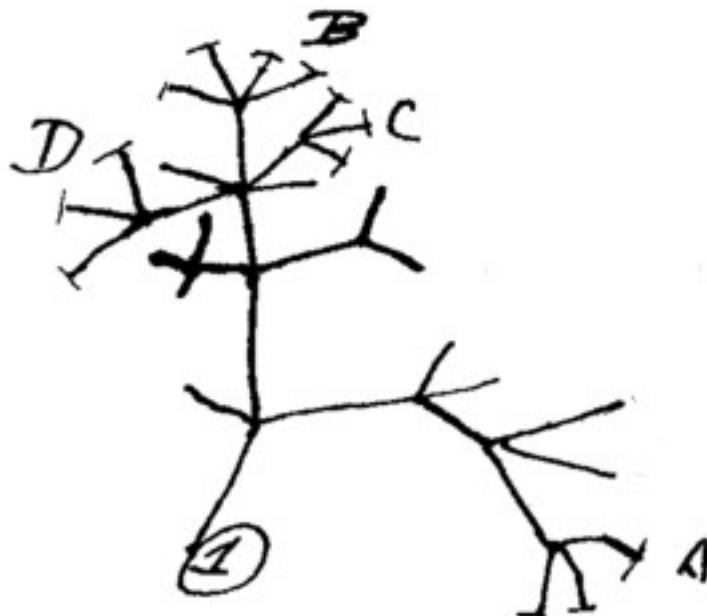


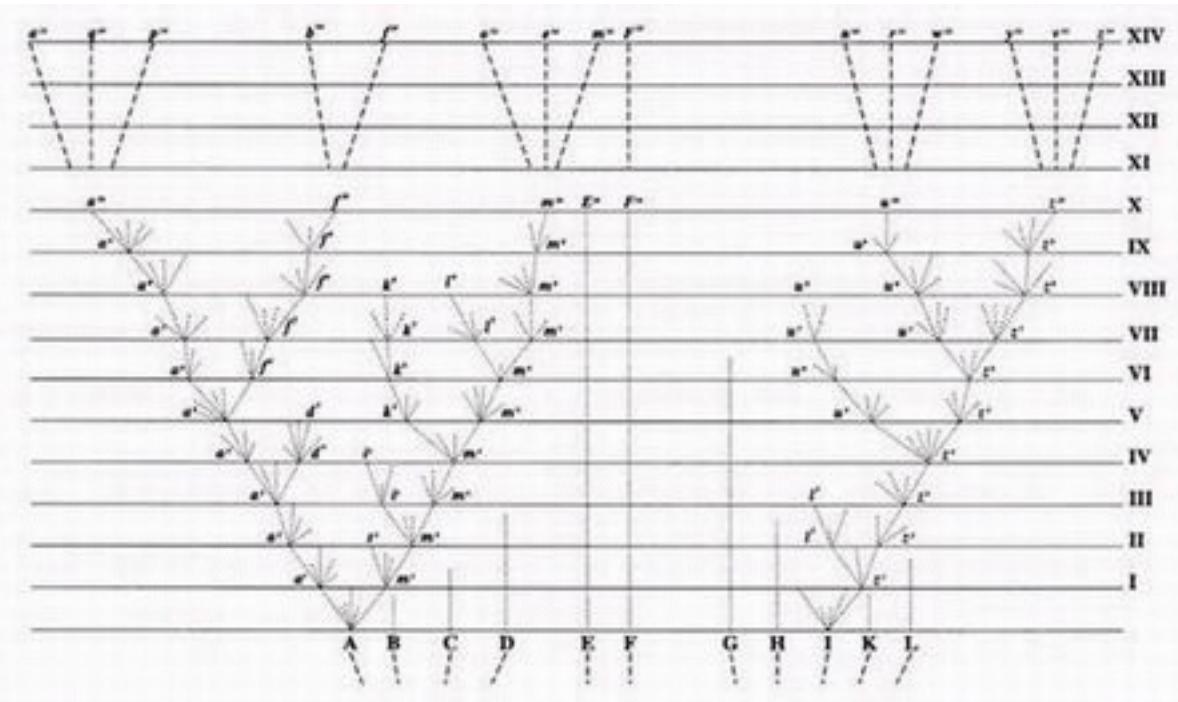
# “What are we even talking about when we say “species tree’?”

I think



Anne D. Yoder  
Department of Biology  
Duke University  
August 6, 2019

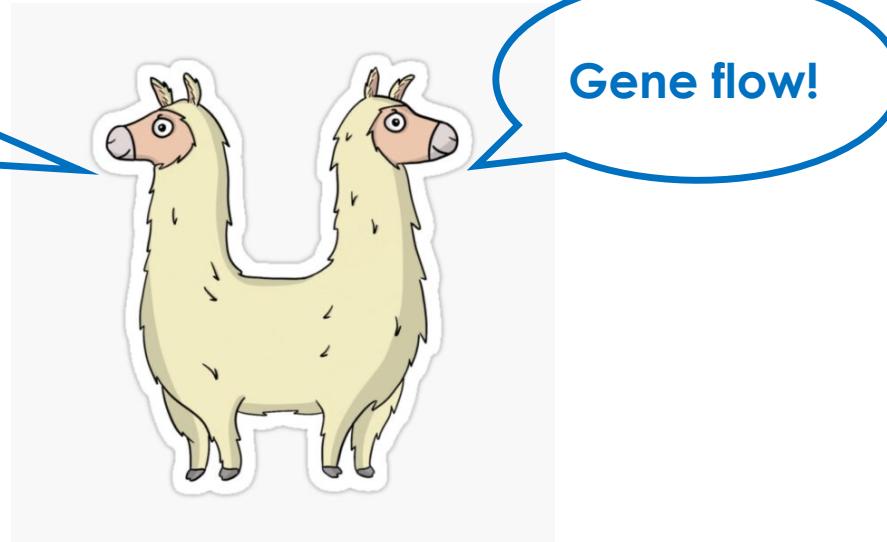
# The one and only figure in Darwin's *Origin of Species*



Ancestor/Descendant Relationships Across Life on Earth

Reproductive  
Barriers!

Gene flow!



Speciation is a “push me pull you”  
between reproductive barriers (of  
all kinds) and gene flow

THE OCEAN AGENCY/CORBIS/SCIENCE PHOTO LIBRARY

But the excitement around cancer immunotherapies — two researchers won a Nobel prize last year for pioneering them — has been tempered after several participants in US clinical trials died from side effects. Regulators around the world have moved slowly to approve the treatments for sale. The US Food and Drug Administration has approved only three cancer immunotherapies so far, and the Chinese drug regulator has approved none.

Before 2016, Chinese regulations for the sale of cell therapies were ambiguous, and many hospitals sold the treatments to patients while safety and efficacy testing was still under way. Ren Jun, an oncologist at the Beijing Shijitan Hospital Cancer Center, estimates that roughly one million people paid for such procedures.

But the market came under scrutiny when it was revealed that a university student with a rare cancer had paid more than 200,000 yuan (US\$30,000) for an experimental immunotherapy, after seeing it promoted by a hospital on the internet. The treatment was unsuccessful, and the patient later died. The government cracked down on hospitals selling cell therapies — although clinical trials in which participants do not pay for treatment were allowed to continue.

#### GATHERING EVIDENCE

Under the proposed regulations, roughly 1,400 elite hospitals that conduct medical research, known as Grade 3A hospitals, would be able to apply for a licence to sell cell therapies, after proving that they have expertise in processing the cells and running clinical trials.

Once the hospital was licensed, its review board would oversee clinical research of experimental therapies, with participants who did not pay. If the board were to decide that these investigations had produced enough evidence that a therapy was safe and effective, the hospital would be able to start selling it.

Ren is confident that the measures would prevent treatments of unknown quality being promoted and sold.

But not everyone agrees. "The proposed regulation is a bad idea," says Michele Teng, who studies cancer immunotherapy at QIMR Berghofer. "It is critical that any new cellular therapy to be administered into patients demonstrates its safety and efficacy in phase III efficacy trials."

Having large, well-funded facilities and trained medical staff is not an adequate substitute for well-designed studies to determine whether a treatment is efficacious or not, says Douglas Sipp, who studies cell-therapy policies at the RIKEN Center for Biosystems Dynamics Research in Kobe, Japan.

The rules could even dissuade companies from doing rigorous studies, he says. ■



Habitats such as coral reefs have been hit hard by pollution and climate change.

#### BIODIVERSITY

# One million species face extinction

*Landmark United Nations report finds that human activities threaten ecosystems around the world.*

BY JEFF TOLLEFSON

Up to one million plant and animal species face extinction, many within decades, because of human activities, says the most comprehensive report yet on the state of global ecosystems.

The rate of species extinctions is already tens to hundreds of times higher than the average across the past ten million years. Without drastic action to conserve habitats, the extinction rate will only increase, says a United Nations-backed panel called the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES).

About 75% of land and 66% of ocean areas have been "significantly altered" by people, driven in large part by agriculture, according to a summary of the work, released on 6 May (see go.nature.com/2v42bn9). The loss of species and habitats poses as much a danger to life on Earth as climate change does, the IPBES report says.

The analysis distills findings from nearly 15,000 studies and government reports, integrating information from the natural and social sciences, Indigenous peoples and traditional agricultural communities. The report is the first major international appraisal of biodiversity since 2005. Representatives of 132 governments met last week

in Paris to finalize and approve it.

"We have never had a single unified statement from the world's governments that unambiguously makes clear the crisis we are facing for life on Earth," says Thomas Brooks, chief scientist at the International Union for Conservation of Nature in Gland, Switzerland, who helped to edit the report. "That is really the absolutely key novelty that we see here."

Without "transformative changes" to the world's economic, social and political systems to address this crisis, the IPBES panel projects that major biodiversity losses will continue to 2050 and beyond.

The analysis, which will be released in full later this year, also inextricably links biodiversity loss and climate change. An estimated 5% of all species would be threatened with extinction by 2 °C of warming above pre-industrial levels — a threshold that the world could breach in the next few decades, unless greenhouse-gas emissions are drastically reduced.

The biodiversity crisis should be at the top of the global agenda alongside climate, said Anne Larigauderie, IPBES executive secretary, at a 6 May press conference in Paris. "We can no longer say that we did not know," she said.

Brooks says that the IPBES report will help to set the agenda when governments negotiate conservation goals for the next decade at the UN Convention on Biodiversity next year. ■

Madagascar is one of the Earth's hottest biodiversity hotspots



- 80 – 90% species endemism across all groups
- Severely threatened by human impacts

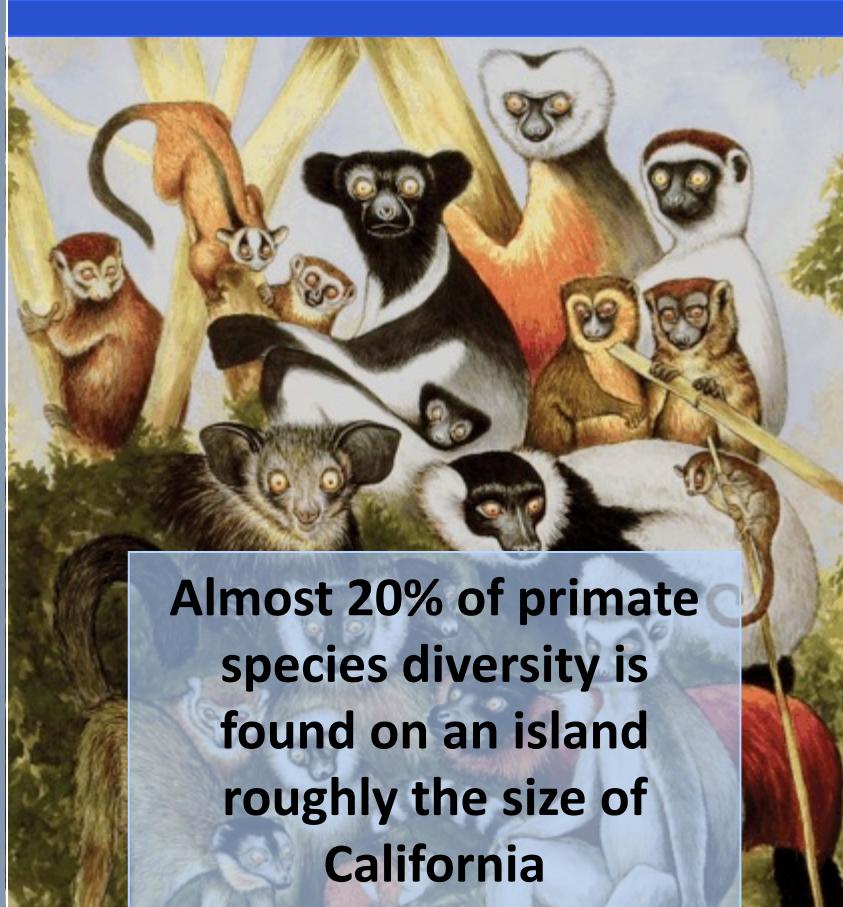


**Madagascar = 0.0001 (0.01%) of Earth's surface**



May 3, 2005  
vol. 102 no. suppl 1

PNAS



Almost 20% of primate  
species diversity is  
found on an island  
roughly the size of  
California



IUCN has declared lemurs to be the most threatened mammals on earth



A close-up photograph of two brown lemurs with large, prominent blue eyes. They are perched on a tree branch, facing towards the camera. The background is blurred green foliage.

**Species, populations, and their genomes are vanishing ...**

*Proc. Natl. Acad. Sci. USA*  
Vol. 93, pp. 5122–5126, May 1996  
Evolution

# Ancient single origin for Malagasy primates

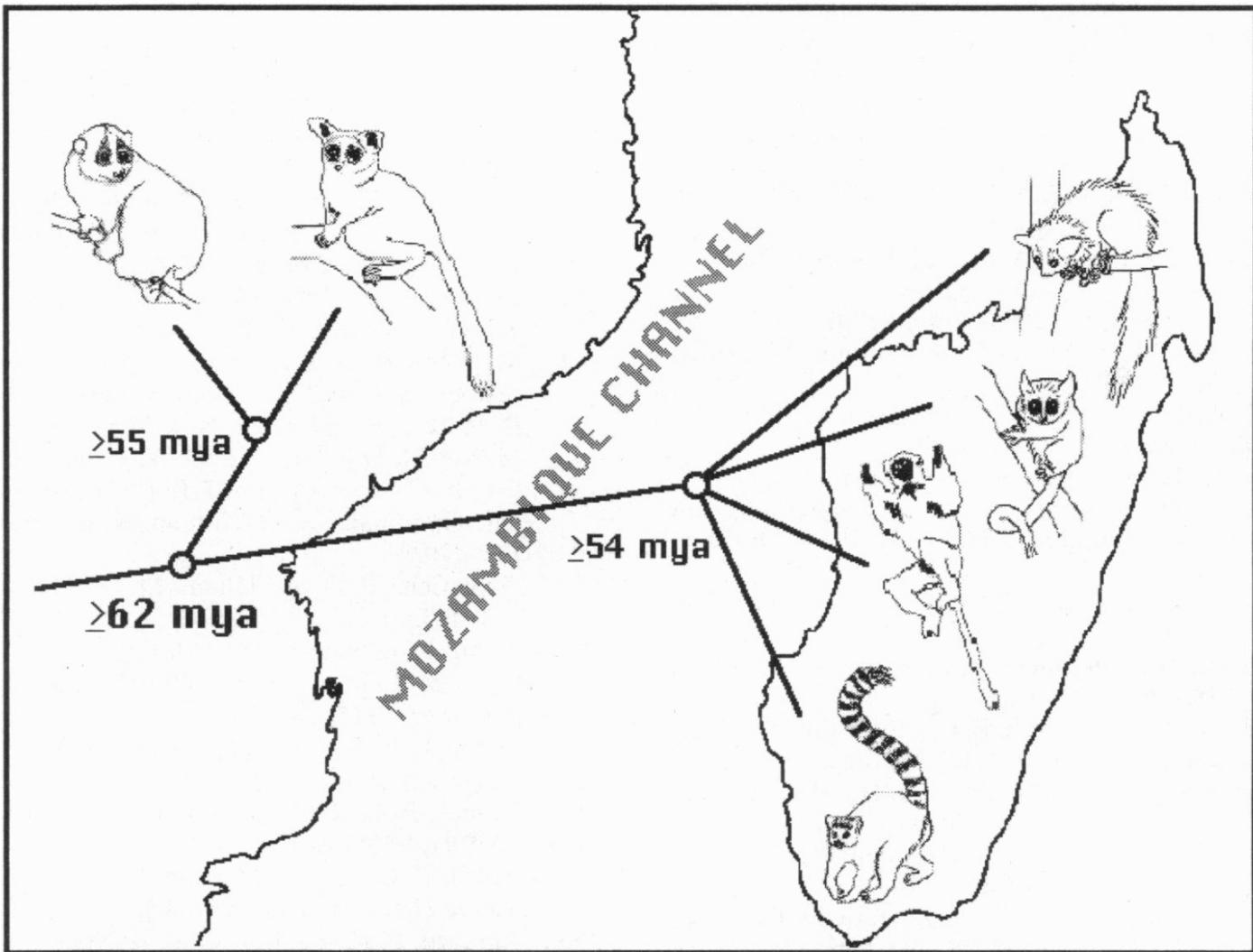
(primate origins/cytochrome *b*/molecular evolution)

ANNE D. YODER\*†‡, MATT CARTMILL§, MARYELLEN RUVOLO\*, KATHLEEN SMITH§, AND RYTAS VILGALYS¶

Departments of \*Anthropology and †Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138;

§Department of Biological Anthropology and Anatomy, Duke University Medical School, Durham, NC 27710; and ¶Department of Botany, Duke University, Durham, NC 27708

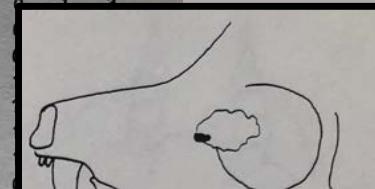
*Communicated by Andrew H. Knoll, Harvard University, Cambridge, MA, January 16, 1996 (received for review July 20, 1995)*



147 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	HYPANC
L. fulvus	0	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	
L. rubriventer	0	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?		
L. macaco	0	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	
L. mongoz	0	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?	
L. coronatus	0	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	
L. catia	0	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	
V. variegata	0	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0/1	2	
H. griseus	0	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	
H. simus	0	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/1	2	
L. mustelinus	0	1	0	0	0	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
A. laniger	1	2	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	
P. verreauxi	1	2	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	
I. indri	1	2	0	1	0	1	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0/1	3	
D. madagascariensis	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	
C. major	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	
M. murinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	
P. furcifer	0	1	0	0	0	0	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
M. coquereli	0	1	0	0	0	0	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?
A. trichotis	0	1	0	0	0	0	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?
G. senegalensis	0	1	0	0	0	0	2	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	
G. crassicaudata	0	1	0	0	0	0	2	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
G. allenii	0	1	Q	0	0	0	2	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
G. demidovii	0	1	0	0	0	0	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
P. poliocephalus	0	1	0	0	0	0	2	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
A. calabarensis	0	1	0	0	0	0	2	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
N. coucang	0	1	0	0	0	0	2	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
L. tardigradus	0	1	0	0	0	0	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
T. bancanus	0	1	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1		
C. apella	0	1	0	0	0	0	2	2	0	0	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
C. polykomos	1	1	0	1	0	2	2	0	0	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
C. aethiops	1	1	0	1	0	2	2	0	0	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
T. gilesi	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
HYPANC	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

## 92 morphological characters



B

# SCIENTIFIC REPORTS



OPEN

## Snoozing through the storm: torpor use during a natural disaster

Received: 24 February 2015

Accepted: 14 May 2015

Julia Nowack, A. Daniella Rojas, Gerhard Körtner & Fritz Geiser



SCIENTIFIC  
REPORTS



## Underground hibernation in a primate

Marina B. Blanco<sup>1,2</sup>, Kathrin H. Dausmann<sup>2</sup>, Jean F. Ranaivoarisoa<sup>3</sup> & Anne D. Yoder<sup>1,4</sup>



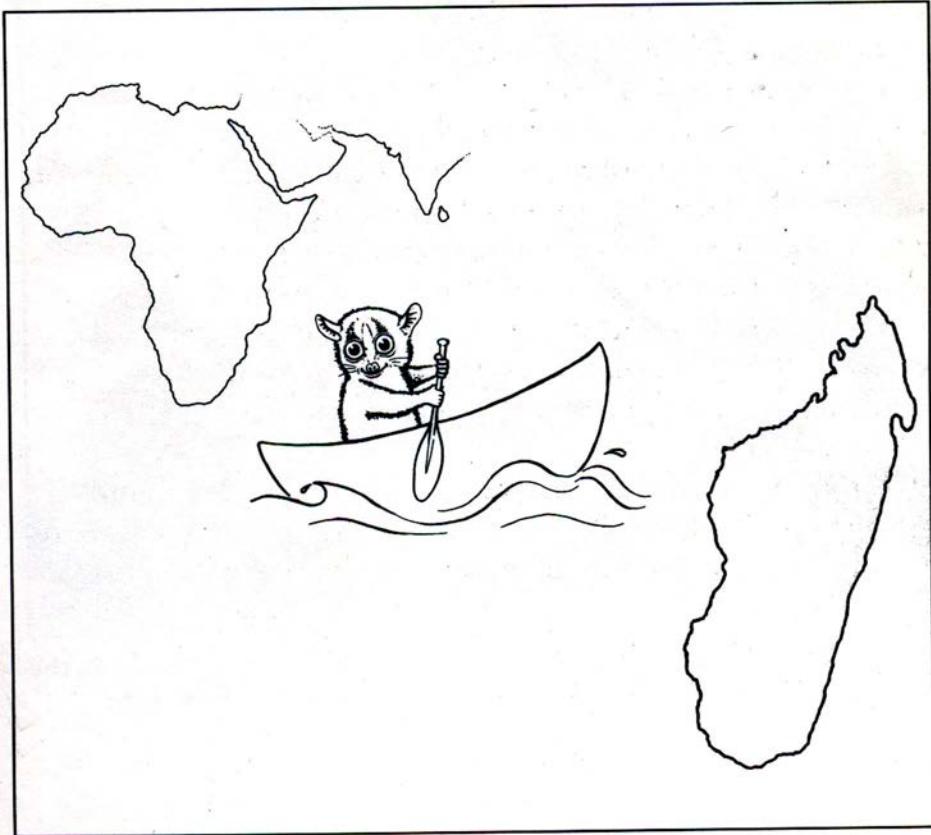


ILLUSTRATION BY STEPHEN D. NASH

**Fig. 2.2: An ancestral lemur makes its way across to Madagascar.**



ARTICLE



## Conservation genomic analysis reveals ancient introgression and declining levels of genetic diversity in Madagascar's hibernating dwarf lemurs

Rachel Williams

Rachel C. Williams<sup>1,2</sup> · Marina B. Blanco<sup>1,2</sup> · Jelmer W. Poelstra<sup>1</sup> · Kelsie E. Hunnicutt<sup>3</sup> · Aaron A. Comeault<sup>4,5</sup> · Anne D. Yoder<sup>1,2</sup>

● *Cheirogaleus medius*

C



● *Cheirogaleus major*



● *Cheirogaleus crossleyi*



● *Cheirogaleus sibreei*



**Table S4.** Gene ontology results for introgressed regions of the genome for tests between *Cheirogaleus medius/C. major*, and *C. medius/C. sibreei*. All results are significantly over enriched for both tests respectively ( $p<0.00006$ ,  $p<0.00009$ ).

GO category	FDR	Count	Total	GO description
<i>C. med/C. maj</i>				
GO:0000122	7.30E-20	73	505	negative regulation of transcription from RNA polymerase II promoter
GO:0000978	1.00E-13	50	328	RNA polymerase II core promoter proximal region sequence-specific DNA binding
GO:0001078	1.70E-13	27	93	transcriptional repressor activity, RNA polymerase II core promoter proximal region sequence-specific binding
GO:0043565	1.27E-09	39	270	sequence-specific DNA binding
GO:0000977	1.88E-08	28	160	RNA polymerase II regulatory region sequence-specific DNA binding
GO:0001227	1.88E-08	17	55	transcriptional repressor activity, RNA polymerase II transcription regulatory region sequence-specific binding
GO:0005634	1.88E-08	154	2496	nucleus
GO:0004993	1.90E-07	7	7	G-protein coupled serotonin receptor activity
GO:0001228	4.44E-05	18	99	transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific binding
GO:0045892	7.19E-05			
GO:0045944	8.23E-05			
GO:0045893	8.25E-05			
GO:0044212	0.000135577			
GO:0051378	0.000444776	5	6	serotonin binding
GO:0003677	0.000762117	35	375	DNA binding
GO:0003700	0.00134417	27	256	transcription factor activity, sequence-specific DNA binding
GO:0016339	0.003062617	5	8	calcium-dependent cell-cell adhesion via plasma membrane cell adhesion molecules
GO:0070577	0.003062617	6	13	lysine-acetylated histone binding
GO:0007156	0.004020354	7	20	homophilic cell adhesion via plasma membrane adhesion molecules
GO:0008202	0.005942195	5	9	steroid metabolic process
GO:0035240	0.00624982	4	5	dopamine binding
GO:0051923	0.00624982	4	5	sulfation
GO:0006355	0.006679222	21	190	regulation of transcription, DNA-templated
GO:0007193	0.022140053	7	26	adenylate cyclase-inhibiting G-protein coupled receptor signaling pathway
GO:0008146	0.02581555	5	12	sulfotransferase activity
GO:0001077	0.02581555	20	195	transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding
GO:0004952	0.02581555	3	3	dopamine neurotransmitter receptor activity
GO:0004985	0.02581555	3	3	opioid receptor activity
GO:0008504	0.02581555	3	3	monoamine transmembrane transporter activity
GO:0042613	0.030175566	4	7	MHC class II protein complex

$p < 0.00006$ ,  $p < 0.00009$

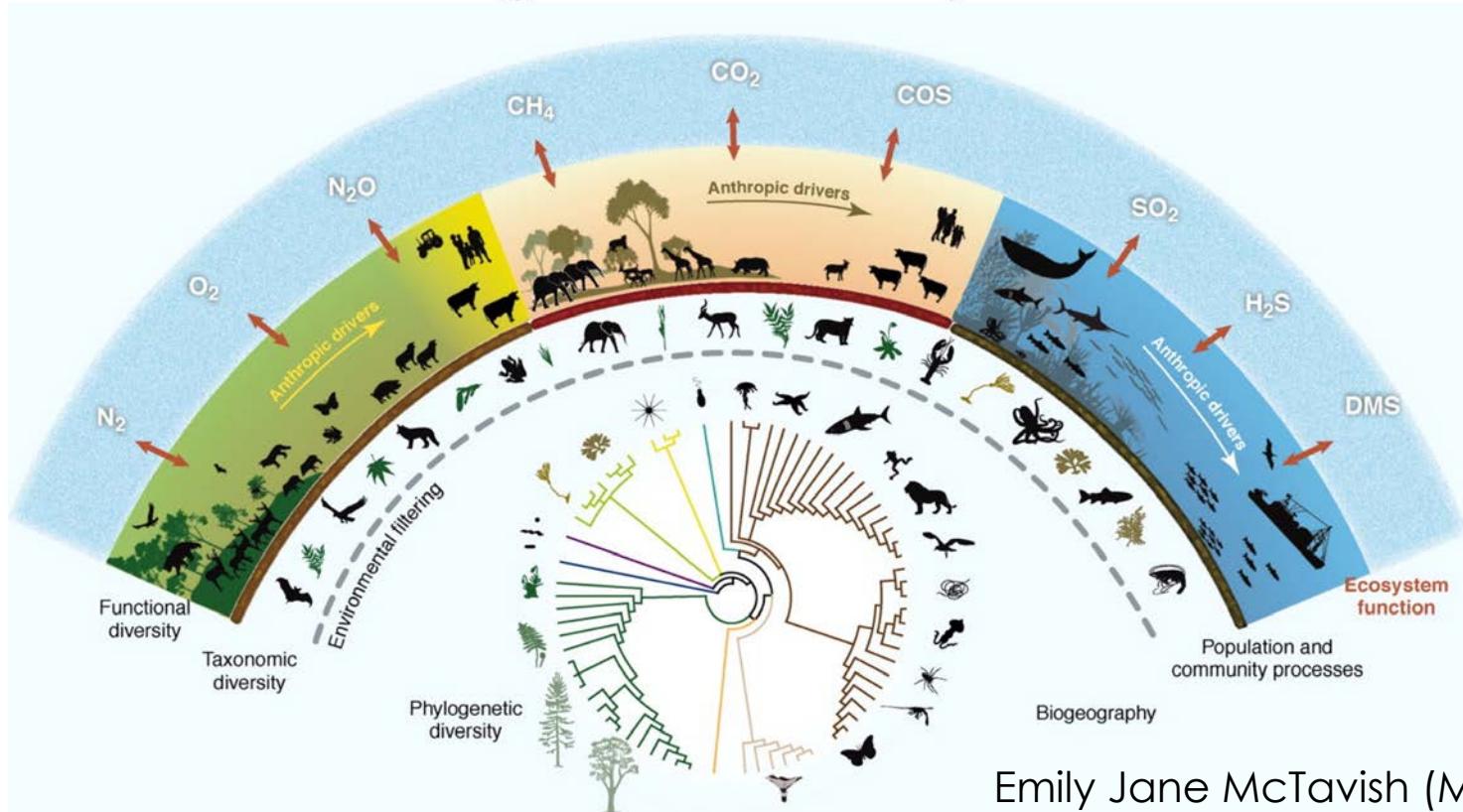
**THE FOLLOWING PREVIEW HAS BEEN APPROVED FOR  
APPROPRIATE AUDIENCES  
BY THE MOTION PICTURE ASSOCIATION OF AMERICA, INC.**

[www.filmratings.com](http://www.filmratings.com)

[www.mpaa.org](http://www.mpaa.org)

**(funny story)**

# Evolutionary context provides a framework for understanding and conserving global biodiversity



Emily Jane McTavish (MOLE, 2022)

# We want to know things like:

## **Conservation concern:**

- How many species are out there and where do we find them?
- Are their populations growing, declining, holding steady?
- How does present-day demography relate to ancestral demography? (this is another way of saying the same thing)

## **Basic science:**

- When did species diverge from one another?
- Why and how did they diverge?
- What is maintaining species boundaries?
- Are they exchanging genes?
- And if so, is this a neutral ("speciation with gene flow") or selection-driven ("adaptive introgression") process?

**Why mouse lemurs???**

# MAKE WAY FOR THE MOUSE LEMUR

*If a US biochemist has his way, the world's tiniest primate could become a top research animal for genetics.*

BY LESLIE ROBERTS

**O**nja is struggling tonight — her hands keep slipping off a miniature grip bar used to measure her strength. “Come on, you can do better,” coos Zeph Pendleton, who is gently supporting the mouse lemur as she tries to get a firm hold. Finally, the animal gets her fingers around the bar and gives it a tug. It records a force of 1 kilogram, impressive for a creature weighing only 41 grams. “Good,” says Pendleton, a research assistant who is working here in the rainforest at Centre ValBio, a research station at Ranomafana National Park in Madagascar.

RIASOLD/RIVA PRESS

## **Tabula Microcebus: A transcriptomic cell atlas of mouse lemur, an emerging primate model organism**

Mouse lemurs are the smallest, fastest reproducing, and among the most abundant primates, and an emerging model organism for primate biology, behavior, health and conservation. Although much has been learned about their physiology and their Madagascar ecology and phylogeny, little is known about their cellular and molecular biology. Here we used droplet- and plate-based single cell RNA sequencing to profile 226,000 cells from 27 mouse lemur organs and tissues opportunistically procured from four donors clinically and histologically characterized. With our computational cell clustering, integration, and expert cell annotation pipeline, **we defined and biologically organized over 750 mouse lemur molecular cell types and their full gene expression profiles.**

**(Collaboration with Mark Krasnow and Steve Quake, Stanford University)**

RESEARCH ARTICLE

Open Access

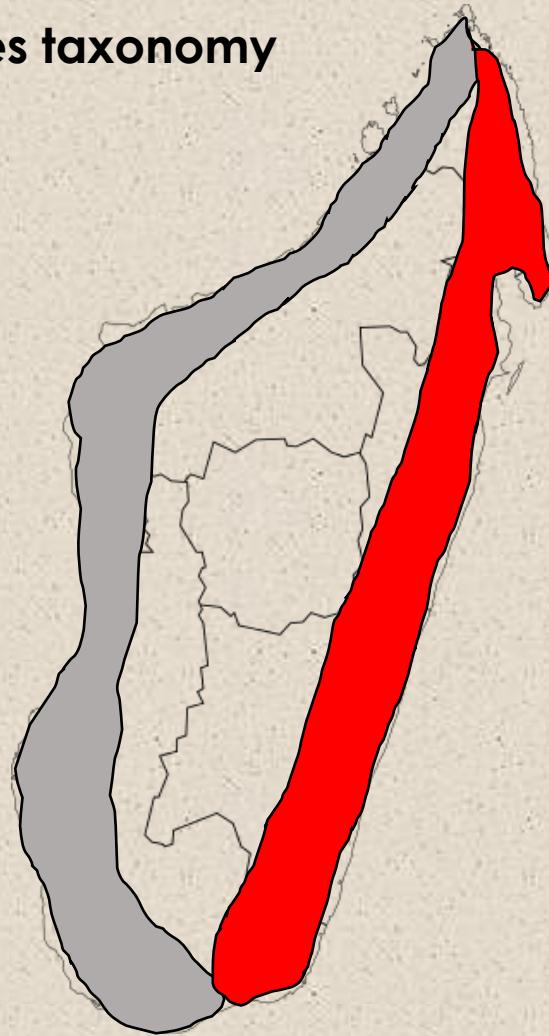


# Comparative analyses of two primate species diverged by more than 60 million years show different rates but similar distribution of genome-wide UV repair events

Umit Akkose<sup>1†</sup>, Veysel Ogulcan Kaya<sup>1†</sup>, Laura Lindsey-Boltz<sup>2†</sup>, Zeynep Karagoz<sup>1</sup>, Adam D. Brown<sup>3</sup>, Peter A. Larsen<sup>4,5</sup>, Anne D. Yoder<sup>4</sup>, Aziz Sancar<sup>2</sup> and Ogun Adebali<sup>1\*</sup> 

## 1973 – 1993: two species taxonomy

*Microcebus murinus* (grey mouse lemur); dry deciduous habitat; distributed throughout the west.



*Microcebus rufus* (roufus mouse lemur); wet forest habitat; distributed throughout the east.



Joerg Ganzhorn

Steve Goodman



Dear Anne,

For the past year or two Rodin, a student that works closely with Joerg Ganzhorn, has been collecting *Microcebus* in western Madagascar for a morphological study. He has done very well and **the specimens seem to include four or five types (*myoxinus*, *murinus*, a rufous *murinus* type that is not *myoxinus* [too big], and two that are probably undescribed)**. He has religiously collected tissues ... [more details]

Cheers for now,  
Steve

January 27, 1997



Rodin Rasoloarison

# 1999: D-loop sequences – barely alignable!

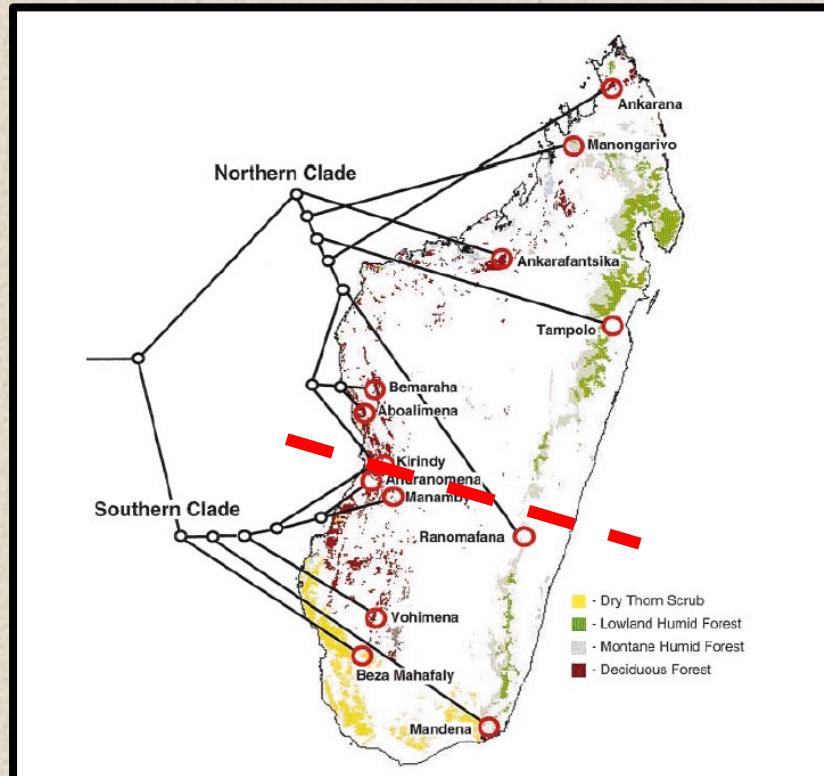


[t.29.c.468 s.c] Character 468; Sequence site 419 [in taxon "#RMR120"]  
Color of cell: Colored to show state of character

# Remarkable species diversity in Malagasy mouse lemurs (primates, *Microcebus*)

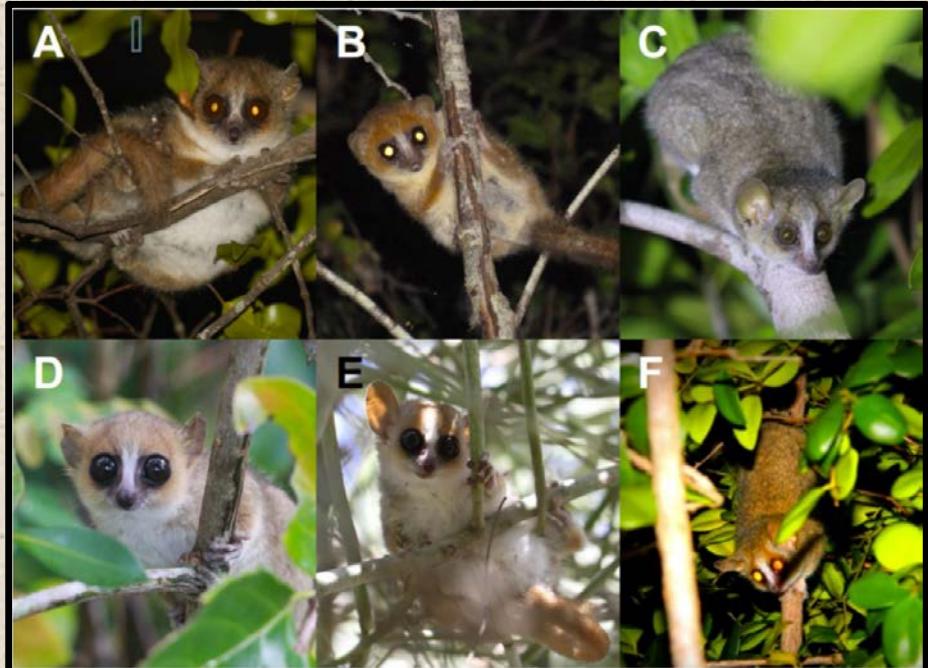
Anne D. Yoder\*<sup>†‡</sup>, Rodin M. Rasoloarison<sup>§</sup>, Steven M. Goodman<sup>†¶</sup>, Jodi A. Irwin\*<sup>||</sup>, Sylvia Atsalis\*, Matthew J. Ravosa\*<sup>†</sup>, and Jörg U. Ganzhorn\*\*

- Much more phylogenetic diversity than expected



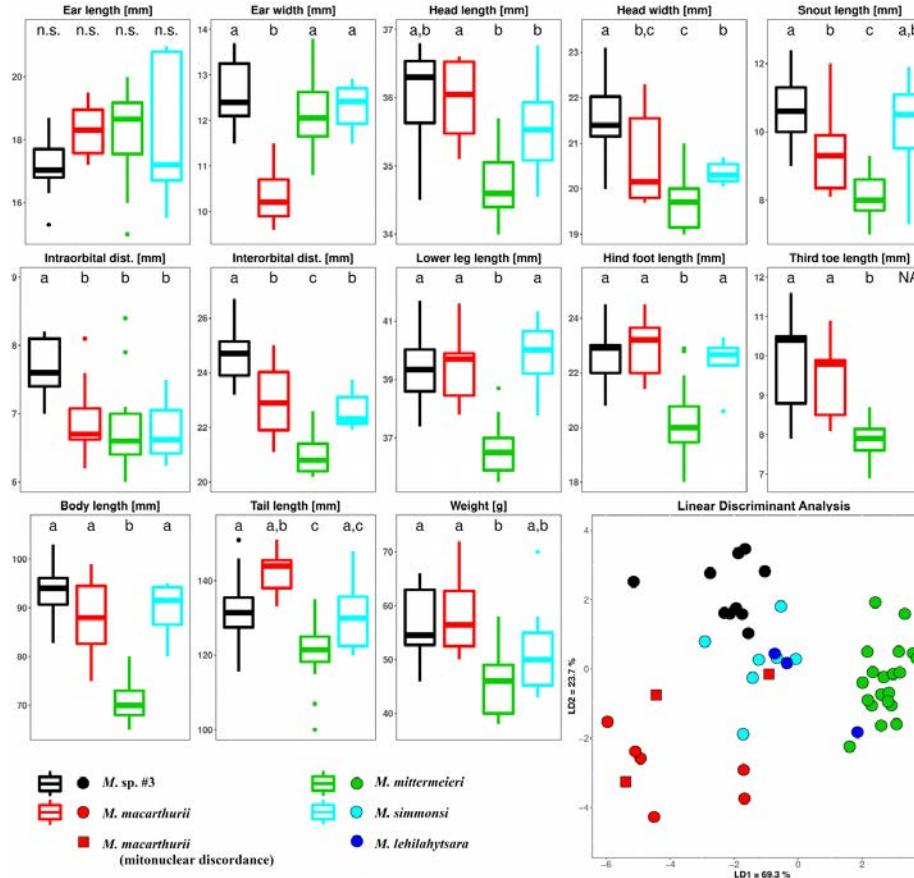
- Phylogeographic patterns don't fit previous east v. west assumptions

Mouse lemurs are morphologically and ecologically cryptic



**Figure 2 – The *Microcebus* spp. of Southeast Madagascar.** Figure illustrates cryptic morphology of diverged lineages currently recognized as named species. **A)** *M. manitatra*, **B)** *M. tanosi*, **C)** *M. ganzhorni*, **D)** *M. tanosi* (pale variant), **E)** *M. griseorufus*, **F)** *Microcebus* sp. (pending DNA barcode). Photographs by Sam Hyde Roberts. See Figure 3 for geographic distribution and habitat preferences of named species.

# Overlapping distributions for virtually all characters measured





M. ravelobensis



M. murinus



M. griseorufus

**Can you tell them apart???**



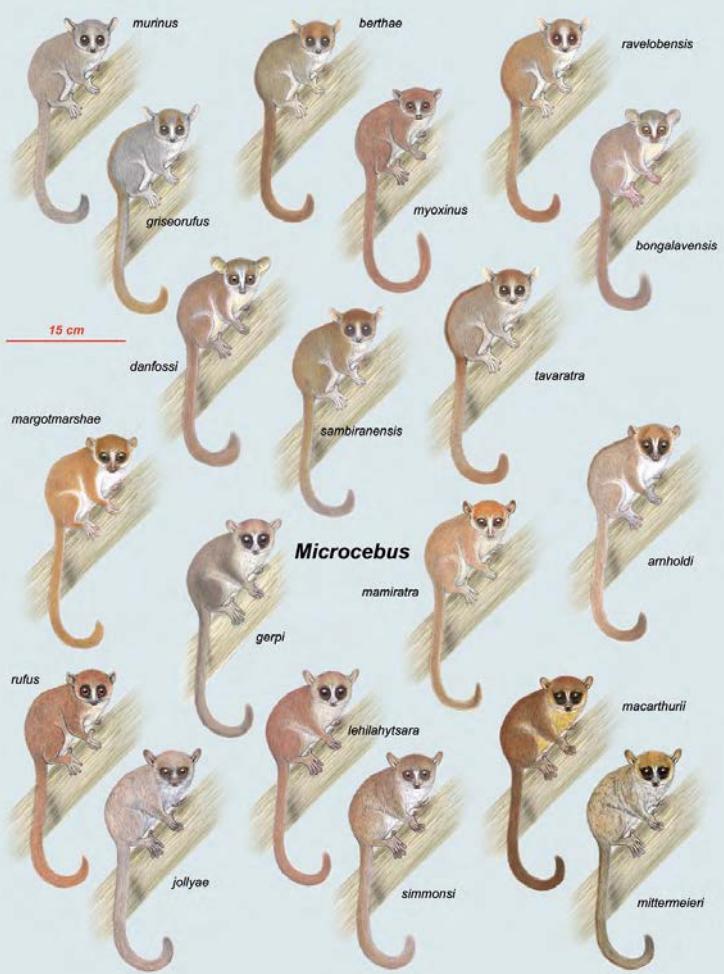
M. tavaratra



M. berthae



M. rufus

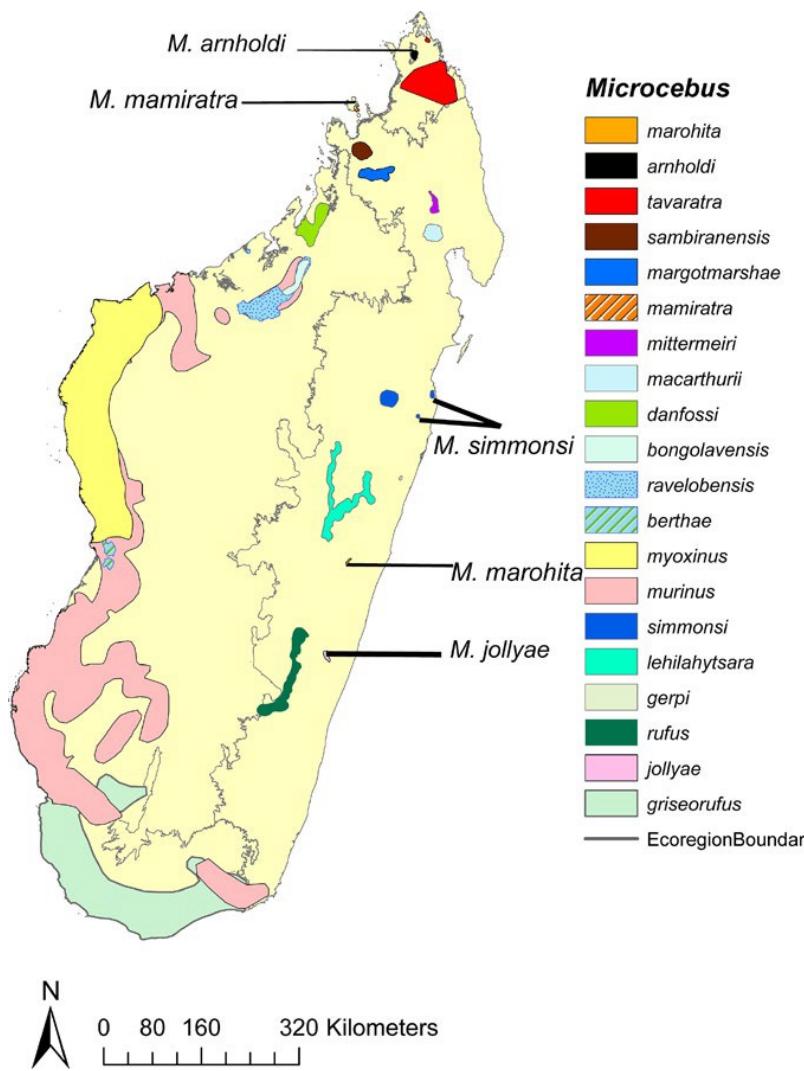


Recognized species diversity has gone from two species (1972 – 1993) to ~ 25 species (2020).

# Madagascar's Lemurs: Cryptic Diversity or Taxonomic Inflation?

IAN TATTERSALL

We live in inflationary times. A quarter of a century ago, cigarettes were about \$1 a pack in New York City, a bottle of Château Beaucastel set you back \$15, and there were 36 different species of lemur alive in Madagascar<sup>1</sup> (Table 1). Today the equivalent figures are \$7.40, \$95, and 83 lemur species<sup>2</sup> (Table 1).



The majority of named species appear to be ***microendemics***, though others have much broader geographic distributions ...

# Questions:

- Are these things actually different species?
- How can we even tell? (Especially when one is agnostic about species concepts?!!)
- What is driving/has driven lineage diversification?
- What is maintaining lineage diversification?  
Biology (i.e., RI)? Ecology? Both??
- Can our approach to these problems be “generalized” so that it can be applied to other cryptic organismal systems?

## The ideal species concept – and why we can't get it

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*D. L. Hull*

Contacting address: Department of Philosophy, Northwestern University, Evanston,  
IL 60208, USA

Cohesion Species Concept

Biological Species Concept

Non-dimensional Species Concept

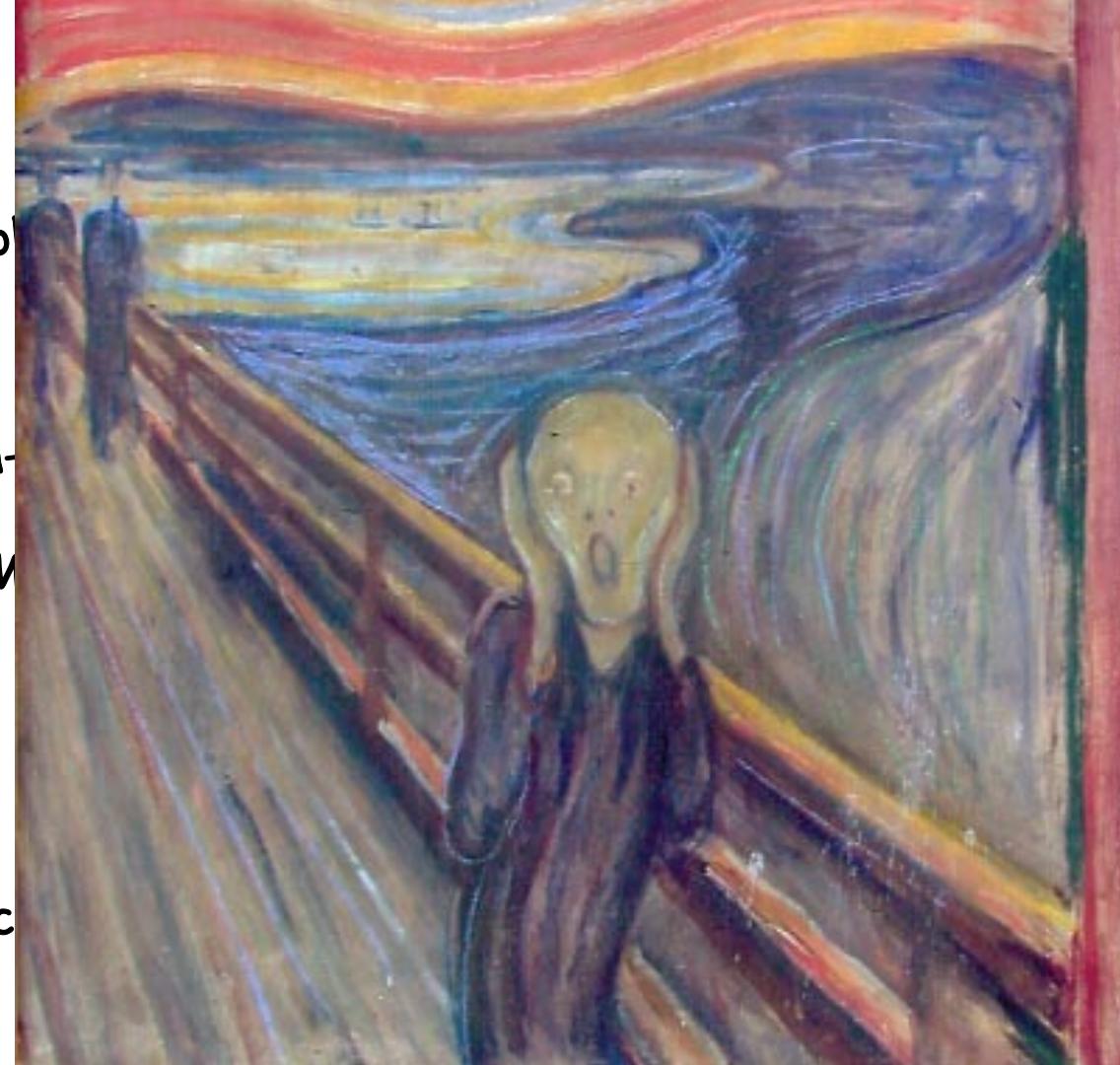
Ecological Species Concept

Morphological Species Concept

Genetic Species Concept

Recognition Species Concept

Phylogenetic Species Concept



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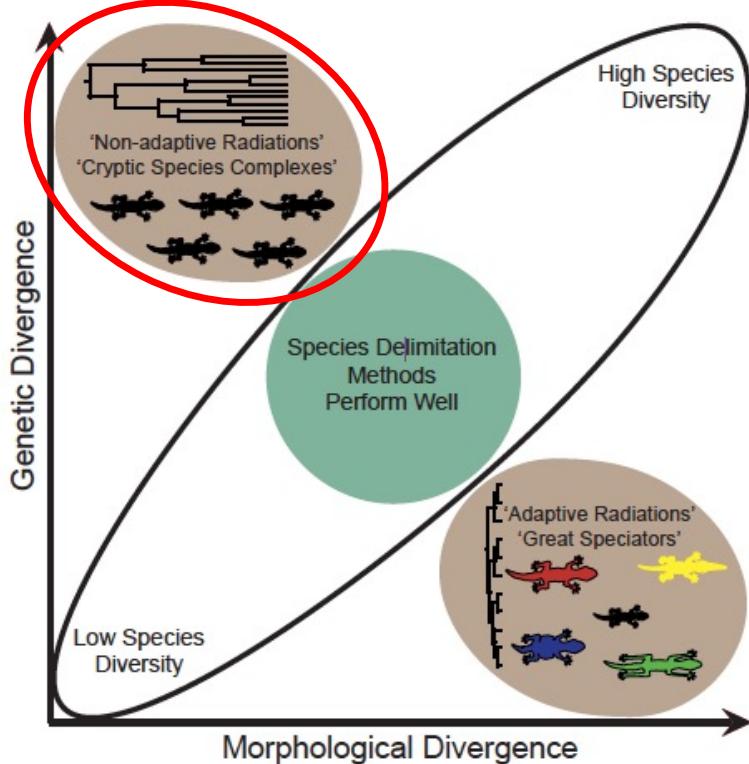
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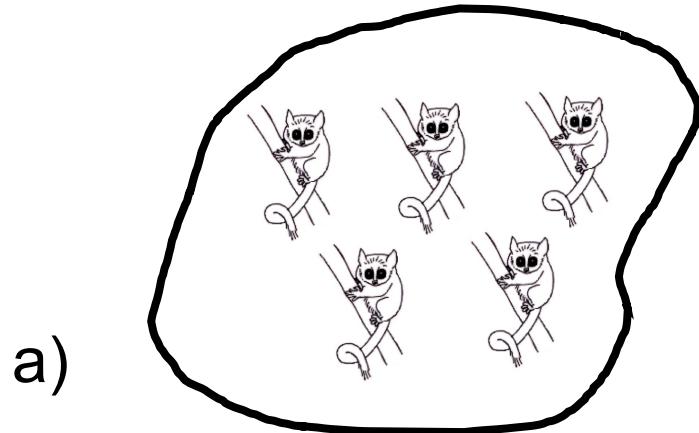
**Figure 1.** Hypothetical axes of morphological and genetic diversity within a species group. Area circumscribed by black ellipse represents systems where species delimitation is usually simple.

The two areas circumscribed in brown represent conditions under which species delineation is often problematic, but conceptually interesting.

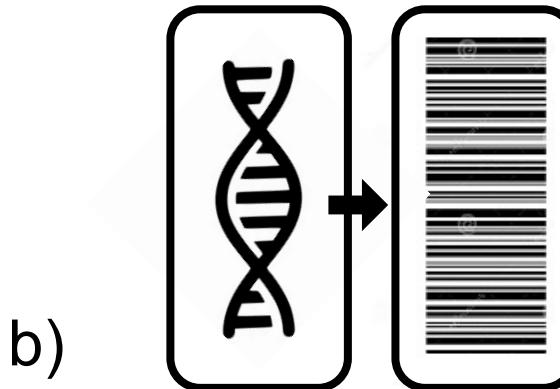
# Tools along the way:

- Phylogenomics
- Phylogeography
- Demography
- Statistical genetics (MSC)
- Divergence time estimation
- Sensory biology
- Ecology (e.g., movement)

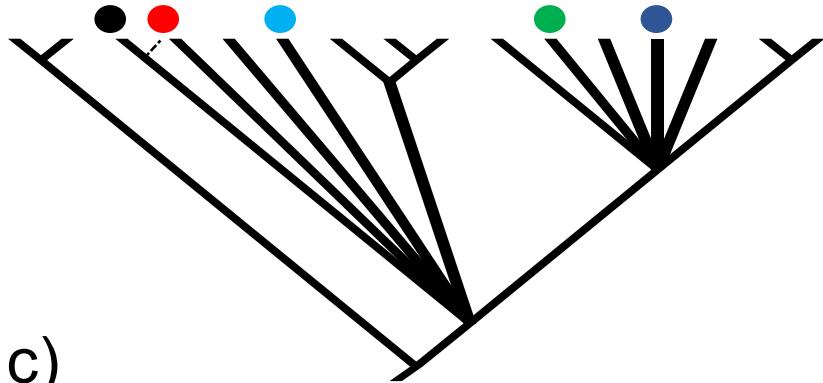
# Geographic Sampling & Phenotypic Analysis



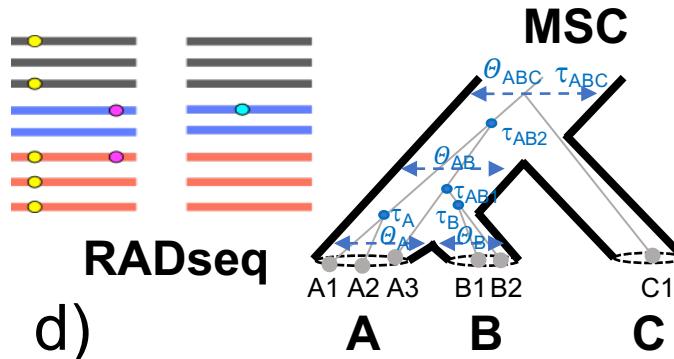
# mtDNA barcoding



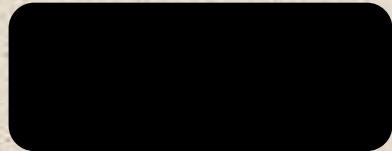
# Phylogenetically-guided Hypotheses



# Genomic Species Delimitation

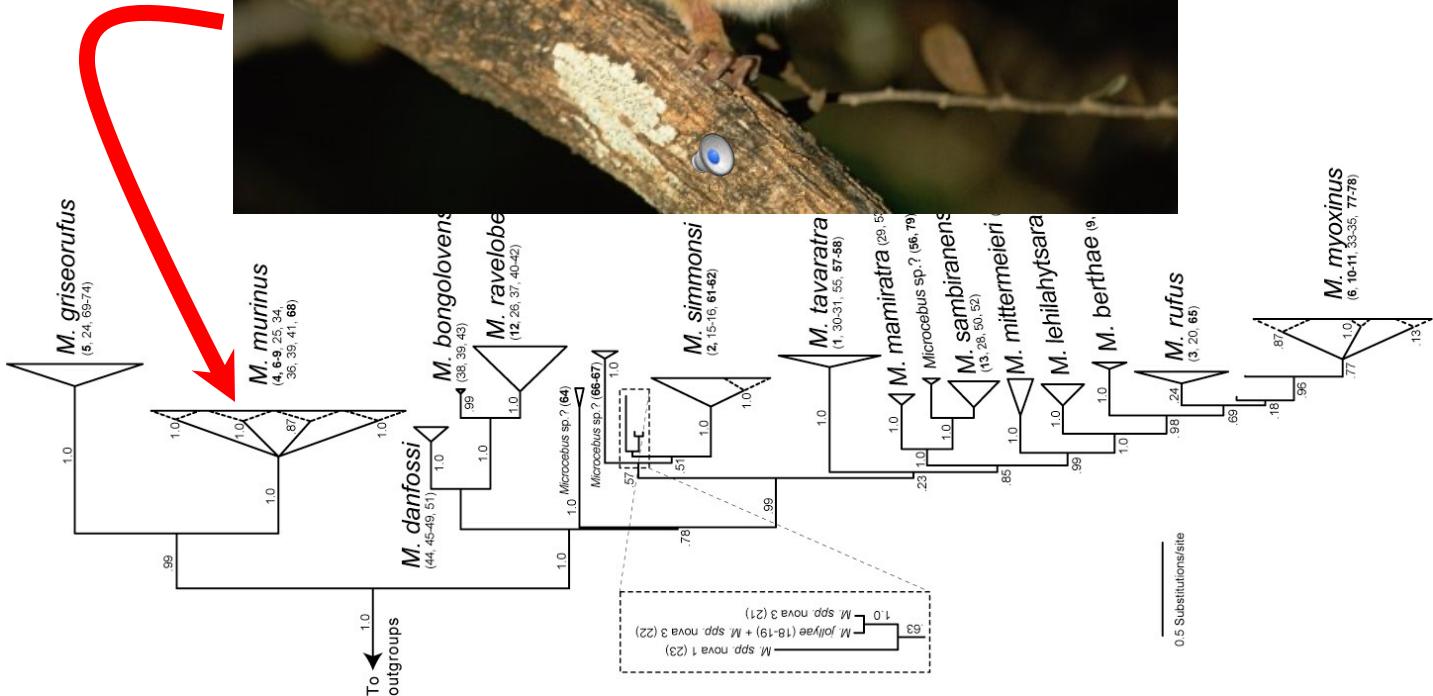


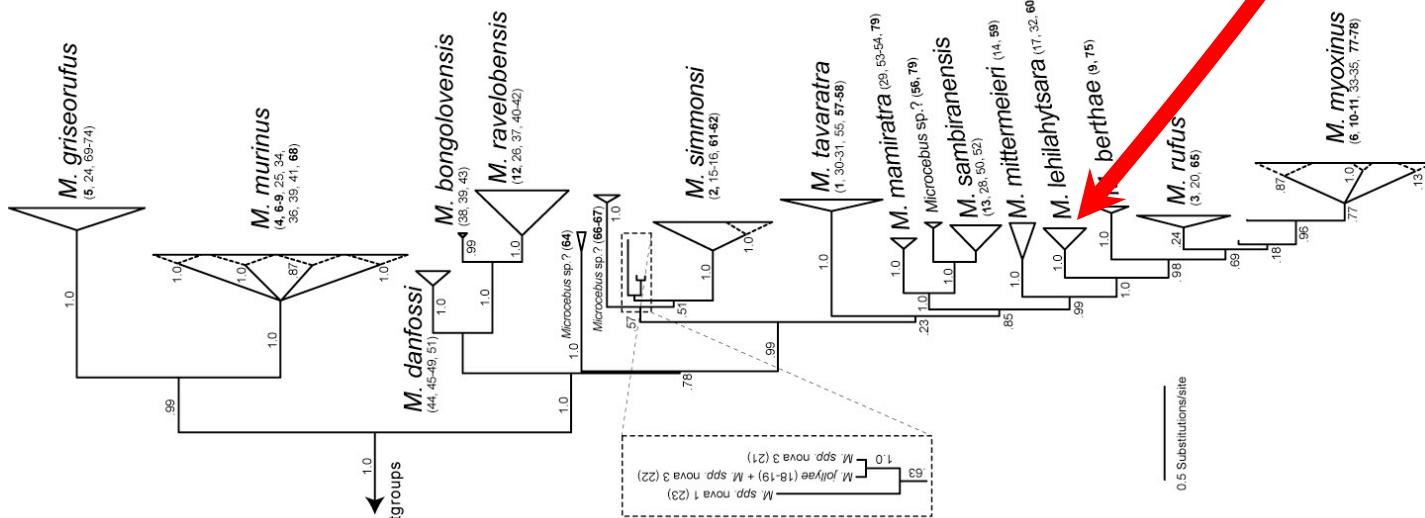


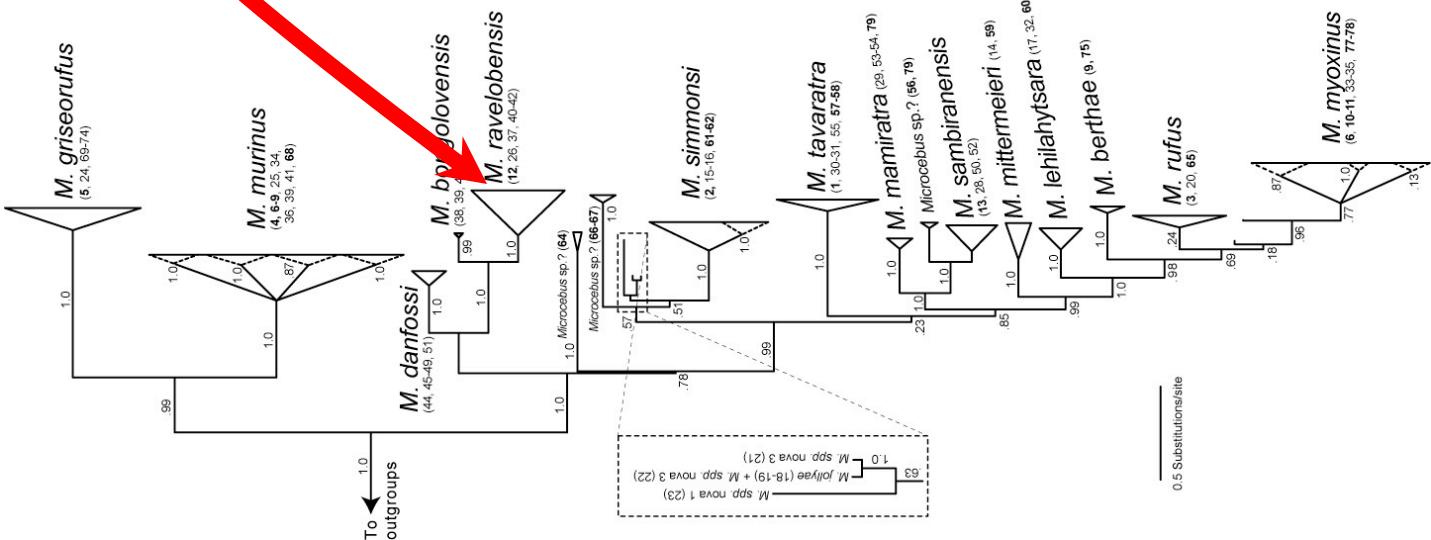


Sound A red arrow pointing to the left, positioned to the right of the word "Sound".

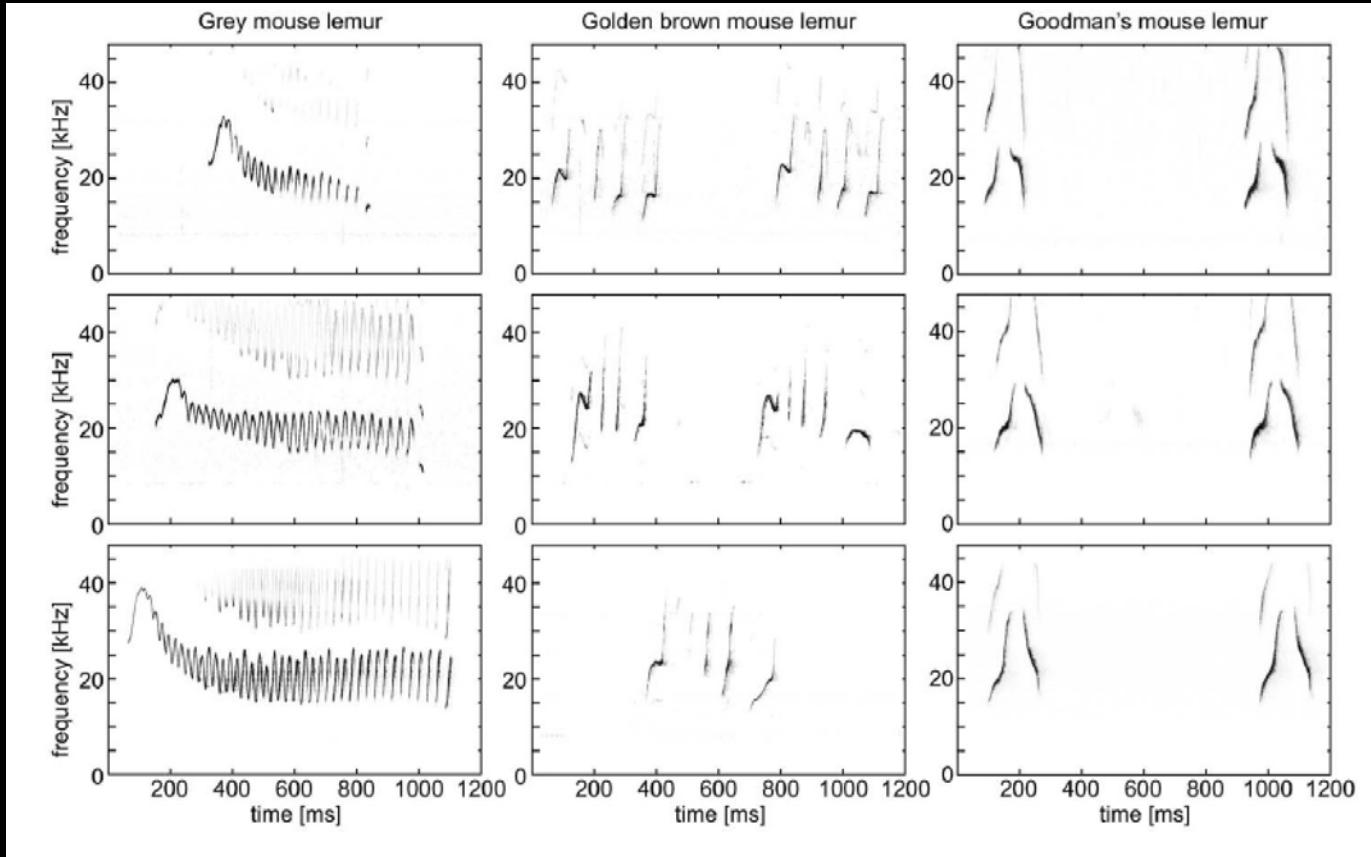
Smell

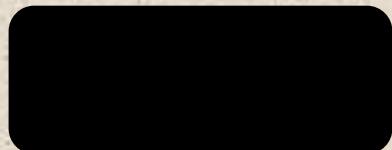






# Male advertisement calls for three species





Sound

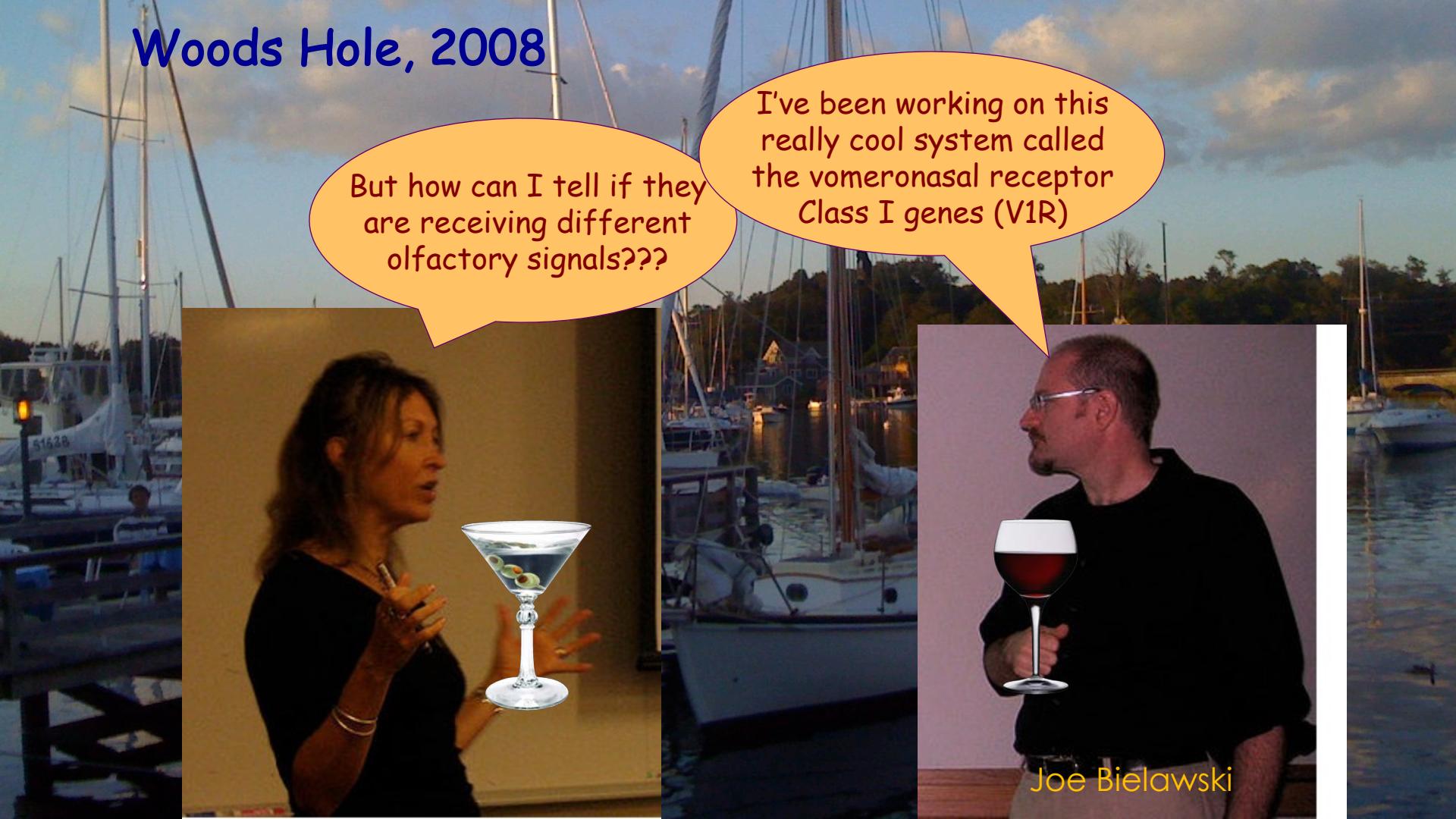


Smell





# Woods Hole, 2008



But how can I tell if they  
are receiving different  
olfactory signals???

I've been working on this  
really cool system called  
the vomeronasal receptor  
Class I genes (V1R)

Joe Bielawski

# Molecular Evolutionary Characterization of a V1R Subfamily Unique to Strepsirrhine Primates

Anne D. Yoder<sup>1,\*</sup>, Lauren M. Chan<sup>1,†</sup>, Mario dos Reis<sup>2,†</sup>, Peter A. Larsen<sup>1,†</sup>, C. Ryan Campbell<sup>1</sup>, Rodin Rasoloarison<sup>3,4</sup>, Meredith Barrett<sup>5</sup>, Christian Roos<sup>4</sup>, Peter Kappeler<sup>6</sup>, Joseph Bielawski<sup>7</sup>, and Ziheng Yang<sup>2</sup>

<sup>1</sup>Department of Biology, Duke University

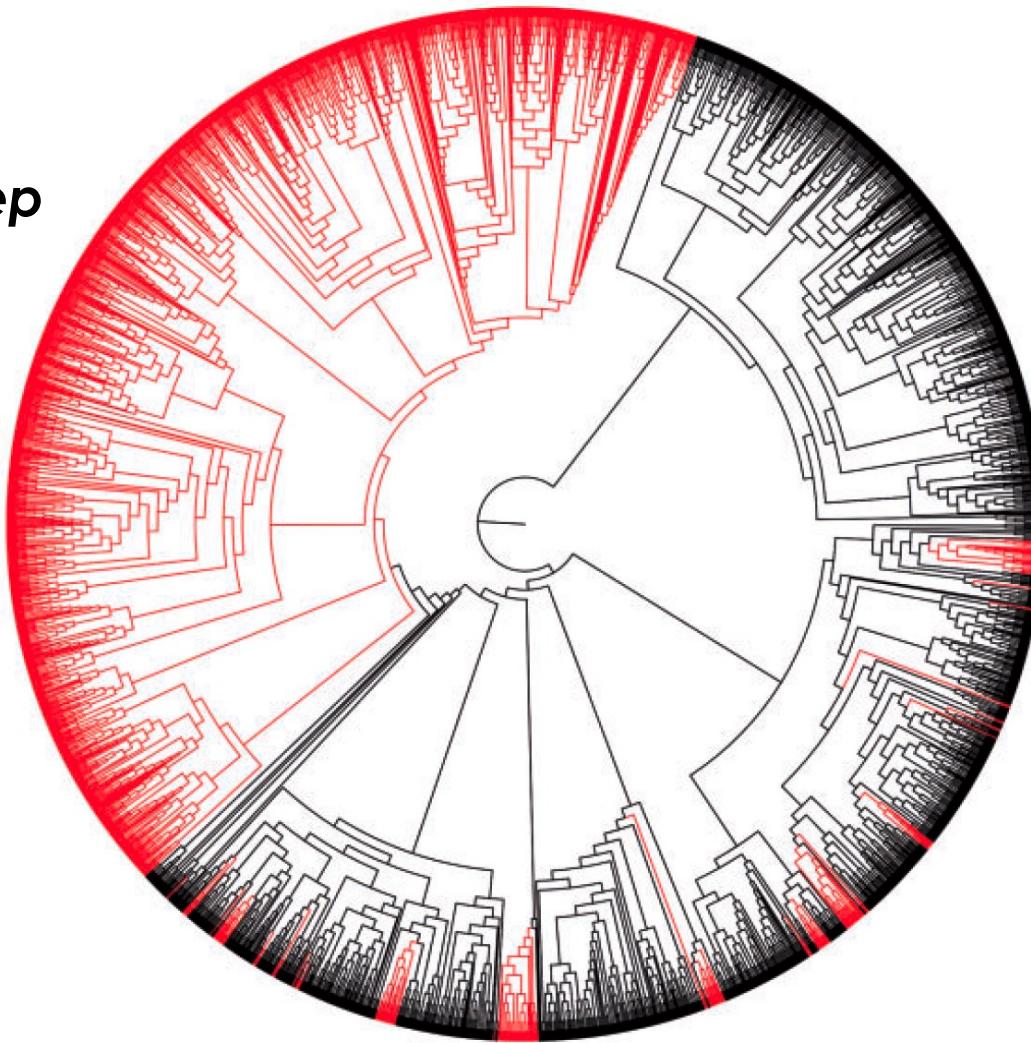
<sup>2</sup>Department of Genetics, Evolution and Environment, University College London, London, United Kingdom

<sup>3</sup>Département de Biologie Animale, Université d'Antananarivo, Antananarivo, Madagascar

<sup>4</sup>Gene Bank of Primates and Primate Genetics Laboratory, German Primate Center (DPZ), Göttingen, Germany

<sup>5</sup>UCSF Center for Health & Community

**V1Rstrep**



**Our hypothesis:** nocturnal primates (like mouse lemurs) will show higher diversity of V1Rs than will diurnal primates, and these gene copies will be under strong positive selection

**Our results:** based on targeted PCR, cloning, and Sanger sequencing we discovered a gene family, apparently unique to the strepsirrhines, that was under strong positive selection but did NOT show any particularly compelling patterns regarding nocturnality and diurnality



George Tiley



Kelsie Hunnicutt

GBE

## Comparative Genomic Analysis of the Pheromone Receptor Class 1 Family (V1R) Reveals Extreme Complexity in Mouse Lemurs (Genus, *Microcebus*) and a Chromosomal Hotspot across Mammals

Kelsie E. Hunnicutt<sup>1,8,†</sup>, George P. Tiley<sup>1,†</sup>, Rachel C. Williams<sup>1,2</sup>, Peter A. Larsen<sup>1,9</sup>, Marina B. Blanco<sup>2</sup>, Rodin M. Rasolosarison<sup>3,4</sup>, C. Ryan Campbell<sup>1</sup>, Kevin Zhu<sup>5</sup>, David W. Weisrock<sup>6</sup>, Hiroaki Matsunami<sup>5,7</sup>, and Anne D. Yoder<sup>1,\*</sup>

2019

# Sensory drive speciation and patterns of variation at selectively neutral genes

Authors

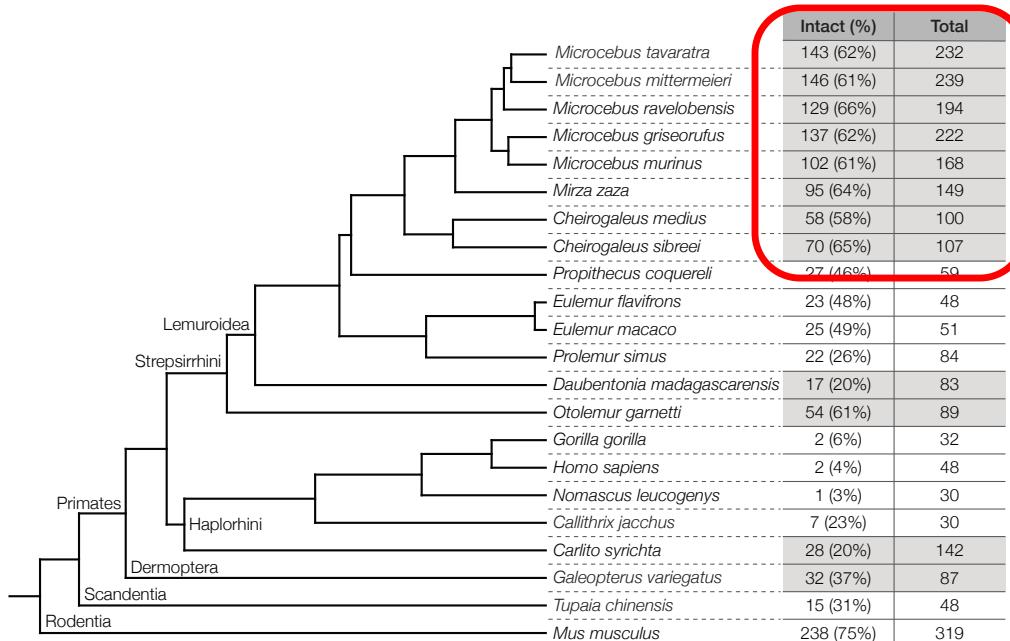
[Authors and affiliations](#)

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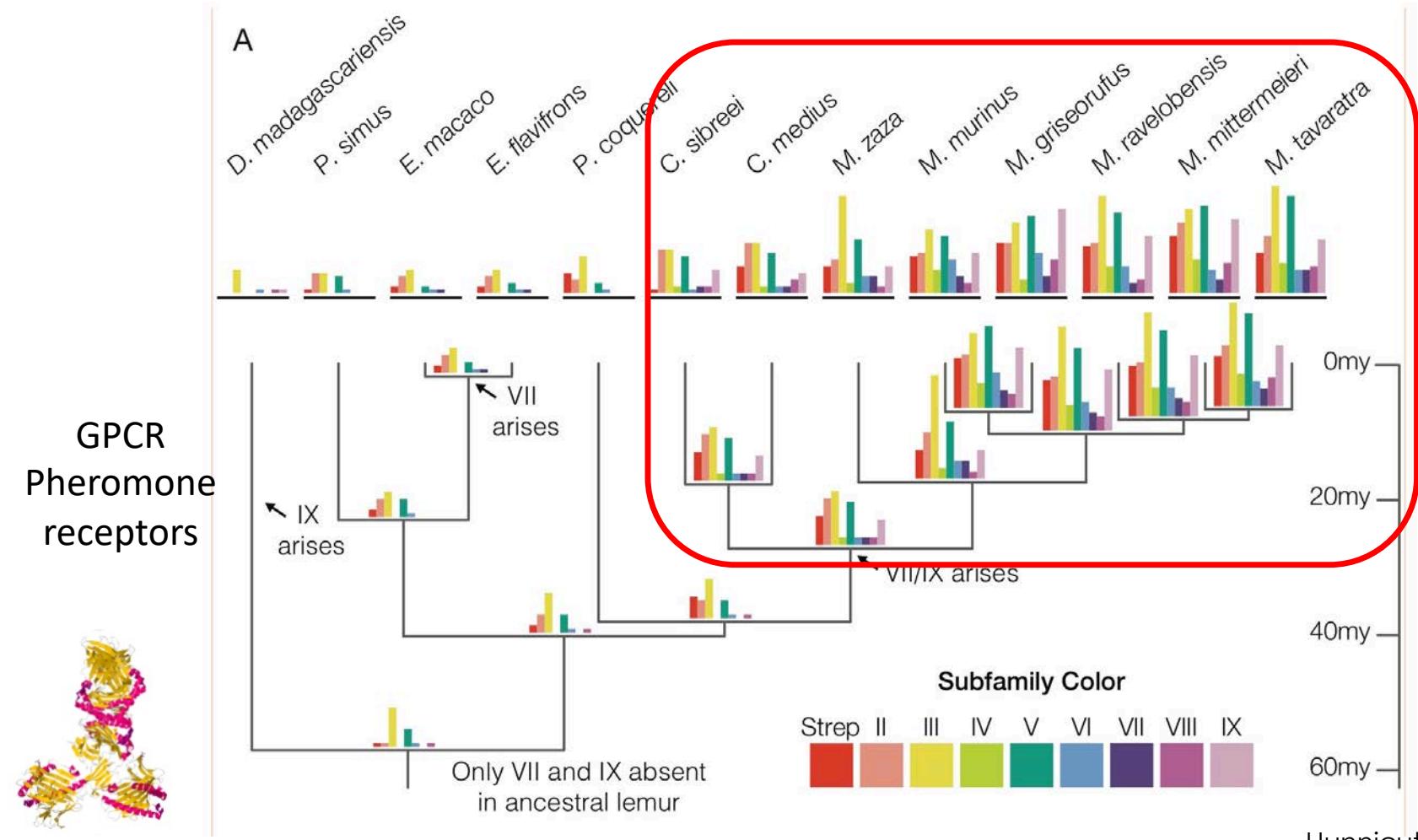
Tomotaka Matsumoto , Yohey Terai, Norihiro Okada, Hidenori Tachida

“Speciation by sensory drive can occur if divergent adaptation of sensory systems causes rapid evolution of mating traits and the resulting development of assortative mating.”

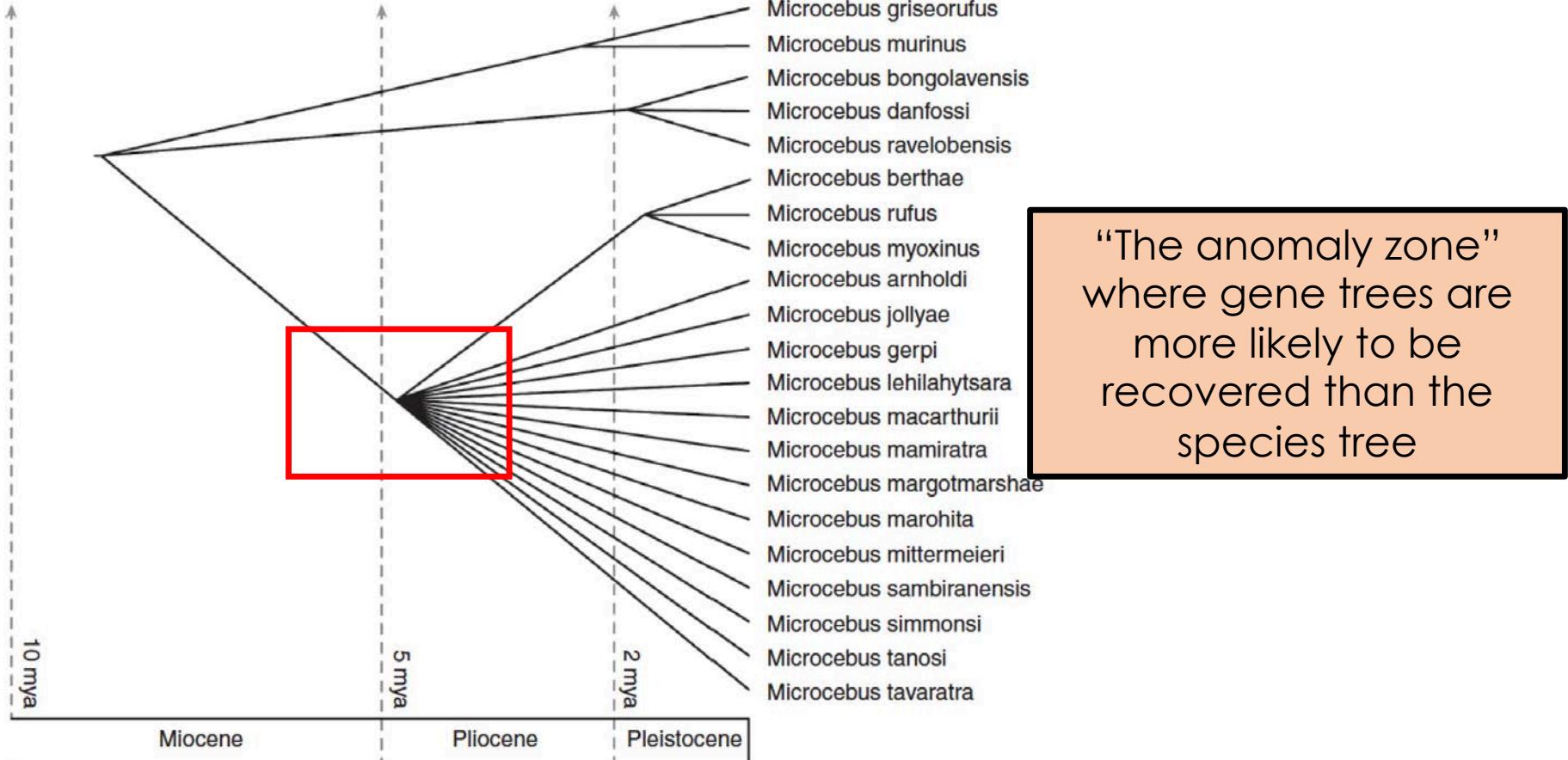
# Hunnicutt, Tiley et al. (2019)



Whole genome analysis indicates that mouse and dwarf lemurs have the most diverse portfolio of V1Rs, with highest proportion of intact copies, in all of primates.



So how are all these guys  
related to each other???

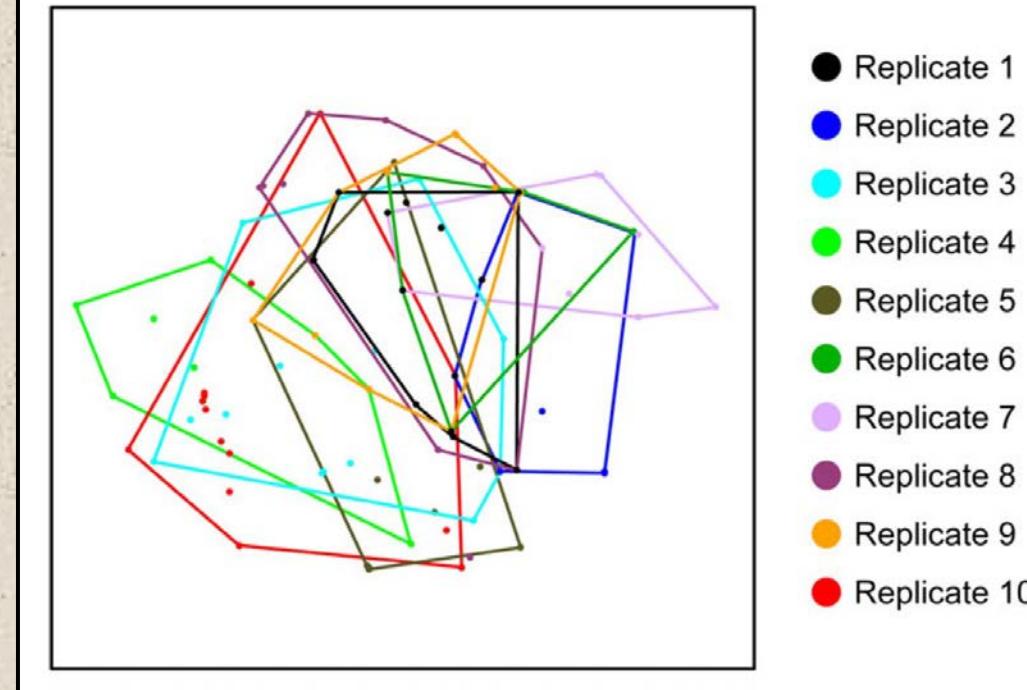


# Phylogenetic resolution is highly sensitive to sample of chosen loci in concatenated phylogenetic analysis (in mouse lemurs)



Dave Weisrock

B) Concatenated nuclear and mitochondrial data



## The MSC Defined

Coalescent theory describes the genealogical histories of a sample of alleles from a population, with time running backwards from the present until the most recent common ancestor of the sample. The **Multispecies Coalescent (MSC)** is an extension of this process to multiple species.

# GENE TREES IN SPECIES TREES

WAYNE P. MADDISON

*Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, USA*

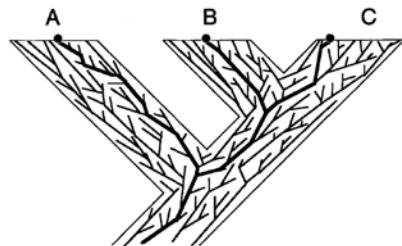


FIGURE 1. A gene tree contained within a species tree leading to three extant species: A, B, and C. Bold branches of gene tree show relationships among the sampled copies of the gene (●). Sampled copies from sister species B and C are sister copies.

A “species tree” can contain many “gene trees”

Gene trees often don't match the species tree

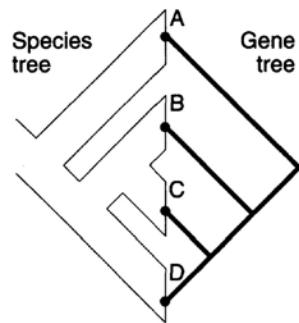
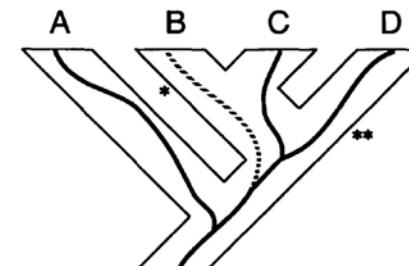


FIGURE 2. Discord between gene and species trees. At left is the species tree of four species, A, B, C, and D, and at right is the tree of a gene sampled one copy per species. Species B and C are sister species, but their gene copies are not sister copies.



Branch short and wide:  
Deep coalescence likely

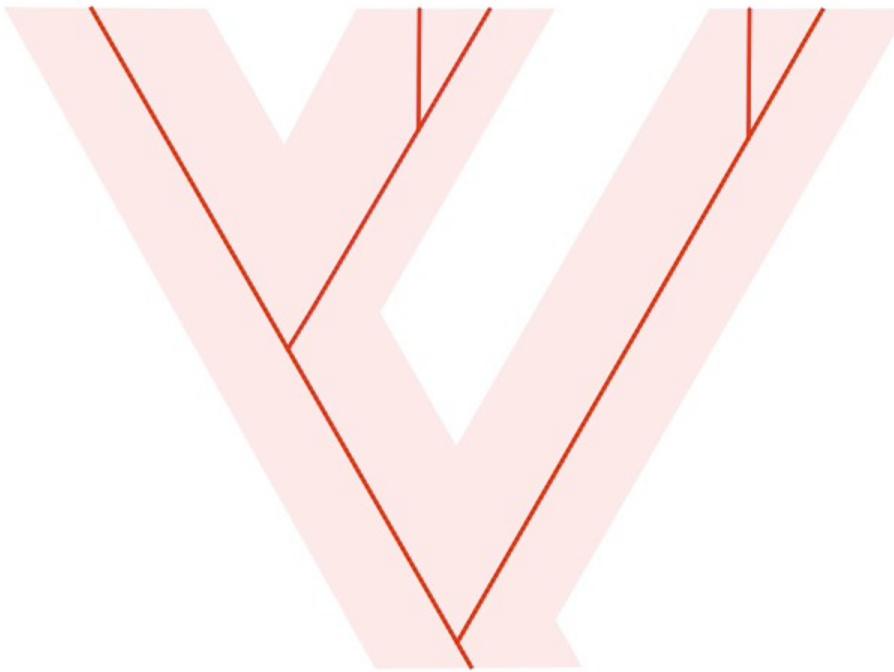
Behavior of gene trees relates to MANY factors, including ancestral population sizes

# Thank you, Peter Beerli (et al.)!

In no particular order ...

- The coalescent is a powerful tool for understanding historical demography (e.g., ancestral Ne and divergence times)
- Branch lengths can have an enormous impact on recovering the species tree from gene trees
- Rapid radiations with short internal branches can really F\* U up!
- Ancestral population size (the size of “the box”) can have a huge impact on coalescent rates
- Assumed mutation rate can have HUGE impact on estimation of Ne (recall Peter’s whales)
- Increasingly clear that concatenation is inferior to MSC for estimating species tree
- Theoretically, coalescent times should be as old or older than speciation events

AGCACTGACC CGGGATGC TACGATCGACATCGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT  
AGCACTGACC CGGGATGC TACGATCGACATCGACGACACTAGCCGGGT TCATCGATCACGCAT GCGACT  
AGCACTGACC CGGGATGC TACGATCGACATGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT  
AGCACTGAC CGGGATGC TACGATCGACATCGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT  
AGCACTGAC CGGGATGC ACGATCGACATCGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT  
AGCACTGAC CGGGATGC TACGATCGACATCGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT



Animation courtesy of Kelsie Hunnicutt

## **Summary of Beerli (31 May 2022):**

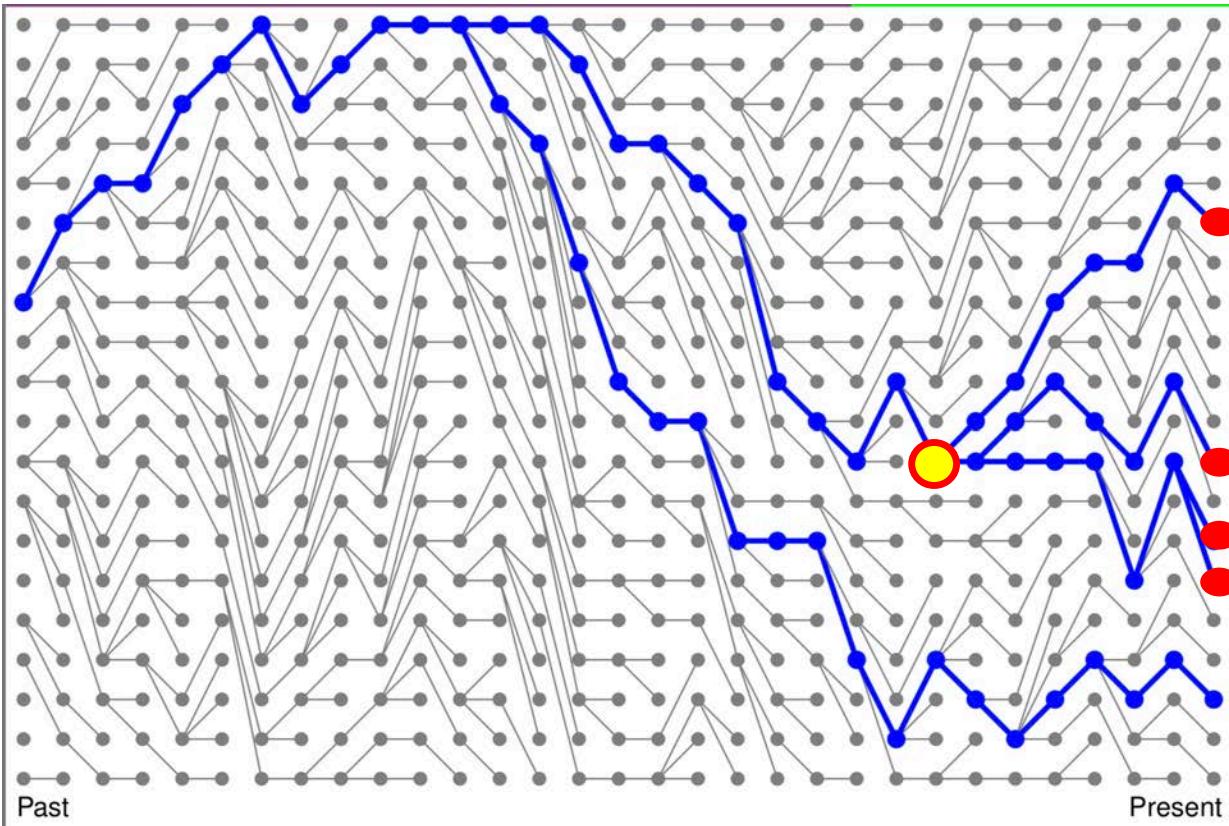
**BOTTOM LINE:**

**IT'S BLOODY COMPLICATED!!!**

## **The GOOD NEWS:**

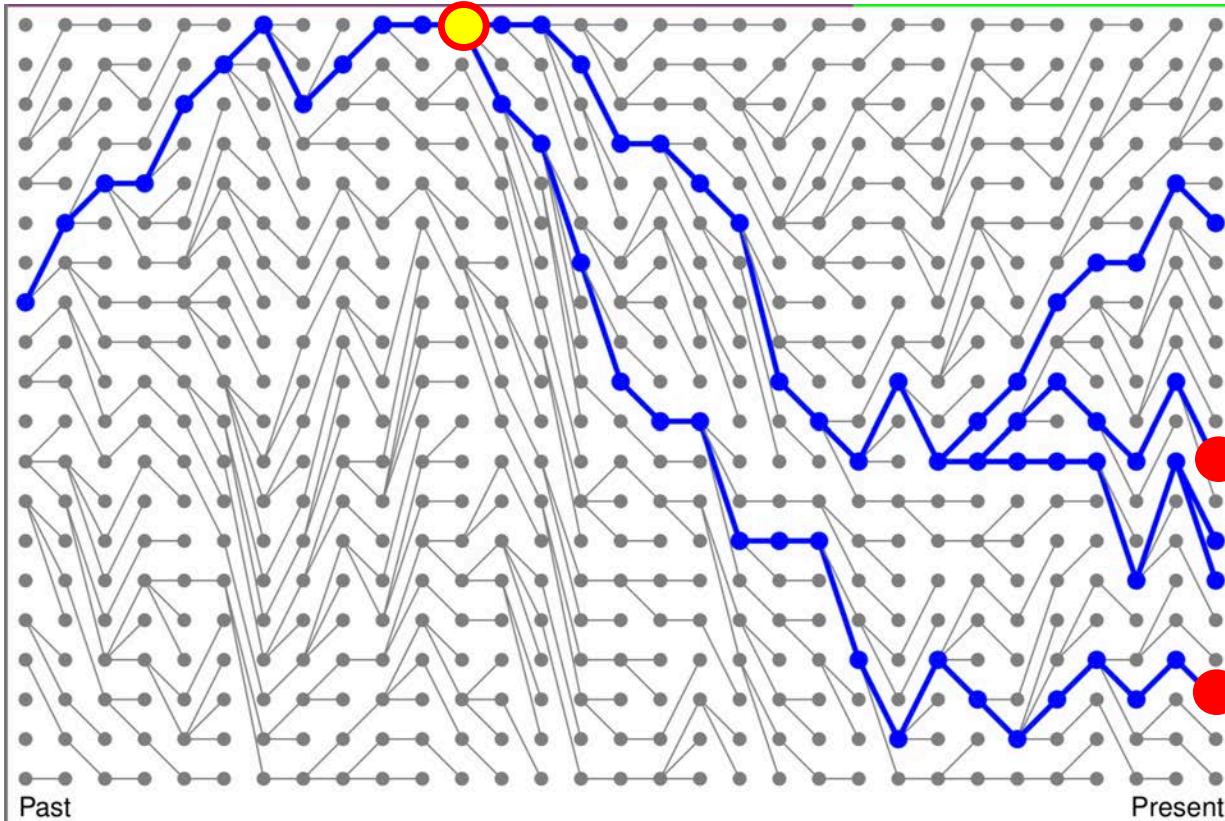
- Small sample sizes can work!

**Though sampling strategy must be carefully considered!**  
**(E.g., frogs in a single pond ...)**



Thanks to  
Peter Beerli  
MOLE 2022)

**But sampling from TWO ponds can yield older coalescent events, even with fewer individuals**



Thanks to  
Peter Beerli  
MOLE 2022)

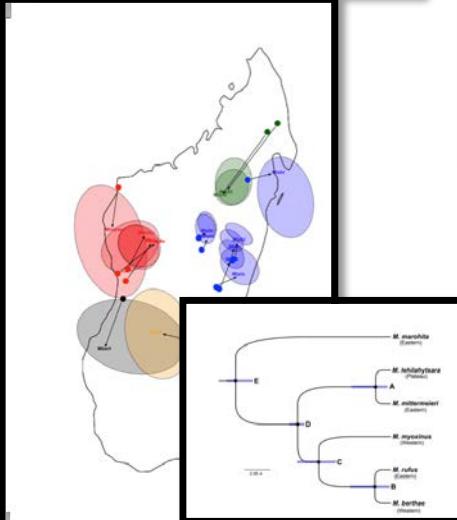
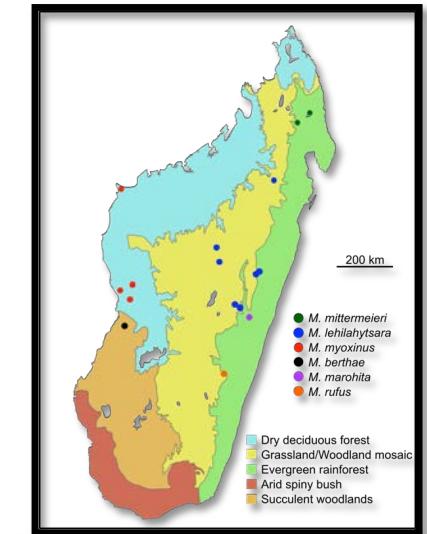
# The MSC is increasingly used for:

- Species delimitation
- Reconstructing historical demography  
(e.g., ancestral population size)
- Patterns of gene flow among and  
between phylogenetic lineages
- Phylogeny reconstruction
- Divergence time estimation

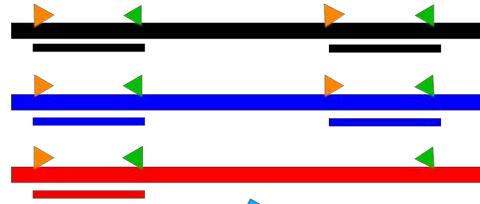
The entire field of population genetics will benefit from increased exchange between coalescents and phylogeography ... [and] the future availability of multilocus genetic data will serve as a bridge between these two approaches."

**Wakeley, 2003**

Ideally, we want multiple loci  
that are independently  
segregating in the genome

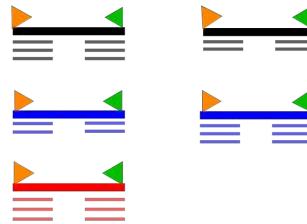


Library Prep

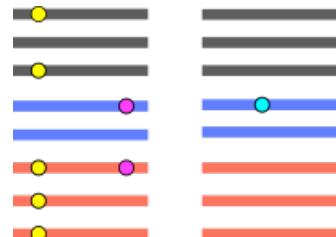


NextGen Sequencing

RADseq: Tens of thousands of unlinked SNPs from across the genome!



Data Types



Cluster Data/Call SNPs

Slide courtesy of C. Ryan Campbell

# RADseq Stats

*Filtering thresholds:*

- Invariant loci are retained; minimum locus size: 100; maximum % of missing data: 10.
- Total number of loci (that passed filtering): 15,267.
- # of invariant loci: 1,822.

*Mean locus length: 231 bp.*

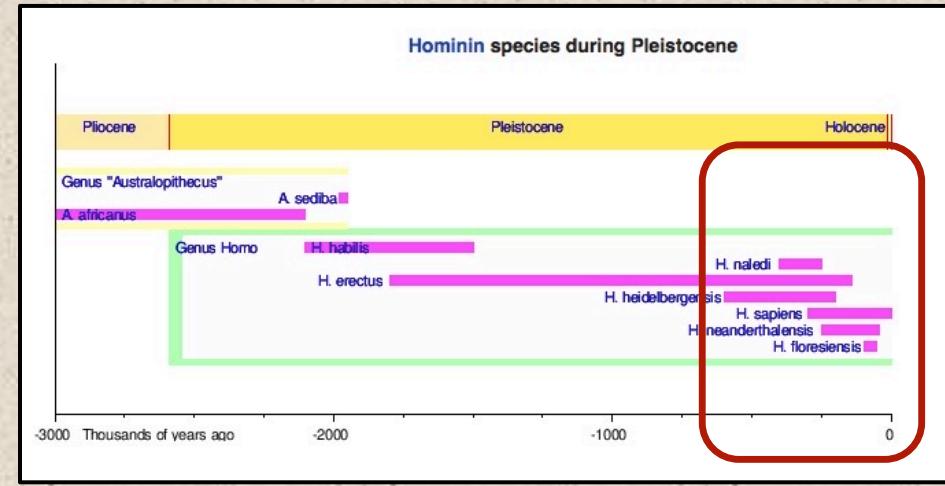
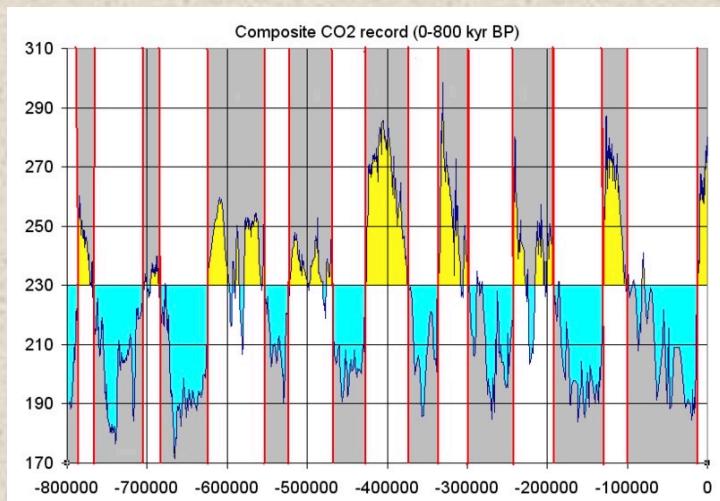
*Mean # of variable sites: 4.7.*

*Mean # of parsimony-informative sites: 3.29.*

*Mean % of missing data (Ns): 5.53.*

# Has climate change been a driver of speciation?

## Climate Fluctuation during the Pleistocene



Lineage diversification within hominins

~ 2.6 mya through ~ 12 kya

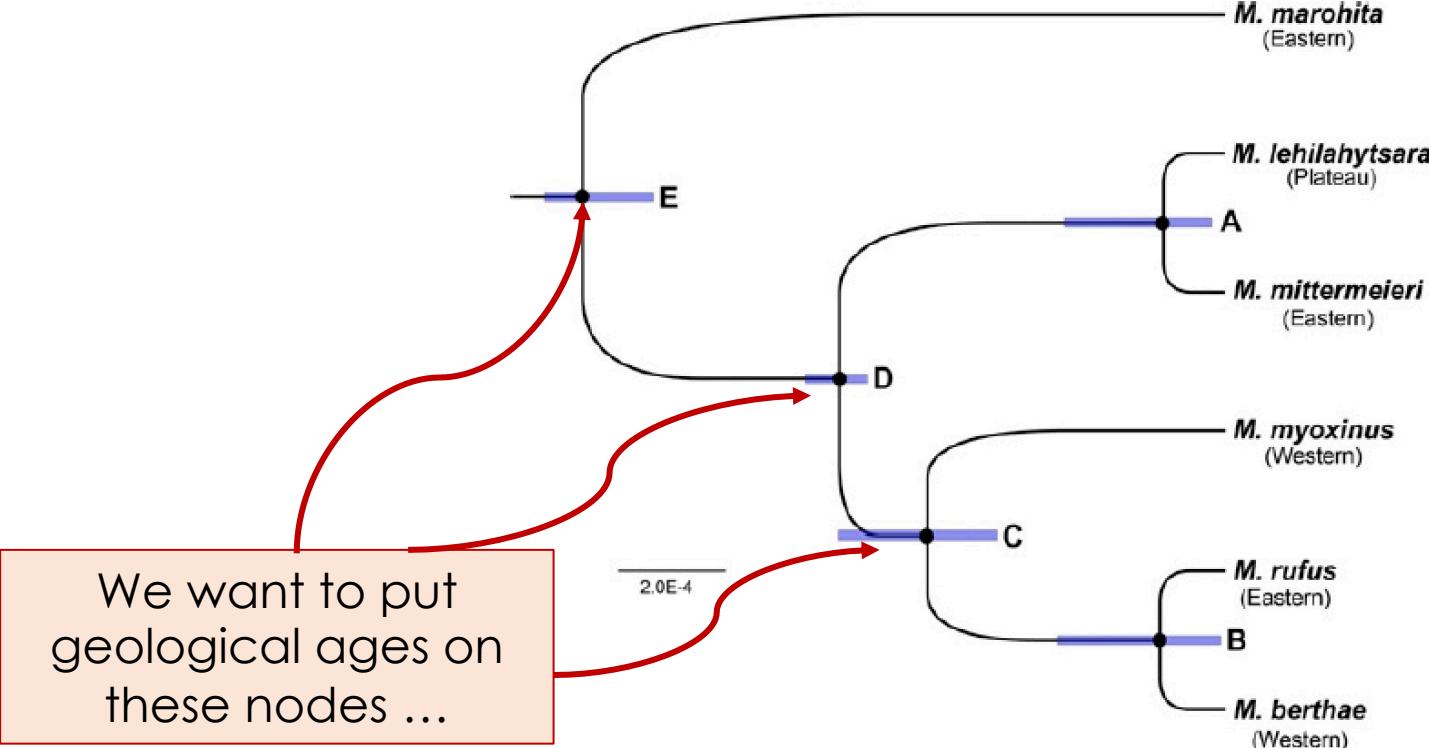
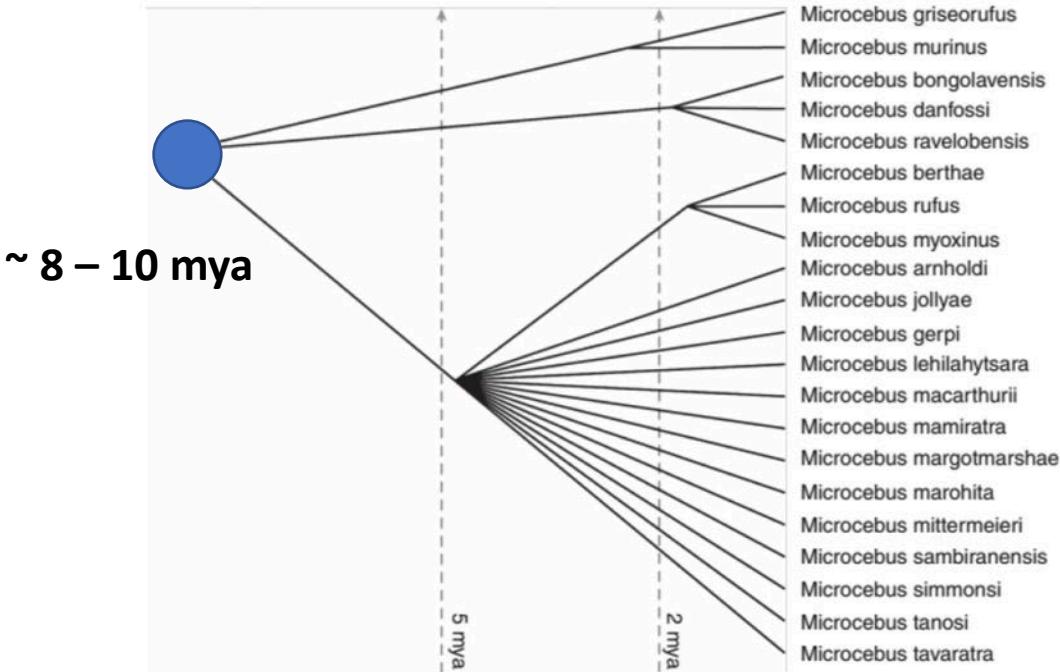


Fig. 3. Maximum clade credibility species tree from a BPP analysis of nuclear RAD-seq data. Node labels correspond to those in Table 1. All nodes in the tree have 100% statistical support. Blue bars are the 95% highest posterior densities of node heights. Species are identified by their contemporary geographic range (eastern versus western); plateau, Central Highlands plateau.

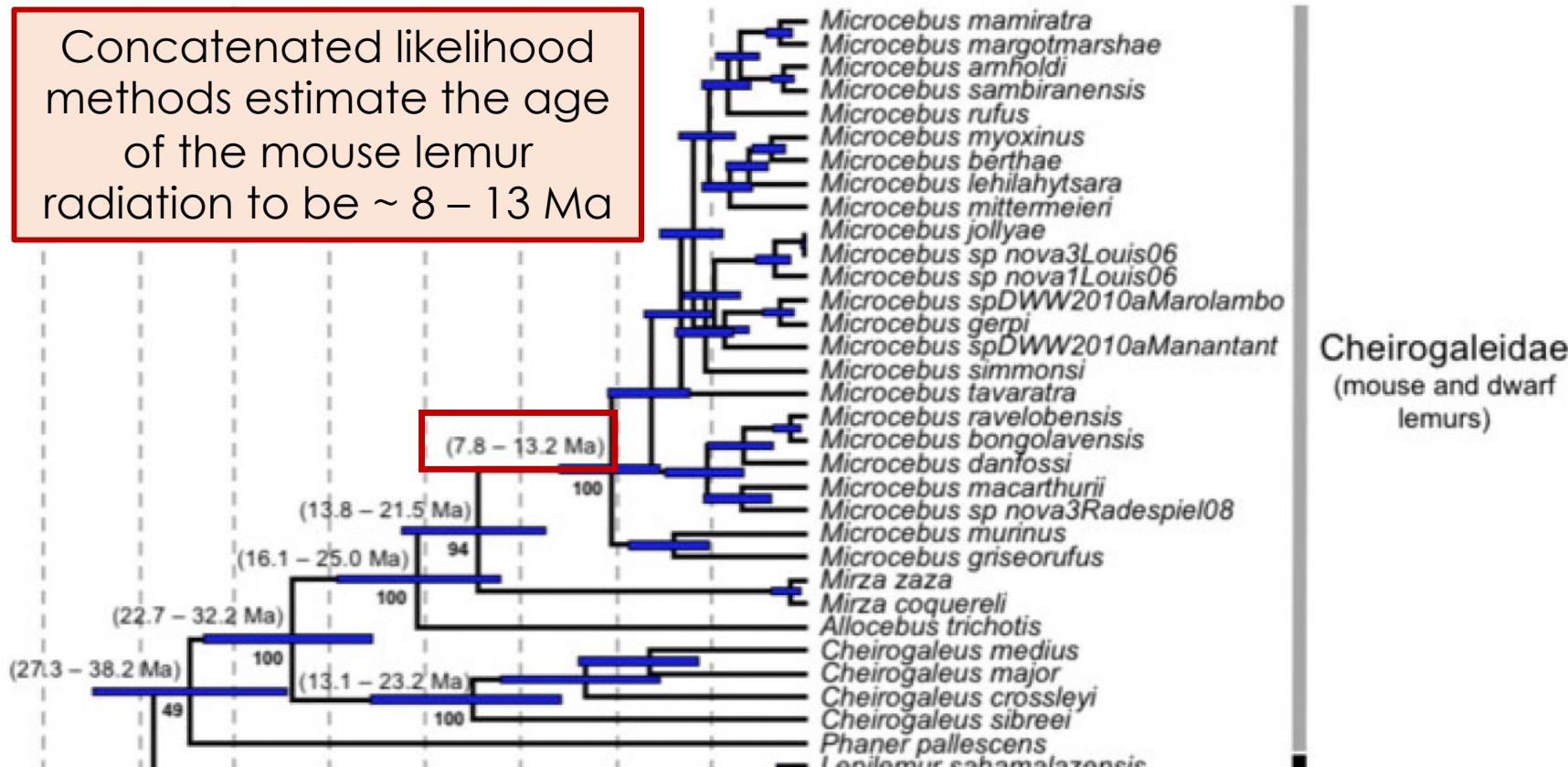
# Traditional concatenated methods

## Unresolved phylogeny...

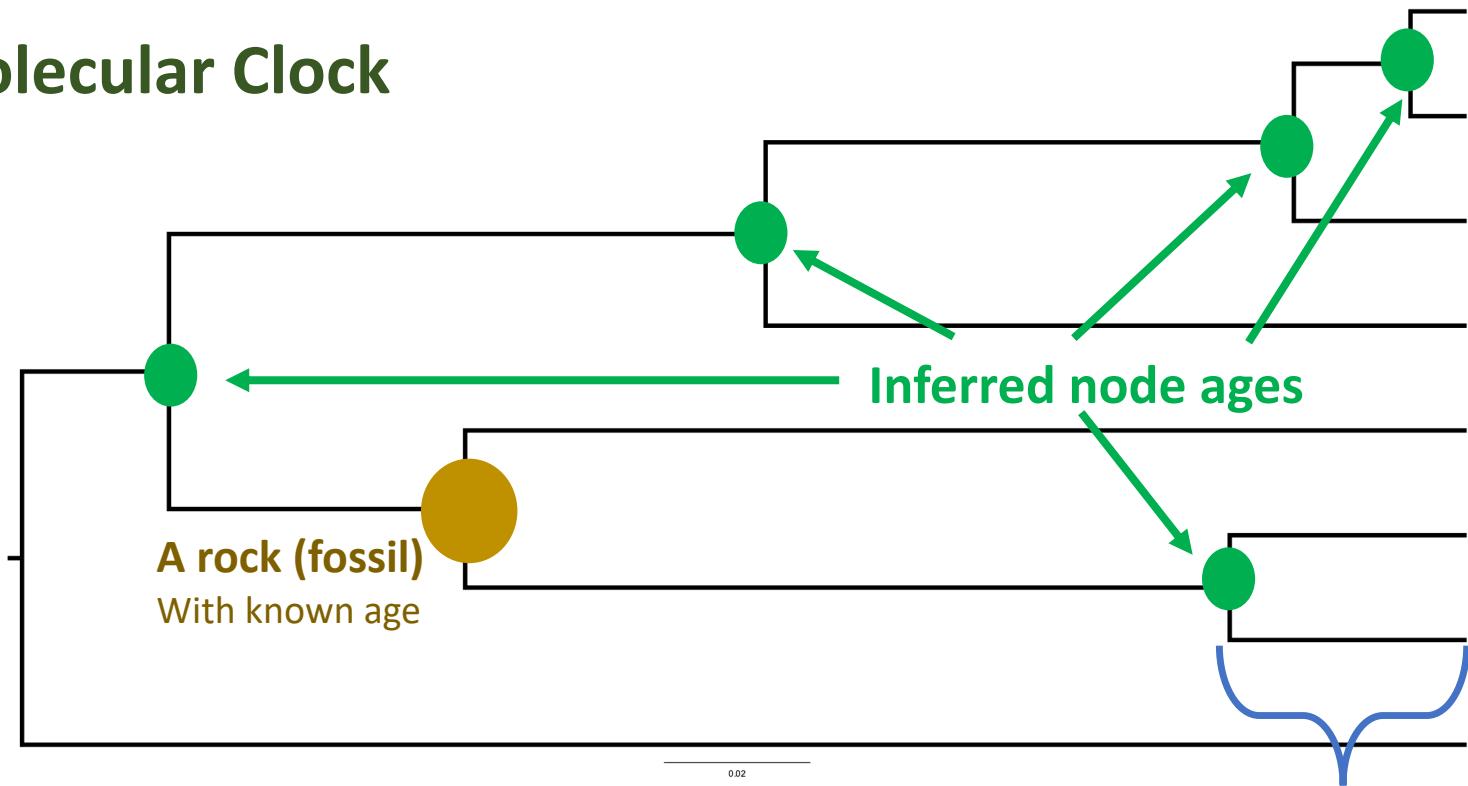


Yoder (2016)

Concatenated likelihood methods estimate the age of the mouse lemur radiation to be ~ 8 – 13 Ma

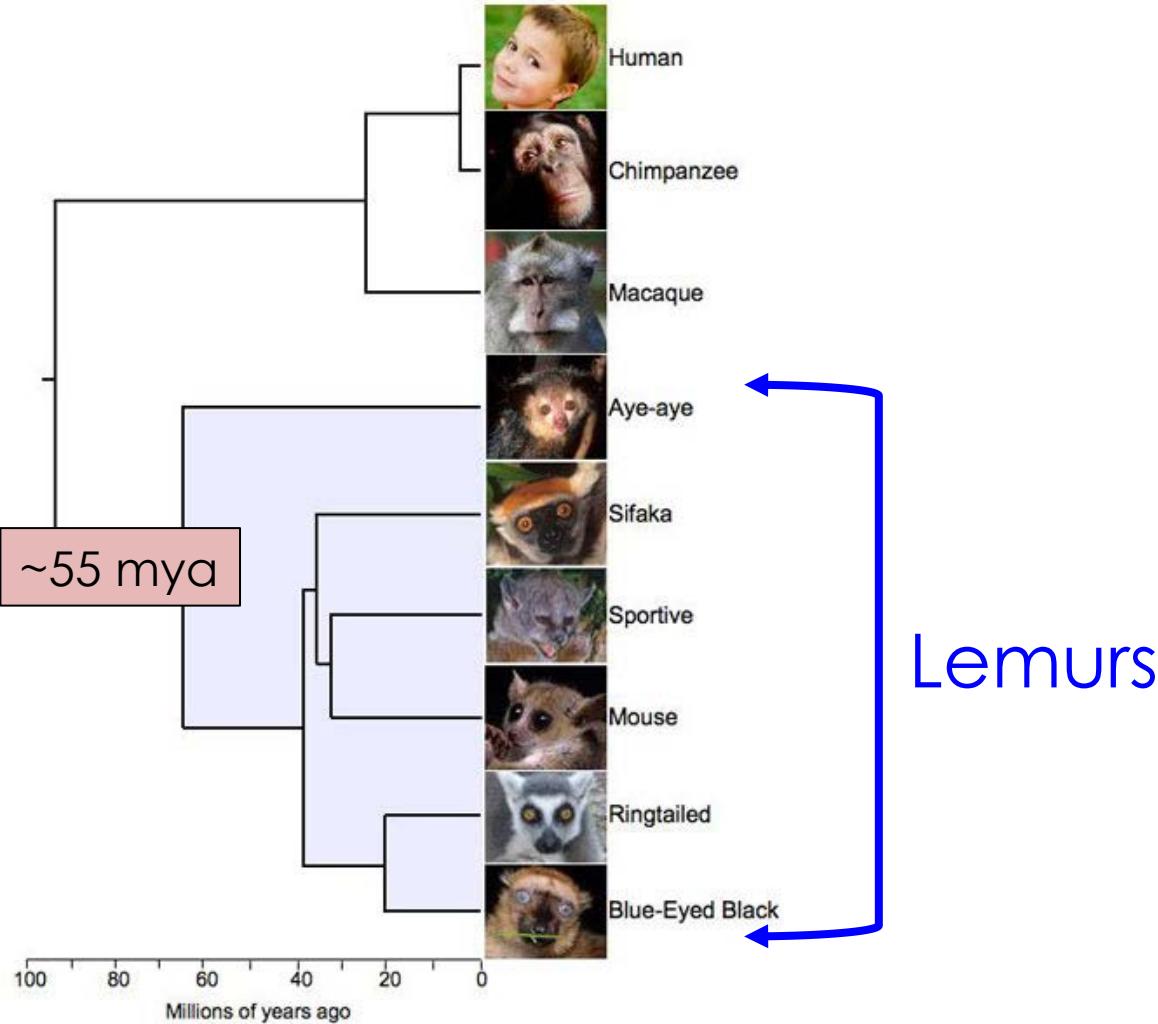


# Molecular Clock

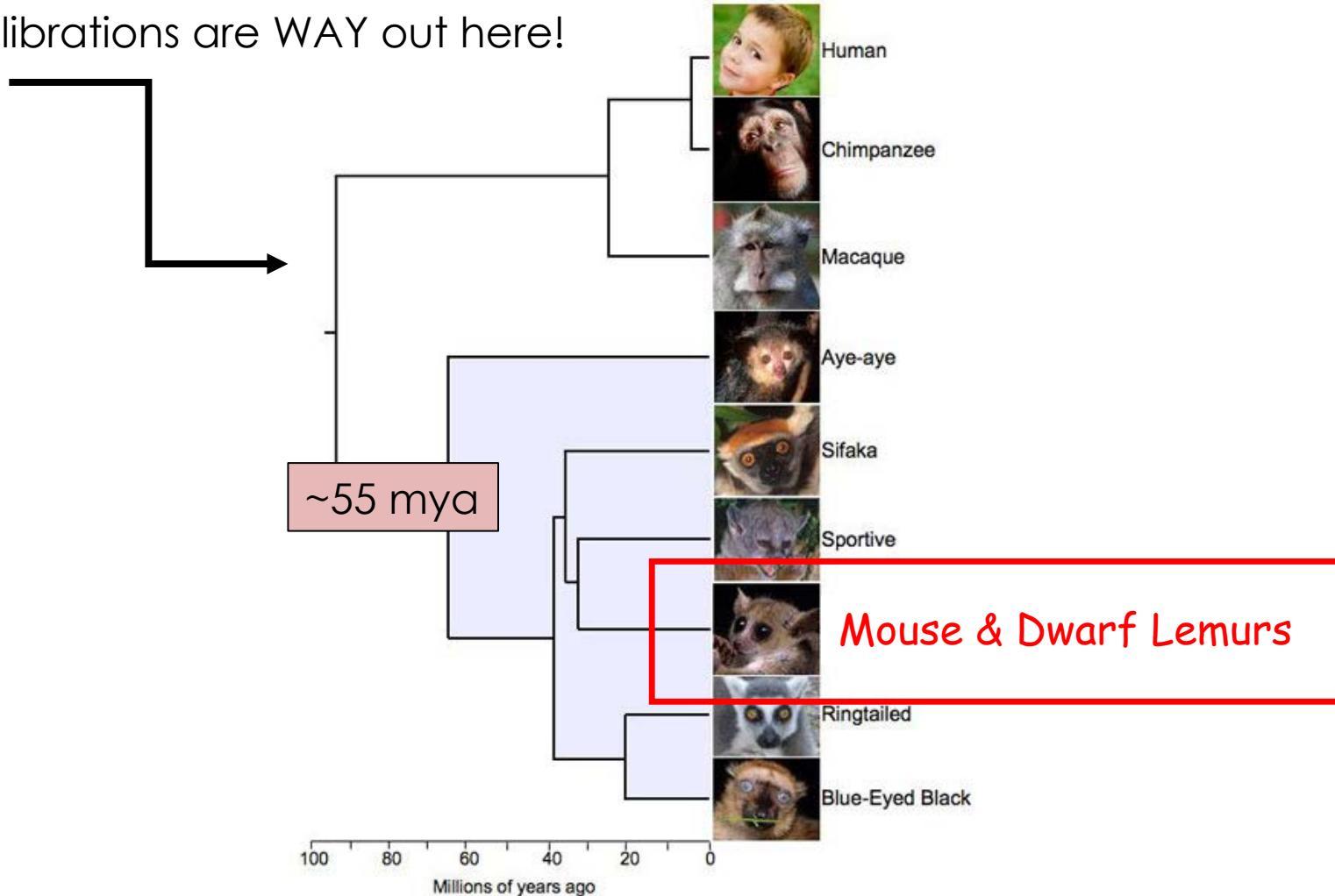


**Branch length**  
(in substitution per site, coalescent units, etc.)  
proportional to [mutation rate \* time]

**No fossil record!!!**



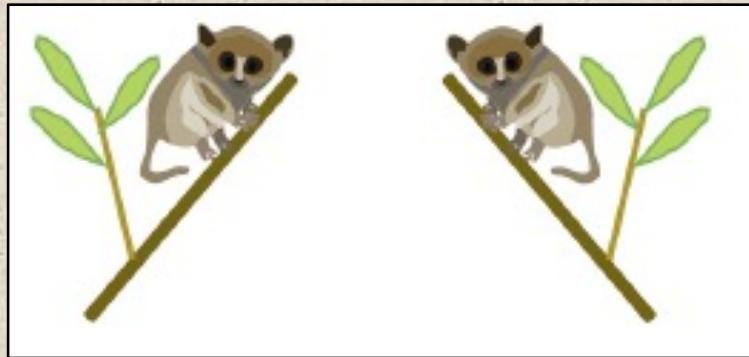
Fossil calibrations are WAY out here!





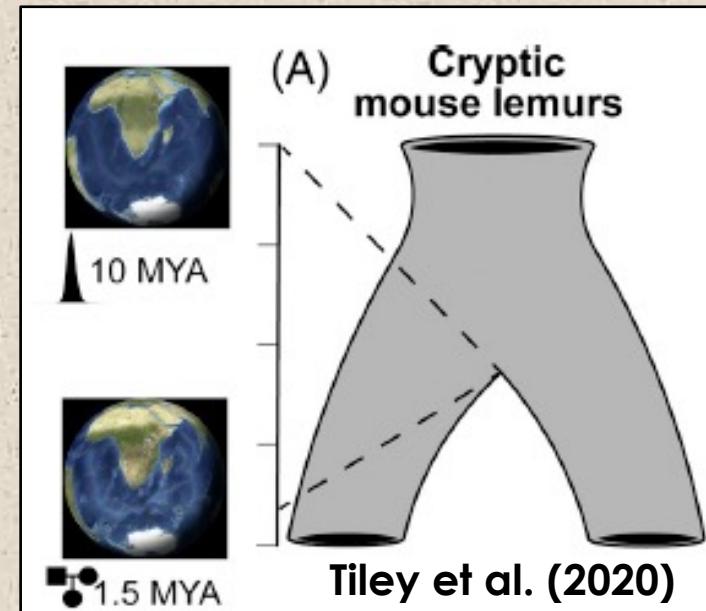
## Review

## Molecular Clocks without Rocks: New Solutions for Old Problems

George P. Tiley ,<sup>1</sup> Jelmer W. Poelstra,<sup>1</sup> Mario dos Reis,<sup>2</sup> Zheng Yang ,<sup>3</sup> and Anne D. Yoder<sup>1,\*</sup>

MRCA = 8 – 10 Ma  
(concatenation)

MRCA ~ 1.5 Ma  
(MSC)

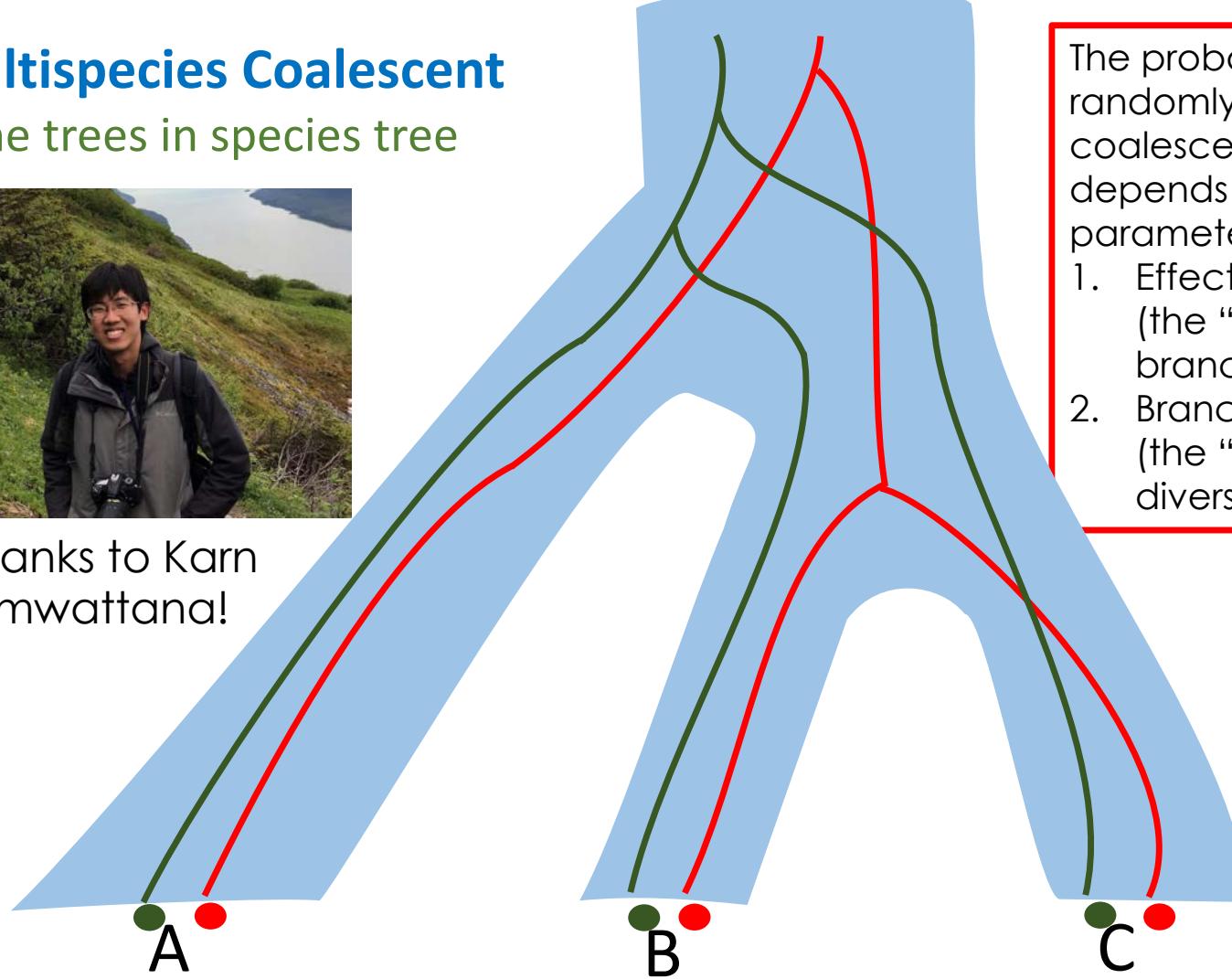


# Multispecies Coalescent

Gene trees in species tree



Thanks to Karn  
Imwattana!



The probability that two randomly chosen alleles will coalesce at a given time depends upon two key parameters:

1. Effective population size (the “width” of the branch)
2. Branch length distributions (the “speed” of diversification)

## Concatenation methods

“Multiple loci are treated as a single nonrecombining locus with a single underlying topology.”



## Coalescent methods

“Stochastic process of lineage joining when one traces the genealogical history of a sample of sequences from a population backwards in time.”

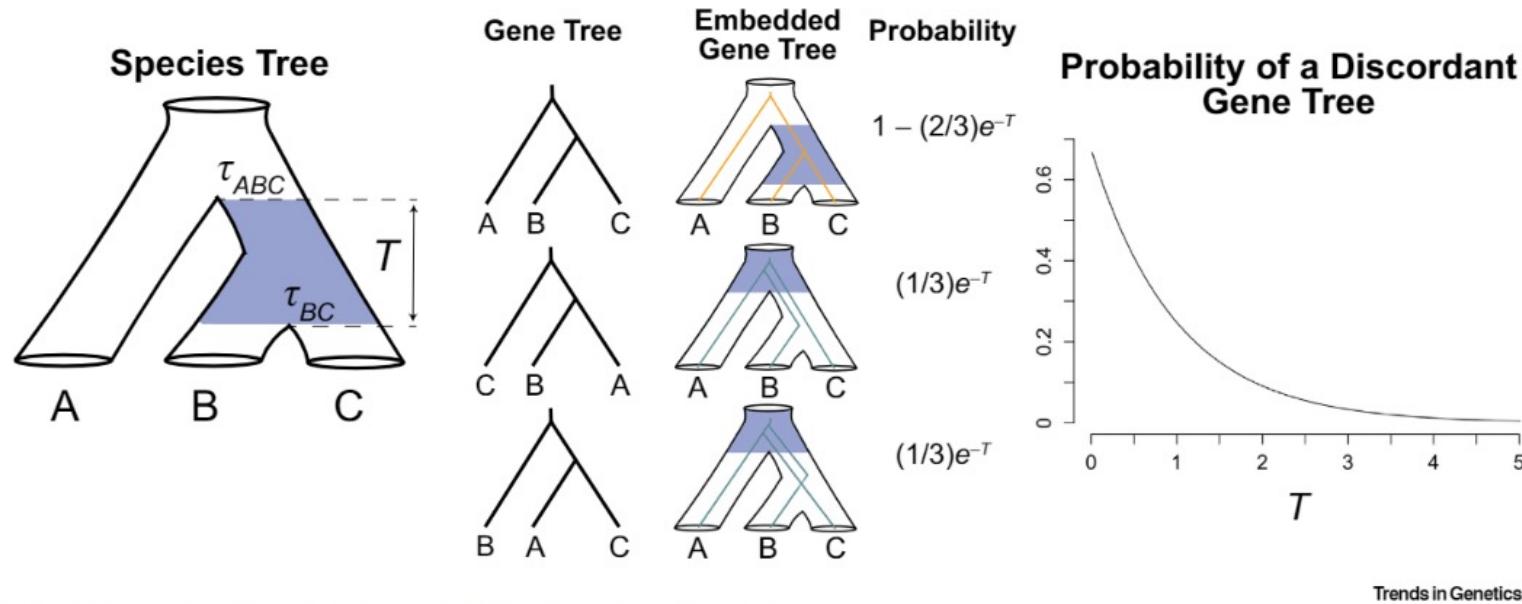
Under coalescent framework,  
each non-recombining locus has its  
own phylogeny (gene tree).

- Coalescent processes can generate discordances between gene trees and species tree (both in topology and branch lengths), **especially when population sizes are big and/or divergence times are short.**
- MSC methods can account for the coalescent processes.
- With **mutation rate** and **generation time**,  $\tau$  and  $\theta$  parameters inferred from MSC can be converted to absolute divergence time and effective population size, without the need for fossil calibration.



# The effects of ILS on the frequency of discordant gene trees

(Figure 2.)



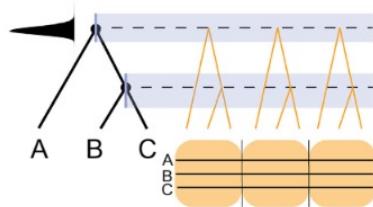
Trends in Genetics

- Low  $T$  (short internal branch) → lots of ILS (can cause errors if concatenation method is used)
- High  $T$  (long internal branch) → low ILS

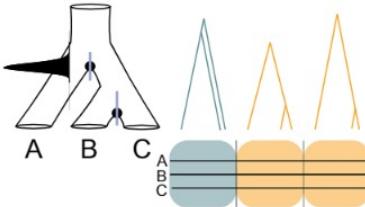


# Differences between Bayesian Methods for Divergence Time Estimation and Programs for Implementing Them (Figure 5.)

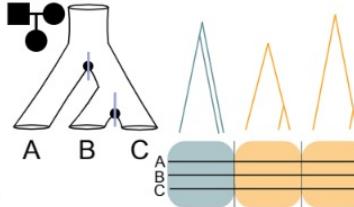
## Concatenation with Fossil Calibrations



## MSC with Fossil Calibrations



## Mutation Rate Calibrated MSC



### Strengths

Computationally efficient for large numbers of tips and loci

Considers discordance between gene trees and species trees

Does not require calibrations on nodes from external information such as fossils

### Weaknesses

May produce biased estimates when ILS is high or when gene sequence divergence is far from species divergence

Increased computational complexity from averaging over gene trees to estimate species tree parameters

Requires external mutation rate estimates from sequenced pedigrees and potentially not appropriate for distant taxa

### Common Programs

BEAST2 [96]  
MCMCTREE [97]  
MrBayes [98]  
PhyloBayes [99]

BPP [5,71]  
StarBEAST2 [6]

BPP [5,71]  
StarBEAST2 [6]



# How to calibrate coalescent trees to geological time

Jul 3, 2016 • Mario dos Reis



Simple overview:

By using estimates of **mutation rate** and **generation time** we can calculate absolute time of divergence (without fossil calibrations!)

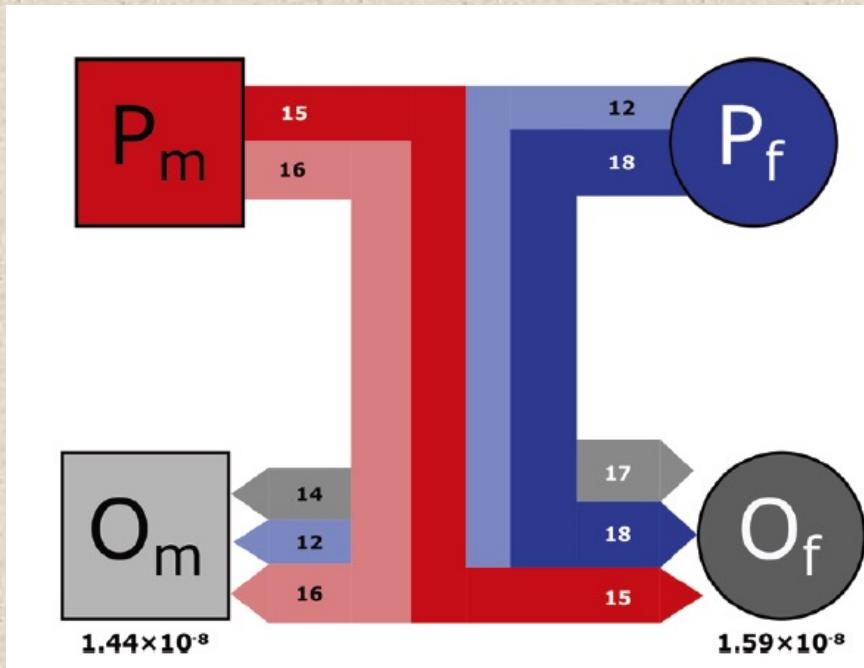
ars.

**Remember what Peter told us yesterday, that if we know the mutation rate we can differentiate population size**

Different  $N$  and appropriate  $\mu$  can give the same number of mutations. For example, for 100 loci sampled from 20 individuals with 1000bp each, we get :

$N$	$\mu$	$4N\mu$	$\hat{S}$	$\sigma_S^2$
1250	$10^{-5}$	0.05	153.95	16.25
12500	$10^{-6}$	0.05	152.89	16.05

# Estimating mutation rate



**"Trio" studies:** sequence the genomes of parents and their offspring, and “count” the mutations in offspring that are not observed in the parents.

## **Measuring de novo mutation rate:**

- Count number of mutations
- Divide by size of the genome
- # mutations/size of genome = rate

**Easy, right?**

## Problems (just a few):

- Depth of coverage (some mutations will be called with more confidence than others)
- Genome assembly really, really matters (need long contigs to avoid, as much as possible, missing or misassembled regions due to highly repetitive regions)
- The number of “mutations” caused by sequencing error will VASTLY exceed biological mutations (replication errors)
- Ironically then, the false negative rate can be just as error prone (if not more so) than the false positive rate due to extreme filtering stringency

# Estimating the de novo mutation rate for the gray mouse lemur (*Microcebus murinus*)

the  
**genetics**society

[www.nature.com/hdy](http://www.nature.com/hdy)

## ARTICLE

Pedigree-based and phylogenetic methods support surprising patterns of mutation rate and spectrum in the gray mouse lemur

 Check for updates

C. Ryan Campbell<sup>1,2,8</sup>, George P. Tiley<sup>1,8</sup>, Jelmer W. Poelstra<sup>1</sup>, Kelsie E. Hunnicutt<sup>1,6</sup>, Peter A. Larsen<sup>1,7</sup>, Hui-Jie Lee<sup>1,3</sup>, Jeffrey L. Thome<sup>4</sup>, Mario dos Reis<sup>1,5</sup> and Anne D. Yoder<sup>1,6</sup>

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C. Ryan Campbell

2021



[www.nature.com/hdy](http://www.nature.com/hdy)

ARTICLE



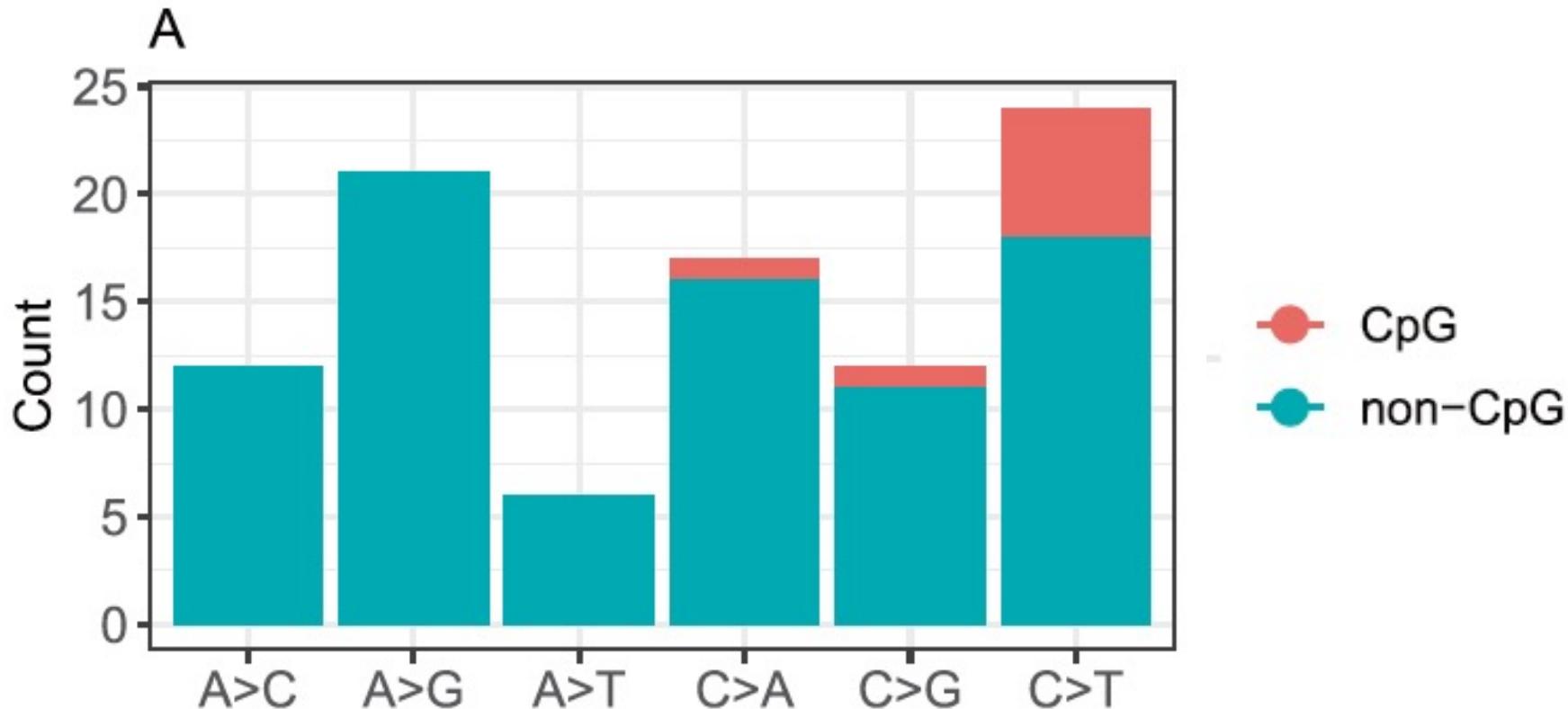
~~Pedigree-based and phylogenetic methods support surprising patterns of mutation rate and spectrum in the gray mouse lemur~~

Rate is higher than expected and mutational spectrum surprising!

C. Ryan Campbell<sup>1,2,8</sup>, George P. Tiley<sup>1,8</sup>, Jelmer W. Poelstra<sup>1</sup>, Kelsie E. Hunnicutt<sup>1,6</sup>, Peter A. Larsen<sup>1,7</sup>, Hui-Jie Lee<sup>1,3</sup>, Jeffrey L. Thorne<sup>4</sup>, Mario dos Reis<sup>5</sup> and Anne D. Yoder<sup>1</sup>✉

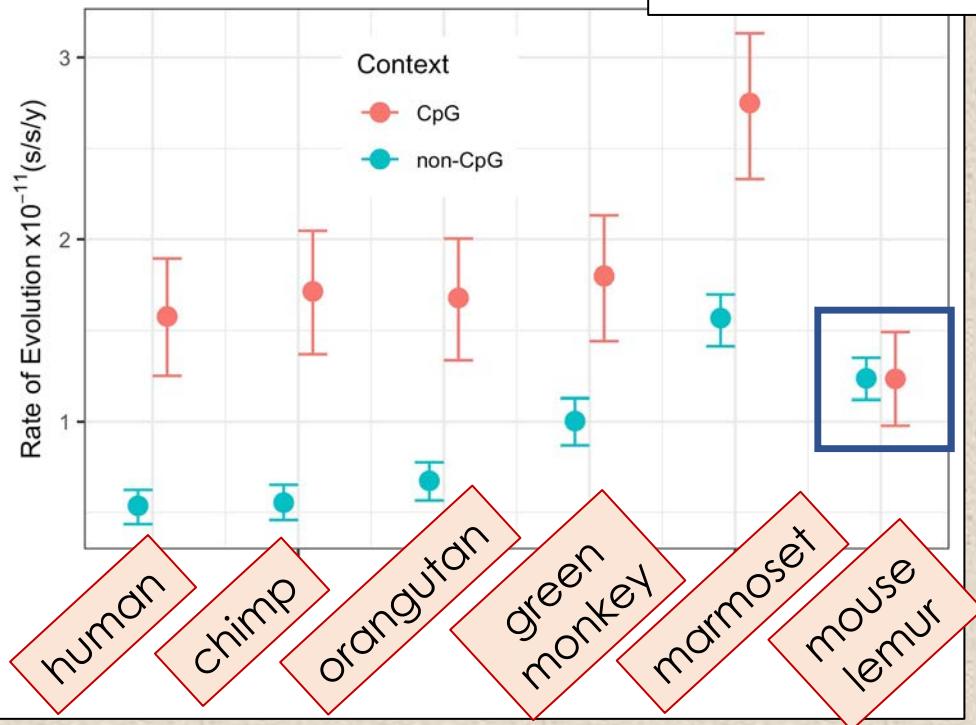
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# Mouse Lemur Mutation Spectrum

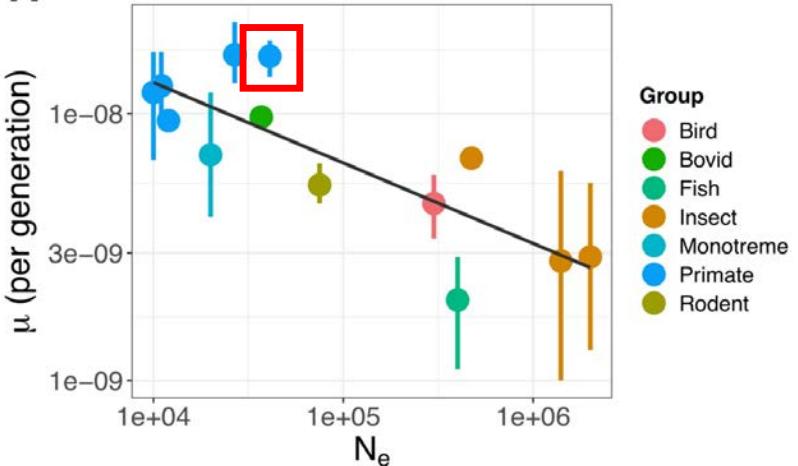


**Substitution rate analysis validates surprising results from a pedigree-based measurement of the de novo mutation rate in gray mouse lemurs.**

\*C. Ryan Campbell<sup>1,2</sup>, \*George P. Tiley<sup>1</sup>, Jelmer W. Poelstra<sup>1</sup>, Kelsie E. Hunnicutt<sup>1,†</sup>, Peter A. Larsen<sup>1,‡</sup>, Hui-Jie Lee<sup>3</sup>, Jeffrey L. Thorne<sup>4</sup>, Mario dos Reis<sup>5</sup>, Anne D. Yoder<sup>1</sup>

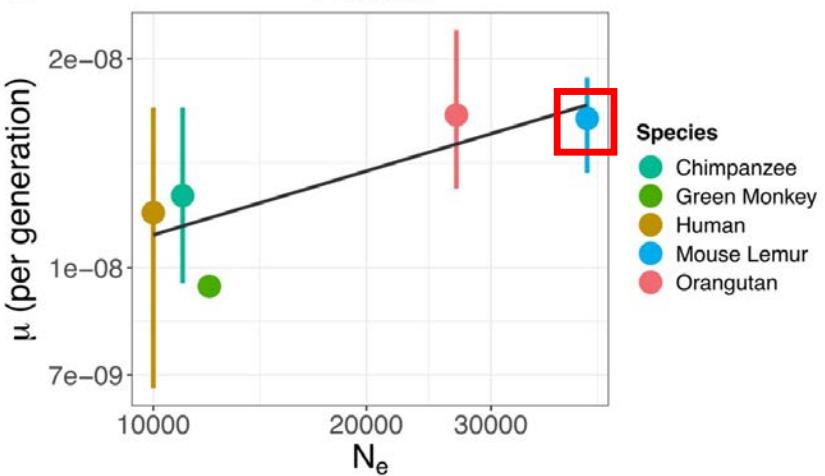


**Punch line:** substitution rate analysis yields same result regarding low values of CpG rate

**A** All animals

$$1.57 \times 10^{-8}$$

(one of the highest  
rates yet measured  
for a mammal)

**B** Primates

# Key Findings

- High de novo rate – makes sense, right? 😎
- Low paternal bias – getting a little worrisome 🙄
- Very low CpG bias – biological nonsense? 😛



Even with sequencing accuracy of **99.999%** you will have ~ 28,000 errors in 2.8 Gb genome ...



Molly Przeworski

False  
positives ...



Priya Moorjani

False  
positives ...

Meredith Yeager



False  
positives ...

# Playing around with computational filters

		Callable Sites Filter											
		Allele Drop		10x	11x	12x	15x	20x	22x	24x	25x		
Allelic Balance Filter	None	Pat:Mat Ratio	CpG Rate	de novo $\mu$	83.55%	88.46%	88.05%	87.55%	85.30%	77.27%	72.00%	65.52%	61.91%
	20-80	1.11	8.41%	107	1.70E-08	1.60E-08	1.61E-08	1.62E-08	1.66E-08	1.84E-08	1.97E-08	2.17E-08	2.30E-08
	25-75	1.11	8.41%	107	1.70E-08	1.60E-08	1.61E-08	1.62E-08	1.66E-08	1.84E-08	1.97E-08	2.17E-08	2.30E-08
	25-65	1.06	8.57%	105	1.70E-08	1.59E-08	1.60E-08	1.61E-08	1.65E-08	1.83E-08	1.96E-08	2.15E-08	2.28E-08
	30-70	1.03	8.70%	92	1.52E-08	1.43E-08	1.44E-08	1.44E-08	1.48E-08	1.64E-08	1.76E-08	1.93E-08	2.05E-08
	Total Sites												

**Fig. 2 Effect of filtering thresholds on mutation-rate estimation.** The mutation rate and spectrum of the gray mouse lemur, as a product of two main filtering decisions: (1) an allelic balance filter (along the rows) and (2) a callable site filter (along the columns). The first three columns display how the parent-of-origin, the mutation rate at CpG sites, and total number of mutations vary. The remainder of the table shows the combined effect of these filters on the calculated rate. Cells for lower rates are shaded blue and higher rates are shaded red. All mutation rates have been corrected for the estimated number of false positives and false negatives with their respective number of mutations and callable sites.

# Playing around with computational filters

		Callable Sites Filter											
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	20-80	1.11	8.41%	107	1.70E-08	1.60E-08	1.61E-08	1.62E-08	1.66E-08	1.84E-08	1.97E-08	2.17E-08	2.30E-08
	25-75	1.11	8.41%	107	1.70E-08	1.60E-08	1.61E-08	1.62E-08	1.66E-08	1.84E-08	1.97E-08	2.17E-08	2.30E-08
	25-65	1.06	8.57%	105	1.70E-08	1.59E-08	1.60E-08	1.61E-08	1.65E-08	1.83E-08	1.96E-08	2.15E-08	2.28E-08
	30-70	1.03	8.70%	92	1.52E-08	1.43E-08	1.44E-08	1.44E-08	1.48E-08	1.64E-08	1.76E-08	1.93E-08	2.05E-08

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Can have enormous impact on estimated rate

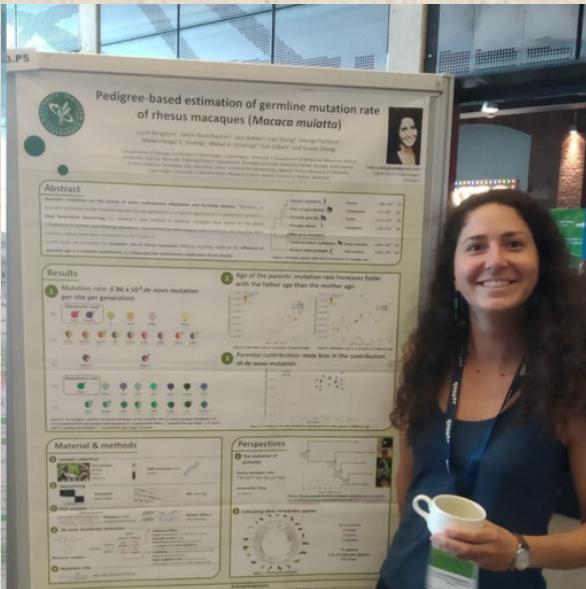
# Playing around with computational filters

		Callable Sites Filter											
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	20-80	1.11	8.41%	107	1.70E-08	1.60E-08	1.61E-08	1.62E-08	1.66E-08	1.84E-08	1.97E-08	2.17E-08	2.30E-08
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**Fig. 2 Effect of filtering thresholds on mutation-rate estimation.** The mutation rate and spectrum of the gray mouse lemur, as a product of two main filtering decisions: (1) an allelic balance filter (along the rows) and (2) a callable site filter (along the columns). The first three columns display how the parent-of-origin, the mutation rate at CpG sites, and total number of mutations vary. The remainder of the table shows the combined effect of these filters on the calculated rate. Cells for lower rates are shaded blue and higher rates are shaded red. All mutation rates have been corrected for the estimated number of false positives and false negatives with their respective number of mutations and callable sites.

Has little impact on estimate of mutational spectrum

# Working towards best practices: standardize methods so that results are comparable!



Lucie Bergeron



The Mutationathon highlights the importance of reaching standardization in estimates of pedigree-based germline mutation rates

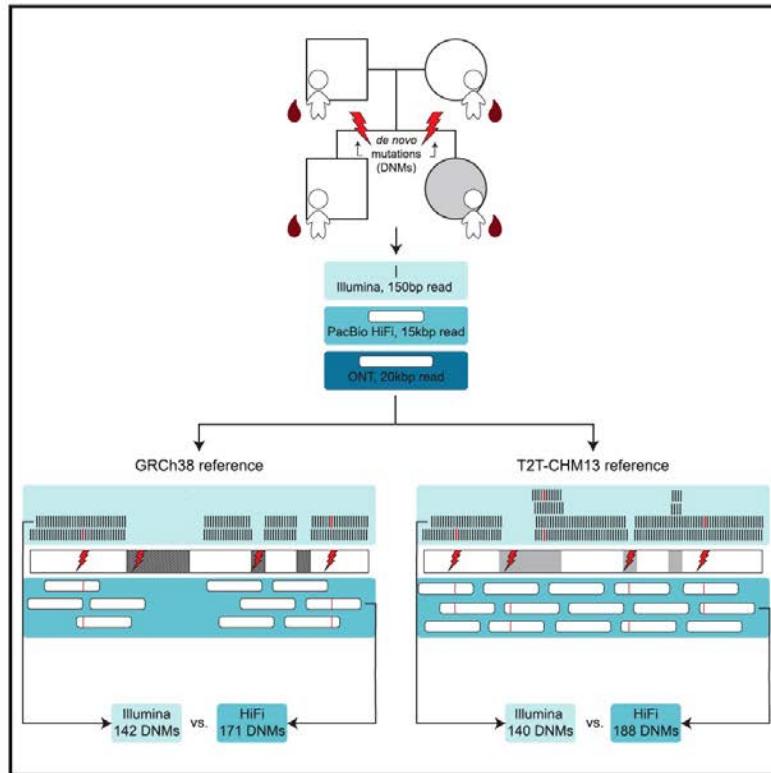
Lucie A Bergeron Søren Besenbacher, Tychele Turner, Cyril J Versoza, Richard J Wang, Alivia Lee Price, Ellie Armstrong, Meritxell Riera, Jedidiah Carlson, Hwei-yan Chen, Matthew W Hahn, Kelley Harris, April Snøfrid Kleppe, Elora H López-Nandam, Priya Moorjani, Susanne P Pfeifer, George P Tiley, Anne D Yoder, Guojie Zhang, Mikkel H Schierup « see less

“We report **almost a two-fold variation** in the final estimated rate among groups using different post-alignment processing, calling, and filtering criteria and provide details into the sources of variation across studies.”



# Familial long-read sequencing increases yield of *de novo* mutations

## Graphical abstract



## Authors

Michelle D. Noyes, William T. Harvey,  
David Porubsky, ..., Jan O. Korbel,  
W. Richard McCombie, Evan E. Eichler

## Correspondence

[eee@gs.washington.edu](mailto:eee@gs.washington.edu)

"We demonstrate that long-read sequencing and assembly, especially when combined with a more complete reference genome, increases the number of DNMs by >25% compared to previous studies, providing a more complete catalog of DNM compared to short-read data alone."

Complexities of calculating generation  
times (a few) ...

# **Estimating generation times:**

## **Key parameters**

- Age at onset of first offspring to last reproductive event
- Number of offspring produced from first to last reproduction
- Probability of surviving to age of last reproduction

# Stable, high-quality environments

- Low mortality (oldest old ~ 8 years)
- Breed at age = 1 year
- Only one litter per year



# Low-quality, unpredictable environments

- High mortality (oldest old ~ 3 years)
- Breed very early (  $\leq$  four months)
- Multiple litters per year (i.e., overlapping generations within a single year)





These behaviors have a complex relationship with the capacity for torpor in mouse lemurs; they can undergo a “**metabolic switch**” that leads to fattening and prolonged torpor/hibernation ... seems to be highly flexible by species and environment



## "The beauty and the horror of mouse lemurs"

Marina Blanco, pers. com.



These behaviors have a complex relationship with the capacity for torpor in mouse lemurs; they can undergo a "**metabolic switch**" that leads to fattening and prolonged torpor/hibernation ... seems to be highly flexible by species and environment

**Take a break???**





Portugal  
(Oeiras)



Lounès Chikhi



USA  
(Durham)

France  
(Toulouse)



Jordi Salmona

Ute Radespiel



Germany  
(Hannover)

RADseq Consortium



N > 400  
individuals from  
> 20 species

- M. sp. - Montagne d'Ambre from Montagne d'Ambre M. sp. - Montagne d'Ambre from Montagne d'Ambre  
M. sp. - Montagne d'Ambre from Montagne d'Ambre M. sp. - Montagne d'Ambre from Ambanja  
M. mittermeieri from Anjanaharibe Sud M. mittermeieri from Anjanaharibe Sud  
M. myoxinus from Andranomanitsy Marojejy Anjanaharibe Sud  
M. myoxinus from Andranomanitsy M. ravelobensis from Ankarafantsika  
C. crossleyi from Ankafofe Mlehi (4)  
M. lehilahysara from Ankafofe  
M. myoxinus from Aboalimena M. myoxinus from Aboalimena  
M. myoxinus from Aboalimena M. myoxinus from Aboalimena  
M. murinus from Andranomena M. lehilahysara from Tsinjoarivo  
M. murinus from Mananay M. murinus from Belo-sur-mer  
M. rufus from Andrambovato Mruf (3)  
M. rufus from Andrambovato M. rufus from Andrambovato  
M. murinus from Vohimena M. murinus from Vohimena  
M. murinus from Vohimena M. murinus from Vohimena  
M. griseorufus from Beza Mahafaly Mmyox (1)  
M. griseorufus from Vombositse M. griseorufus from Vombositse  
M. griseorufus from Tsimanampetsotsa M. griseorufus from Vohondava  
M. griseorufus from Tongaenoro M. manitatra from Bemanasy  
M. griseorufus from Mahavelo

## Consortium efforts

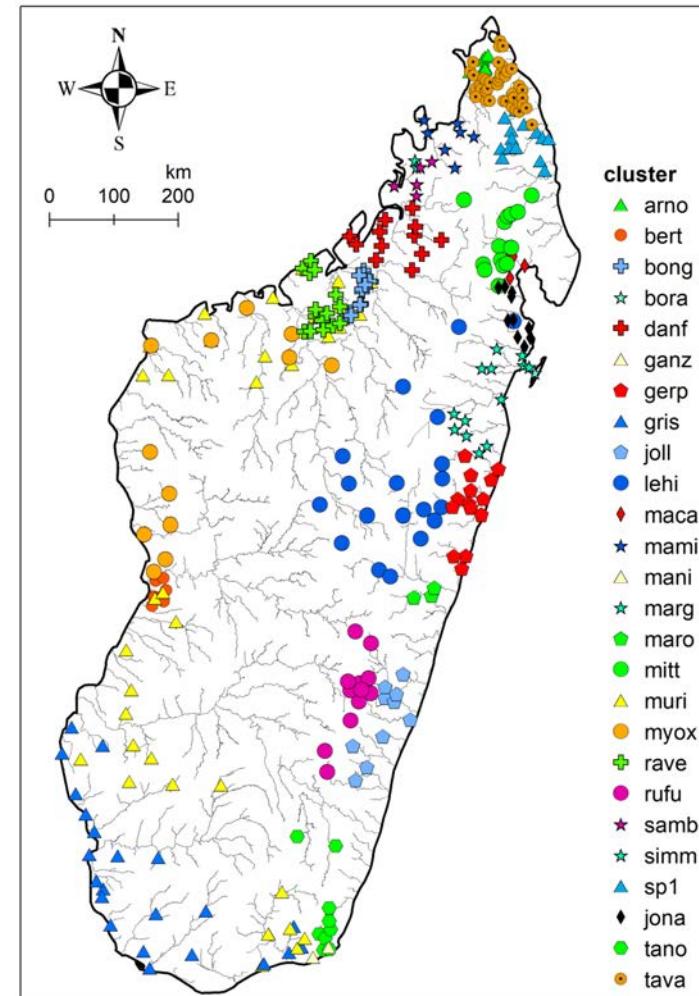
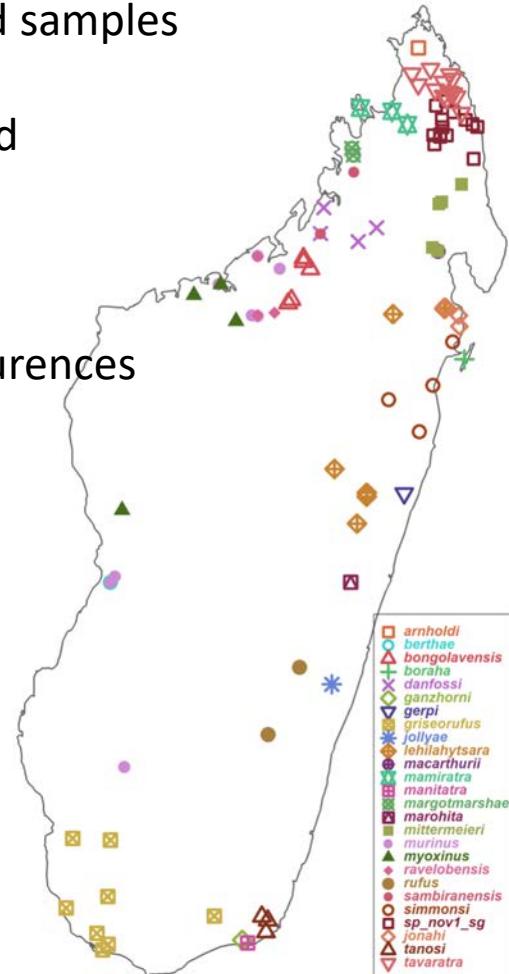
>400 RAD genotyped samples

>25 lineages included

>1 new lineages

>390 localities / occurrences

>3k morphometrics



## Three case studies:

1. Intraspecific divergence driven by natural climate change (**incipient?**)
2. Lineage diversification in close geographic proximity (**ongoing?**)
3. Reproductive isolation in sympatry (**complete?**)

# Story # 1

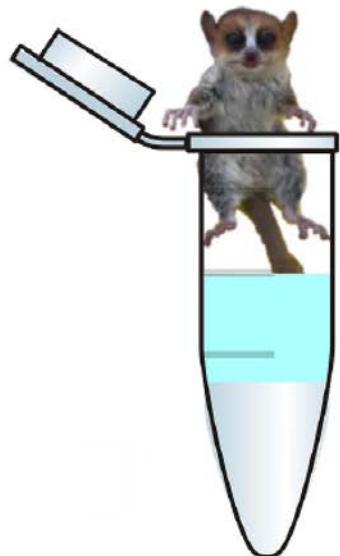


## Geogenetic patterns in mouse lemurs (genus *Microcebus*) reveal the ghosts of Madagascar's forests past

Anne D. Yoder<sup>a,b,1</sup>, C. Ryan Campbell<sup>a</sup>, Marina B. Blanco<sup>b</sup>, Mario dos Reis<sup>c</sup>, Jörg U. Ganzhorn<sup>d</sup>, Steven M. Goodman<sup>e,f</sup>, Kelsie E. Hunnicutt<sup>a</sup>, Peter A. Larsen<sup>a</sup>, Peter M. Kappeler<sup>g</sup>, Rodin M. Rasoloarison<sup>g,h</sup>, José M. Ralison<sup>h</sup>, David L. Swofford<sup>a</sup>, and David W. Weisrock<sup>i</sup>

<sup>a</sup>Department of Biology, Duke University, Durham, NC 27708; <sup>b</sup>Duke Lemur Center, Duke University, Durham, NC 27705; <sup>c</sup>School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, United Kingdom; <sup>d</sup>Tierökologie und Naturschutz, Universität Hamburg, 20146 Hamburg, Germany; <sup>e</sup>Field Museum of Natural History, Chicago, IL 60605; <sup>f</sup>Association Vahatra, BP 3972, Antananarivo 101, Madagascar; <sup>g</sup>Behavioral Ecology and Sociobiology Unit, German Primate Centre, 37077 Goettingen, Germany; <sup>h</sup>Département de Biologie Animale, Université d'Antananarivo, BP 906, Antananarivo 101, Madagascar; and <sup>i</sup>Department of Biology, University of Kentucky, Lexington, KY 40506

# Mouse lemur sampling (TCI grant)



5 field sites

29 mouse lemurs

7 dwarf lemurs

4 flights

4200Km by road





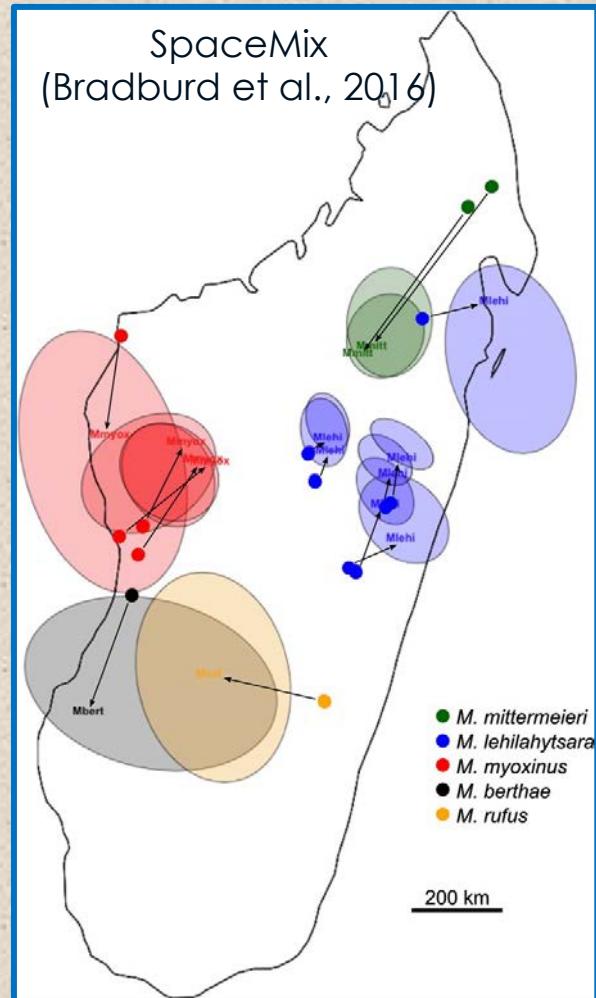


370 mi

Data SIO, NOAA, U.S. Navy, NGA, GEBCO  
Image Landsat

Google earth

“Geogenetic patterns” indicate that there were forest “bridges” across Madagascar that must have entirely disappeared by ~ 50K ybp

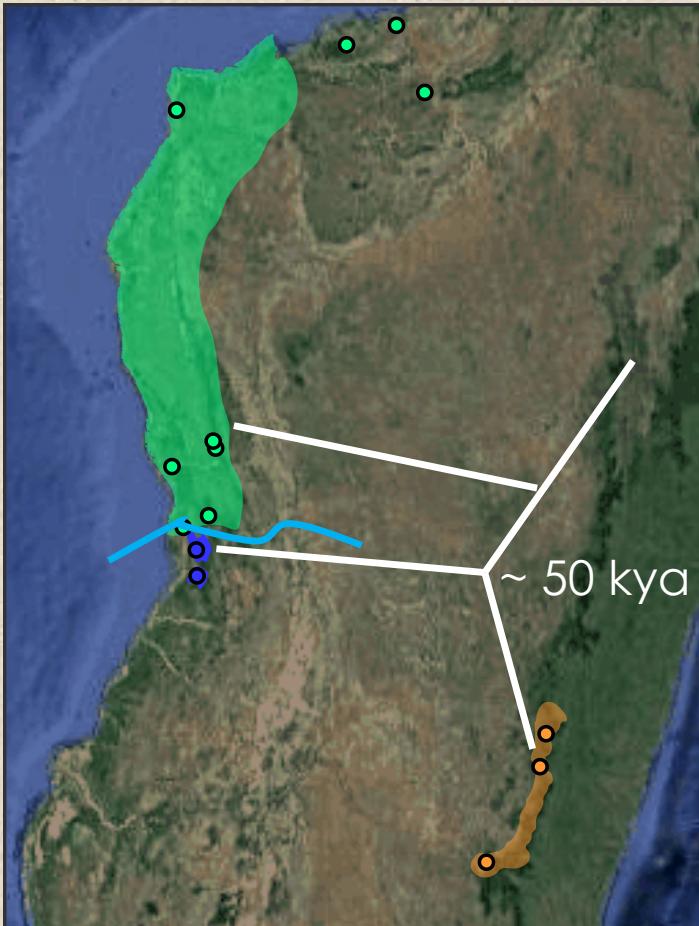




*Microcebus berthae*



*Microcebus rufus*



# *berthae-myoxinus-rufus*

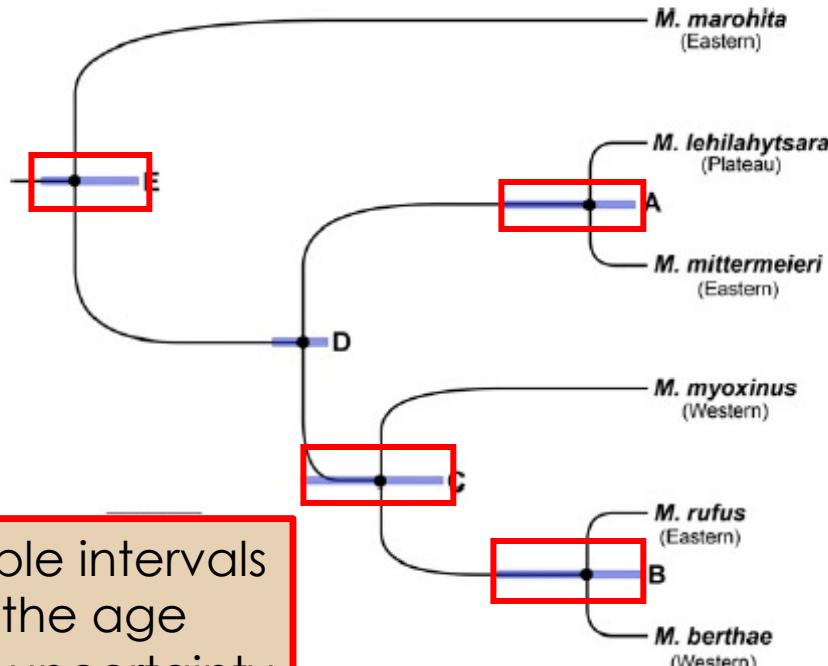


Adult Body  
Weight = **30**  
**grams!**





# Geogenetic patterns in mouse lemurs (genus *Microcebus*) reveal the ghosts of Madagascar's forests past



Wide credible intervals  
around the age  
estimates = uncertainty

## **Summary of Story 1:**

- Forest fragments that are believed to be anthropogenetic may actually be “museums” of genetic diversity
- The Central Plateau of Madagascar may have long been characterized by mixed savannah and woodlands
- Either way, there must have been forest corridors across the island as recently as 50 kya that no longer exist
- Rivers appear to be potent biogeographic barriers (at least, for mouse lemurs)

# Story # 2

*Syst. Biol.* 70(2):203–218, 2021

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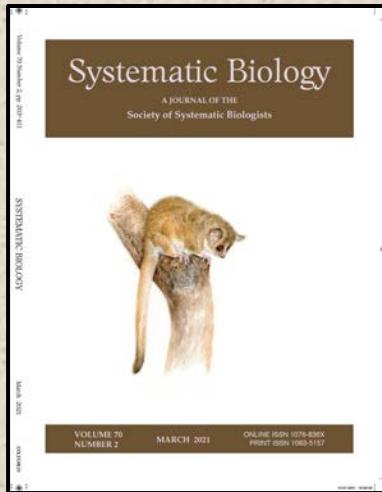
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DOI:10.1093/sysbio/syaa053

Advance Access publication July 8, 2020

## Cryptic Patterns of Speciation in Cryptic Primates: Microendemic Mouse Lemurs and the Multispecies Coalescent

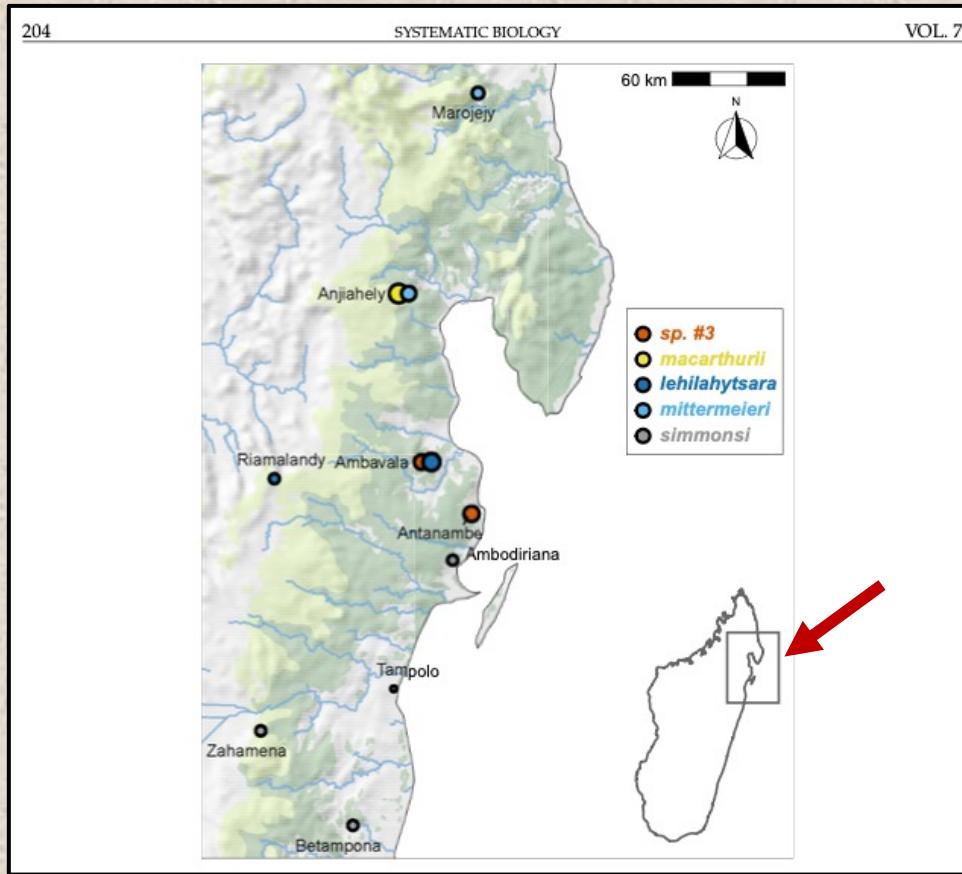
JELMER W. POELSTRA<sup>1</sup>, JORDI SALMONA<sup>2</sup>, GEORGE P. TILEY<sup>1</sup>, DOMINIK SCHÜSSLER<sup>3</sup>, MARINA B. BLANCO<sup>1,4</sup>, JEAN B. ANDRIAMBELOSON<sup>5</sup>, OLIVIER BOUCHEZ<sup>6</sup>, C. RYAN CAMPBELL<sup>1,7</sup>, PAUL D. ETTER<sup>8</sup>, PAUL A. HOHENLOHE<sup>9</sup>, KELSIE E. HUNNICKUTT<sup>1,10</sup>, AMAIA IRIBAR<sup>2</sup>, ERIC A. JOHNSON<sup>8</sup>, PETER M. KAPPELER<sup>11</sup>, PETER A. LARSEN<sup>1,12</sup>, SOPHIE MANZI<sup>2</sup>, JOSÉ M. RALISON<sup>5</sup>, BLANCHARD RANDRIANAMBININA<sup>13,14</sup>, RODIN M. RASOLOARISON<sup>11</sup>, DAVID W. RASOLOFOSON<sup>13</sup>, AMANDA R. STAHLKE<sup>9</sup>, DAVID W. WEISROCK<sup>15</sup>, RACHEL C. WILLIAMS<sup>1,4</sup>, LOUNÈS CHIKHI<sup>2,16</sup>, EDWARD E LOUIS JR.<sup>17</sup>, UTE RADESPIEL<sup>18,\*</sup>, AND ANNE D. YODER<sup>1,\*</sup>

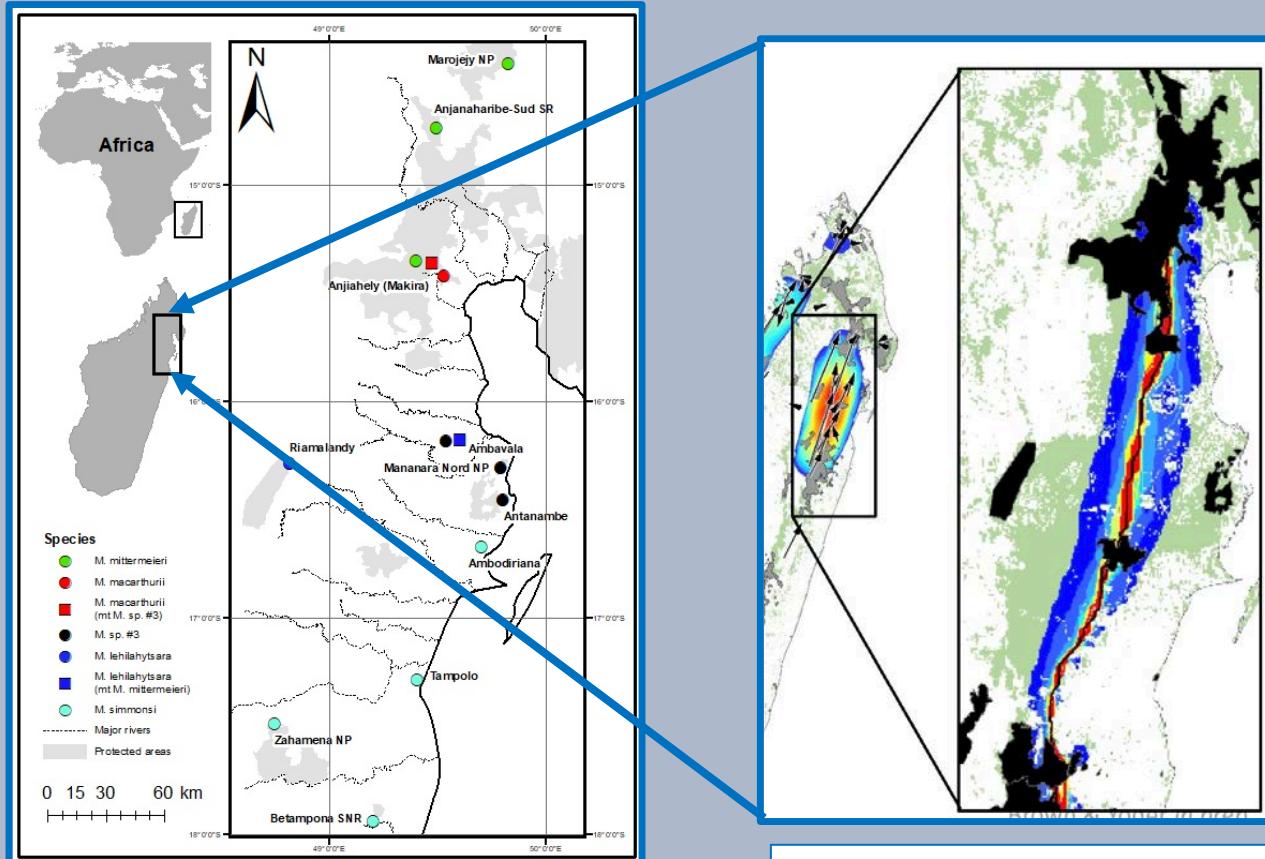


Jelmer Poelstra

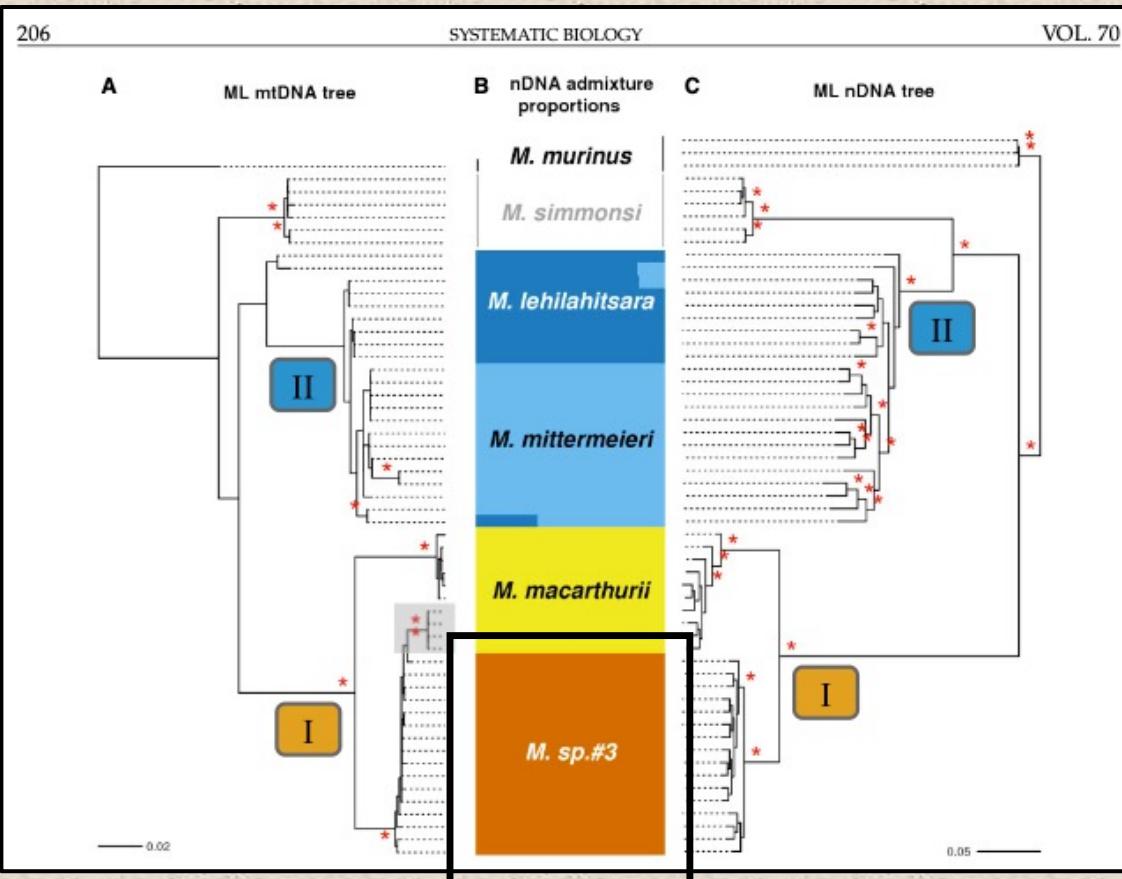
2021

Five metapopulation lineages appear to be independently evolving in small geographic area



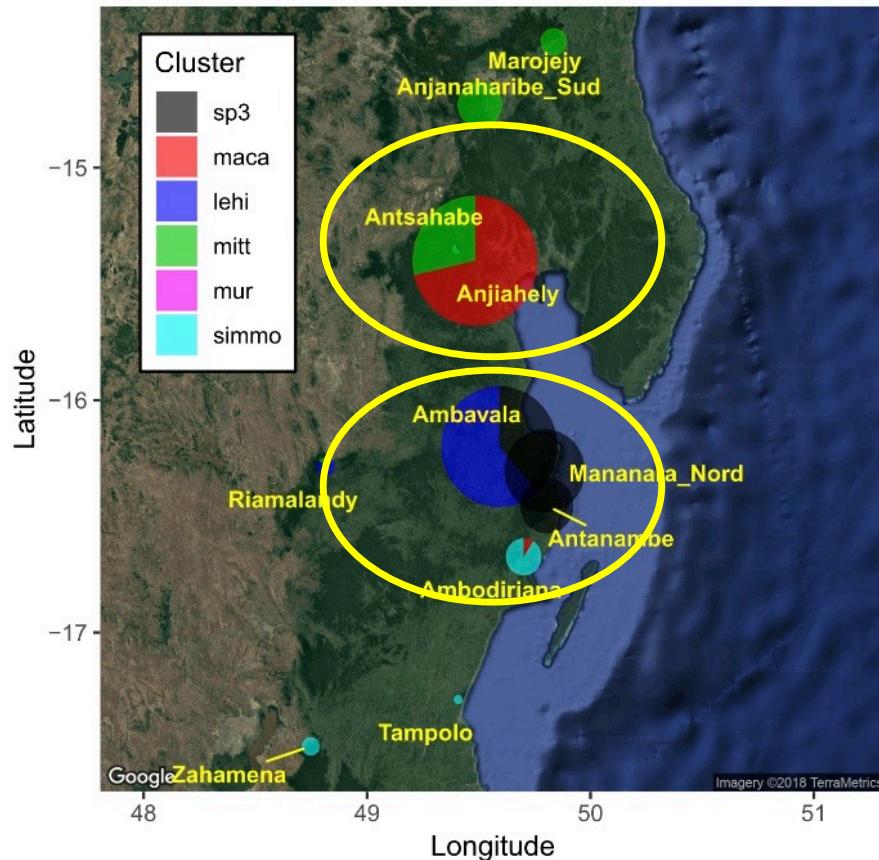


# Phylogenetic analysis of RADseq data revealed brand-new unnamed lineage



# Two areas of sympatric co-occurrence:

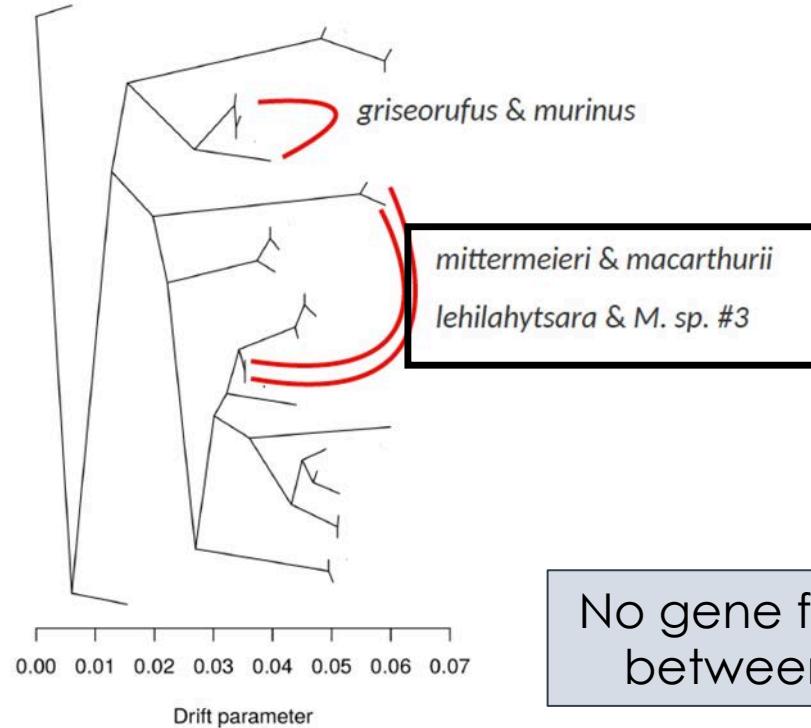
C) All 6 lineages K = 6



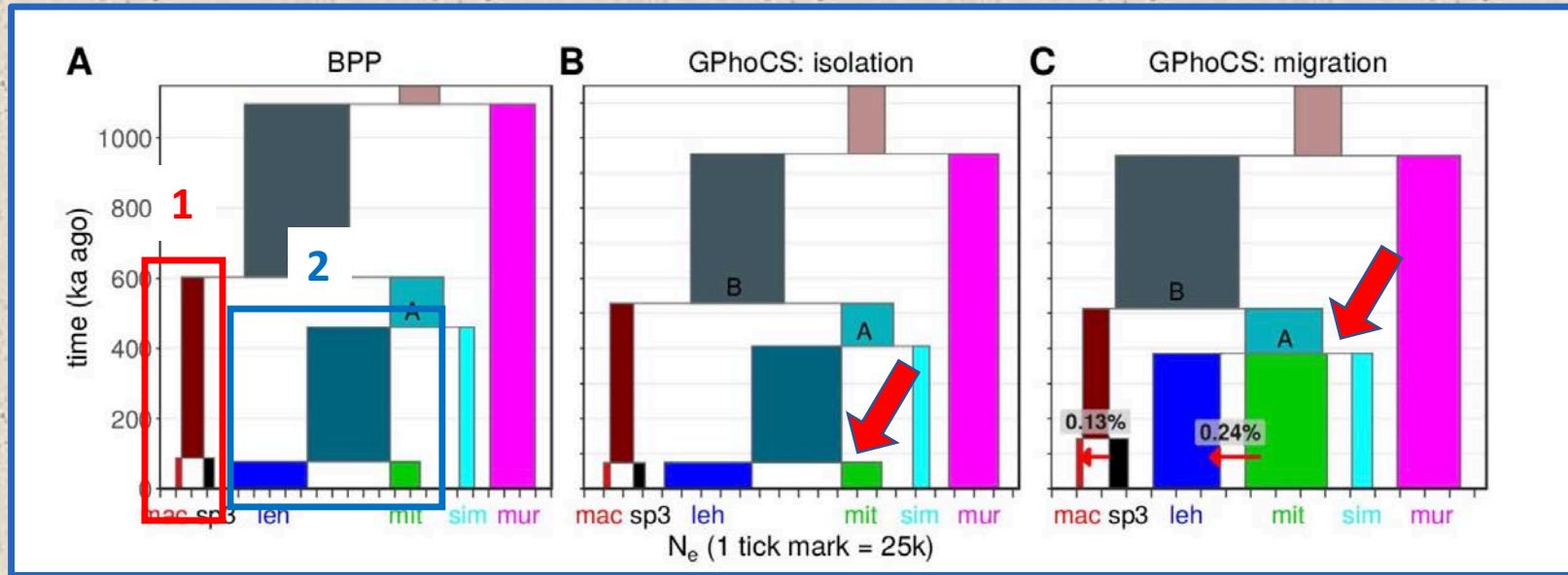
***M. mittermeieri***  
&  
***M. macarthuri***

***M. lehilahyisara***  
&  
***M. sp.nov3***

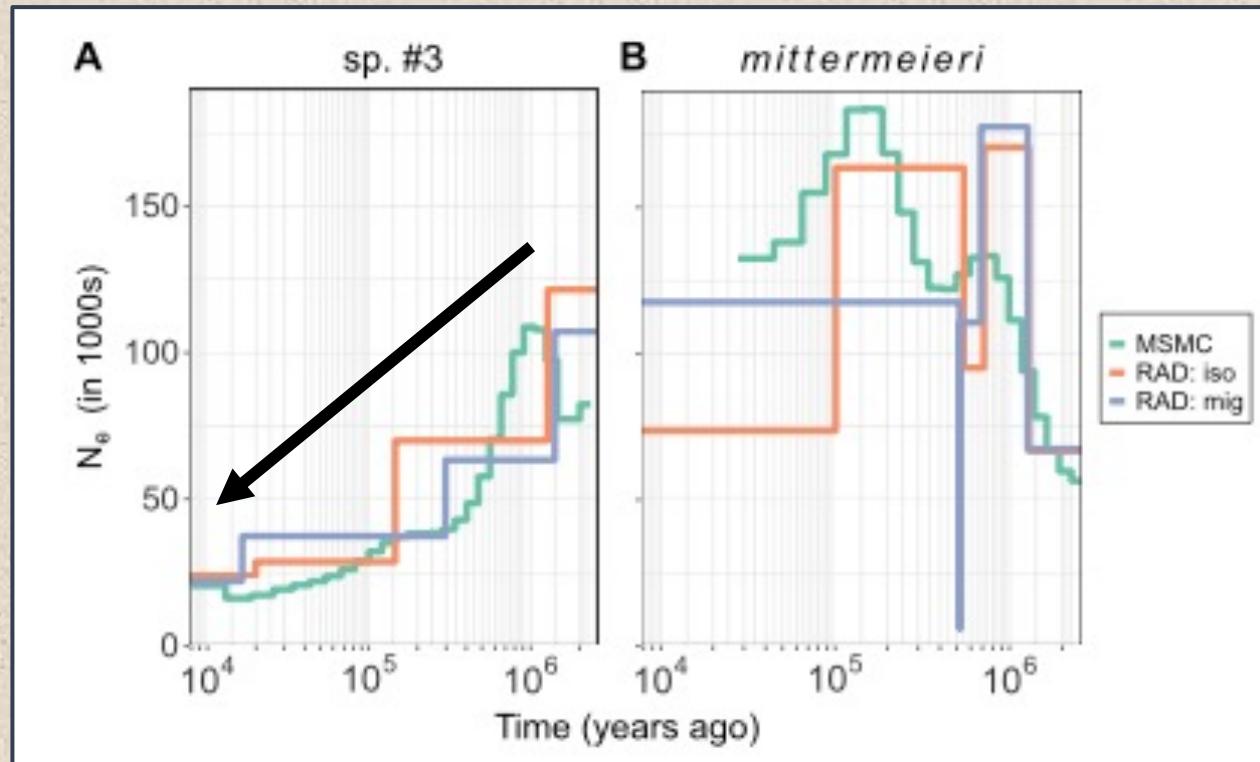
## Phylogenetic context of sympatric species pairs



**GPhoCS without migration yields very different divergence time for species pair #2 versus *with* migration  
(~100 ka ago versus 400 ka ago)**



# PSMC analysis reveals that new species (*M. jonahi*) is in steep decline



**AMERICAN JOURNAL OF PRIMATOLOGY**  
Official Journal of the American Society of Primatologists  
Volume 82 • Issue 8 • September 2020

Received: 5 April 2020 | Revised: 3 June 2020 | Accepted: 9 July 2020  
DOI: 10.1002/ajp.23180

**RESEARCH ARTICLE**

**Ecology and morphology of mouse lemurs (*Microcebus* spp.)  
a hotspot of microendemism in northeastern Madagascar,  
with the description of a new species**

Dominik Schüßler<sup>1</sup> | Marina B. Blanco<sup>2,3</sup> | Jordi Salmona<sup>4</sup> | Jelmer Poelstra<sup>3</sup>  
Jean B. Andriambeloson<sup>5</sup> | Alex Miller<sup>6</sup> | Blanchard Randrianambinina<sup>7,8</sup> |  
David W. Rasolofoson<sup>7</sup> | Jasmin Mantilla-Contreras<sup>1</sup> | Lounès Chikhi<sup>4,6</sup> |  
Edward E. Louis Jr.<sup>9</sup> | D. Yoder<sup>3</sup> | Ute Radespiel<sup>10</sup>

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**ACCEPTED MANUSCRIPT**  
**Cryptic Patterns of Speciation in Cryptic Primates: Microendemic Mouse Lemurs and the Multispecies Coalescent**  
Jelmer Poelstra, Jordi Salmona, George P Tiley, Dominik Schüßler, Marina B Blanco, Jean B Andriambeloson, Olivier Bouchez, C Ryan Campbell, Paul D Ettier, Paul A Hohenlohe ... Show more  
Systematic Biology, syaa053, <https://doi.org/10.1093/sysbio/syaa053>  
Received: 08 July 2020 Article history ▾

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A JOURNAL OF THE  
Society of Systematic Biologists

**ASP**

**Species**  
● *M. mittermeieri*  
● *M. macarthurii*  
● *M. sp. #3*  
● *M. lehilahy*  
● *M. boraha*  
● *M. simo*  
● *M. gerpi*  
■ Protected areas  
— Major rivers  
— Forest  
— Non-forest  
0 15 30 60 km

- 27 authors
- Six countries
- Three continents
- RADseq (120,000 loci per individual)
- Many years of field effort
- Thousands of hours of CPU time

## **Summary of Story 2:**

- Have discovered a new microendemic species of acute conservation concern
- We discovered two areas of microendemism where two lineages co-occur without gene flow
- Divergence time estimates suggest that reproductive isolation (RI) can evolve rapidly ... < 700 kya in this case

# Story #3

bioRxiv preprint doi: <https://doi.org/10.1101/2021.08.12.455854>; this version posted August 13, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1           RADseq data reveal a lack of admixture  
2               in a mouse lemur contact zone  
3               contrary to previous microsatellite results  
4

5           Jelmer Poelstra<sup>1,2\*</sup>, B. Karina Montero<sup>3</sup>, Jan Lüdemann<sup>3</sup>, Ziheng Yang<sup>4</sup>, S. Jacques

6           Rakotondranary<sup>3,5</sup>, Paul Hohenlohe<sup>6</sup>, Nadine Stetter<sup>3,7</sup>, Jörg U. Ganzhorn<sup>3,8</sup>, Anne D. Yoder<sup>1,8</sup>



Jelmer Poelstra

## Hybridization between mouse lemurs in an ecological transition zone in southern Madagascar

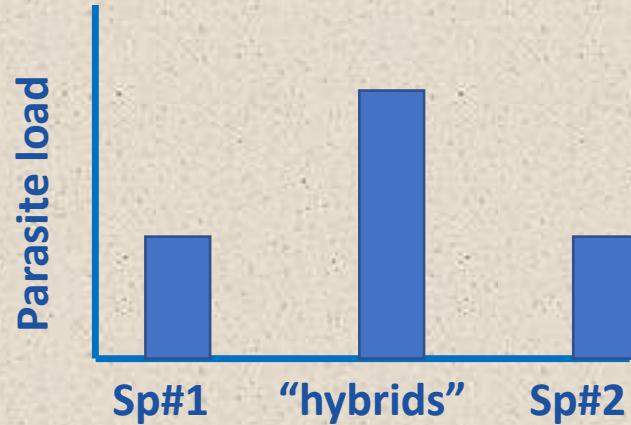
M. GLIGOR,\* J. U. GANZHORN,† D. RAKOTONDRAVONY,‡ O. R. RAMILIJAONA,‡  
E. RAZAFIMAHATRATA,† H. ZISCHLER\* and A. HAPKE\*

Gligor et al. 2009, Mol Ecol

## Hybridization of mouse lemurs: different patterns under different ecological conditions

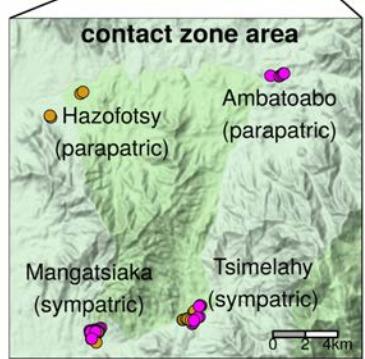
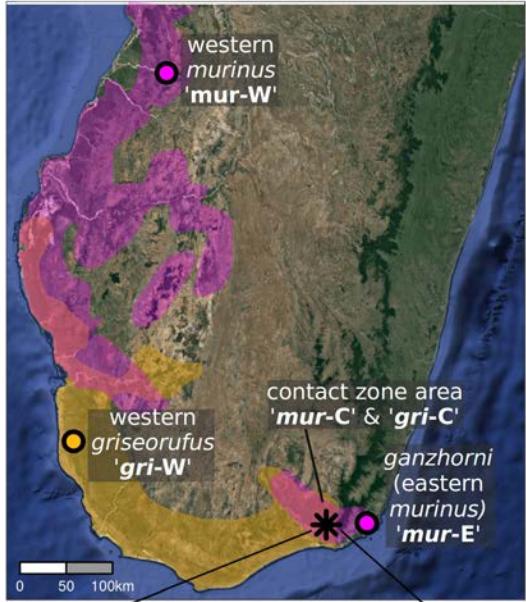
Andreas Hapke<sup>1\*</sup>, Mark Gligor<sup>1,2</sup>, S Jacques Rakotondranary<sup>3</sup>, David Rosenkranz<sup>1</sup> and Oliver Zupke<sup>1,4</sup>

Hapke et al. 2011, BMC Ev Biol



Jörg Ganzhorn



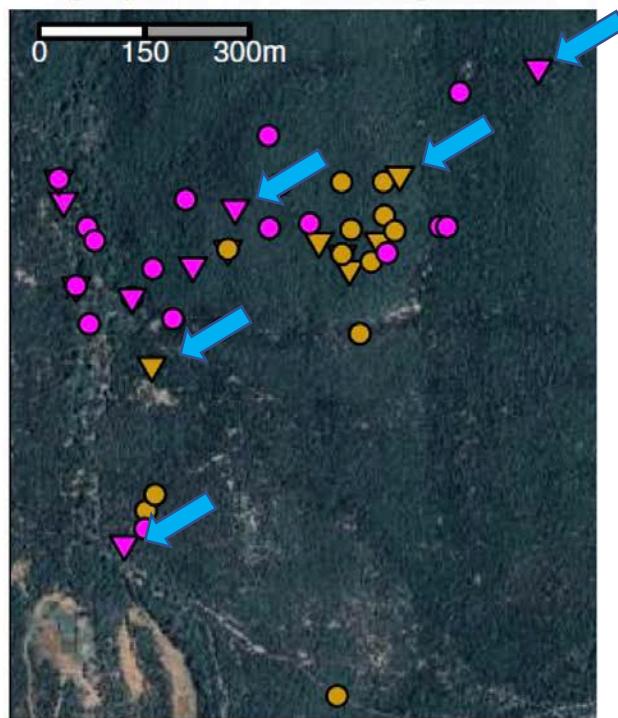


- 80 individuals from two contact zone sites
- 20 individuals from nearby micro-allopatric sites
- 38 individuals from distant allopatric sites
- 3 outgroup individuals (*M. rufus*)

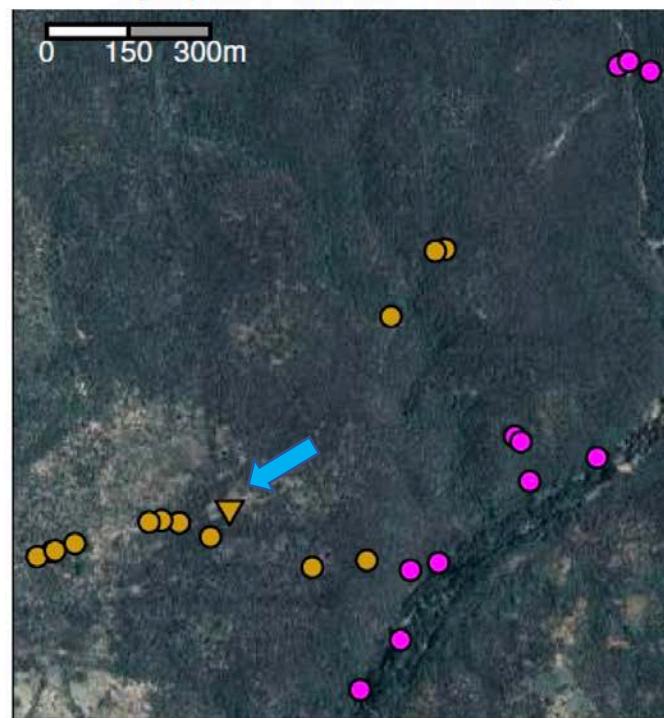
## Site 1: syntopic overlap

## Site 2: “micro-allopatry”

C sympatric site 1: Mangatsiaka



sympatric site 2: Tsimelahy



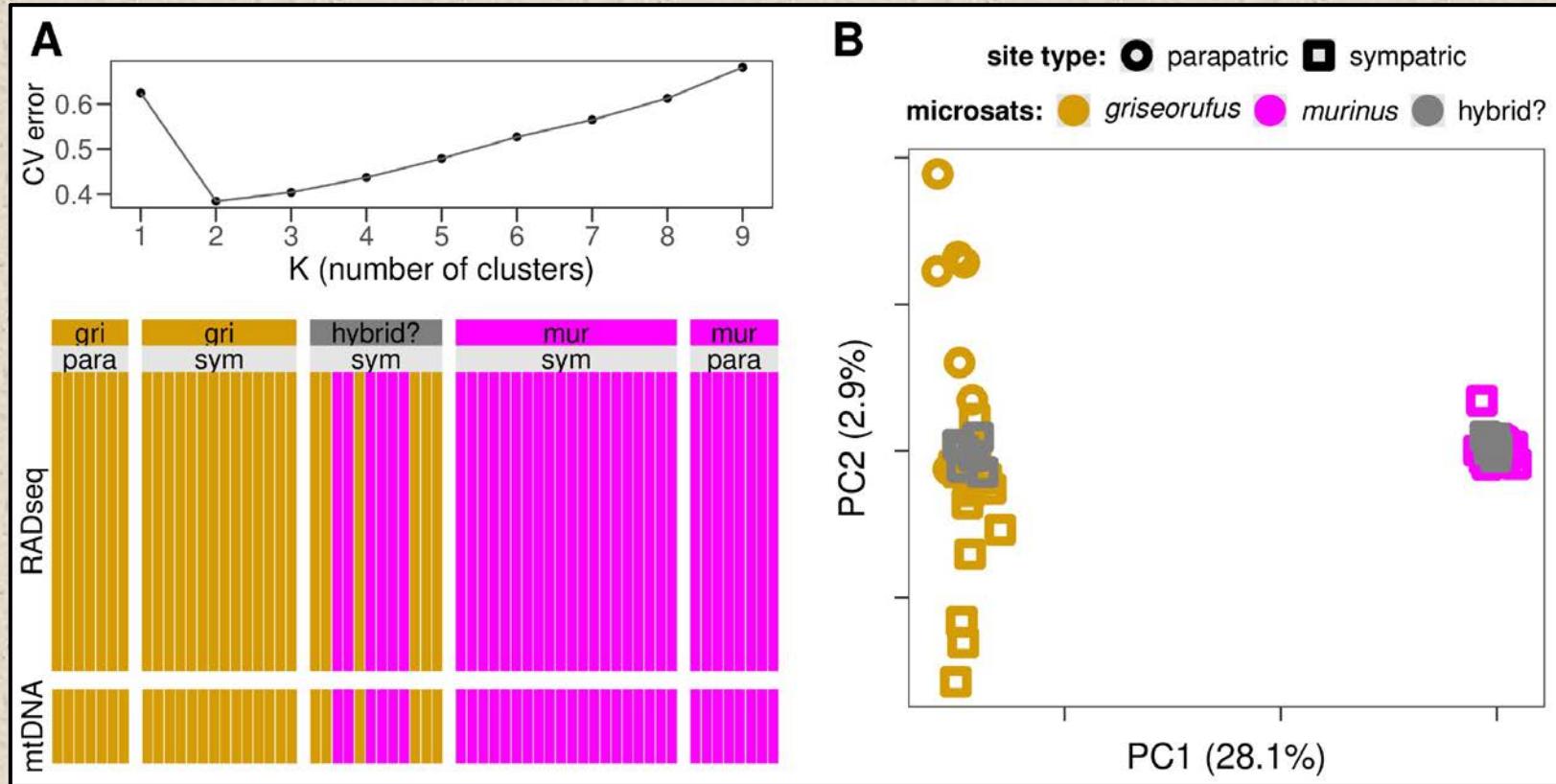
**microsat  
assignment:**

▽ hybrid  
○ non-hybrid

**RADseq  
assignment:**

● *griseorufus*  
● *murinus*

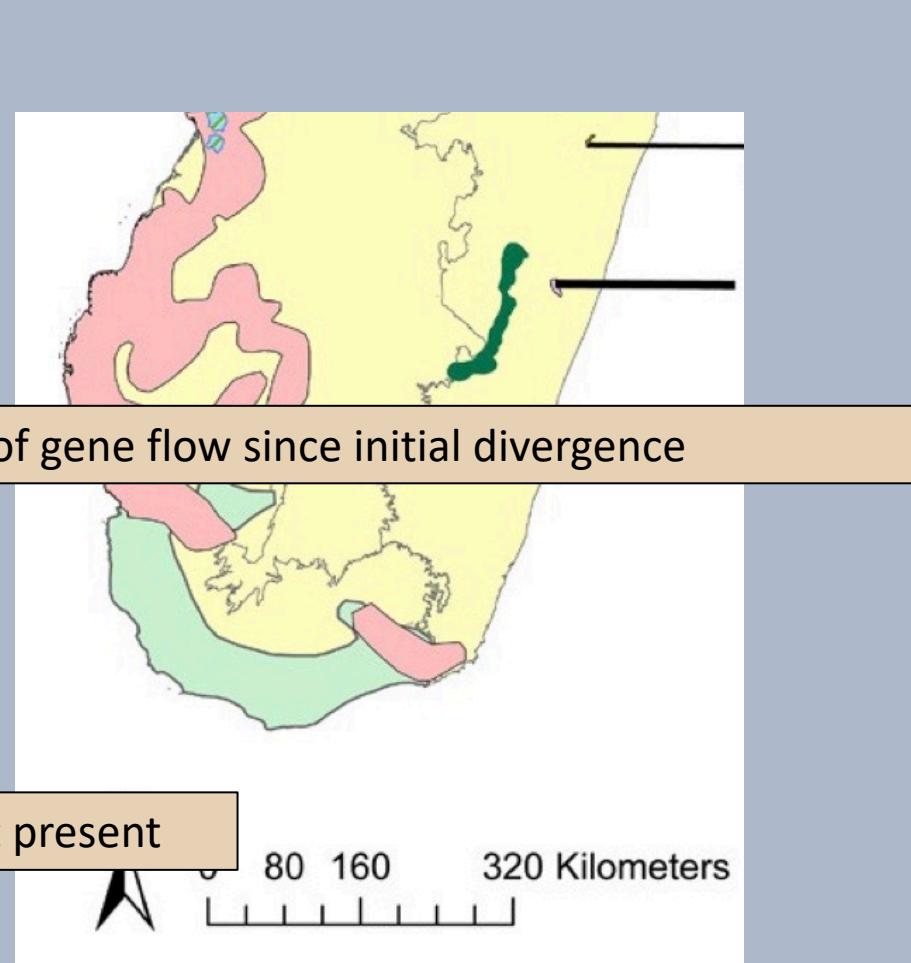
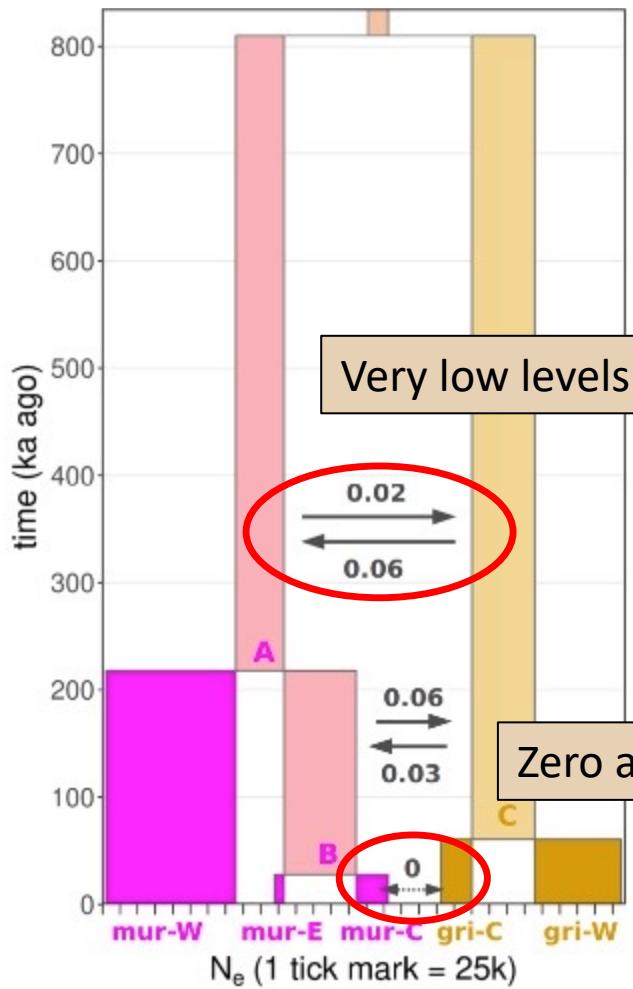
# Both ADMIXTURE and PCA indicate that putative hybrids are pure parental species



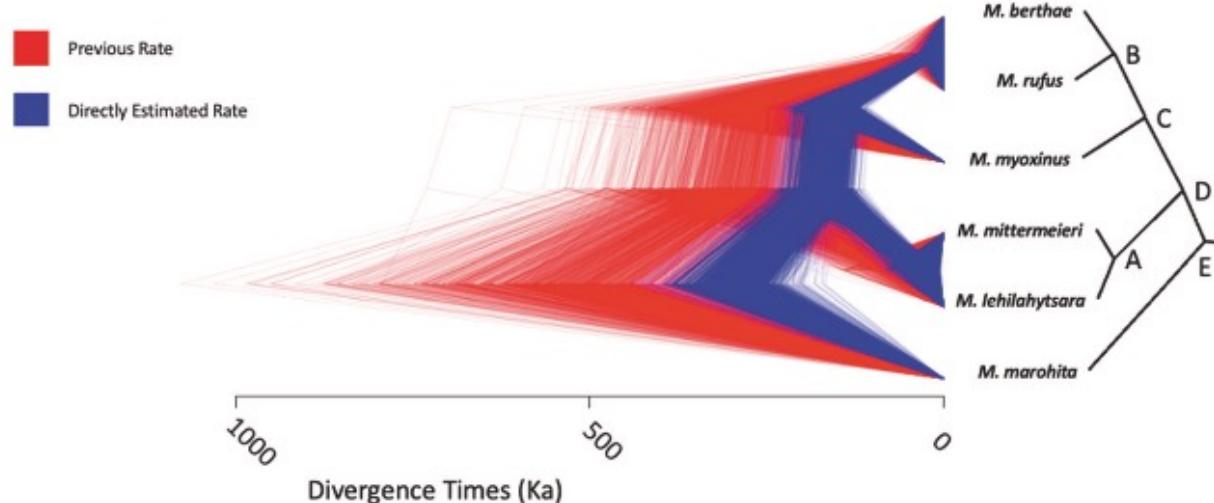


Slide courtesy of Jörg Ganzhorn

# GPhoCS (with migration)



Using directly estimated mutation rate has a big impact on estimated divergence times ...



**Fig. 6 Estimated divergence times among mouse lemur species.** Trees are posterior samples from BPP based on a fixed previously published topology. The directly estimated mutation rate (blue) is nearly twice as high as the previously assumed rate (red). Divergence times estimated with the new mutation rate are nearly half of the previous estimates. Summary statistics are given in Table S4, matched by node labels (A–E).

**Complete RI in < 800,000 ybp!**

## **Summary of Story 3:**

- Microsatellite data can be misleading
- Genome-scale data and MSC analysis have provided new and contradictory insights
- Two species that diverged less than 1 mya ago appear to have achieved complete reproductive isolation (RI)
- We have no idea what are the mechanisms driving and maintaining RI

# **Follow-up study: Intraspecific divergence driven by natural climate change? (Goodman's mouse lemur, *M. lehilahytsara*)**



**George Tiley**

1   **Running Title: Demographic history of mouse lemurs in Madagascar's Central Highlands**

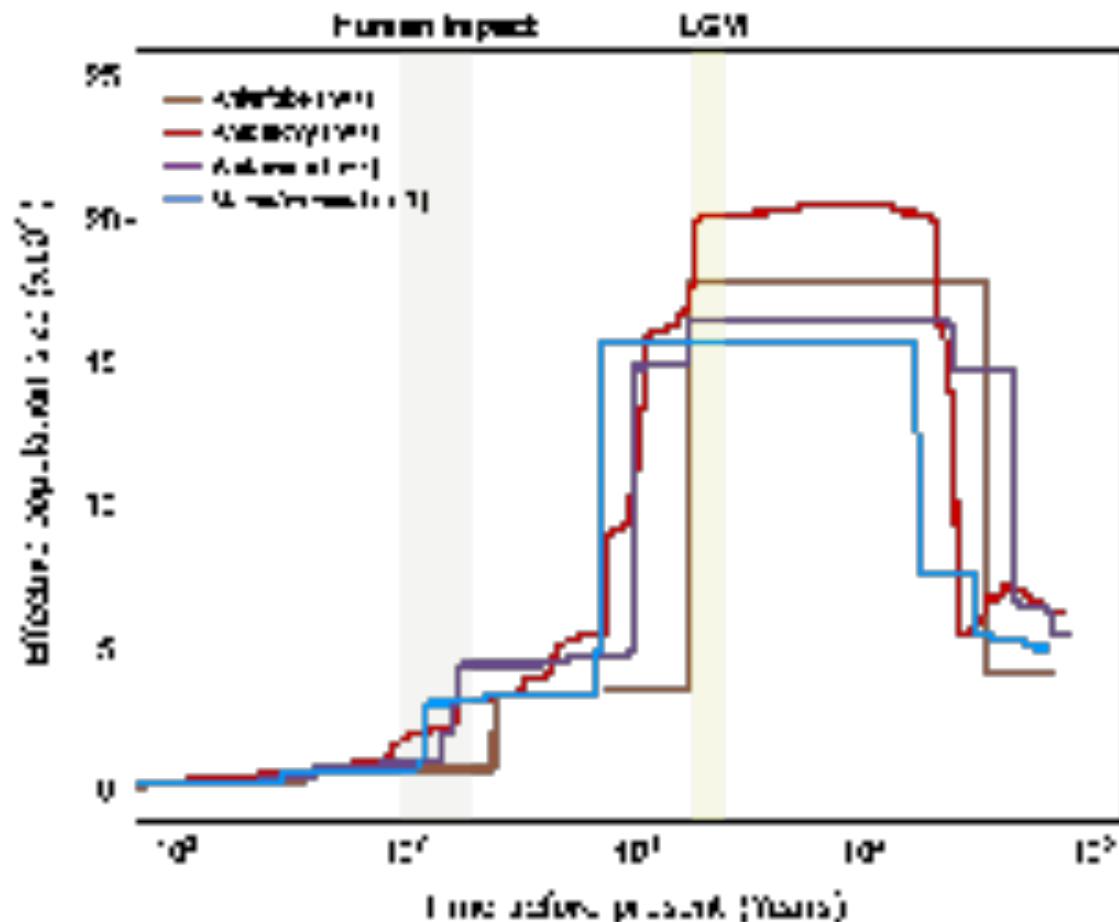
2

3   **Population genomic structure in Goodman's mouse lemur reveals long-standing  
4    separation of Madagascar's Central Highlands and eastern rainforests**

5

6   George P. Tiley<sup>1,†,‡</sup>, Tobias van Elst<sup>2,†</sup>, Helena Teixeira<sup>2</sup>, Dominik Schüßler<sup>3</sup>, Jordi Salmona<sup>4</sup>,  
7   Marina B. Blanco<sup>5</sup>, José M. Ralison<sup>6</sup>, Blanchard Randrianambinina<sup>7,8</sup>, Rodin M. Rasoloarison<sup>6,7</sup>,  
8   Amanda R. Stahlke<sup>10</sup>, Paul A. Hohenlohe<sup>10</sup>, Lounès Chikhi<sup>4,11</sup>, Edward E Louis<sup>12</sup>, Ute  
9    Radespiel<sup>2</sup>, Anne D. Yoder<sup>1</sup>

10



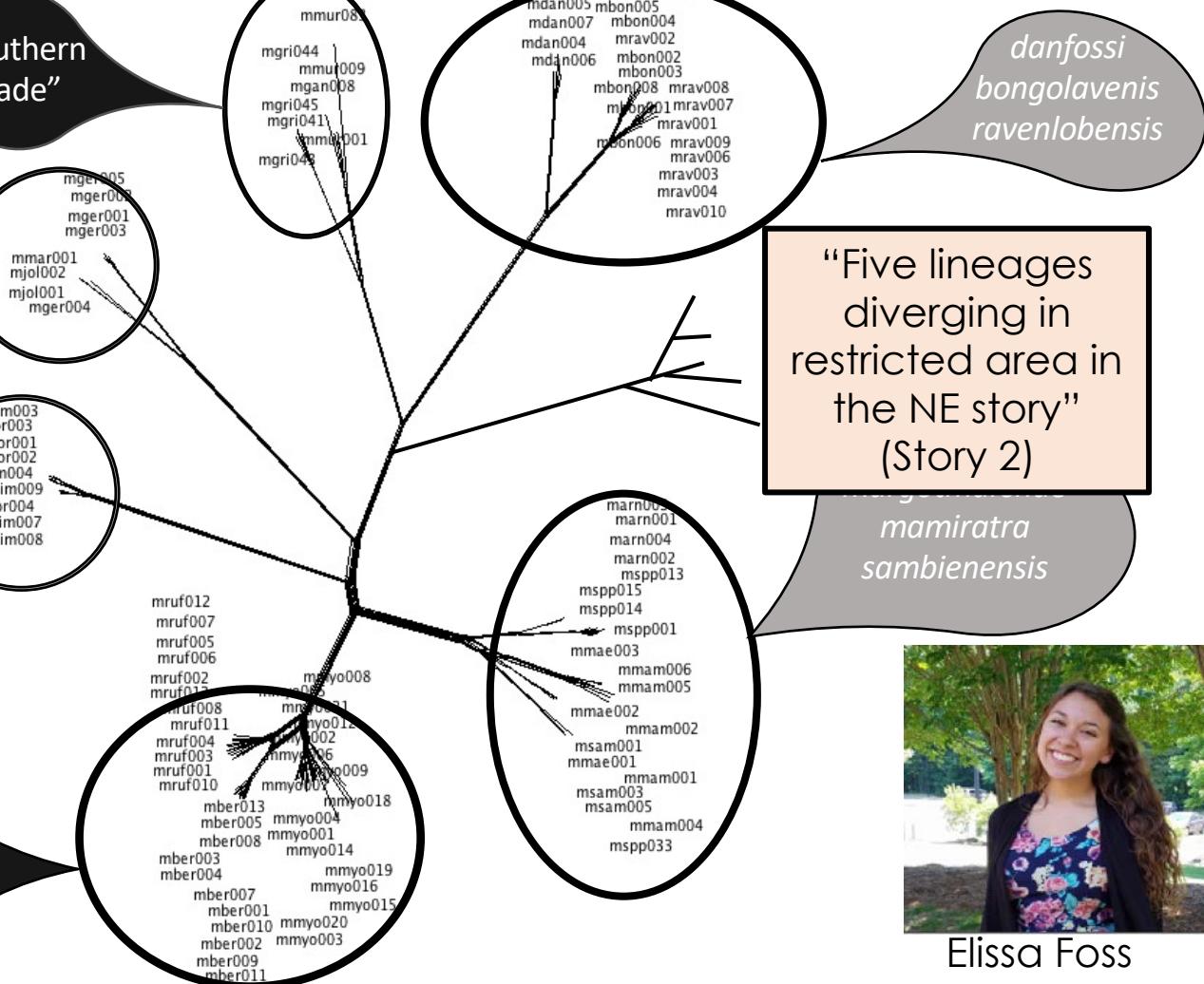
“Sympatric RI  
from the SE story”  
(Story 3)

“Southern  
Clade”

*jollyae*  
*marohita*  
*gerpi*

“Sister species  
separated by  
entire central  
plateau story”  
(Story 1)

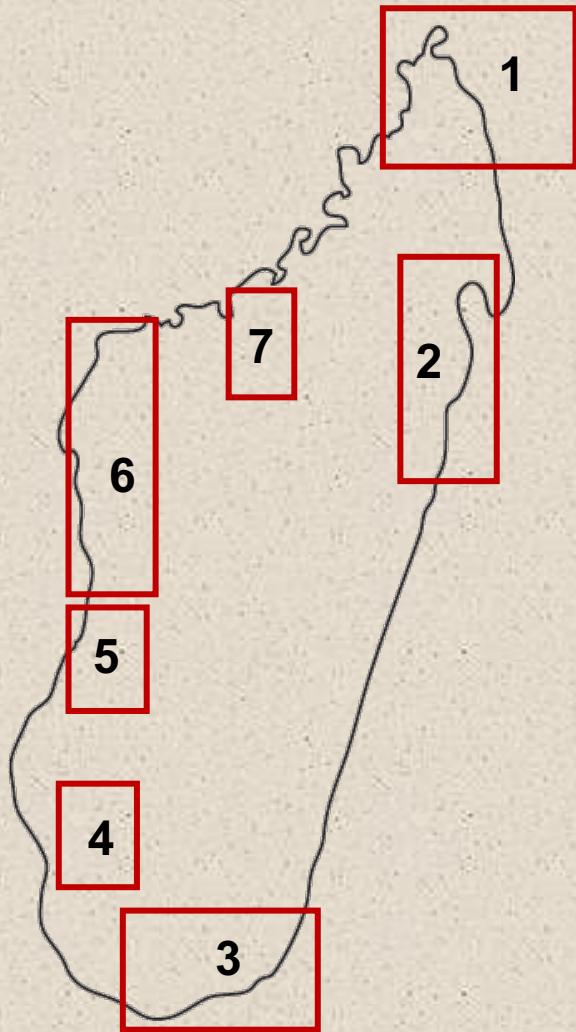
*berthae*  
*myoxinus*  
*rufus*

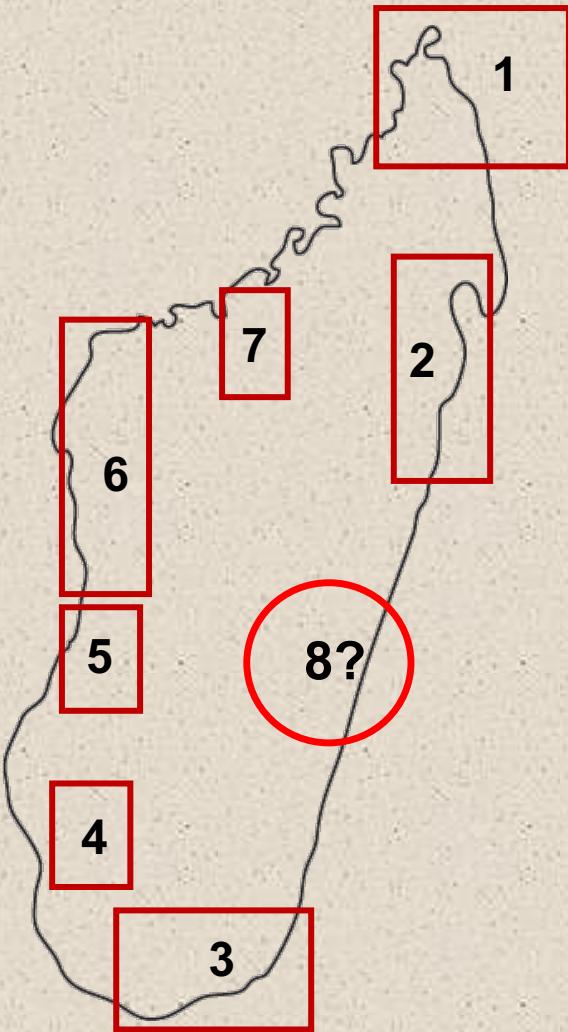


Elissa Foss

## Known areas of sympatric overlap between named species of mouse lemur.

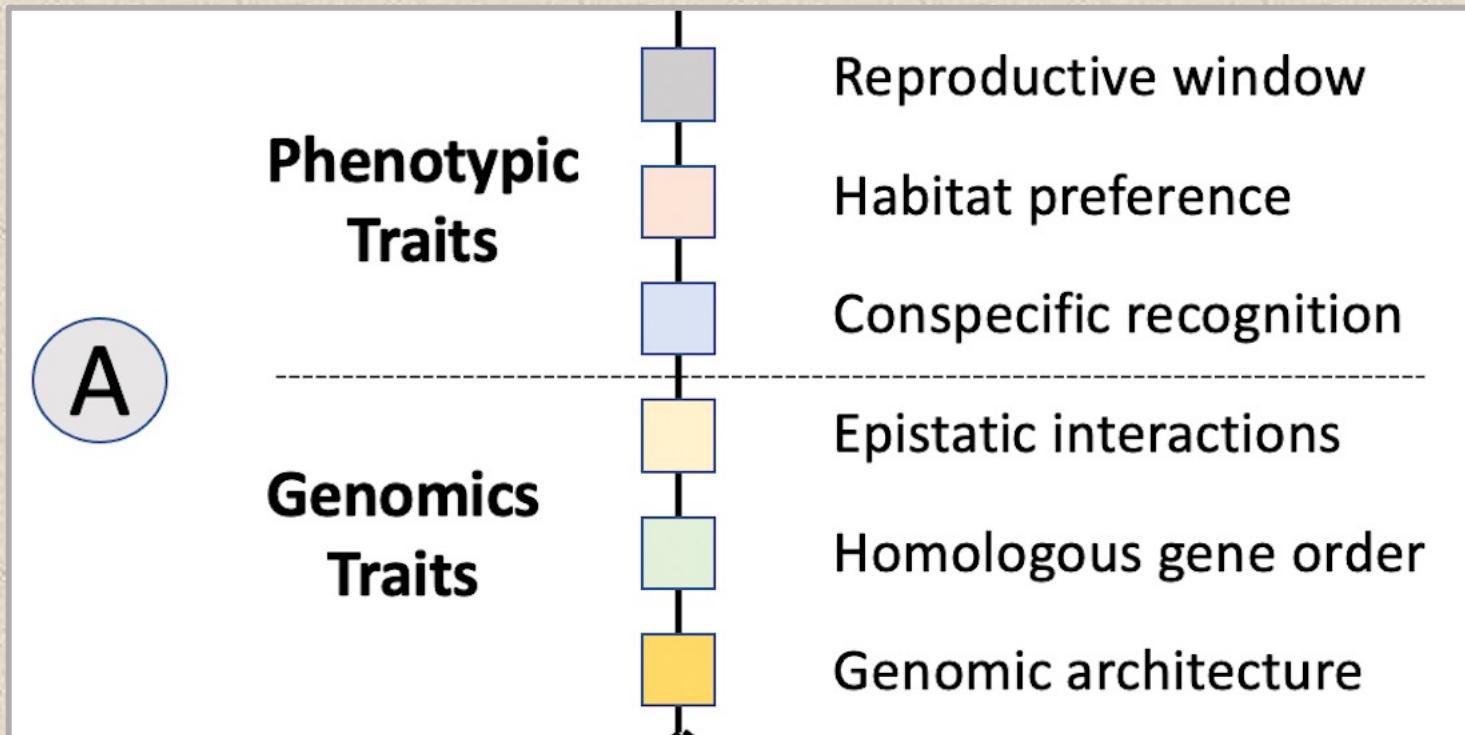
- 1) *M. margotmarshae* and *M. sambariensis*; *M. mamilatra* and *M. arholdi*
- 2) *M. jonahi* and *M. lehilahytsara*; *M. macarthurii* and *M. mittermeieri*
- 3) *M. murinus*, *M. griseorufus*, *M. manitatra*, *M. ganzhorni*, *M. tanosi*
- 4) *M. murinus* and *M. griseorufus*
- 5) *M. murinus* and *M. berthae*
- 6) *M. murinus* and *M. myoxinus*
- 7) *M. murinus* and *M. ravelobensis*



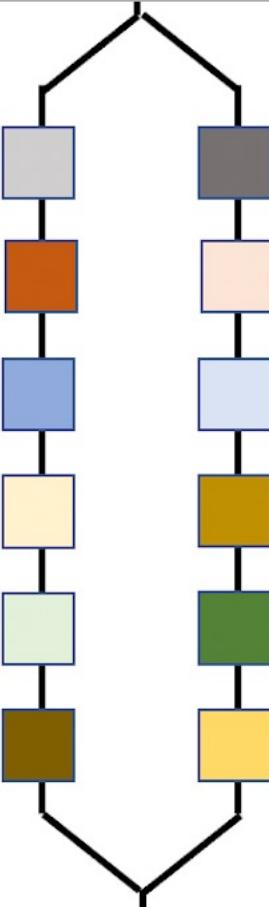


# Future Directions

# Ancestral Lineage

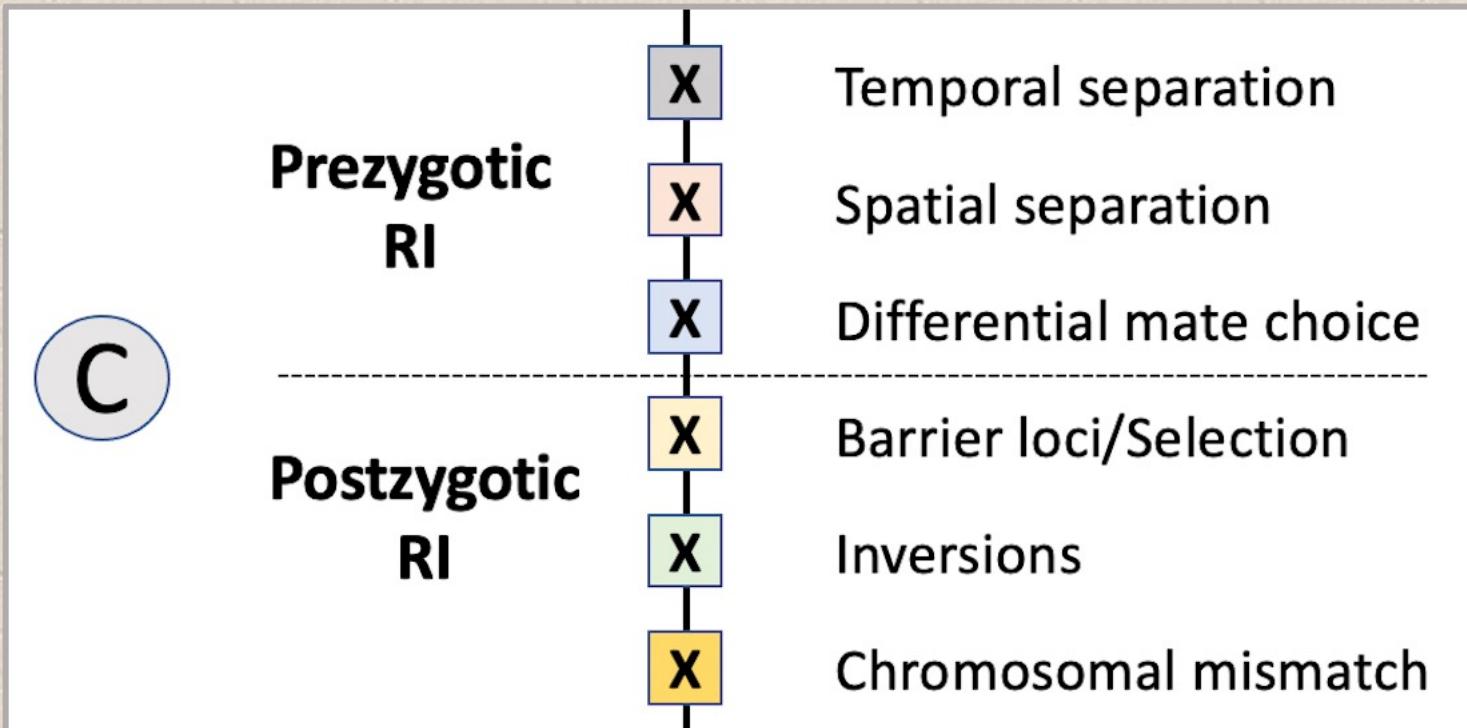


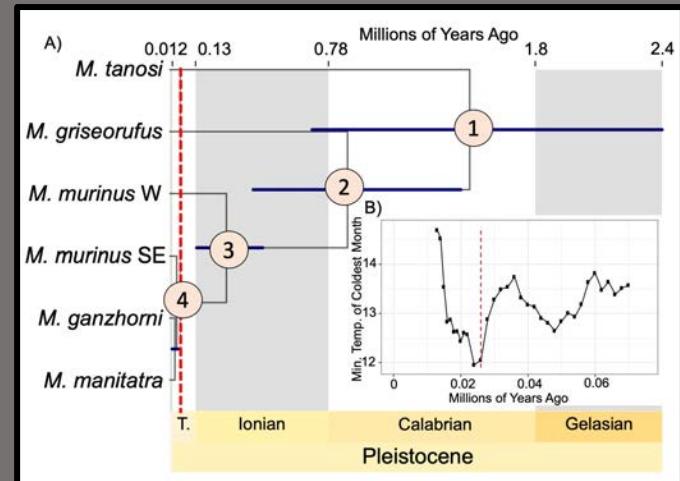
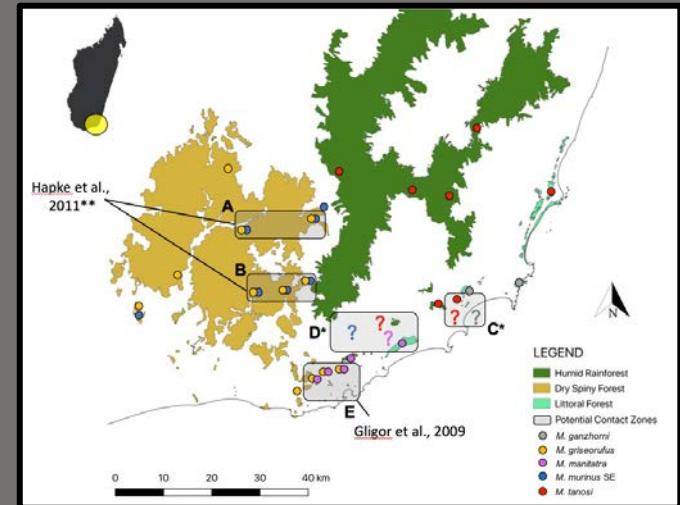
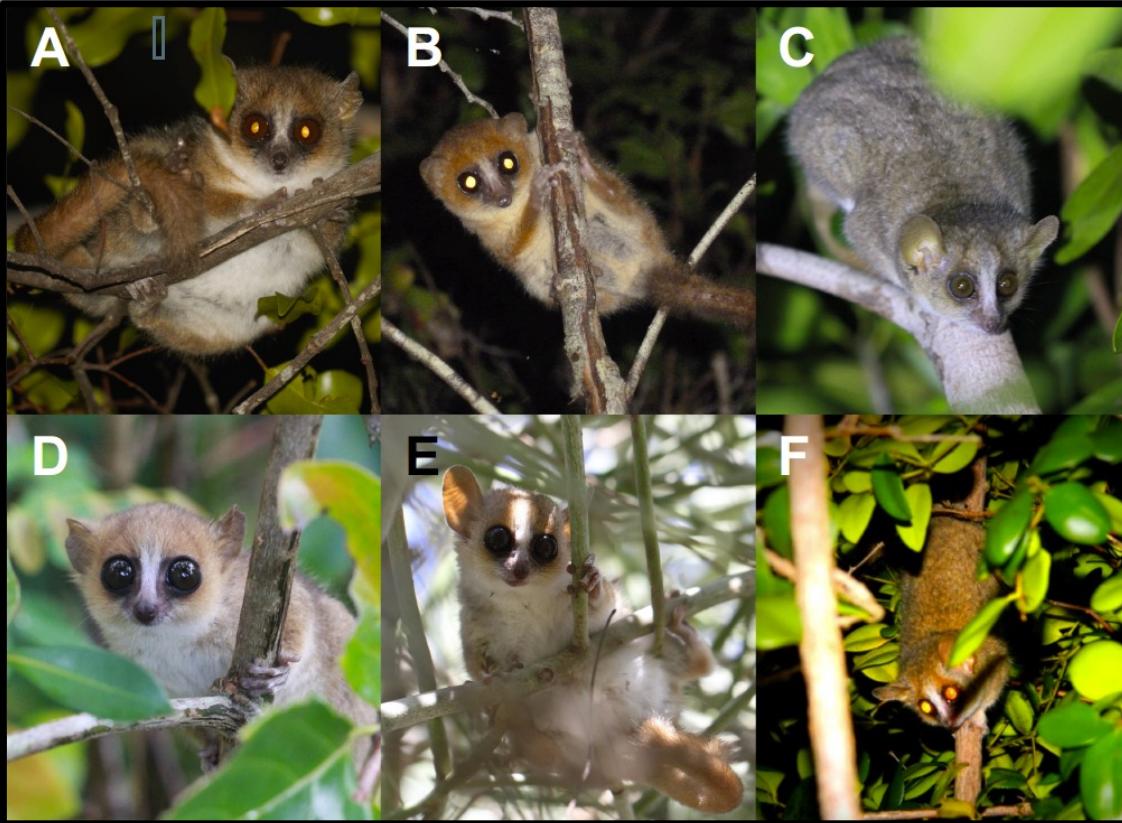
B

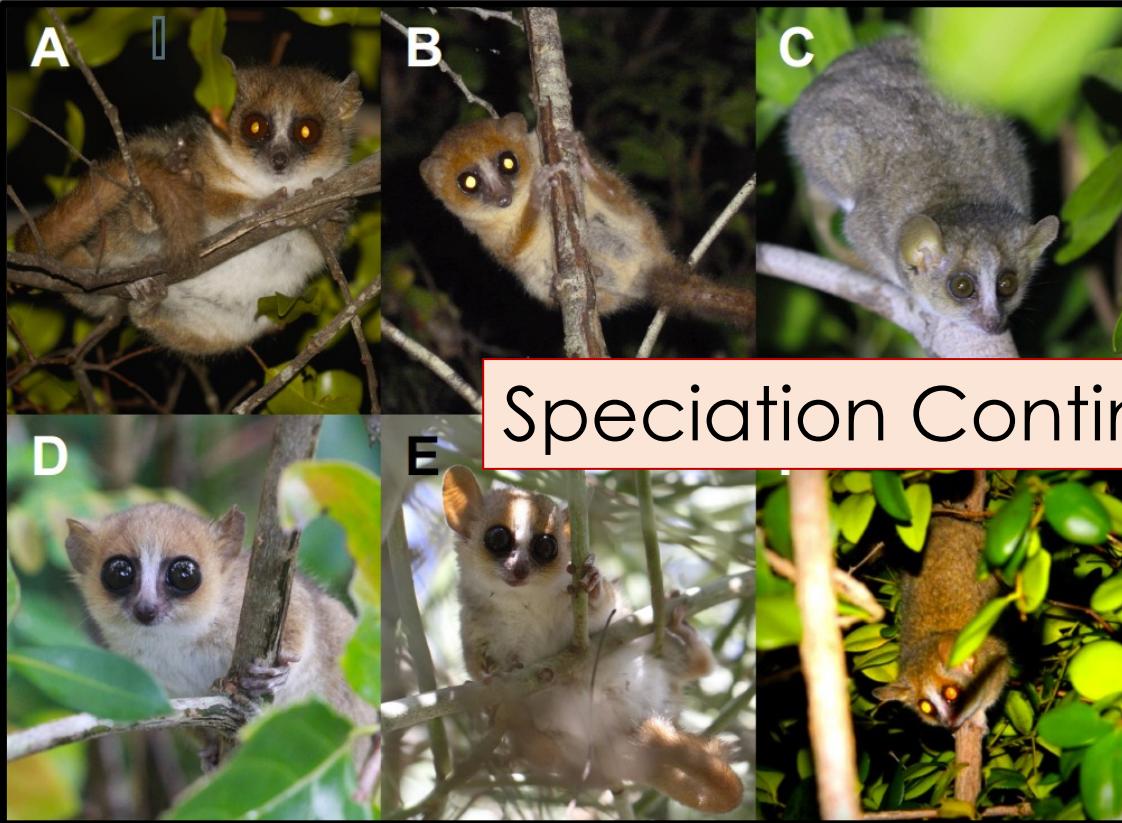


**Geographic  
Separation**

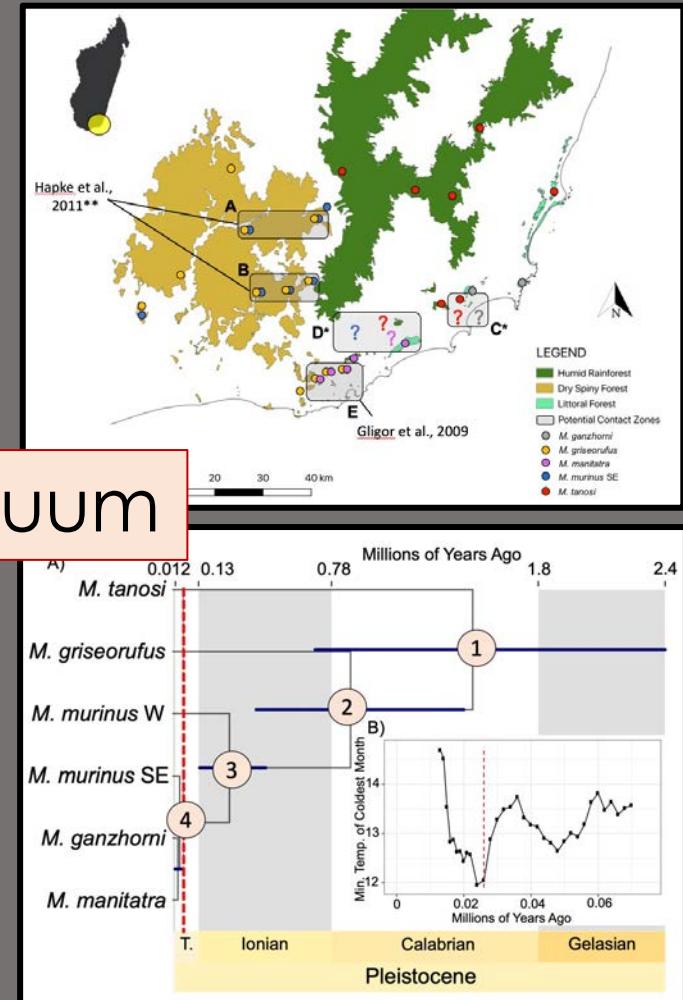
# Secondary Contact



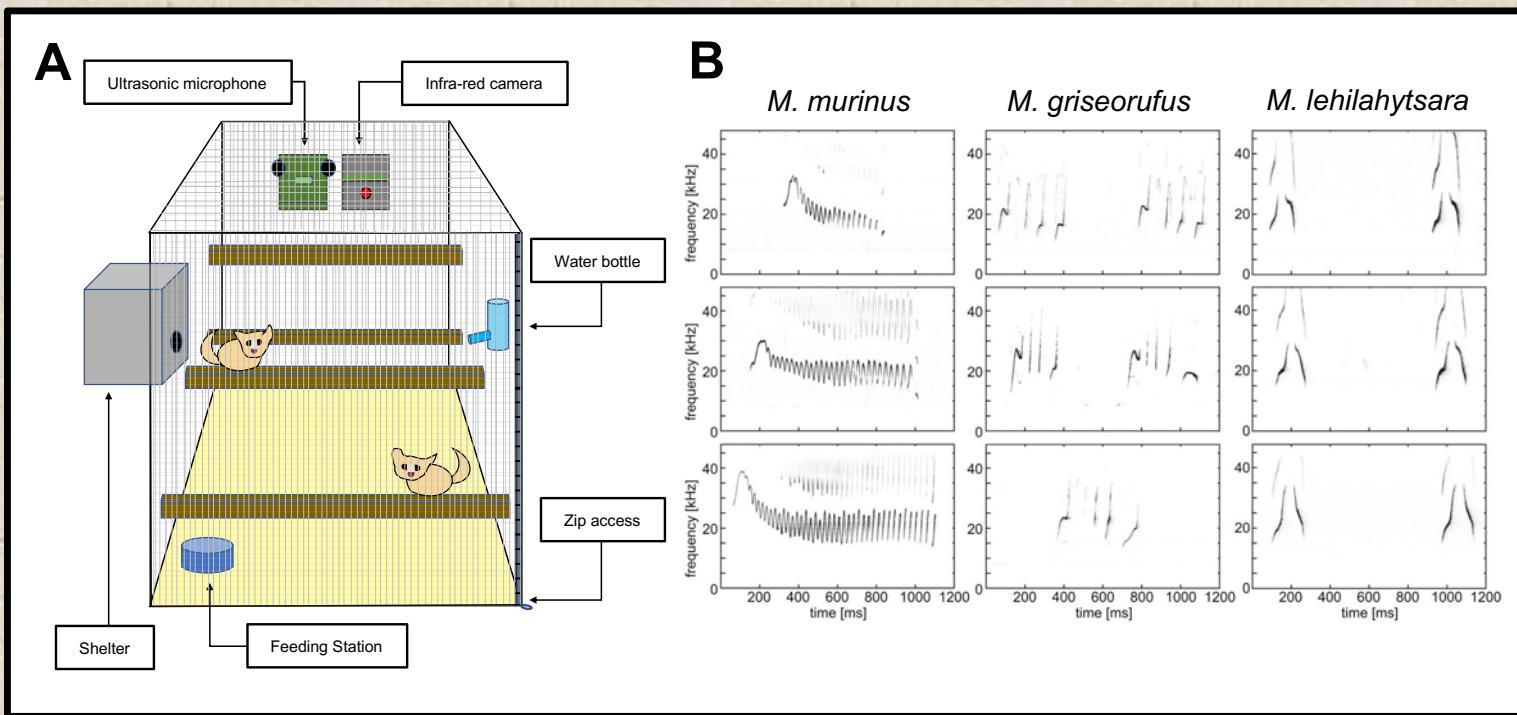




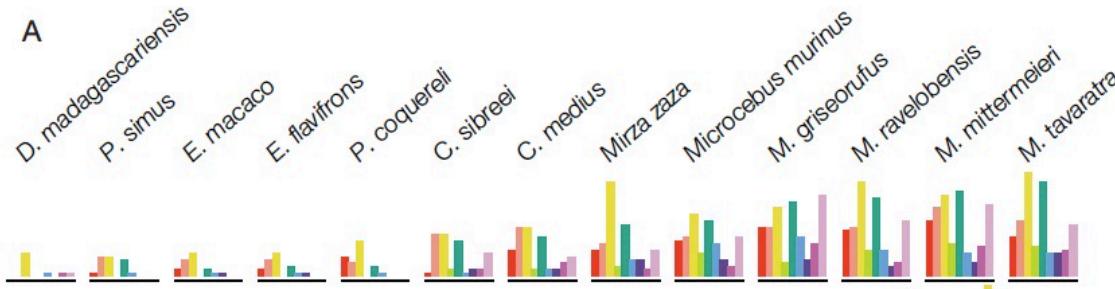
## Speciation Continuum



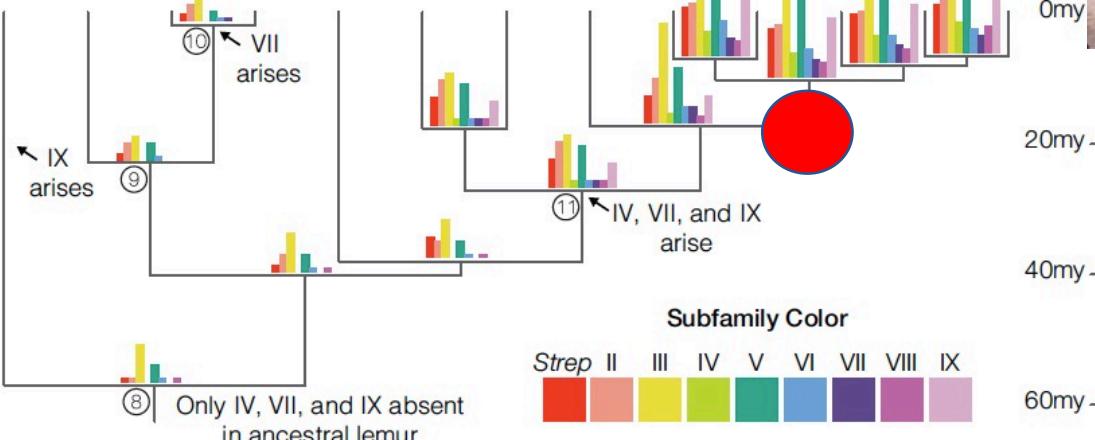
# Auditory Communication



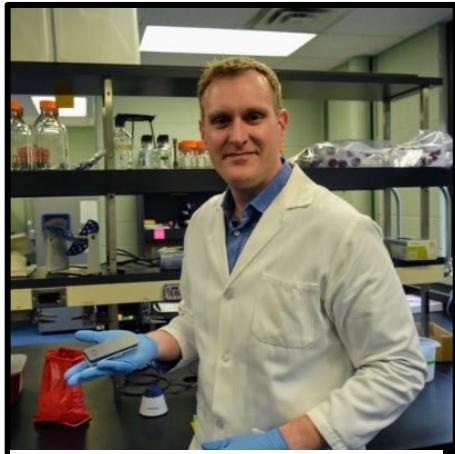
# Olfactory Communication



GPCR  
Pheromone  
receptors



Jmol

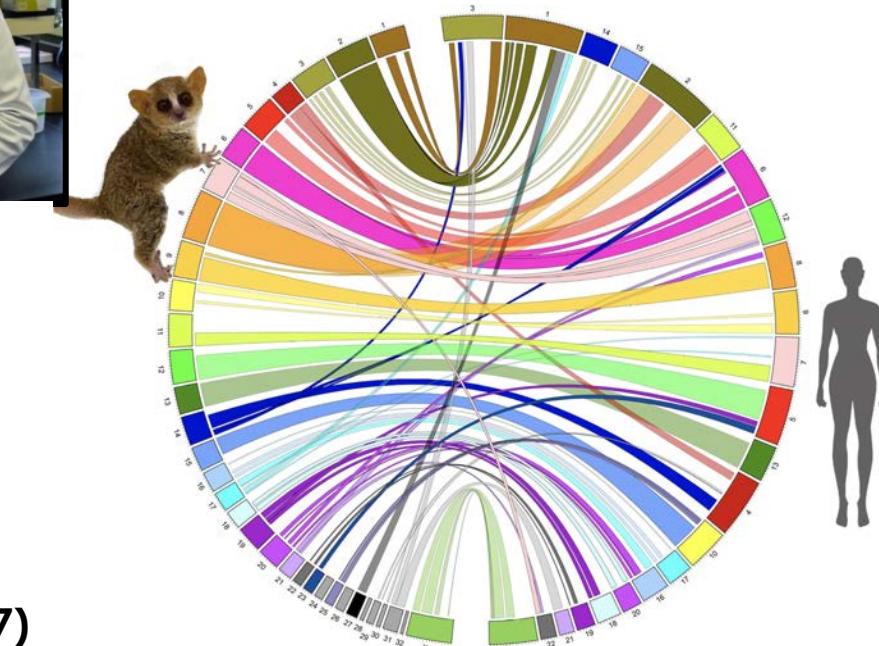


Peter Larsen

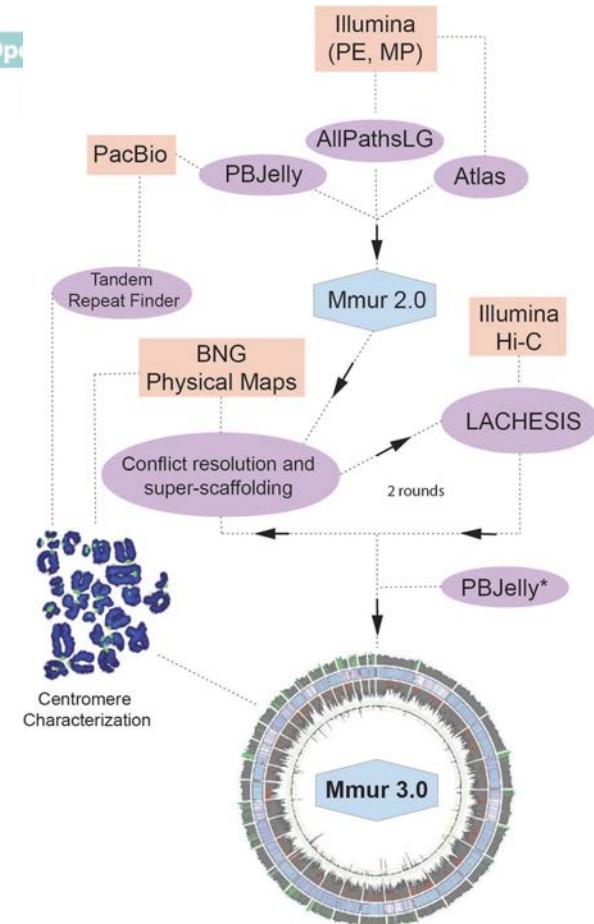
METHODOLOGY ARTICLE

Open

## Hybrid de novo genome assembly and centromere characterization of the gray mouse lemur (*Microcebus murinus*)

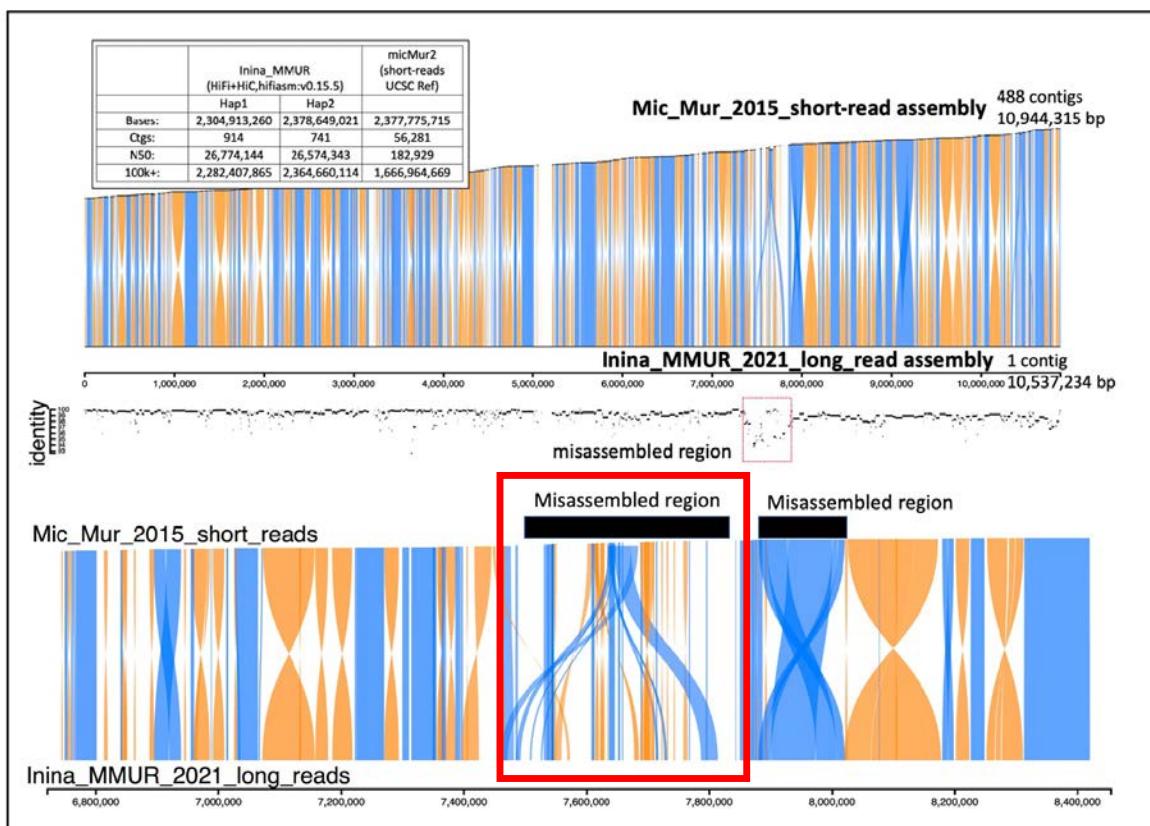


Larsen et al. (2017)

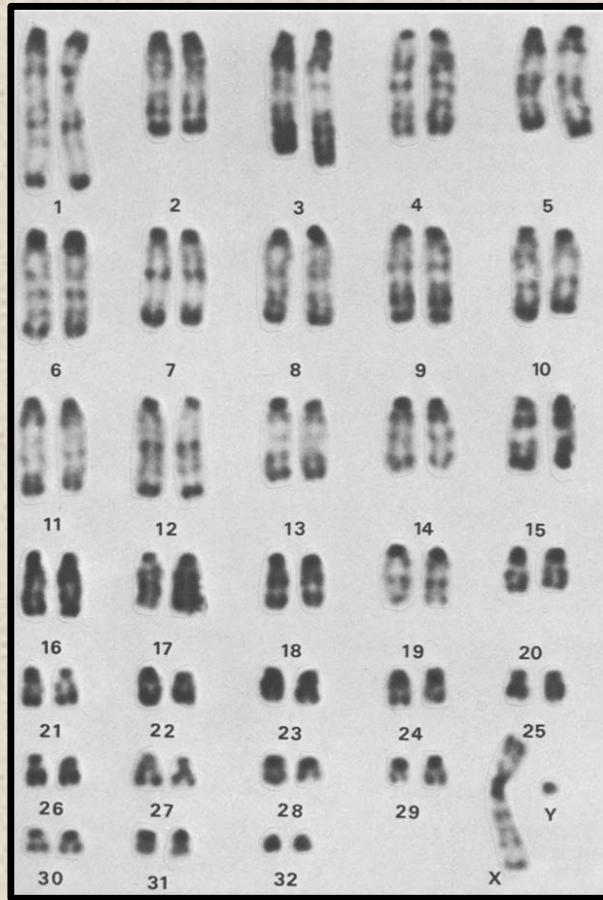




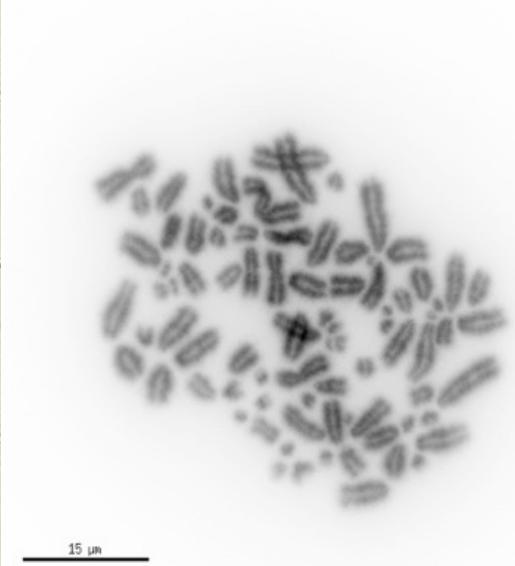
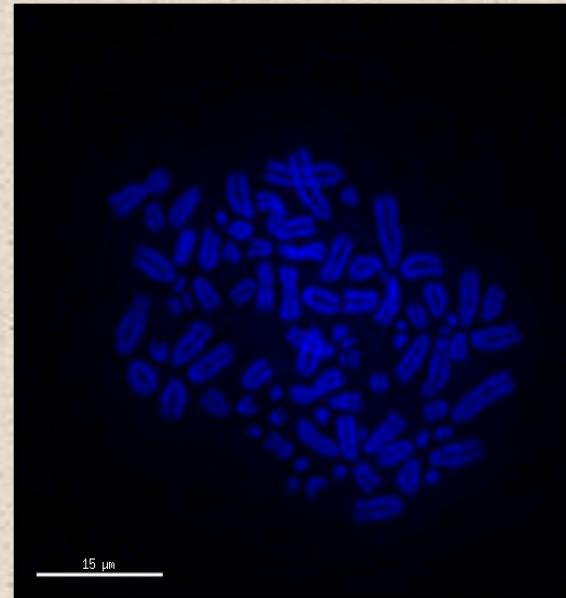
Evan Eichler



**Figure 3.** Comparison of applicant Eichler's new T2T long-read mouse lemur assembly vs current ref. genome. Top panel: A single contig of 10.5 Mbp from HiFiasm assembly (*Inina\_MMUR*) of a mouse lemur (*Microcebus murinus*) is compared to the current reference on UCSC (*Mic\_Mur*) which consists of 488 contigs where orientation is often in error. Each break in reference where there is an orientation flip is colored (blue/orange) while missing sequence is depicted (white space). QV accuracy for the long-read is estimated at >45, however, alignment to the existing reference identifies regions of reduced identity (red dashed box) corresponding most likely to low quality and/or misassembled regions in the current reference. Bottom panel: expanded view of red box showing multiple assembly errors and breaks in the existing reference making it virtually useless for DNM detection.



Dutrillaux (1979)  
Human Genetics



66 chromosomes (acrocentric, except for X)

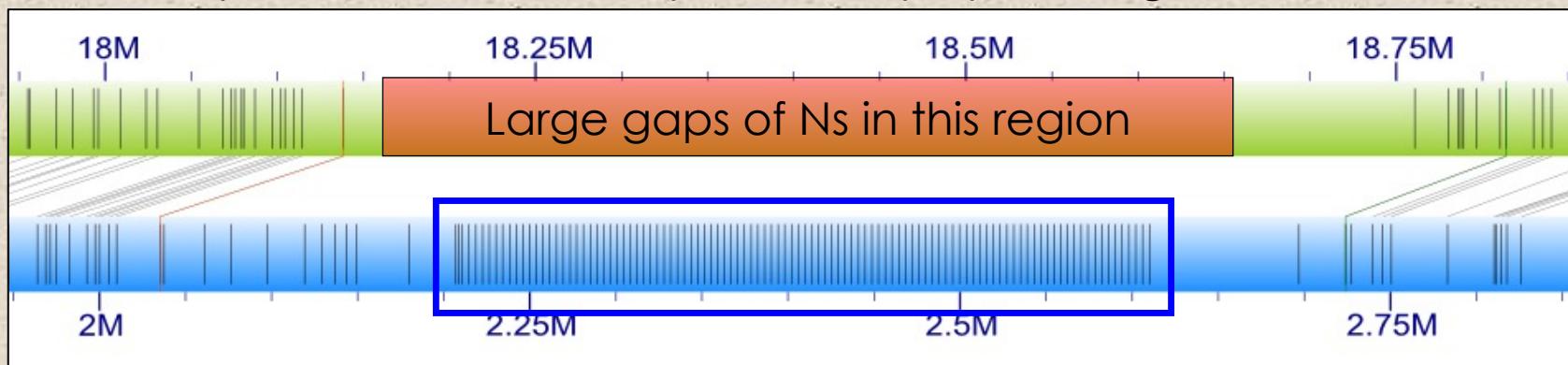


Beth Sullivan

# Centromeres

- Confirmed optical map nick-site present in centromere (*incredible luck*)

Super-scaffold and optical map spanning centromere

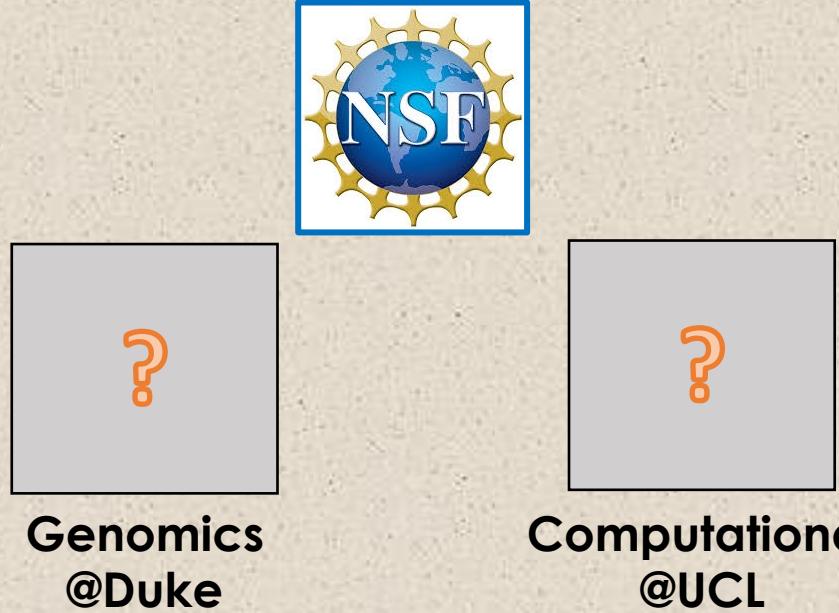


Illustrates 3 million bp region of Optical Map

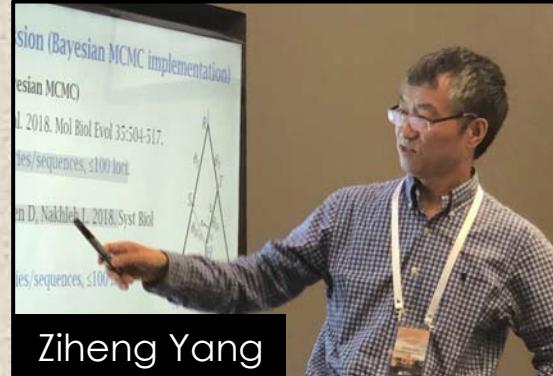




**Lydia Greene**  
**(sensory biology;  
outreach)**



**Sam Hyde Roberts**  
**(field methods;  
conservation)**







minipcr

Conservation Genetics  
<https://doi.org/10.1007/s10592-020-01296-0>

PERSPECTIVE

Check for updates

Next-generation technologies applied to age-old challenges in Madagascar

Marina B. Blanco<sup>1,2</sup> · Lydia K. Greene<sup>1,2,3</sup> · Fidisoa Rasambainarivo<sup>4</sup> · Elizabeth Toomey<sup>4</sup> · Rachel C. Williams<sup>1,2</sup> · Lanto Andrianandrasana<sup>5</sup> · Peter A. Larsen<sup>2,6</sup> · Anne D. Yoder<sup>2</sup>

Received: 27 April 2020 / Accepted: 20 July 2020  
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Oxford  
**NANOPORE**  
Technologies



1b



2b

3



Achille Raselimana  
Fidisoa Rasambainarivo



Marina Blanco



Lydia Greene





