is considered a vulnerable species, though it is known to still live in several forests throughout the central highlands. But there have been reports of lemurs living at Tsinjoarivo that seem to more closely resemble a different species, *Cheirogaleus sibreei (Care-o-gal-ee-us sib-ree-eye)*, sometimes called Sibree's dwarf lemur. Having two such similar species living in the same forest would be rare, but not unheard of. If species' lifestyles are just different enough, they can avoid competition for resources, allowing both species to survive.

If *C. sibreei* were living in Tsinjoarivo, it would be big news. This is because *C. sibreei* is a species of dwarf lemur that scientists have long considered to be extinct. The last known population was wiped out when its forest habitat was destroyed many years ago. Today, the only example we have is a single preserved museum specimen. Unfortunately, due to regulations, taking samples from that one specimen is prohibited, so we can only make hypotheses about the species' genetics.

The problem is that from what we know of *C. sibreei*, the two species look very similar (Figure 7). This is true of all dwarf lemur species. In other words, dwarf lemur species are cryptic. Until just recently scientists identified only two species of dwarf lemur based on their morphology and geographical range. But as scientists have sampled more and more dwarf lemur populations, accumulated new ecological data, and used new genetic tools, they have realized that there are more dwarf lemur species than they previously thought. Today, we know that what scientists initially characterized as two species of dwarf lemur are actually at least nine unique species.

Could it be that the population of C. crossleyi at Tsinjoarivo just contains a little more morphological variation than usual? Or, have we truly rediscovered a species, *C. sibreei*, once thought lost to history? You will need to analyze data, both morphological and genetic, to make the call.

The field site

We will be working at a field site in the forest of Tsinjoarivo. The forest of Tsinjoarivo is one of the largest forest patches of the Central Highlands left today. The forests of the central highlands (a mountainous region that runs through the center of the island) have been a big target for agricultural cultivation. As such, the high-altitude forests are now very fragmented. Instead of the large connected forests that once dominated the landscape, today there are only a few separated pockets of forest left. Animals living in these pockets are separated from other animals of the same species. Each small isolated population has fewer individuals that can reproduce and therefore are much more vulnerable when compared to larger connected groups. Because of this, the pressures of ecological disturbance from humans and climate change are of particular concern for animals living in the forests of the Central Highlands.

Figure 7: *C. crossleyi* lemurs (the furry-eared dwarf lemur, top left) are common at Tsinjoarivo, but some animals (top right) look less like *C. crossleyi* and more like the extinct species *C. sibreei* (Sibreei's dwarf lemur). An artist's rendition of the two species are shown for comparison (*C. crossleyi*, bottom left; *C sibreei*, bottom right).

Photos courtesy of Dr. Marina Blanco.
Illustrations courtesy of Sally Bornbusch.

Dwarf lemurs

This research expedition is specifically focused on dwarf lemurs at Tsinjoarivo. Dwarf lemurs are small (about the size of a squirrel), nocturnal lemurs. Dwarf lemurs are also the only primates that are obligate hibernators, which means that every dwarf lemur hibernates every year, no matter what. Being small nocturnal animals that hibernate for a significant part of the year, dwarf lemurs can be much more difficult to observe than many other lemur species. Doing so requires miles of hiking through rugged terrain, setting traps high in the trees, and lots of patience.

Data collection and analysis

To determine whether the lemurs living at Tsinjoarivo are all *C. crossleyi* or whether some of them are *C. sibreei*, we start by trapping lemurs in the forest. To catch the lemurs, we set traps in the trees baited with fermented fruit. We check the traps every morning, and bring any lemurs we catch back to the field station. We then take photos and detailed morphological measurements of

each lemur. You will use these detailed measurements to establish how many species you think are present based on morphological data. Finally, we take a small tissue sample to test the animals' DNA. These DNA samples will be used to make a final determination of how many species are present. After sampling, we return the lemurs to the same spot where we caught them, ensuring as little disturbance as possible to the animals.

Identifying species using DNA

cytb: The cytochrome b gene (*cytb* for short, pronounced 'sight-bee') is commonly used to identify different species. *cytb* is a mitochondrial gene. Mitochondria are organelles that convert energy in the cell, and they have their own small circular genome that is separate from the DNA in the cell nucleus (Figure 8). Scientists use *cytb* for species identification. This is because mitochondrial genes tend to evolve at such a rate that we observe meaningful differences in the DNA when comparing mitochondrial genes across species, but not when looking at members of the same species. We can figure out how many different lemur species we have captured by looking at the similarities and differences between the *cytb* DNA sequences from different lemurs. Eventually, we will sequence the *cytb* gene, identifying the exact order of every nucleotide, to identify all the differences between each lemur's *cytb* sequences.

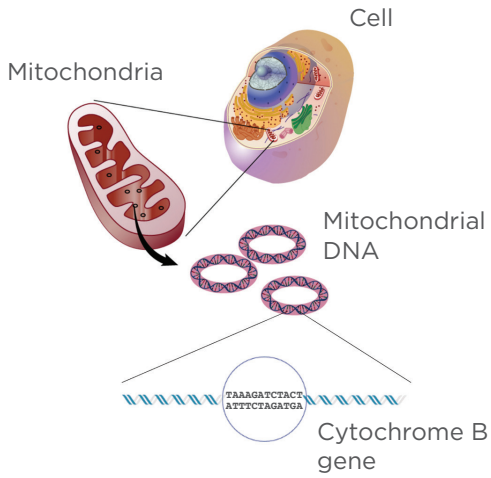


Figure 8: Cytochrome B (*cytb*) is located in the mitochondrial genome. The mitochondrial genome is a circular genome located outside the cell's nucleus. Unlike other DNA, mitochondrial DNA is almost always inherited only from the mother.

PCR-RFLP: Today we're going to use a faster way to test many lemurs without having to sequence the *cytb* gene. This method is called PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism). This technique starts by using *PCR* (polymerase chain reaction), a method for making many copies of a specific DNA sequence. Using PCR, the field team will make copies of a 1,200 base pair (bp) segment of the lemur mitochondrial genome. The resulting 1,200 bp PCR product will contain the entire 1,140 bp of the *cytb* gene. Then, the team will use a *restriction enzyme* to cut those 1,200 bp PCR products at a particular point in their DNA sequence.

Restriction enzymes recognize specific, short DNA sequences (typically 4-8 base pairs long) and cut the DNA there. We have previously identified two unique mutations in the *cytb* gene of *C. crossleyi* (the furry-eared dwarf lemur) that are not present in any other lemur species. These mutations allow the enzyme BglII (pronounced like "bagel 2"), to cut the *cytb* PCR product from *C. crossleyi* lemurs, but not from other species. In our experiment, if the 1,200 bp product is cut into a 500 bp fragment and a 700 bp fragment, we will know that the DNA belonged to *C. crossleyi*. If the restriction enzyme does not cut our *cytb* fragment, we will know the DNA came from a different lemur species (Figure 10). To see if the DNA is cut, we run our samples on an agarose gel. The DNA that you will run on your gel today is the 1,200 bp PCR product from *cytb* after it has been incubated with BglII.

Figure 9: The restriction enzyme BglII cuts the sequence AGATCT at the points marked in red.

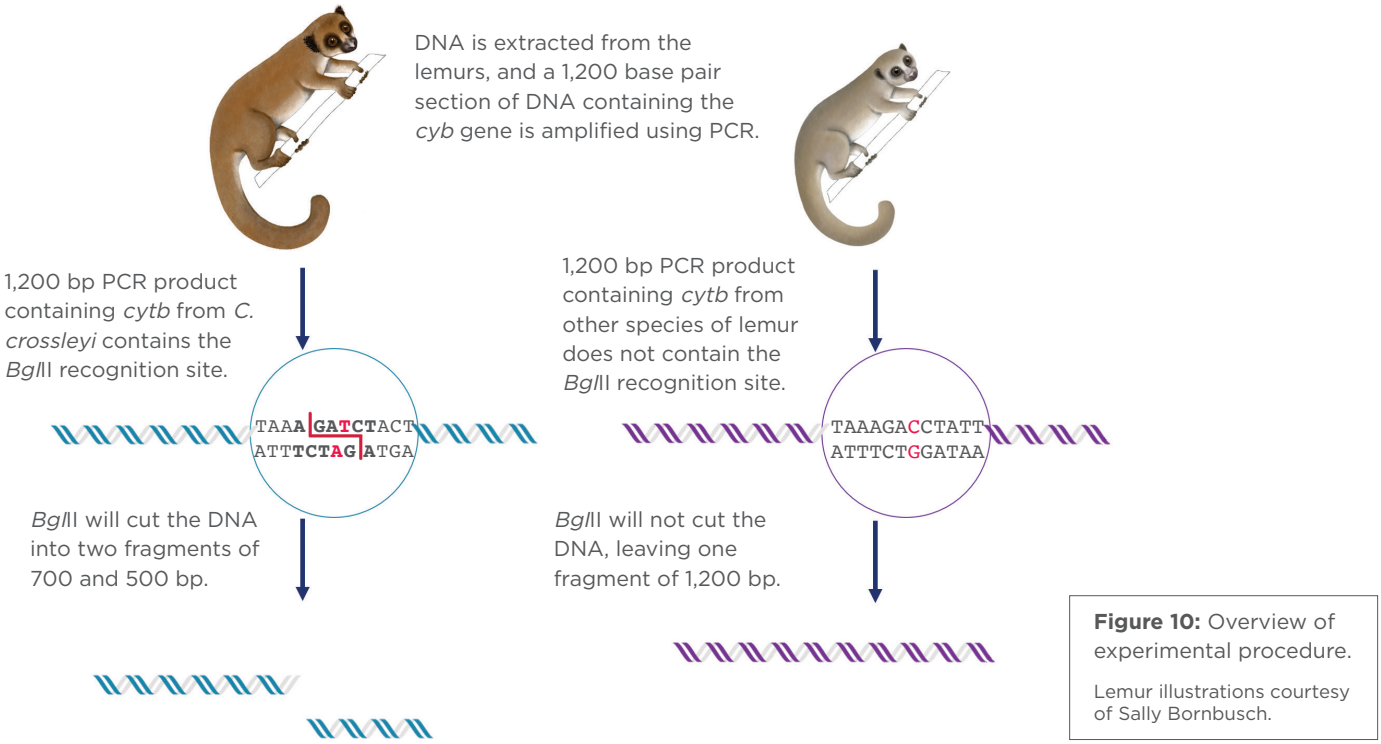


Figure 10: Overview of experimental procedure.

Lemur illustrations courtesy of Sally Bornbusch.

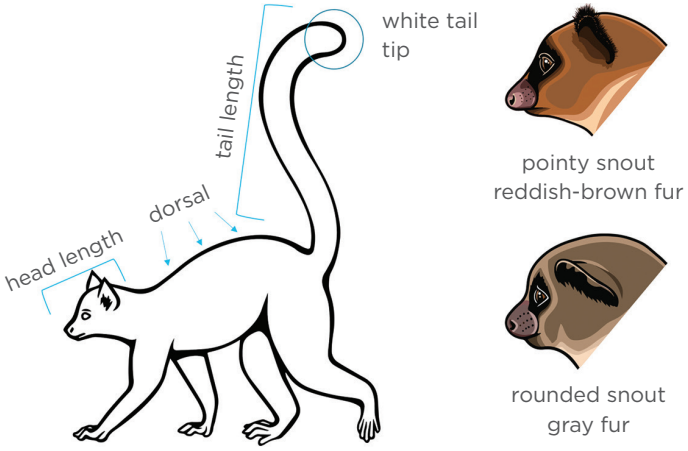
Are there Sibree's dwarf lemurs living in Tsinjoarivo?

Your job today is to analyze the data the researchers gathered. First, you will analyze the morphological data from several lemurs sampled by our field team at Tsinjoarivo. Then, based on your analysis, you will decide on which lemurs you will perform genetic analyses and ultimately determine whether you have rediscovered a lost species of lemur!

Pre-lab activity: Morphological analysis

The team has trapped eight dwarf lemurs and brought them back to the field station for analysis Remember that scientists originally characterized species mostly based on their morphology, or how they looked. Species of dwarf lemurs tend to look very similar. But there are some key differences that we look for and can measure. These are traits that often differ between different dwarf lemur species and include:

- Mass
- Tail length
- Head length
- Presence or absence of a dorsal line (a stripe that runs down the center of the back)
- Presence or absence of a white tail tip
- Snout shape (pointy or rounded)
- Coat color
- Face color



Lemur illustrations courtesy of Sally Bornbusch.

The full range of traits the team observed is summarized in the following table. Your job is to look at the data for each lemur individually and and decide which may belong to *C. crossleyi* (the furry-eared dwarf lemur) and which may belong to the thought-to-be-extinct species *C. sibreei* (Sibree's dwarf lemur).

Trait	Summary of observations
Mass	between 293 g and 466 g
Tail length	between 21.5 cm and 30 cm
Head length	between 49.1 mm and 65.4 mm
Dorsal line	some present; some absent
White tail tip	some present; some absent
Snout shape	rounded or pointy
Coat color	silvery gray or reddish-brown
Face color	white, silvery gray, or reddish-brown

When comparing *C. crossleyi* to the historical descriptions of *C. sibreei*, we expect *C. crossleyi* to be larger and to have longer tails than *C. sibreei*. They are also usually a reddish-brown color, with a white underbelly and black around their eyes, snout, and tips of their ears. Sometimes there is also some white around their noses and eyes and a white tip on the end of their tails. Like *C. crossleyi*, *C. sibreei* have a white underbelly, with black around their eyes and snout, but the rest of their fur is thought to be a little grayer in color.

Morphological analysis

The data collected from eight dwarf lemurs from Tsinjoarivo are presented on pages 22-23. From these data, you need to decide whether you think all of the lemurs belong to the species *C. crossleyi* (the furry-eared dwarf lemur) or whether some may belong to a different species, likely the thought-to-be extinct species *C. sibreei* (Sibree's dwarf lemur).

Decide how you want to group the lemurs that were found. Use the lemur data cards (pages 22-23) for this activity. You may choose to cut out each individual card in order to group the lemurs more easily. You are trying to identify what you think are groups of lemurs that may represent the same species. You may make as many or few groups as you choose, but you must justify your groups based on the data. Remember, researchers are particularly interested in whether there are two different species present, *C. crossleyi* and *C. sibreei*.

Q1. Name the lemurs you think may be *C. crossleyi*:

I believe the individuals in this group belong to the same species because:

Q2. Name the lemurs you think may be *C. sibreei* (if you think there is more than one species present):

I believe the individuals in this group belong to the same species because:

Q3. Based on the information provided, do you feel confident in the groups you made? Explain your answer.

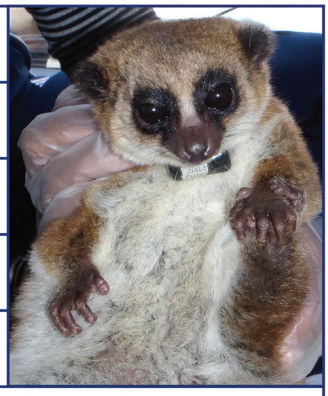
Q4. Do any individuals not fit in these two groups? If so, explain here.

Lemur data cards

Use these *Lemur data cards* to complete the activity on the following pages. You may wish to cut out the eight individual cards.

(Lemur photos courtesy Dr. Marina Blanco)

Alicia



Sex: ***female***	
Age category: ***adult***	
Capture month: ***March***	
Capture season: ***pre-hibernation***	
Coat color: ***reddish brown***	Face color: ***white***
White tail tip: ***yes***	Dorsal line: ***no***
Face shape: ***pointy***	Head length: ***58.6 mm***
Tail length: ***27.6 cm***	Mass: ***458 g***

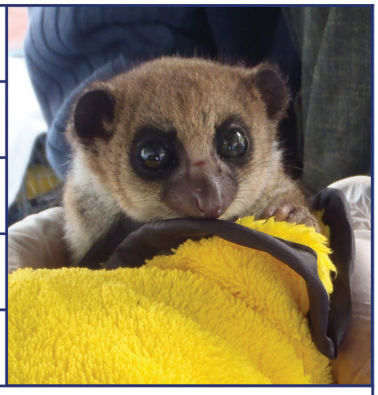
Bema

Sex: ***male***	
Age category: ***adult***	
Capture month: ***October***	
Capture season: ***post-hibernation***	
Coat color: ***reddish brown***	Face color: ***white***
White tail tip: ***yes***	Dorsal line: ***no***
Face shape: ***pointy***	Head length: ***52.2 mm***
Tail length: ***23 cm***	Mass: ***303 g***

Garafa

Sex: ***male***	
Age category: ***adult***	
Capture month: ***March***	
Capture season: ***pre-hibernation***	
Coat color: ***silvery gray***	Face color: ***silvery gray***
White tail tip: ***no***	Dorsal line: ***no***
Face shape: ***rounded***	Head length: ***54.9 mm***
Tail length: ***24.5 cm***	Mass: ***351 g***

Ihary



Sex: ***male***	
Age category: ***juvenile***	
Capture month: ***March***	
Capture season: ***pre-hibernation***	
Coat color: ***reddish brown***	Face color: ***reddish brown***
White tail tip: ***no***	Dorsal line: ***no***
Face shape: ***pointy***	Head length: ***56.6 mm***
Tail length: ***24 cm***	Mass: ***281 g***

Lemur data cards (cont.)

Use these *Lemur data cards* to complete the activity on the following pages. You may wish to cut out the eight individual cards.

(Lemur photos courtesy Dr. Marina Blanco)

Malala

Sex: ***female***	
Age category: ***sub-adult***	
Capture month: ***March***	
Capture season: ***pre-hibernation***	
Coat color: ***silvery gray***	Face color: ***silvery gray***
White tail tip: ***no***	Dorsal line: ***no***
Face shape: ***rounded***	Head length: ***57 mm***
Tail length: ***22 cm***	Mass: ***338 g***

Mbola

Sex: ***male***	
Age category: ***adult***	
Capture month: ***March***	
Capture season: ***pre-hibernation***	
Coat color: ***reddish brown***	Face color: ***white***
White tail tip: ***no***	Dorsal line: ***no***
Face shape: ***pointy***	Head length: ***49.1 mm***
Tail length: ***30 cm***	Mass: ***370 g***

Razafy

Sex: ***female***	
Age category: ***adult***	
Capture month: ***October***	
Capture season: ***post-hibernation***	
Coat color: ***reddish brown***	Face color: ***reddish brown***
White tail tip: ***no***	Dorsal line: ***no***
Face shape: ***pointy***	Head length: ***65.4 mm***
Tail length: ***27 cm***	Mass: ***466 g***

Rodolfo

Sex: ***male***	
Age category: ***juvenile***	
Capture month: ***March***	
Capture season: ***pre-hibernation***	
Coat color: ***silvery gray***	Face color: ***silvery gray***
White tail tip: ***no***	Dorsal line: ***yes***
Face shape: ***rounded***	Head length: ***57 mm***
Tail length: ***21.5 cm***	Mass: ***293 g***

Choose lemurs to test

You have collected data from eight lemurs, but at the field site, you have limited resources. For your initial lab analysis, you will test four of the lemurs using genetic techniques. If these results look promising, you will go ahead and analyze the entire *cytb* sequence from all of the lemurs in the study.

Q5. Discuss with your team which four lemurs you would like to test genetically in order to evaluate your hypothesis.
- In the pre-lab exercise, you made a hypothesis based on data from eight individual lemurs.
- Here, you will test your hypothesis by running DNA from four of those individuals on your gel.
- Your job is to identify whether a lemur species other than *C. crossleyi* is living at the Tsinjoarivo field site. If you find a species other than *C. crossleyi* we can assume that you have rediscovered the species *C. sibreei*. You will be able to confirm this finding in the extension: "Making phylogenetic trees using sequence data."

Sample	Animal name (from pre-lab activity)	Justification for including this sample in your analysis
1		
2		
3		
4		

Q6. Above, you justified each sample individually. Now explain in a couple of sentences your overall strategy for choosing the samples you chose. For example, did you look at any specific characteristics? Did you try to capture a range of animals; did you try to key in on very different ones?

Q7. Justify why you took the approach you did.

Student lab protocol

Protective gloves and eyewear should be worn for the entirety of this experiment.

1. Place the prepared gel into the electrophoresis chamber.

2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
   - You will need 30 ml of TBE buffer for a blueGel™ or Bandit™ electrophoresis system. Do not overfill the chamber.
   - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.

3. Use a micropipette to load samples in the following order. To prevent contamination, use a new tip for each sample.

   Be sure to record which sample you load in wells 2-5, so that you can properly identify them when you interpret the final results.
   - Well 1: 10 µl Fast DNA Ladder 1 (tube L)
   - Well 2: 10 µl Sample 1 - Lemur Name: _____
   - Well 3: 10 µl Sample 2 - Lemur Name: _____
   - Well 4: 10 µl Sample 3 - Lemur Name: _____
   - Well 5: 10 µl Sample 4 - Lemur Name: _____

4. Run the gel for 15-25 minutes.
   - The blueGel™ and Bandit™ electrophoresis systems run at a fixed voltage.
   - If using another gel electrophoresis system, set the voltage in the 70-90 V range.

5. To visualize the DNA samples, turn on the blue light in your electrophoresis system, or move the gel to a transilluminator.

6. If needed, continue to run the gel until there is sufficient separation between the bands in the 500-1,200 bp range to interpret the results.

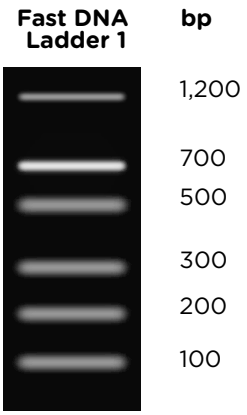
7. If desired, take a photo to document the results.

8. Compare the bands from the DNA samples to the Fast DNA Ladder 1 to obtain size estimates.

Detailed operating instructions for miniPCR electrophoresis systems

blueGel
https://links.minipcr.com/blueGelRun

Bandit
https://links.minipcr.com/BanditViewit



Pre-lab questions

Review

1. The majority of the species that live on Madagascar live nowhere else on earth. Give at least one reason why scientists think Madagascar contains such a unique assemblage of species.

2. How many species of lemur live on Madagascar? How many species of lemur live in other places in the world?

3. Biodiversity is a measure of how much biological variation there is in a particular area. What specific measures of biodiversity are being used in this lab?

4. What do we mean when we say species are “cryptic species”?

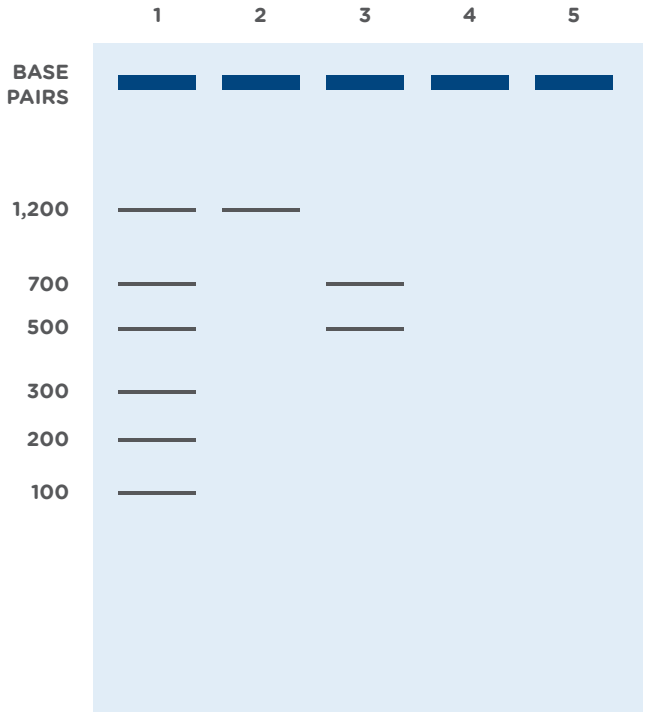
5. Why must we use genetics to tell some species apart?

6. What about dwarf lemurs makes them more difficult to study compared to some other species of lemur?

7. What are two reasons why the cytochrome b (*cytb*) gene sequence is often used for identifying species?

8. There are more species of dwarf lemur recognized today than there were 20 years ago. How were these species identified, and why weren't they recognized previously?

9. The gel on the right shows possible results from today's experiment. Lane 1 shows the DNA ladder. For lanes 2 and 3, identify whether you think the BglII restriction site was present in the DNA fragment. Then, based on that answer, say whether you think the DNA came from a *C. crossleyi* (the furry-eared dwarf lemur) individual or some other species of lemur.



   Lane 2: Did BglII cut?

   - Reasoning:

   - Did this DNA come from a *C. crossleyi* lemur?

   - Reasoning:

Lane 3: Did BglII cut?

- Reasoning:

- Did this DNA come from a *C. crossleyi* lemur?

- Reasoning:

10. In lane 4, draw what you think a result for a *C. sibreei* lemur would be.

    - Explain your reasoning.

11. *Challenge question:* In lane 5, imagine that there was a lemur that was a hybrid offspring of a *C. crossleyi* mother and a father that was another species of lemur. Draw in the results you would expect for this hybrid offspring of lemur. (Hint: remember where in the genome the *cytb* gene is found).

    - Justify your answer.

Critical thinking

12. There are over 100 species of lemur living on Madagascar today. These lemurs are all descended from a small original population that landed on the island 40-55 million years ago. Imagine if Madagascar were all just one type of habitat, say a rainforest. Do you think more or fewer species of lemur would have evolved if Madagascar were all one type of habitat? Explain your answer.

13. In this lab, we defined species as distinct breeding populations that can be distinguished using genetic tools. Different biologists will sometimes use different definitions of species, or "species concepts," depending on what they are studying. For example, paleobiologists (scientists that study fossils), microbiologists (scientists that study microscopic life including asexual bacteria), and conservation biologists likely do not use the same species concept in their work. Using these examples or others of your choosing, why do you think biologists who study different things may need to use different species concepts?

14. In this lab, we will use PCR-RFLP to tell the two species apart. Based on the description of PCR-RFLP in this lab, do you think you could always use that technique to identify any two species? Explain your answer.

Post-lab questions

Interpreting results

1. Use the schematic gel on the right to draw what your gel looks like. For each sample, draw the bands that you see on your actual gel. Label each lane with the name of the lemur that you tested.

2. Next to each band write approximately how long (in base pairs) the DNA in that band is. Use the image of the ladder from page 25 to help you.

3. Record your results in the table below.
   a. Record the names of the lemurs you tested in the top row.
   b. Use check marks to record each lemur's gel electrophoresis result in the second and third rows.

	Lemur name:	**Lemur name:**	**Lemur name:**	**Lemur name:**
700 bp + 500 bp ***C. crossleyi***				
1,200 bp other dwarf lemur species				

Critical thinking

4. Do your results match your prediction from the morphology analysis section of this lab?

   If not, how was it different?

5. Look back at the data used in the morphological analysis. Which types of data now seem to be reliable factors on which to base your species designations?

6. Which morphological data now seem to be less reliable factors on which to base your species designations?

CER table

Fill in the table based on your results from the lab. Use the rubric on the next page to help your answers.

Question:

Is there more than one species of dwarf lemur living at Tsinjoarivo?

Claim

Make a clear statement that answers the above question.

Evidence

Provide data from the lab that supports your claim.

Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

Score	4	3	2	1
CLAIM A statement that answers the original question/ problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent Grade	55	60	65	70	75	80	85	90	95	100

Extension: Using DNA sequence data to identify species

In this lab, you used molecular techniques to investigate whether individual lemurs from Tsinjoarivo belonged to the same species of dwarf lemur. The RFLP analysis you did is often done as a preliminary analysis when comparing DNA sequences. To be more comprehensive, scientists can sequence the entire *cytb* gene.

Today, you will look at the *cytb* DNA sequences of the lemurs you analyzed in your lab activity. Using their *cytb* DNA sequences, you will establish how these individuals are related to each other and to other species of lemurs. This type of analysis will answer whether the groups you identified previously are indeed evolutionarily distinct. That means that the groups have been reproducing separately for long enough that we can recognize differences in their DNA. You will use this analysis to further evaluate the conclusions you drew from the RFLP analysis in the lab exercise.

Using DNA to build relationships

Establishing how species are related to each other evolutionarily is a field of biology called *phylogenetics*, and the description of how a species is related to other species evolutionarily is called a phylogeny. Using DNA sequences, we can build a more complete picture of how individuals are related to each other. We usually display these relationships by building a *phylogenetic tree*—sometimes just called an evolutionary tree. Building a phylogenetic tree is based on a relatively simple idea. If two organisms' DNA sequences share a unique mutation, it is likely that they share a common ancestor in which that mutation occurred (Figure 1). By looking for these shared variants, we can start to build relationships, understanding how individuals and groups of individuals are connected through their common ancestors. These connections are then presented in the form of a tree.

```
A GTGGCGTATCTGTCCACAAAGCCGAACTAG
B GTCACGTATCCGTCCAGATAGCCGCACTAA
C GTCGCGTATCCGTCCAGAAGGCTGCACTAA
D GTAGCGATTCCGTGGATAAAGCCAGACCAA
E GTTGCGATTACGTGGATAAAGCCACACCAA
F GTCGCGATTCCGTGGATAAAGCCACACCAA
G AACGGGTTTCAGTGAAGAAAGCGACACTAA
H GACGGGTTTCAGTGGAGACAGCGAGTCTAA
```

Figure 1: The eight sequences presented here are all different. But by looking at similarities between sequences we can start to put them into groups. Sequences A, B, and C share mutations highlighted in blue. We can make the assumption that they share ancestors in which those mutations occured. Sequences D, E, and F share mutations highlighted in pink, making it likely that they share common ancestors that carried these mutations. Sequences G and H share mutations highlighted in orange.

Tree thinking

All phylogenetic trees are made of two basic features: *branches* and *nodes*. Branches are the lines of the tree and represent a population as it evolves over long periods of time and many, many generations. Nodes are the places where branches diverge. A node represents the last common ancestor of the branches that are connected to it. Understanding at which nodes two branches connect will tell you how organisms are related to each other.

You can draw these connections in different styles, but their meanings are the same. Sometimes lines are drawn at an angle, making a series of connected "V"s (Figure 2, top). Sometimes lines look more rectangular and advance from left to right (Figure 2, bottom) as in the trees you will use today, but they can be oriented in any direction. Sometimes they are even drawn as a circle.

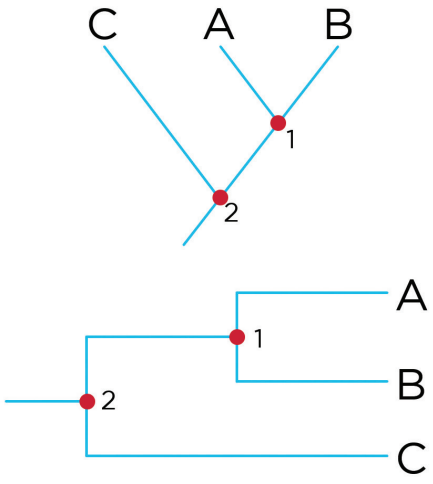


Figure 2: In these trees, the branches are drawn with blue lines. The nodes are numbered and marked with red dots, though, in most trees, the nodes are not labeled. The nodes are places where one branch splits into two.

The two trees to the left may look different, but to a phylogeneticist, they are identical. In both trees, organisms A and B are most closely related to each other. They share a common ancestor at node 1. Organism C is more distantly related to A and B. All three organisms share a common ancestor at node 2. Node 2 is deeper in the tree than node 1, meaning the organisms represented by node 2 lived before the organisms that lived at node 1. In fact, node 2 represents the ancestor of all the organisms on the tree. For this reason, we call node 2 the *root* of the tree.

Continued on the next page

Interpreting a tree

Interpreting a phylogenetic tree involves putting organisms into groups based on relatedness. We call these groups clades. A clade includes all the organisms descended from a single node on a tree, and only those organisms. A clade may be very large if a node represents an organism that lived a long time ago and has many, many species that are descended from it. Or a clade may be as small as a single species. In the tree to the right (Figure 3), using the genus *Panthera* (also known as the big cats) as an example, you can see several clades. For example, lions and leopards form a clade (highlighted in pink), because they share an ancestor at node 1. Lions, leopards, and jaguars form a larger clade (highlighted in orange) because they are all of the organisms descended from node 2. Tigers and snow leopards form a clade (highlighted in purple) because they are all of the organisms descended from node 4, while all the big cats together form a larger clade (highlighted in yellow). In this tree, the domestic cat, which is not a member of the genus *Panthera*, is also included. The domestic cat represents an *outgroup*, meaning that all the other organisms on the tree are part of a clade (highlighted in yellow) to which the domestic cat does not belong. In other words, all the other cats on this tree are more closely related to each other than they are to a domestic cat. An outgroup is often included in a tree intentionally. Scientists do this by choosing an organism they know in advance to be more distantly related. In some software programs used to build phylogenies, this is needed for technical reasons, but generally, it can help to give the tree some perspective. The outgroup will branch off of the deepest node in the tree, node 5 in this example. The deepest node in your tree represents what is called the *root* of the tree. The root represents the common ancestor to all the organisms in the tree.

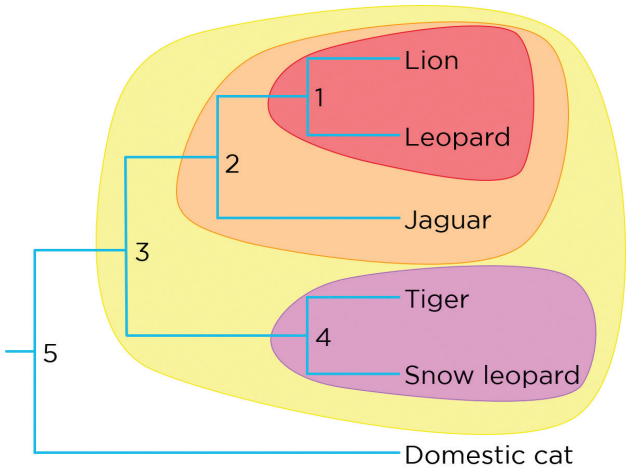


Figure 3: Phylogeny of the genus *Panthera*, the big cats. Here, nodes are numbered 1-5 for reference. Different clades are shaded in different colors. Domestic cats are included in the tree as an outgroup, meaning we knew in advance that they do not belong to the same clade as the other organisms.

1. *Panthera* is the genus that includes lions, leopards, jaguars, tigers, and snow leopards. Which node on this tree tells you that all those organisms form a clade?

2. According to this tree, is a jaguar more closely related to a tiger or a lion? Justify your answer with evidence from the tree.

Dwarf lemur evolutionary tree

Bioinformatics is the field of biology that uses computers to analyze biological data, including genetic sequences. Bioinformatics combines computer science, statistics, and biology to look at large complex data in ways that would be impossible to do by hand. Today, you will analyze a tree generated by the program Simple Phylogeny, which, as its name suggests, is designed to give relatively quick results to tree-building problems. Often, scientists will use much more powerful algorithms that take hours or even days to run. Simple Phylogeny can build trees in a couple of minutes.

On the following pages, you will find the 1,140 bp *cytb* sequence for the following lemurs:

- The eight individual lemurs that you analyzed in the first part of this lab. These sequences were obtained by sequencing the DNA of organisms caught in the field in Madagascar.
- Seven other lemurs. These other sequences were collected from the online sequence database GenBank.
  - Three Greater dwarf lemurs (*C. major*) labeled lemurs A-C
  - Three Fat-tailed dwarf lemur (*C. medius*) labeled lemurs D-F
  - One Gray mouse lemur (*Microcebus murinus*) labeled lemur G

3. Quickly scan through the lemur DNA sequences. Upon your first impression, do these *cytb* sequences look very similar or very different? Explain your answer.

4. If you were given just these sequences with no additional computer programs, how would you go about trying to put them in groups? Do you think you would be successful?

ALICIA

ATGACTAACATTCGAAAAACCCACCCCCTCATAAAAATCATAAACAGCTCATTCATTGACTTACCAGCACCATCCAATATCTCCTCCTGATG
AAATTTCGGTTCCCTCCTAGGAGCTTGTCTAGCTATTCAAATCATCACAGGCCTATTCTTAGCTATGCATTACACAGCAGATACAACAACCG
CATTCTCTTCCGTAACCCATATTTGCCGGGACGTAAACCACGGCTGAATCATCCGCTATCTCCACGCCAACGGAGCATCTATATTTTTTCTA
TGCCTATTTATTCACGTAGGACGTGGTATATACTACGGATCTTTTACCATATCAGAAACCTGAAACATCGGTATTATCCTACTATTTACAGTA
ATAGCAACCGCTTTTATAGGTTATGTACTTCCATGAGGACAAATATCATTTTGAGGAGCCACAGTCATCACAAACCTACTCTCAGCAATCCC
ATACATCGGTACTATATTAGTAGAATGAATCTGAGGAGGGTTCTCAGTTGATAAAGCCACACTAACCCGATTCTTCGCATTCCACTTTATCC
TACCTTTTATCATTACAGCCTTAGTCATAGTCCACCTTCTCTTTCTTCACGAAACAGGATCCAACAACCCACTAGGCACCTCATCAGACTCC
GACAAAATCCCATTCCACCCTTATTACACCATTAAAGATCTACTAGGACTTTTATTTCTCTTAATTCTACTCATAATCCTAGTACTTTTCTCCC
CTGACCTATTAGGGGACCCCGACAATTATACACCAGCCAATCCACTTAACACCCCACCCCATATTAAACCAGAATGATATTTCCTCTTCGCT
TATGCTATCCTACGATCCATCCCTAATAAACTAGGAGGCGTGCTAGCCTTAATTATATCTATTCTCATCCTAGCGATTATCCCTATACTTCAA
ACAACTAAACAACGAAGCATAATATTCCGACCCTTTAGCCAGATCCTATTCTGAATCCTCACAGCGGATTTATTTATTCTTACATGAATTGGA
GGCCAACCCGTTGAATATCCCTTCATTACTATTGGTCAAATAGCATCCATCCTATACTTCTTTCTAATTCTCATTATTATACCAACCGCAAGC
CTTATAGAAAACAAAATACTCAAATGAAGA

BEMA

ATGACTAACATTCGAAAAACTCACCCCCTCATAAAAATCATAAACAGCTCATTCATTGACTTACCAGCACCATCCAATATCTCCTCCTGATG
AAATTTCGGTTCCCTCCTAGGAGCTTGTCTAGCTATTCAAATCATCACAGGCCTATTCTTAGCTATGCATTACACAGCAGATACAACAACCG
CATTCTCTTCCGTAACCCATATTTGCCGAGACGTAAACCACGGCTGAATCATCCGCTATCTCCACGCCAACGGAGCATCTATATTTTTTCTA
TGCCTATTTATTCACGTAGGACGTGGTATATACTACGGATCTTTTACCATATCAGAAACCTGAAACATCGGTATTATCCTACTATTTACAGTA
ATAGCAACCGCTTTTATAGGTTATGTACTTCCATGAGGACAAATATCATTTTGAGGAGCCACAGTCATCACAAACCTACTCTCAGCAATCCC
ATACATCGGTACTATAATAGTAGAATGAATCTGAGGAGGGTTCTCAGTTGATAAAGCCACACTAACCCGATTCTTCGCATTCCACTTTATCC
TACCTTTTATCATTACAGCCTTAGTCATAGTCCACCTTCTCTTTCTTCACGAAACAGGATCCAACAACCCACTAGGCACCTCATCAGACTCC
GACAAAATCCCATTCCACCCTTATTACACCATTAAAGATCTACTAGGACTTTTATTTCTCTTAATTCTACTCATAATCCTAGTACTTTTCTCCC
CTGACCTATTAGGGGACCCCGACAATTATACACCAGCCAATCCACTTAACACCCCACCCCATATTAAACCAGAATGATATTTCCTCTTCGCT
TATGCTATCCTACGATCCATCCCTAATAAACTAGGAGGCGTGCTAGCCTTAATTATATCTATTCTCATCCTAGCGATTATCCCTATACTTCAA
ACAACTAAACAACGAAGCATAATATTCCGACCCTTTAGCCAGATCCTATTCTGAATCCTCACAGCGGATCTATTTATTCTTACATGAATTGG
AGGCCAACCCGTTGAATATCCCTTCATTACTATTGGTCAAATAGCATCCATCCTATACTTCTTTCTAATTCTCATTATTATACCAACCGCAAG
CCTTATAGAAAACAAAATACTCAAATGAAGA

GARAFA

ATGACCAACATTCGAAAAACCCACCCCTTAATAAAAATTATAAATAGCTCATTCATTGATCTCCCAGCACCATCTAACATCTCCTCTTGATGA
AATTTTGGTTCCCTCCTAGGAGCCTGCTTAATTATTCAAATTATTACAGGCTTATTCTTAGCAATACATTATACAGCAGACACAACAACCGCA
TTTTCTTCCGTGACCCATATCTGCCGAGATGTAAATCACGGATGAATCATTCGATATCTTCACGCCAACGGAGCATCTATATTTTTCCTGTG
TTTATTCATTCATGTAGGCCGCGGCATATACTACGGCTCCTTTACCATATCAGAAACCTGAAACATCGGCATTATTCTCTTATTTACAGTTAT
AGCAACTGCTTTCATAGGCTATGTACTCCCATGAGGACAAATATCATTTTGAGGAGCCACAGTCATCACAAATTTACTCTCAGCAATTCCAT
ATATCGGCACTAGCCTGGTAGAATGAATTTGAGGTGGCTTTTCCGTGGACAAAGCCACACTAACCCGATTCTTCGCATTCCACTTTATCCT
ACCCTTCATCATCGCAGCCCTAGTTTTAATTCACCTATTATTTCTCCACGAAACAGGATCTAACAACCCACTAGGTACCTCATCAGACTCAG
ATAAAATTCCATTCCACCCCTATTACACAATTAAAGACCTATTAGGATTTCTATTTTTCCTAATCTTACTAATAACTCTAGTACTTTTCTCCCC
CGATTTACTAGGAGACCCTGATAATTATACACCAGCCAATCCACTTAGCACTCCACCCCATATTAAACCAGAATGATATTTTCTCTTCGCCT
ACGCTATTCTACGATCCATCCCTAACAAACTAGGAGGAGTACTAGCCTTAGTCCTATCCATTCTTATCCTAGCAATCGTACCCATACTCCAA
ACAACTAAACAACGAAGCATAATATTCCGACCCCTAAGTCAAATTCTATTCTGAATCCTAGCAGCAGACCTATTTACTCTTACATGAATTGG
AGGTCAACCCGTCGAATACCCTTTCGTTACCATTGGACAAATAGCATCTATCCTATACTTTTCCCTTATCCTTATTATTATACCAATCGTAAG
TCTTATAGAAAATAAAATGCTCAAATGAAGA

IHARY

ATGACCAACATTCGAAAAACCCACCCCTTAATAAAAATTATAAATAGCTCATTCATTGATCTCCCAGCACCATCTAACATCTCCTCTTGATGA
AATTTTGGTTCCCTCCTAGGAGCCTGCTTAATTATTCAAATTATTACAGGCTTATTCTTAGCAATACATTATACAGCAGACACAACAACCGCA
TTTTCTTCCGTGACCCATATCTGCCGAGATGTAAATCACGGATGAATCATTCGATATCTTCACGCCAACGGAGCATCTATATTTTTCCTGTG
TTTATTCATTCATGTAGGCCGCGGCATATACTACGGCTCCTTTACCATATCAGAAACCTGAAACATCGGCATTATTCTCTTATTTACAGTTAT
AGCAACTGCTTTCATAGGCTATGTACTCCCATGAGGACAAATATCATTTTGAGGAGCCACAGTCATCACAAATTTACTCTCAGCAATTCCAT
ATATCGGCACTAGCCTGGTAGAATGAATTTGAGGTGGCTTTTCCGTGGACAAAGCCACACTAACCCGATTCTTCGCATTCCACTTTATCCT
ACCCTTCATCATCGCAGCCCTAGTTTTAATTCACCTATTATTTCTCCACGAAACAGGATCTAACAACCCACTAGGTACCTCATCAGACTCAG
ATAAAATTCCATTCCACCCCTATTACACAATTAAAGACCTATTAGGATTTCTATTTTTCCTAATCTTACTAATAACTCTAGTACTTTTCTCCCC
CGATTTACTAGGAGACCCTGATAATTATACACCAGCCAATCCACTTAGCACTCCACCCCATATTAAACCAGAATGATATTTTCTCTTCGCCT
ACGCTATTCTACGATCCATCCCTAACAAACTAGGAGGAGTACTAGCCTTAGTCCTATCCATTCTTATCCTAGCAATCGTACCCATACTCCAA
ACAACTAAACAACGAAGCATAATATTCCGACCCCTAAGTCAAATTCTATTCTGAATCCTAGCAGCAGACCTATTTACTCTTACATGAATTGG
AGGTCAACCCGTCGAATACCCTTTCGTTACCATTGGACAAATAGCATCTATCCTATACTTTTCCCTTATCCTTATTATTATACCAATCGTAAG
TCTTATAGAAAATAAAATGCTCAAATGAAGA

MALALA

ATGACCAACATTCGAAAAACCCACCCCTTAATAAAAATTATAAATAGCTCATTCATTGATCTCCCAGCACCATCTAACATCTCCTCTTGATGA
AATTTTGGTTCCCTCCTAGGAGCCTGCTTAATTATTCAAATTATTACAGGCTTATTCTTAGCAATACATTATACAGCAGACACAACAACCGCA
TTTTCTTCCGTGACCCATATCTGCCGAGATGTAAATCACGGATGAATCATTCGATATCTTCACGCCAACGGAGCATCTATATTTTTCCTGTG
TTTATTCATTCATGTAGGCCGCGGCATATACTACGGCTCCTTTACCATATCAGAAACCTGAAACATCGGCATTATTCTCTTATTTACAGTTAT
AGCAACTGCTTTCATAGGCTATGTACTCCCATGAGGACAAATATCATTTTGAGGAGCCACAGTCATCACAAATTTACTCTCAGCAATTCCAT
ATATCGGCACTAGCCTGGTAGAATGAATTTGAGGTGGCTTTTCCGTGGACAAAGCCACACTAACCCGATTCTTCGCATTCCACTTTATCCT
ACCCTTCATCATCGCAGCCCTAGTTTTAATTCACCTATTATTTCTCCACGAAACAGGATCTAACAACCCACTAGGTACCTCATCAGACTCAG
ATAAAATTCCATTCCACCCCTATTACACAATTAAAGACCTATTAGGATTTCTATTTTTCCTAATCTTACTAATAACTCTAGTACTTTTCTCCCC
CGATTTACTAGGAGACCCTGATAATTATACACCAGCCAATCCACTTAGCACTCCACCCCATATTAAACCAGAATGATATTTTCTCTTCGCCT
ACGCTATTCTACGATCCATCCCTAACAAACTAGGAGGAGTACTAGCCTTAGTCCTATCCATTCTTATCCTAGCAATCGTTCCCATACTCCAA
ACAACTAAACAACGAAGCATAATATTCCGACCCCTAAGTCAAATTCTATTCTGAATCCTAGCAGCAGACCTATTTACTCTTACATGAATTGG
AGGTCAACCCGTCGAATACCCTTTCGTTACCATTGGACAAATAGCATCTATCCTATACTTTTCCCTTATCCTTATTATTATACCAATCGTAAG
TCTTATAGAAAATAAAATGCTCAAATGAAGA

MBOLA

ATGACTAACATTCGAAAAACTCACCCCCTCATAAAAATCATAAACAGCTCATTCATTGACTTACCAGCACCATCCAATATCTCCTCCTGATG
AAATTTCGGTTCCCTCCTAGGAGCTTGTCTAGCTATTCAAATCATCACAGGCCTATTCTTAGCTATGCATTACACAGCAGATACAACAACCG
CATTCTCTTCCGTAACCCATATTTGCCGAGACGTAAACCACGGCTGAATCATCCGTTATCTCCACGCCAACGGAGCATCCATATTTTTTCTA
TGCCTATTTATTCACGTAGGACGTGGTATATACTACGGATCTTTTACCATATCAGAAACCTGAAACATCGGTATTATCCTACTATTTACAGTA
ATAGCAACCGCTTTCATAGGTTATGTACTTCCATGAGGACAAATATCATTTTGAGGAGCCACAGTCATCACAAACCTACTCTCAGCAATCCC
ATACATCGGCACTATAATAGTAGAATGAATCTGAGGAGGGTTCTCAGTTGATAAAGCCACACTAACCCGATTCTTCGCATTCCACTTTATCC
TACCTTTTATCATTACAGCCCTAGTCATAGTCCACCTTCTCTTTCTTCACGAAACAGGATCCAACAACCCACTAGGCACCTCATCAGACTCC
GACAAAATCCCATTCCACCCTTATTACACCATTAAAGATCTACTAGGACTTTTATTTCTCTTAATTCTACTCATAATCCTAGTACTTTTCTCCC
CTGACCTATTAGGGGACCCCGACAATTATACACCAGCCAATCCACTTAACACCCCACCCCATATTAAACCAGAATGATATTTCCTCTTCGCT
TATGCTATCCTACGATCCATCCCTAATAAACTAGGAGGCGTGCTAGCCTTAGTTATATCTATTCTCATCCTAGCGATTATCCCTATACTTCAA
ACAACCAAACAACGAAGCATAATATTCCGACCCTTTAGCCAGATCCTATTCTGAATCCTCACAGCAGATCTATTTATTCTTACATGAATTGG
AGGCCAACCCGTCGAATATCCCTTCATTACTATTGGTCAAATAGCATCCATCCTATACTTCTTTCTAATTCTCATTATTATACCAACCGCAAG
CCTTATAGAAAACAAAATACTCAAATGAAGA

RAZAFY

ATGACTAACATTCGAAAAACTCACCCCCTCATAAAAATCATAAACAGCTCATTCATTGACTTACCAGCACCATCCAATATCTCCTCCTGATG
AAATTTCGGTTCCCTCCTAGGAGCTTGTCTAGCTATTCAAATCATCACAGGCCTATTCTTAGCTATGCATTACACAGCAGATACAACAACCG
CATTCTCTTCCGTAACCCATATTTGCCGAGACGTAAACCACGGCTGAATCATCCGTTATCTCCACGCCAACGGAGCATCCATATTTTTTCTA
TGCCTATTTATTCACGTAGGACGTGGTATATACTACGGATCTTTTACCATATCAGAAACCTGAAACATCGGTATTATCCTACTATTTACAGTA
ATAGCAACCGCTTTCATAGGTTATGTACTTCCATGAGGACAAATATCATTTTGAGGAGCCACAGTCATCACAAACCTACTCTCAGCAATCCC
ATACATCGGCACTATAATAGTAGAATGAATCTGAGGAGGGTTCTCAGTTGATAAAGCCACACTAACCCGATTCTTCGCATTCCACTTTATCC
TACCTTTTATCATTACAGCCCTAGTCATAGTCCACCTTCTCTTTCTTCACGAAACAGGATCCAACAACCCACTAGGCACCTCATCAGACTCC
GACAAAATCCCATTCCACCCTTATTACACCATTAAAGATCTACTAGGACTTTTATTTCTCTTAATTCTACTCATAATCCTAGTACTTTTCTCCC
CTGACCTATTAGGGGACCCCGACAATTATACACCAGCCAATCCACTTAACACCCCACCCCATATTAAACCAGAATGATATTTCCTCTTCGCT
TATGCTATCCTACGATCCATCCCTAATAAACTAGGAGGCGTGCTAGCCTTAGTTATATCTATTCTCATCCTAGCGATTATCCCTATACTTCAA
ACAACCAAACAACGAAGCATAATATTCCGACCCTTTAGCCAGATCCTATTCTGAATCCTCACAGCAGATCTATTTATTCTTACATGAATTGG
AGGCCAACCCGTCGAATATCCCTTCATTACTATTGGTCAAATAGCATCCATCCTATACTTCTTTCTAATTCTCATTATTATACCAACCGCAAG
CCTTATAGAAAACAAAATACTCAAATGAAGA

RODOLFO

ATGACCAACATTCGAAAAACCCACCCCTTAATAAAAATTATAAATAGCTCATTCATTGATCTCCCAGCACCATCTAACATCTCCTCTTGATGA
AATTTTGGTTCCCTCCTAGGAGCCTGCTTAATTATTCAAATTATTACAGGCTTATTCTTAGCAATACATTATACAGCAGACACAACAACCGCA
TTTTCTTCCGTGACCCATATCTGCCGAGATGTAAATCACGGATGAATCATTCGATATCTTCACGCCAACGGAGCATCTATATTTTTCCTGTG
TTTATTCATTCATGTAGGCCGCGGCATATACTACGGCTCCTTTACCATATCAGAAACCTGAAACATCGGCATTATTCTCTTATTTACAGTTAT
AGCAACTGCTTTCATAGGCTATGTACTCCCATGAGGACAAATATCATTTTGAGGAGCCACAGTCATCACAAATTTACTCTCAGCAATTCCAT
ATATCGGCACTAGCCTGGTAGAATGAATTTGAGGTGGCTTTTCCGTGGACAAAGCCACACTAACCCGATTCTTCGCATTCCACTTTATCCT
ACCCTTCATCATCGCAGCCCTAGTTTTAATTCACCTATTATTTCTCCACGAAACAGGATCTAACAACCCACTAGGTACCTCATCAGACTCAG
ATAAAATTCCATTCCACCCCTATTACACAATTAAAGACCTATTAGGATTTCTATTTTTCCTAATCTTACTAATAACTCTAGTACTTTTCTCCCC
CGATTTACTAGGAGACCCTGATAATTATACACCAGCCAATCCACTTAGCACTCCACCCCATATTAAACCAGAATGATATTTTCTCTTCGCCT
ACGCTATTCTACGATCCATCCCTAACAAACTAGGAGGAGTACTAGCCTTAGTCCTATCCATTCTTATCCTAGCAATCGTACCCATACTCCAA
ACAACTAAACAACGAAGCATAATATTCCGACCCCTAAGTCAAATTCTATTCTGAATCCTAGCAGCAGACCTATTTACTCTTACATGAATTGG
AGGTCAACCCGTCGAATACCCTTTCGTTACCATTGGACAAATAGCATCTATCCTATACTTTTCCCTTATCCTTATTATTATACCAATCGTAAG
TCTTATAGAAAATAAAATGCTCAAATGAAGA

LEMUR A: Greater dwarf lemur (*C. major*)

ATGACCAACATTCGAAAAACTCACCCCCTTATAAAAATTATAAACAGTTCATTTATTGATCTACCAGCACCATCCAACATCTCCTCCTGATGA
AATTTCGGTTCCCTCCTAGGAGCTTGCCTAGCTATTCAAATTATTACAGGCCTATTCCTAGCTATACATTACACAGCAGATACAACAACAGC
ATTTTCTTCCGTTGCCCACATCTGCCGAGACGTAAACCACGGCTGAATTATTCGATATCTCCATGCCAACGGAGCATCCATATTCTTCCTAT
GCTTATTTATCCATGTAGGCCGCGGCATATACTATGGATCCTTTACTATATCAGAAACCTGAAACATCGGCATTATTTTACTATTTACAGTTA
TAGCAACTGCTTTTATAGGCTATGTCCTCCCATGAGGACAAATATCATTTTGAGGAGCCACAGTCATCACAAATTTACTCTCAGCAATTCCT
TACATCGGCACCATTCTAGTAGAATGAATCTGAGGGGGATTCTCAGTTGACAAAGCTACACTAACCCGATTTTTCGCATTTCACTTTATTTT
ACCCTTTATTATTACAGCCCTAGTTATAATCCATCTCCTCTTTCTCCATGAATCAGGGTCCAACAATCCACTAGGCACTTCATCAGATTCCG
ACAAAATCCCATTTCACCCCTACTACACAATTAAAGACTTACTAGGACTCCTATTTTTCCTAATTCTACTTTTAATCCTAGTGCTTTTCTCCCC
CGACTTATTAGGAGACCCTGACAATTATACACCAGCCAATCCACTTAGCACCCCACCCCATATTAAACCAGAATGATATTTCCTCTTTGCCT
ACGCTATCTTACGATCTATTCCCAATAAACTAGGAGGAGTCCTGGCCCTAGTCCTATCTATTCTTATTCTAGCAATTATTCCCATACTTCAAT
TAACTAAACAACGAAGCATAATGTTCCGGCCTCTTAGCCAAATTCTATTCTGAATTCTAACAGCAGACCTATTTACCCTTACATGAATTGGA
GGCCAGCCCGTTGAACACCCCTTCATCACCATCGGCCAAATAGCATCTATCCTTTATTTCTCCCTTATTCTCATCATTATACCAATCGTAAG
CCTTATAGAGAACAAAATACTCAAATGAAGA

LEMUR B: Greater dwarf lemur (*C. major*)

ATGACCAACATTCGAAAAACTCACCCCCTTATAAAAATTATAAACAGTTCATTTATTGATTTACCAGCACCATCCAACATCTCCTCCTGATGA
AATTTCGGTTCCCTCCTAGGAGCTTGCCTAGCTATTCAAATTATTACAGGTCTATTCCTAGCTATACATTATACAGCAGATACAACAACAGC
ATTTTCTTCCGTTACCCATATTTGCCGAGACGTAAACCACGGCTGAATTATCCGATATCTCCACGCCAACGGAGCATCCATATTCTTCCTAT
GCTTATTTATCCATGTAGGCCGTGGCATATACTATGGATCCTTTACTATATCAGAAACCTGAAACATCGGCATTATTTTACTATTTACAGTTA
TAGCAACTGCTTTTATAGGCTATGTCCTCCCATGAGGACAAATATCATTTTGAGGGGCCACAGTCATCACAAATTTACTCTCAGCAATCCCT
TACATCGGCACCATTCTAGTAGAATGAATCTGAGGGGGATTCTCAGTTGACAAAGCTACGCTAACCCGATTTTTCGCATTTCACTTTATCTT
ACCCTTTATTATTGCAGCCCTAGTTATAATCCATCTCCTCTTTCTACATGAAACAGGATCCAACAATCCACTAGGTACTTCATCAGACTCCG
ACAAAATCCCATTTCACCCTTACTACACAATTAAAGATTTACTAGGACTCCTATTTTTCTTAATTCTACTTTTAACCTTAGTGCTTTTCTCCCC
CGACTTATTAGGAGACCCTGACAATTATACACCAGCCAATCCACTTAGCACCCCACCCCATATTAAACCAGAATGATATTTCCTCTTTGCCT
ACGCTATCTTACGATCTATTCCCAATAAACTAGGAGGCGTCCTGGCCCTAGTTCTATCTATTCTTATCCTAGCAATTATCCCCATACTTCAAT
TAACTAAACAACGAAGCATAATGTTCCGGCCCCTTAGCCAAATTCTATTCTGAGTTCTAACAGCAGACCTATTTACCCTTACATGAATTGGA
GGCCAACCCGTTGAACACCCCTTCATCACCATCGGTCAAATAGCATCTATCCTATATTTCTCCCTTATTCTCATCATTATACCAATCGCAAG
CCTTATAGAAAACAAAATACTCAAATGAAGA

LEMUR C: Greater dwarf lemur (*C. major*)

ATGACCAACATTCGAAAAACTCACCCCCTTATAAAAATTATAAACAGTTCATTTATTGATTTACCAGCACCATCCAACATCTCCTCCTGATGA
AATTTCGGTTCCCTCCTAGGAGCTTGCCTAGCTATTCAAATTATTACAGGTCTATTCCTAGCTATACATTATACAGCAGATACAACAACAGC
ATTTTCTTCCGTTACCCATATTTGCCGAGACGTAAACCACGGCTGAATTATCCGATATCTCCACGCCAACGGAGCATCCATATTCTTCCTAT
GCTTATTTATCCATGTAGGCCGTGGCATATACTATGGATCCTTTACTATATCAGAAACCTGAAACATCGGCATTATTTTACTATTTACAGTTA
TAGCAACTGCTTTTATAGGCTATGTCCTCCCATGAGGACAAATATCATTTTGAGGGGCCACAGTCATCACAAATTTACTCTCAGCAATCCCT
TACATCGGCACCATTCTAGTAGAATGAATCTGAGGGGGATTCTCAGTTGACAAAGCTACGCTAACCCGATTTTTCGCATTTCACTTTATCTT
ACCCTTTATTATTACAGCCCTAGTTATAATCCATCTCCTCTTTCTACATGAAACAGGATCCAACAATCCACTAGGTACTTCATCAGACTCCGA
CAAAATCCCATTTCACCCTTACTACACAATTAAAGATTTACTAGGACTCCTATTTTTCTTAATTCTACTTTTAACCTTAGTGCTTTTCTCCCCC
GACTTATTAGGAGACCCTGACAATTATACTCCAGCCAATCCACTTAGCACCCCACCCCATATTAAACCAGAATGATATTTCCTCTTTGCCTA
CGCTATCTTACGATCTATTCCCAATAAACTAGGAGGCGTCCTGGCCCTAGTTCTATCTATTCTTATCCTAGCAATTATCCCCATACTTCAATT
AACTAAACAACGAAGCATAATGTTCCGGCCCCTTAGCCAAATTCTATTCTGAGTTCTAACAGCAGACCTATTTACCCTTACATGAATTGGAG
GCCAACCCGTTGAACACCCCTTCATCACCATCGGTCAAATAGCATCTATCCTATATTTCTCCCTTATTCTCATCATTATACCAATCGCAAGC
CTTATAGAAAATAAAATACTCAAATGAAGA

LEMUR D: Fat-tailed dwarf lemur (*C. medius*)

ATGACCAACACTCGAAAAACCCACCCTCTAATAAAAATCATGAATAGCTCATTCATTGATCTCCCAGCACCATCCAACATTTCCTCTTGATG
GAACTTTGGTTCTCTCCTAGGAGCCTGCCTAGCAATCCAAATCATTACAGGCCTATTTCTAGCAATACACTACACAGCAGACACAACAACT
GCATTCTCCTCTGTTACCCACATTTGCCGAGACGTAAACCACGGCTGAATTATTCGATACCTCCATGCCAACGGAGCATCTATATTCTTTTT
ATGCCTATTCATTCATGTAGGCCGTGGCATATACTACGGGTCTTTCACTATACTAGAAACCTGAAACATCGGTATTATTCTATTATTTACAGT
TATAGCAACCGCTTTCATAGGATATGTTCTCCCATGAGGACAAATATCATTTTGAGGGGCCACAGTCATCACAAACTTACTTTCAGCAATCC
CATATATCGGTACTGTTCTAGTAGAATGAATCTGAGGGGGATTTTCAGTCGACAAAGCTACACTTACTCGATTCTTTGCATTCCACTTCATT
CTACCCTTTGTTATTGCAGCCCTTGTCATAGTACATCTCCTATTTCTACACGAAACAGGTTCTAACAACCCACTAGGCACCTCATCAGACTC
CGACAAAATTCCATTCCACCCCTACTATACAATCAAAGACTTACTAGGACTTCTATTTTTCCTAATCTTACTCCTAACCCTAGTGCTTTTCTC
CCCCGACTTACTAGGAGACCCAGACAATTACACACCAGCCAATCCGCTAAGCACCCCACCCCATATTAAACCAGAATGATACTTTTTATTC
GCCTACGCCATCCTACGATCTATCCCTAATAAACTAGGAGGAGTTATAGCCTTAGTTCTATCTATTCTAATCTTAGCGATCATCCCAATACT
CCAAACAACCAAACAACGAAGCATATTATTCCGACCTCTTAGCCAAATCCTATTCTGAATTCTAACAGCAGACCTATTTATCCTTACATGAAT
TGGAGGTCAACCAGTCGAATACCCTTTCATCACCATCGGCCAAATAGCATCCATCCTATACTTTTCTATTATTCTTATCATTATGCCTACTGT
AAGTCTTATAGAAAACAAAATACTTAAATGAAGA

LEMUR E: Fat-tailed dwarf lemur (*C. medius*)

ATGACCAACATTCGAAAAACCCACCCCCTAATAAAAATCATGAATAGCTCATTCATTGATCTCCCAGCACCATCCAATATTTCCTCTTGATG
GAACTTCGGTTCTCTCCTAGGAGCTTGCCTAGCAATCCAAATCATTACAGGTCTATTTCTAGCAATACACTATACAGCAGATACAACAACCG
CGTTCTCCTCAGTTACCCACATTTGCCGAGACGTAAACCACGGCTGAATTATTCGATATCTCCATGCCAACGGAGCATCCATATTCTTTCTA
TGCCTATTCATTCATGTAGGCCGCGGCATATACTATGGGTCCTTCACTATACTAGAAACCTGAAACATCGGTATTATTCTATTATTTACAGTT
ATAGCAACCGCTTTCATAGGATATGTCCTCCCATGAGGACAAATATCATTTTGAGGAGCTACAGTCATCACAAACTTACTTTCAGCAATCCC
ATATATCGGCACTGTCCTAGTAGAATGAATCTGAGGGGGATTTTCAGTCGACAAAGCTACACTAACTCGATTCTTTGCATTCCACTTCATTC
TACCCTTTATTATCGCAGCCCTTGTCATAGTGCATCTTCTATTTCTACACGAAACAGGTTCCAACAATCCACTAGGCACCTCATCAGACTCC
GACAAAATTCCATTCCACCCCTATTATACAATCAAAGACTTACTAGGACTTCTATTTTTCTTAATTTTACTCCTATCCCTAGTACTTTTCTCCC
CCGACTTACTAGGAGACCCAGACAATTATACACCAGCTAACCCACTAAGCACACCACCCCCATATTAAACCAGAATGATATTTTCTATTCGCC
TACGCCATCCTACGATCTATCCCCAATAAACTAGGAGGAGTTATGGCCTTAGTCCTATCTATCCTGATCTTAGCATTTATCCCAATACTCCA
AACAACCAAACAACGAAGCATACTATTCCGACCTCTCAGCCAAATCCTATTCTGAATCCTAACAGCAGACCTATTTATCCTTACATGAATTG
GAGGTCAACCAGTCGAATACCCTTTCATCACCATCGGCCAAATAGCATCCATCCTATACTTTTCTATTATTCTTATTATTATACCTACCGTAA
GTCTTATAGAAAACAAAATACTCAAATGAAGA

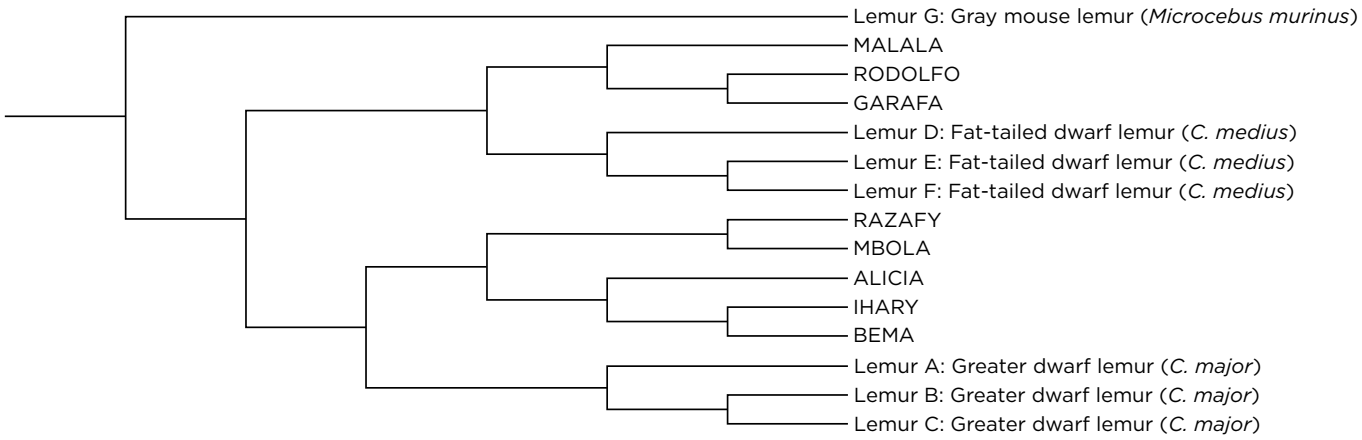
LEMUR F: Fat-tailed dwarf lemur (*C. medius*)

ATGACCAACATTCGAAAAACCCACCCCCTAATAAAAATCATGAATAGCTCATTCATTGATCTCCCAGCACCATCCAATATTTCCTCTTGATG
GAACTTCGGTTCTCTCCTAGGAGCTTGCCTAGCAATCCAAATCATTACAGGTCTATTTCTAGCAATGCACTATACAGCAGATACAACAACCG
CGTTCTCCTCAGTTACCCACATTTGCCGAGACGTAAACCACGGCTGAATTATTCGATATCTCCATGCCAACGGAGCATCCATATTCTTTTTA
TGCCTATTCATTCATGTAGGCCGCGGCATATACTATGGGTCCTTCACTATACTAGAAACCTGAAACATCGGTATTATTCTATTATTTACAGTT
ATAGCAACCGCTTTCATAGGATATGTCCTCCCATGAGGACAAATATCATTTTGAGGAGCTACAGTCATCACAAACTTACTTTCAGCAATCCC
ATATATCGGTACTGTCCTAGTAGAATGAATCTGAGGGGGATTTTCAGTCGACAAAGCTACACTAACTCGATTCTTTGCATTCCACTTCATTT
TACCCTTTATTATCGCAGCCCTTGTCATAGTGCATCTCCTATTTCTACACGAAACAGGTTCCAACAATCCACTAGGCACCTCATCAGACTCC
GACAAAATTCCATTCCACCCCTATTATACAATCAAAGACTTACTAGGTCTTCTTTTTTTCTTAATTTTACTCCTATCCCTAGTGCTTTTCTCCC
CCGACTTACTAGGAGACCCAGACAATTACACACCAGCCAACCCACTAAGCACACCACCCCATATTAAACCAGAATGATATTTTCTATTCGC
CTACGCCATCCTACGATCTATCCCCAATAAACTAGGAGGAGTTATAGCCTTAGTCCTATCTATCCTGATCTTAGCATTTATCCCAATACTCC
AAACAACCAAACAACGAAGCATACTATTCCGACCTCTCAGCCAAATCCTATTCTGAATCCTAACAGCAGACCTATTTATCCTTACATGAATT
GGAGGCCAACCAGTCGAATACCCTTTCATCACCATCGGCCAAATAGCATCCATCCTATACTTTTCTATTATTCTTATTATTATACCTACCGTA
AGTCTTATAGAAAACAAAATACTCAAATGAAGA

LEMUR G: Gray mouse lemur (*Microcebus murinus*)

ATGACCAACATCCGAAAGACCCACCCACTAATGAAAATCATAAACAACTCATTCATTGACCTTCCAGCACCGTCCAATATTTCTTCTTGATG
AAATTTTGGTTCCCTATTAGGAGCTTGCCTAGTCATTCAAATCATCACAGGCTTATTTCTAGCAATACATTACACAGCAGACACAACAACCG
CATTCTCCTCCGTAACTCACATCTGCCGAGATGTAAACCAGGGCTGAATCATTCGCTACCTACACGCTAATGGAGCATCCATATTCTTTCTA
TGCCTTTTCCTCCACGTAGGACGAGGTATGTACTATGGATCCTTTACTCTAACTGAAACCTGAAATATTGGTATCATTTTATTATTTACAGTA
ATAGCAACTGCTTTTATGGGATATGTTCTCCCATGAGGACAAATATCATTTTGAGGCGCAACAGTAATTACTAATTTATTATCAGCAATCCCC
TATATAGGCACTGATCTAGTAGAATGAATCTGAGGCGGCTTTTCCGTCGACAAAGCCACACTCACTCGATTCTTCGCATTCCATTTCATTTT
ACCATTTGTTATCTTAGCCCTAGTTATAGTTCACCTCCTCTTTCTCCATGAAACCGGATCAAATAACCCATTAGGTATTCCATCAGAATCAGA
CAAAATTCCATTTCATCCTTACTACACAATTAAAGATTTACTAGGACTTATATTCCTTTTAATTACACTTATAATTTTAGTACTCTTCTCCCCTG
ACTTACTAGGCGACCCTGACAACTACATGCCGGCCAACCCACTCAGCACCCCTCCCCATATCAAACCAGAGTGATATTTCCTCTTCGCCTA
CGCTATCCTGCGATCTATCCCCAATAAACTAGGAGGAGTCCTAGCTCTAGTTATATCAATTCTAATTCTTGCAATCATCCCTATATTACAAAC
CGCCAAGCAACGAAGCATAACATTCCGACCACTTAGCCAAATTATATTTTGAATCCTTACGGCAGACCTACTTATCCTTACATGAATTGGAG
GCCAACCAGTTGAACACCCCTTCGTAACTATTGGACAAGTAGCCTCTATTCTATATTTTTCTCTGATCCTTATCATTATACCAACCGTAAGCC
TTTTCGAAAATAAAATACTTAAATGAAG

Below is the tree generated by Simple Phylogeny using the *cytb* sequences from the previous pages.



5. Is there an outgroup in the tree? You will recognize it because it will be the only organism to branch off from the leftmost node on the tree.

6. If so, which lemur is it? Does this make sense? Explain your reasoning.

7. Find Garafa, one of the dwarf lemurs from Tsinjoarivo, on your tree. What individual's *cytb* gene is most closely related to Garafa's?

8. Keep following Garafa's branch back until you come to the next node. Looking at this node, which individual's *cytb* gene is second most closely related to Garafa's?

9. According to this phylogeny, is Alicia's *cytb* gene more closely related to Bema, Ihary, or equally related to both? Use evidence from the tree to justify your answer.

The big cats tree that you looked at previously had branches that all ended in a single species. Here we are analyzing a tree that ends in different individuals. We want to decide which of those individuals can be grouped together as one species. We recognize species on a tree because all of the individuals of one species will be part of a single clade. That means that the branches of all individuals of the same species will meet at a node before those branches connect to other individuals on the tree. We included other lemurs (*C. major* and *C. medius*) that we know are different species to help make that distinction.

10. Looking at this tree, do the eight named dwarf lemurs from Tsinjoarivo that you analyzed in part one of this lab converge at a single node before connecting to the branches of other species, or are they separated into different groups?

11. In your own words, describe how the eight lemurs that you analyzed in the first part of this lab are grouped on this tree.

12. Does this tree support the conclusion that there is only one species living in Tsinjoarivo? Or does it support the conclusion that there are multiple species living in Tsinjoarivo and you have rediscovered *C. sibreei* lemurs? Justify your answer using evidence from the tree.

13. Does this tree support or conflict with your conclusion from your gel electrophoresis data in the first part of the lab? Justify your answer.

Final conclusions

Combine the information from this tree with the information from the first part of this lab. Identify all eight named dwarf lemurs from Tsinjoarivo as either *C. crossleyi* or *C. sibreei*

C. crossleyi	*C. sibreei*

14. According to this tree based on *cytb* data, does *C. crossleyi* appear to be more closely related to *C. major* or *C. medius*? Justify your answer.

15. According to this tree based on *cytb* data, does *C. sibreei* appear to be more closely related to *C. major* or *C. medius*? Justify your answer.

Extension: Using ecological data to evaluate the health of species

Tsinjoarivo is the site of continuous environmental change. These changes impact all the living things that call the central highlands home, including the *C. crossleyi* and *C. sibreei* species you studied in this lab. In this exercise, you will analyze how one specific environmental change, deforestation, affects the lemurs that reside in the area, and how different species may respond to that threat differently.

Part 1: Environmental change

Our research group has been studying lemurs at Tsinjoarivo for the last two decades. Even in that relatively short time, the landscape has visibly changed. To support a growing human population, the local community has cleared more low-altitude forest to make room for agricultural crops. In Tsinjoarivo, people also practice selective logging where larger trees of certain valuable species are harvested, either to use for lumber or to sell as precious hardwood. Taking trees in this way leaves more total forest intact but changes the forest habitat by changing the species and age distribution of the trees. For species of lemurs that rely on a very specific ecological niche, even small changes in forest composition can have dramatic effects.

According to the IUCN, *C. sibreei* (Sibree's dwarf lemur) is considered critically endangered, and *C. crossleyi* (the furry-eared dwarf lemur) is considered vulnerable. Changes to the forest could have serious implications for the survival of both species, but we don't know how such changes will affect the two species differently.

1. In terms of managing biodiversity, selective logging is often presented as a positive alternative to clearcutting. What advantages do you think selective logging could have compared to clearcutting?

2. Both clearcutting and selective logging have occurred at Tsinjoarivo over the past decade, but the majority of forest change has been due to selective logging. How do you predict this will impact the lemur populations living there?

3. Ecologists and environmental scientists often identify species as generalists or specialists. Generalists tend to live in a wider variety of habitats and rely on more varied resources. Specialists tend to live in a narrower range of habitats and rely on a more specific range of resources. Based on the description of selective logging above, would you expect specialists or generalists to fare better in a forest where selective logging is being practiced? Explain your answer.

Part 2: Population impact

Our research group monitors the size of the lemur population at Tsinjoarivo on an ongoing basis. There are several different ways to estimate the population size of a particular species in an area. Although it's not possible to directly count all individuals in the area—remember these are small, highly mobile animals that are only out at night for part of the year—we can estimate the size of the lemur population by conducting a trapping study. By laying a set of traps overnight, we can count and then release the lemurs we find the next morning. From the number of lemurs we count, we can estimate the size of the local population. As a population grows, our trapping success rate goes up. That is, we expect to trap individuals more easily. As a population declines, it will become more difficult to trap animals and the trapping success rate will decrease accordingly.

Analysis of population change

First, you will analyze trap data to get an idea of how the different species of lemur are responding to continued change in the Tsinjoarivo forest. The table on the next space summarizes trap data taken periodically over a 13-year span beginning in 2006. This is the same time period during which our research group observed the environmental changes such as deforestation and selective logging that were described above.

We can calculate the trapping success rate by dividing the total number of lemurs trapped by the total number of "trap nights," and expressing this value as a percentage. You can think of trap nights as the total opportunities there were to catch a lemur. To calculate trap nights, the number of traps used is multiplied by the number of nights the traps were in the field. For example, if a single trap is in the field for 5 nights, that equals 5 trap nights. If two traps are in the field for 5 nights, that equals 10 trap nights.

4. Calculate the trapping success rate for each year of our study. To do this, divide the total lemurs trapped (column C) by the trap nights (column B) and multiply by 100. Fill these values into the "Trapping success rate (%)" column below (column D). For now, leave columns F and H blank.

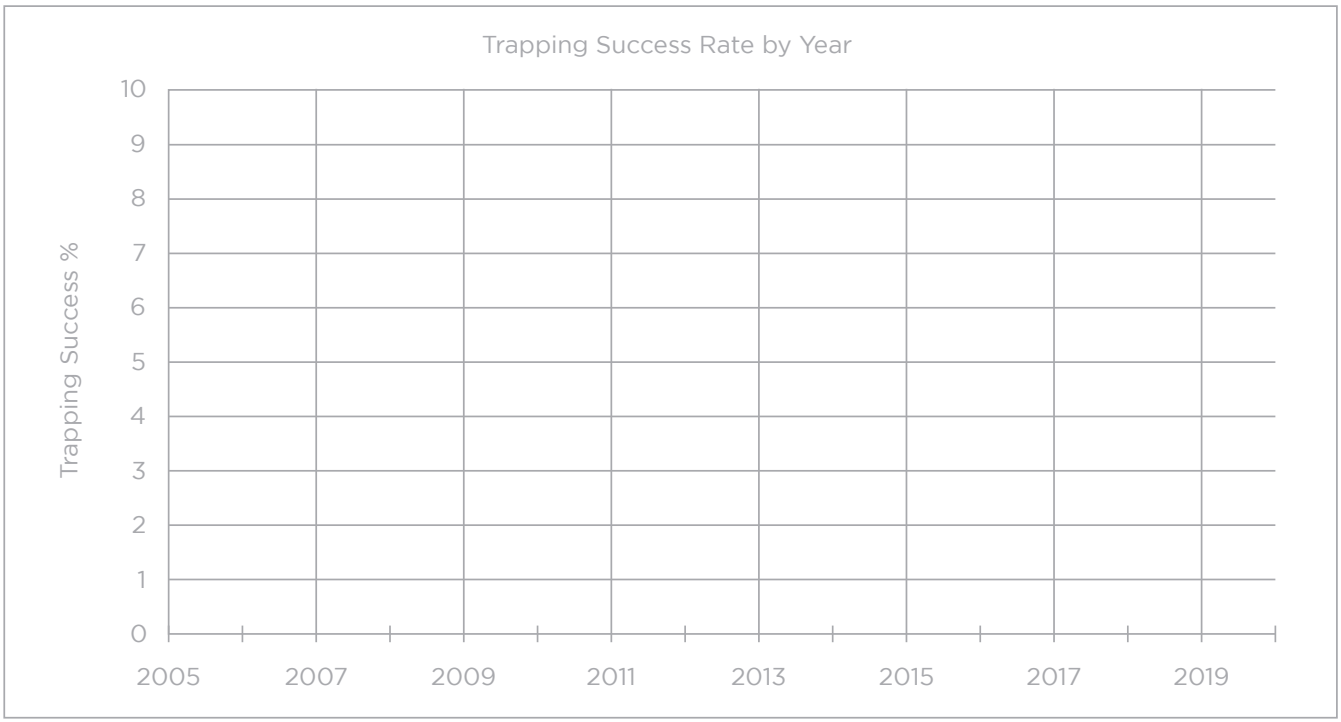
A	B	C	D	E	F	G	H
Year	**Trap nights**	**Total number of lemurs trapped**	**Trapping success rate (%)**	**Number of *C. sibreei* trapped**	**Trapping success rate (%) *C. sibreei***	**Number of *C. crossleyi* trapped**	**Trapping success rate (%) *C. crossleyi***
2006	100	7		7		0	
2007	200	15		12		3	
2009	300	14		8		6	
2011	900	17		10		7	
2013	900	19		9		10	
2014	900	11		0		11	
2019	600	10		2		8	

Use the data from columns A-D to answer questions 5-9. For now you are looking at total lemurs, not individual species.

5. Based on these data, during which year or years would you estimate the lemur population to be the largest? How do you know?

6. Is the year with the largest total lemur population the same year the most lemurs were caught? If not, explain how those two things can be different.

7. Graph the trapping success rate (column D) by year using the grid below.



8. Using your graph as a reference, make a statement about how the lemur population at Tsinjoarivo changed between 2006 and 2019.

9. Does the data support the prediction you made in question 2 on page 45?

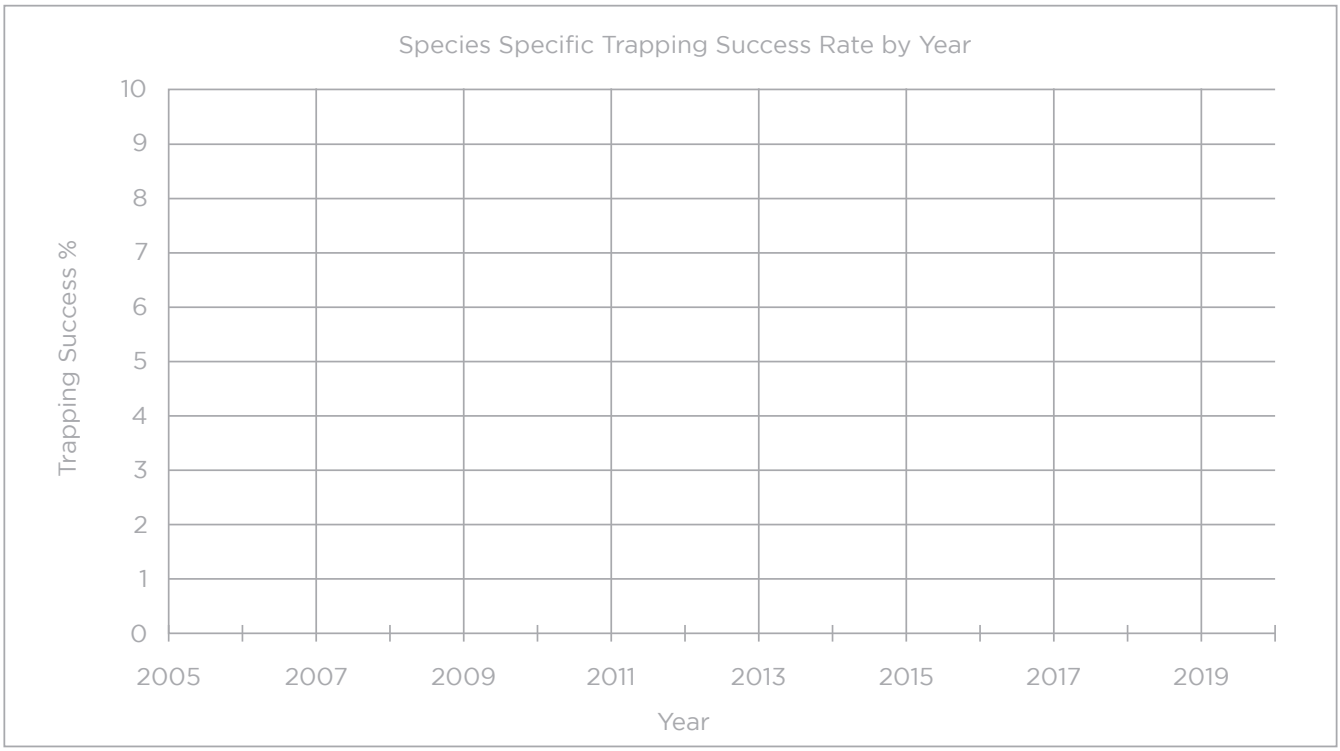
Analysis of species contact

10. Calculate the trapping success for *C. sibreei*. Divide the number of *C. sibreei* lemurs trapped by the trap nights and multiply by 100. Fill these values into the "Trapping success rate (%) – *C. sibreei*" column above (column F).

11. Use the same process to calculate the percent of trapped lemurs belonging to C. crossleyi. Fill these values into the "Trapping success rate (%) – *C. crossleyi*" column above (column H).

12. Graph the trapping success rate for *C. sibreei* (column G) across time on the graph below.

13. On the same graph, plot the trapping success rate for *C. crossleyi* (column H) across time. Because you will have more than one line on the same graph, be sure to make your two data sets look different by using a different color or style of data point than you did previously. Indicate which symbols belong to which species in the graph legend below the graph.



Graph legend:
C. crossleyi
C. sibreei

14. Using your graph as a reference, make a statement about how *C. sibreei* and *C. crossleyi* populations at Tsinjoarivo changed between 2006 and 2019.

    a. *C. sibreei:*

    b. *C. crossleyi:*

15. Does this graph change your impression of what is happening at Tsinjoarivo compared to the first graph on page 48 that did not distinguish between species? Explain your answer.

16. What limitations might this dataset have? Looking at this dataset and based on what you've been told about how this data was collected, identify one potential source of error.

Part 3: Tracking behavior

The population numbers from Tsinoravo that you just analyzed are alarming. But to specifically respond to this threat, we need to try to understand why the different species of lemur are being affected differently. A major driver of how species respond to change is whether they are a generalist or a specialist. Very little data exists on whether *C. crossleyi* or *C. sibreei* are specialists or generalists in relation to each other. To understand whether each species would be recognized as more of a generalist or a specialist, we will need to gather all sorts of behavioral data, from diet, to hibernation sites, to home ranges. All of these data takes a lot of time and labor to gather and is currently being studied by scientists to different degrees. In this section, you will explore some of these data.

Because trees meet a number of needs for forest-dwelling animals like lemurs, there are multiple ways that specialists and generalists could be affected differently by the changes we observed at Tsinjoarivo. In this section, we'll explore one hypothesis: loss of sleeping sites.

Most lemur species sleep in trees. Trees offer shelter from the environment and protection from predators. Some lemurs build nests in branches, while others prefer to take shelter in tree holes (naturally occurring cavities in tree trunks). Sleeping in tree nests tends to be a much more flexible strategy as a tree nest can be made in more types of trees. Tree holes tend to be a more limited resource and are usually found in older, larger trees.

17. Knowing that selective logging is an important factor in the habitat change in the Tsinjoarivo forest, make a hypothesis as to whether species that sleep in tree nests or tree holes will be more affected by selective logging practices.

18. Based on your hypothesis, and looking at the data that you graphed on page 49 in the previous exercise, which species of lemur do you think is more likely to prefer sleeping in tree holes?

To assess whether sleeping site availability might contribute to population changes, our research group conducted a study of lemur sleeping behavior, recording where several lemurs were observed sleeping over the course of several nights. To obtain these data, captured lemurs were fitted with radio tracking collars. These collars help our team track where specific lemurs go and help us find and observe the same individuals repeatedly. Remember, these are animals the size of a squirrel sleeping somewhere high up in the canopy. Even with a radio collar, collecting these data involves miles of hiking and hours looking for hard-to-spot animals. Some of the lemurs in this dataset will be familiar to you from the morphological and genetic analyses you performed in earlier sections of this lab. This dataset is presented below.

A	B	C	D	E	F	G
Name	**Species**	**Total # of observations**	**Observations in tree hole**	**Percent observations in tree hole**	**Observations in nest**	**Percent observations in nest**
Alicia	*C. crossleyi*	4	0		4	
Fidel	*C. crossleyi*	12	4		8	
Meline	*C. sibreei*	5	5		0	
Patricia	*C. sibreei*	3	3		0	
Brigitta	*C. crossleyi*	3	1		2	
Rodolfo	*C. sibreei*	2	2		0	
Narcisa	*C. sibreei*	3	3		0	
Gafara	*C. sibreei*	2	2		0	
Mbola	*C. crossleyi*	7	3		4	
Malala	*C. sibreei*	3	3		0	

19. To make it easier to compare different individuals' sleep site preferences, calculate the percent of the total observations (Divide Column D by column C and multiply by 100) for which each lemur was sleeping in a tree hole. Fill these values into the "percent observations in tree hole" column (column E).

20. Use the same process to calculate the percent of observations where each lemur was sleeping in a nest. Fill these values into the "percent observations in nest" column (column G).

21. Based on the data above, does there seem to be a difference in sleep site preference between species? Make your claim and cite evidence to support your answers below.

    a. *C. crossleyi*?

    b. *C. sibreei*?

22. A biologist might say a species is a "specialist" when it thrives only within a narrow range of environmental conditions. A "generalist," on the other hand, thrives under a variety of environmental conditions. With respect to sleeping behavior, how would you classify *C. sibreei,* as a specialist or a generalist? How would you classify *C. crossleyi*? Explain your reasoning.

    a. *C. crossleyi*?

    b. *C. sibreei*?

23. Generalist species tend to tolerate changes to their environments better than specialist species. Support this claim using the examples of *C. sibreei* and *C. crossleyi* sleeping behavior from this exercise. Incorporate data into your argument.

24. What limitations might this dataset have? Based on what you've been told about how these data were collected, describe one potential source of error.

Critical thinking

Consider the data provided in Part 2: Population impact and Part 3: Tracking behavior to answer the questions below.

25. After reviewing these data, one of your colleagues is confident that selective logging, coupled with species differences in sleeping behavior, directly caused the population changes you observed in part 2 of this exercise. Do you agree? Why might your colleague be mistaken?

26. We know that both clear cutting and selective logging occurred during the period over which we collected the trapping data shared in part 2. Other environmental changes not discussed here may have contributed to *C. sibreei* and *C. crossleyi* population changes, as well. What is one other factor that could have played a part in causing the population changes captured in part 2?

27. Aside from curtailing human activities, can you think of one strategy we might use to mitigate the population losses described in part 2?

Extension: Research at Tsinjoarivo

It takes about a week to travel from the United States to Tsinjoarivo. It's a full day to fly to the capital city of Antananarivo (Tana), a few days of planning meetings and food/supply shopping in Tana, an 8-hour drive to the drop off point, and a 45-min hike to the campsite.

Camp consists of a few tents, a covered kitchen area and a covered dining/laboratory area. Nirina, our camp manager, is an amazing cook and prepares all our food. Our conversations are half in English, half in Malagasy, the national language of Madagascar. Without electricity, everyone turns into bed at sunset (around 7 pm) and gets up at sunrise (around 5 am).

Left, Dr. Marina Blanco at camp. Right, some of the delicious food prepared by Nirina, our camp manager.

The daily routine at camp begins in the afternoon. Each afternoon, we set up about 40 traps baited with bananas, high in the trees, about 15-50 feet up. The team guides, Renee and Jules, are expert tree climbers. The traps have a hinge inside them, such that when an animal enters, the door shuts closed. Importantly, we never snare or harm animals using this trapping method.

Left, hiking out of camp to check the traps. Center, researchers track lemurs with radio collars. Right, Marina and Jean-Basile track lemurs in the forest.

Early the next morning, before breakfast & coffee, we check the traps and bring any lemurs back to camp. Some days we trap nothing at all, some days we trap a few geckos or rats (who are immediately released), and sometimes we get very lucky and trap a few dwarf lemurs. At camp, the dwarf lemurs are weighed, measured, photographed, and sampled. After their exams, they sleep during the day at camp in soft cloth bags. We give them a bit of sugar water and fruit and release them in the same place where we caught them later that same evening, when they would normally wake up to begin their nightly foraging. While half the team goes to release the dwarf lemurs, the other half goes to set up the next round of traps for tomorrow.

Marina and Jean-Basile measure lemurs and prepare biological samples back at camp.

(Photos courtesy of Dr. Marina Blanco)

Meet the team:

Jean-Basile Andriambeloson is a Masters student at the University of Antananarivo in Madagascar's capital city. Basile is an expert in the ecology and behavior of nocturnal lemurs, including mouse lemurs, dwarf lemurs, and aye-ayes.

Marina Blanco, Ph.D., is a senior scientist at the Duke Lemur Center. Marina has spent the past 15 years researching mouse and dwarf lemurs in Madagascar, and is one of the world's leading experts in their hibernation and ecology.

Lydia Greene, Ph.D., is a junior scientist at the Duke Lemur Center. She's an expert in lemur feeding ecology and is also the team member in charge of science communication.

Michelle Larsen is an undergraduate at Duke University working with Rachel, Marina, and Lydia in Anne Yoder's lab. Michelle is learning how to perform laboratory and genetic analyses, and hopes to continue researching genetics in graduate school.

Jules Rafalimanatsoa is a senior guide and research assistant for the Tsinjoarivo Protected Area. Jules is a lemur expert and has decades of experience locating, trapping, and studying dwarf lemurs in the Tsinjoarivo Protected Area.

Noel Rakotoniaina, aka Renee, is a senior guide and research assistant for the Tsinjoarivo Protected Area. He's an expert in the lemurs inhabiting Tsinjoarivo's forests and has decades of experience locating, trapping, and studying dwarf lemurs. Renee is also the local law enforcement agent.

Edmond Razanadrakoto is a senior guide and landowner in the Tsinjoarivo area. He is one of the most respected elders in the community. He has extensive knowledge of the forest, the habitats, and animals, and has good relationships with all the local families. Edmond provides the land for our camp site, and the space for our team to live and work.

Nirina Razanadrakoto manages the field camp and cooks the most delicious food for the team. She always treats us with delicious traditional dishes, like 'mofo akondro', which are battered and fried bananas. Nirina is also Edmond's daughter.

Rachel Williams, Ph.D., is a postdoctoral researcher at Duke University, working in Anne Yoder's lab. Rachel is an expert in genetic analyses, including determining species richness based on genetic information.

Anne Yoder, Ph.D., is a professor of Biology at Duke University. Anne has been studying patterns of mouse and dwarf lemur speciation for the past 30 years, and is one of the world's leading experts in the evolutionary history of lemurs.