

What do we even mean when we talk about “The Species Tree”? (lessons from mouse lemurs)



Anne D. Yoder
Duke University

31 May 2025

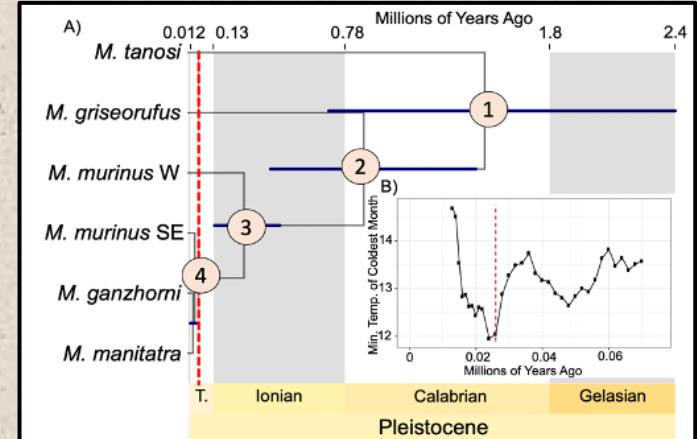
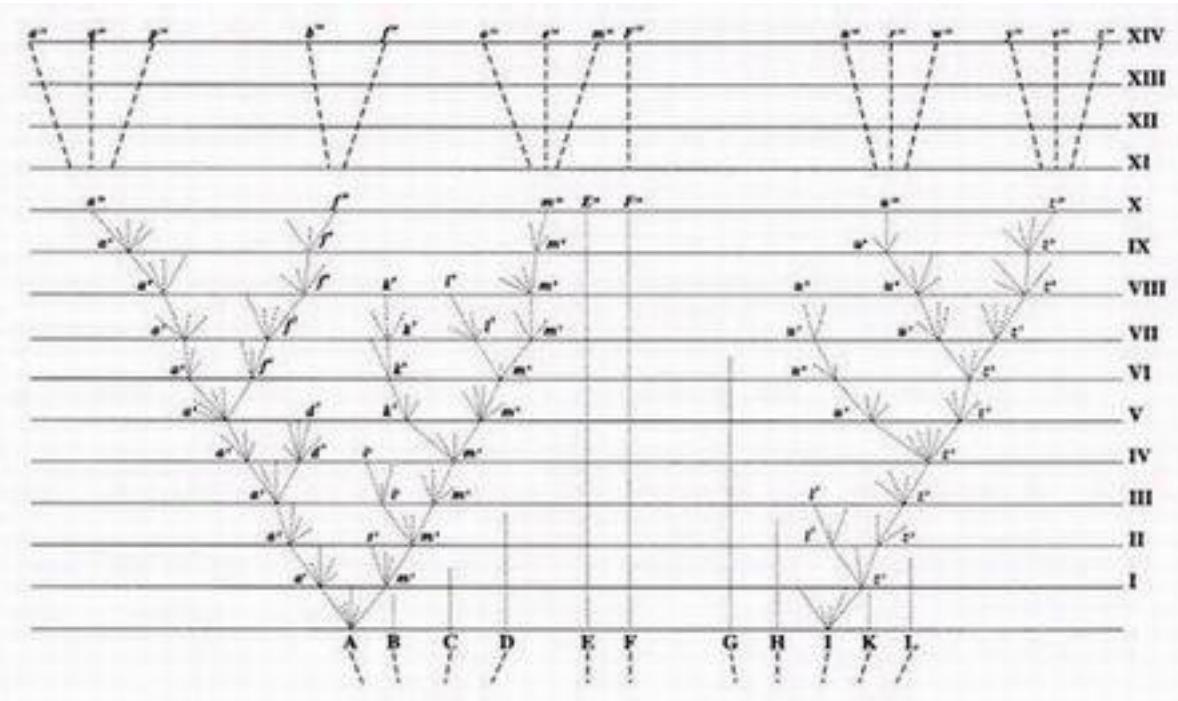


Figure 6: Speciation Continuum of Focal Lineages. A) The radiation among *M. murinus* (SE), *M. ganzhorni*, and *M. manitatra* (Node 4) is estimated to coincide precisely with the Last Glacial Maximum at 26,000 years ago (illustrated with dashed line). B) Paleoclimatic temperatures for SE Madagascar based on simulations under the HadCM3 circulation model (84).

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



Ancestor/Descendant Relationships Across Life on Earth

I have used the species tree for:

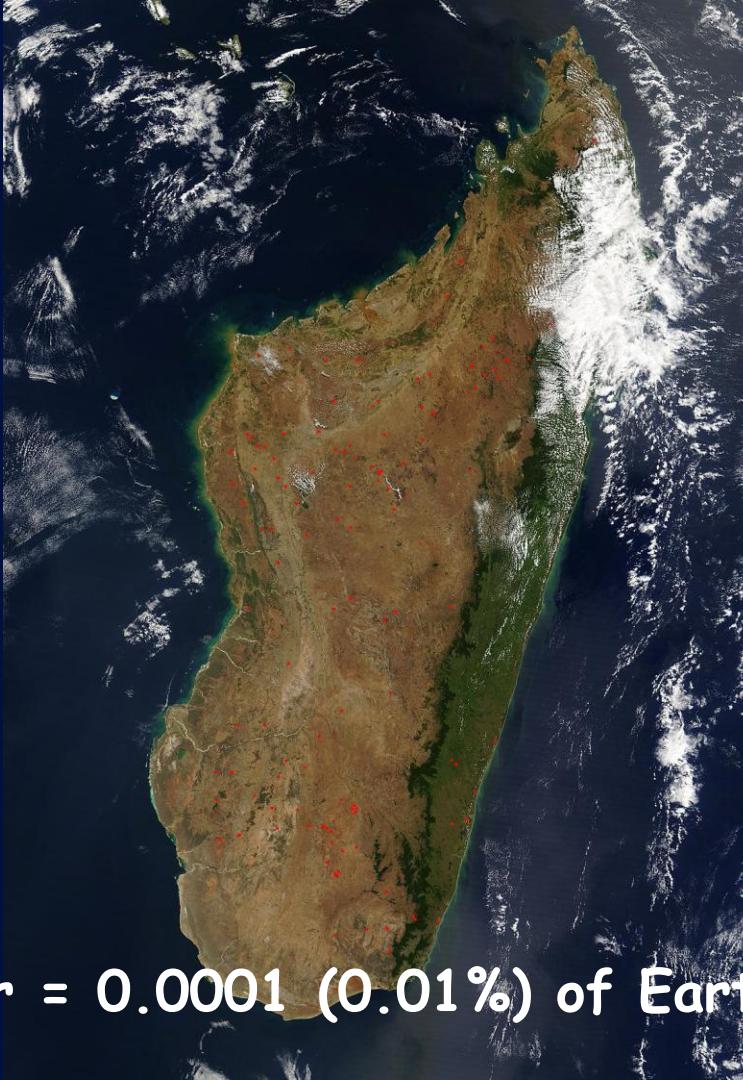
- Biogeography
- Phylogeography
- Species delimitation
- Divergence time analysis
- Speciation genetics/genomics
- Just for the hell of it ...

Biogeography



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

Google earth



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



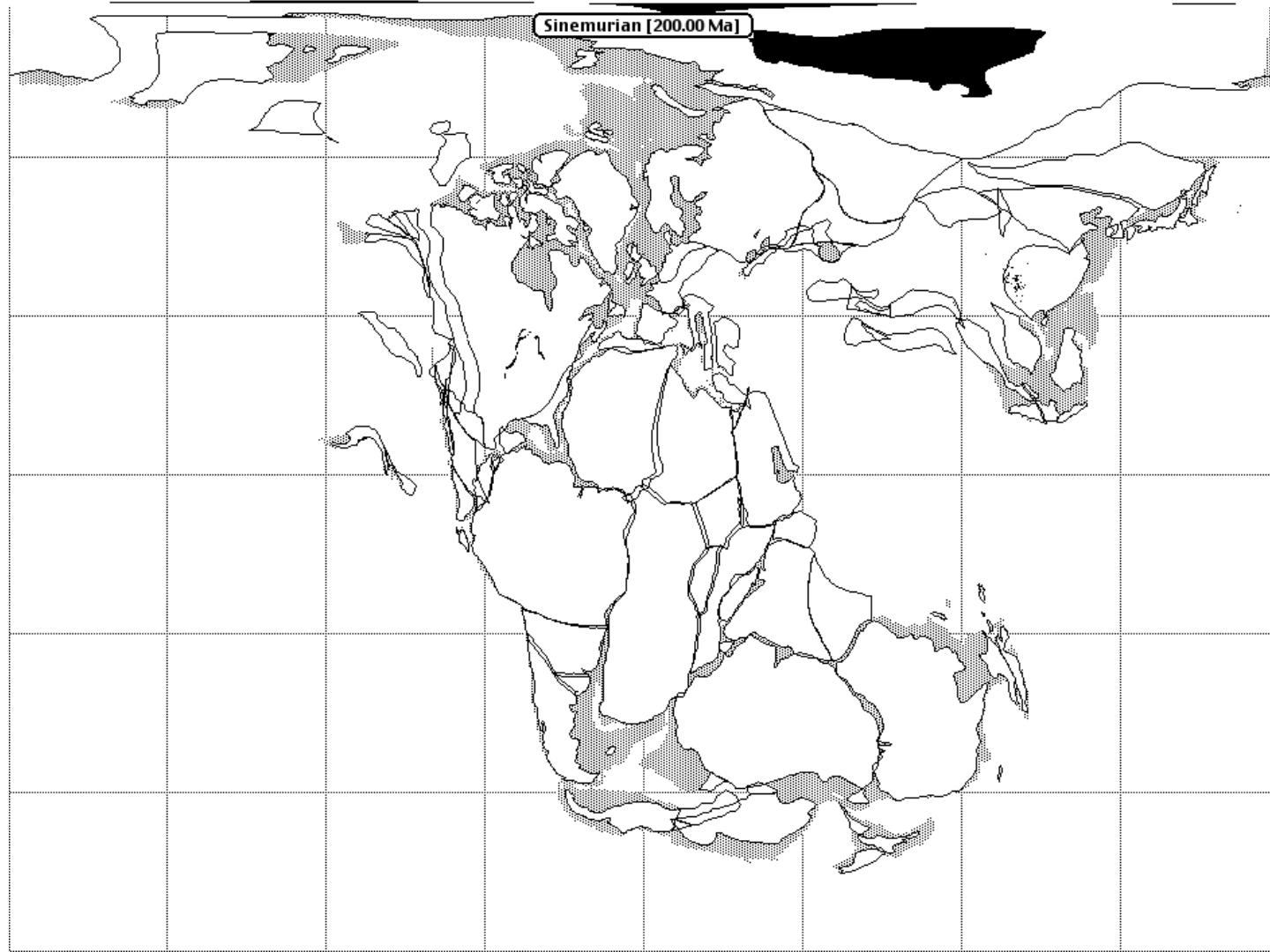
May 3, 2005
vol. 102 no. suppl 1

PNAS



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

Sinemurian [200.00 Ma]



Ancient single origin for Malagasy primates

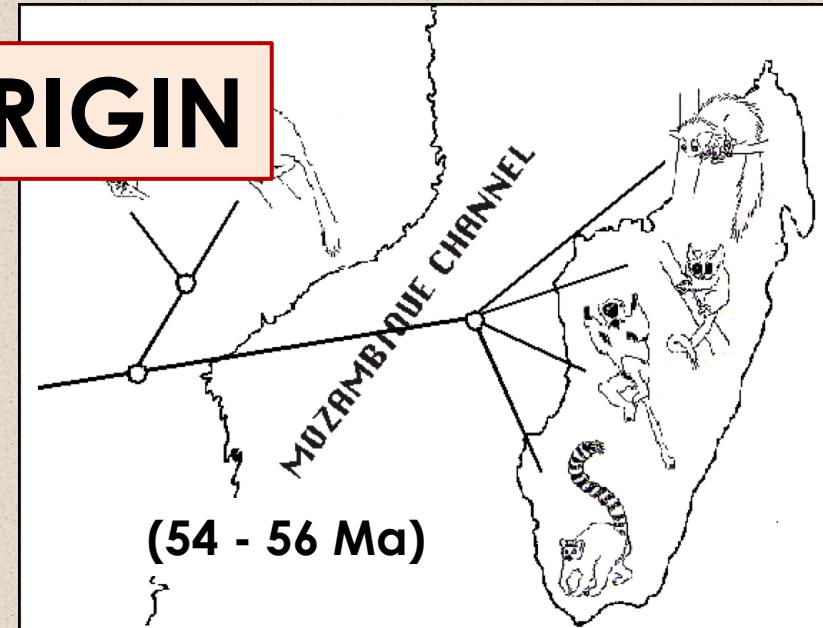
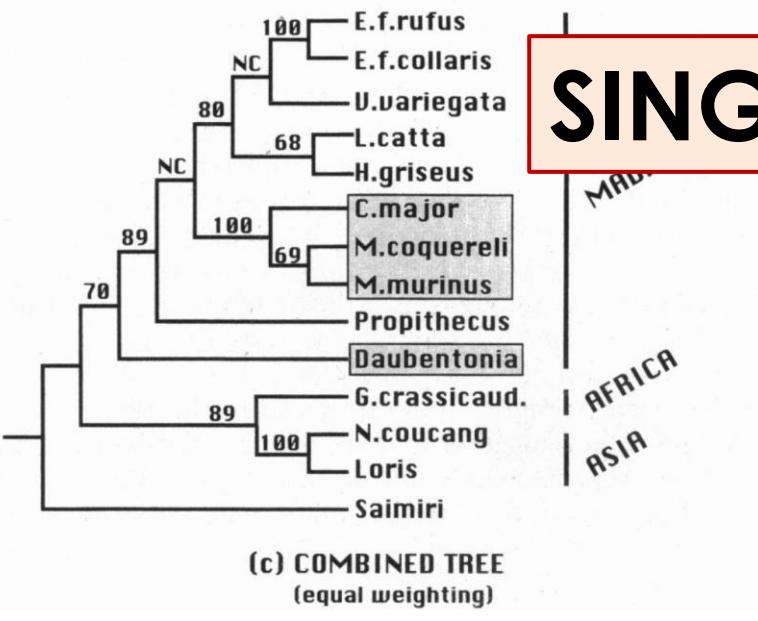
(primate origins/cytochrome b/molecular evolution)

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

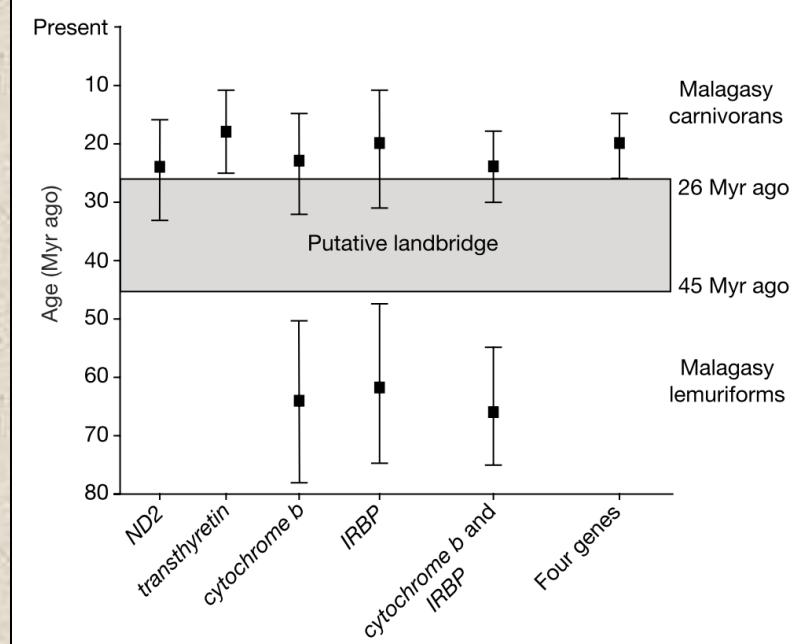
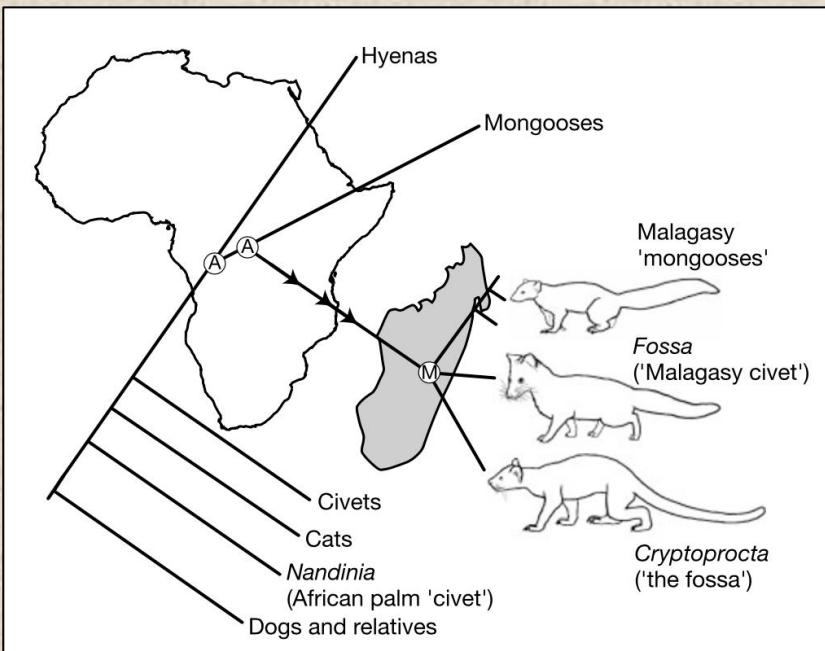
SINGLE ORIGIN



letters to nature

Single origin of Malagasy Carnivora from an African ancestor

Anne D. Yoder^{*†}, Melissa M. Burns[†], Sarah Zehr[‡], Thomas Delefosse^{*}, Geraldine Veron[§], Steven M. Goodman^{†||} & John J. Flynn[‡]



Shout out to Jeff Thorne
and MultiDivTime!!!!



SCIENTIFIC REPORTS



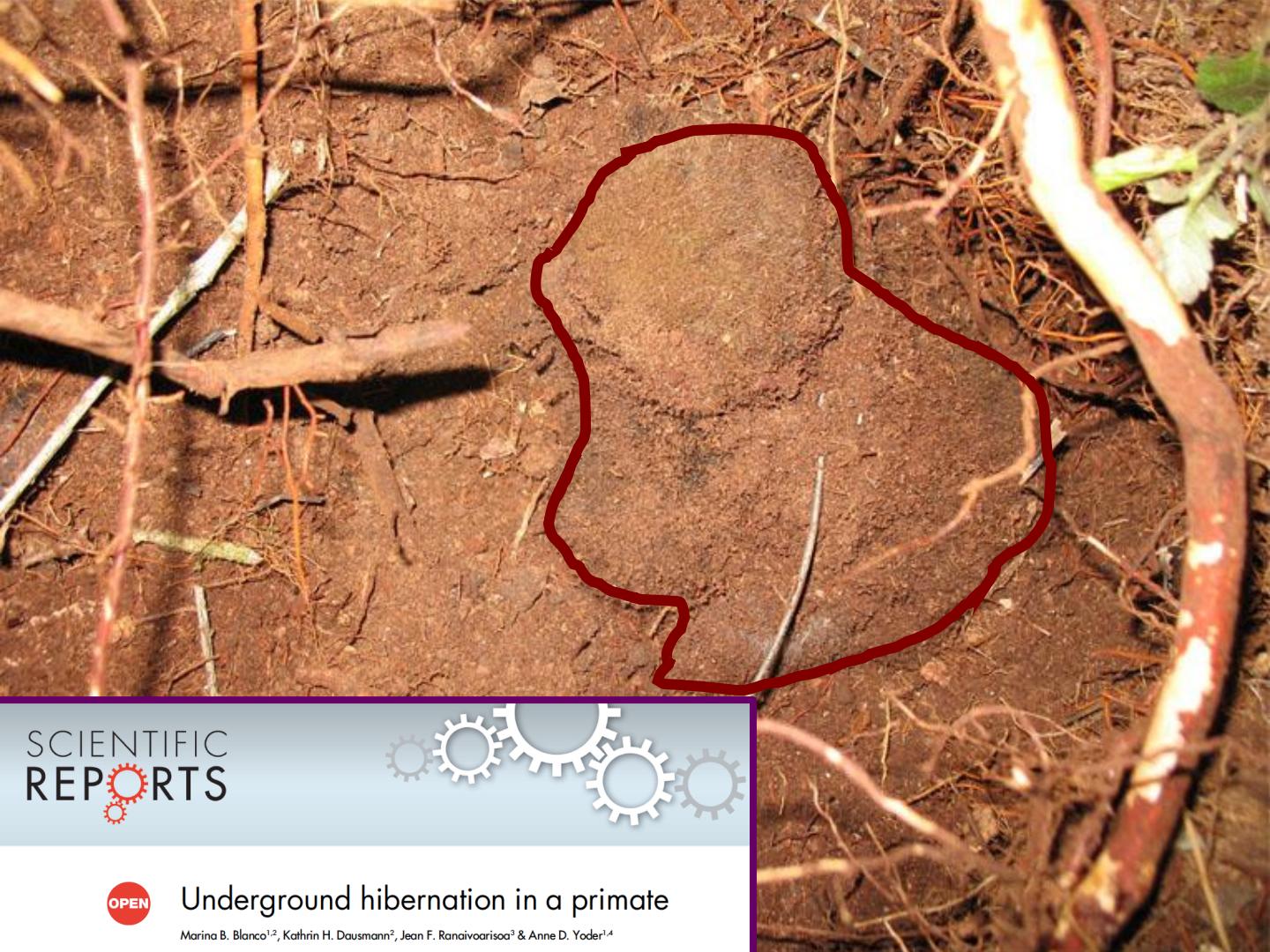
OPEN

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^{1,2}, Kathrin H. Dausmann², Jean F. Ranaivoarisoa³ & Anne D. Yoder^{1,4}



ILLUSTRATION BY STEPHEN D. NASH

SWEEEPSTAKES

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

The colonisation of Madagascar by land-bound vertebrates

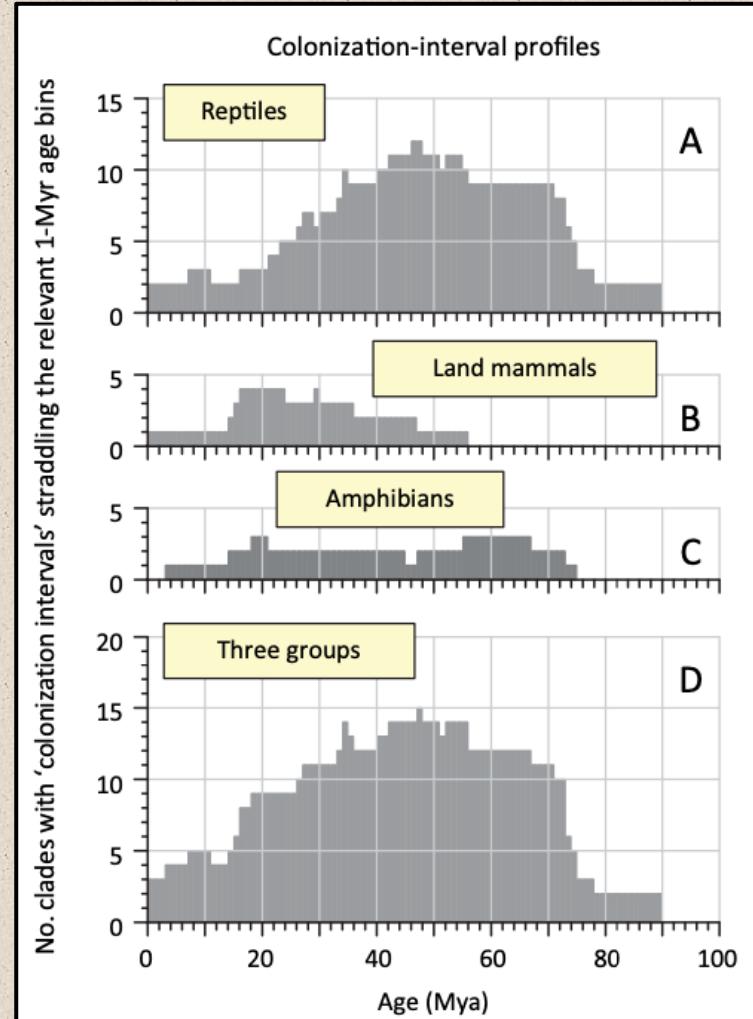
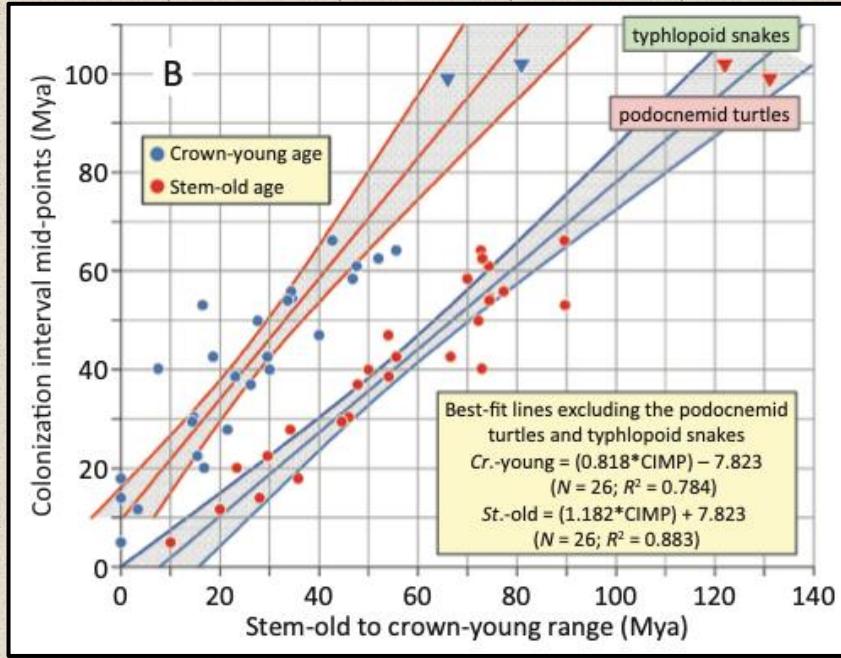
Jason R. Ali^{1,*}  and S. Blair Hedges²

¹*Department of Earth Sciences, University of Hong Kong, Pokfulam Road, Hong Kong, China*

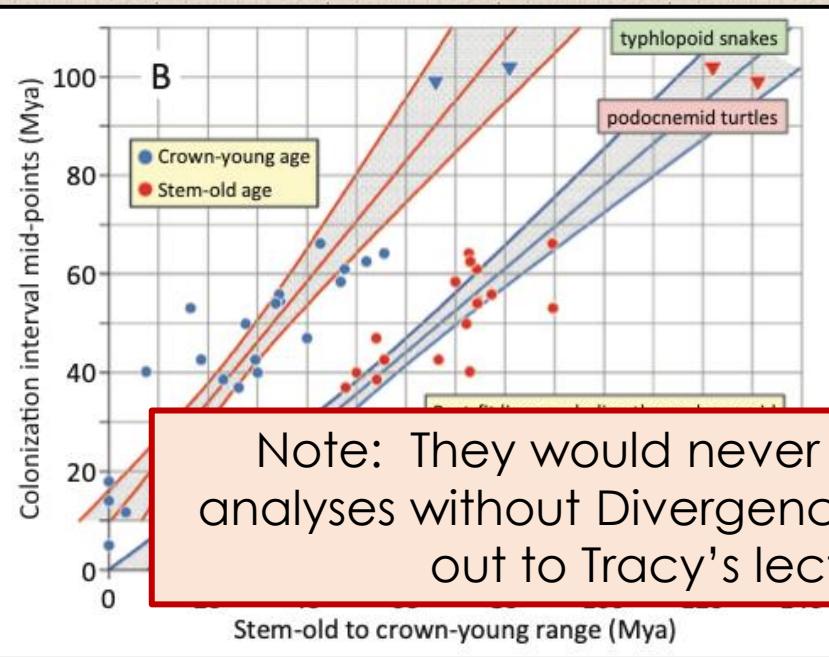
²*Center for Biodiversity, Temple University, 1925 N 12th Street, Suite 502, Philadelphia, PA 19122, USA*

ABSTRACT

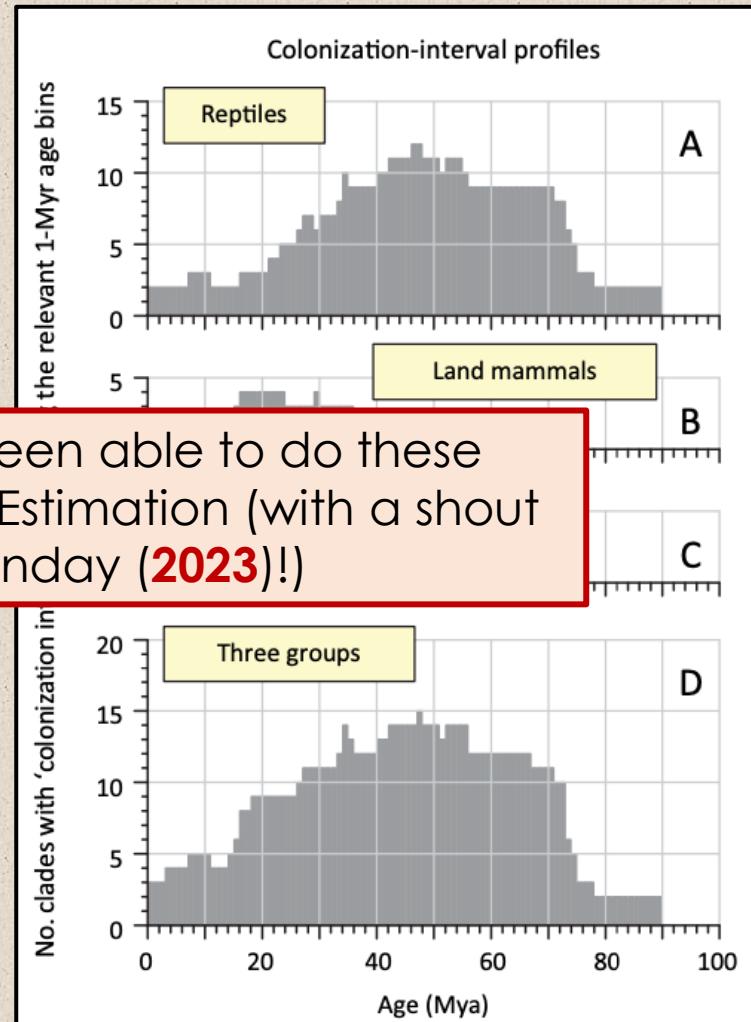
2023



[Ali & Huber, 2023] show “that **stochastic over-water dispersal through time best explains the observed profile**, rather than passage along temporary causeways. This finding is congruent with the geological evidence that rejects the suggestion that Africa and Madagascar were connected by land bridges/stepping-stone chains in the Cenozoic.”



Note: They would never have been able to do these analyses without Divergence Time Estimation (with a shout out to Tracy's lecture Monday (**2023**)!)

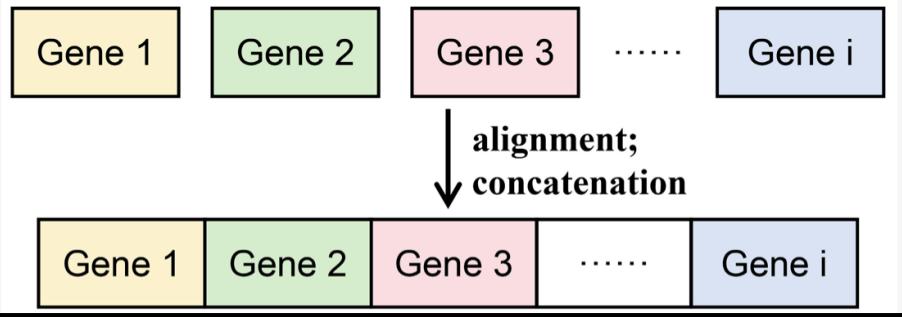


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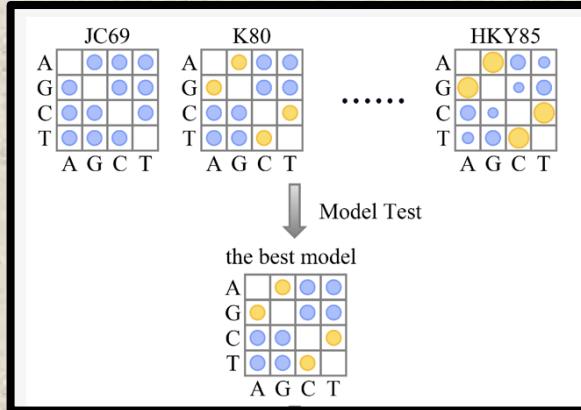
Divergence Time Estimation

Sequence and align DNA data

Figure 7. Strategies and principles of the concatenation phylogeny method for constructing phylogenetic trees.



Model Selection



Concatenation Approach (overview)

Build the tree
(ML or Bayesian, typically)

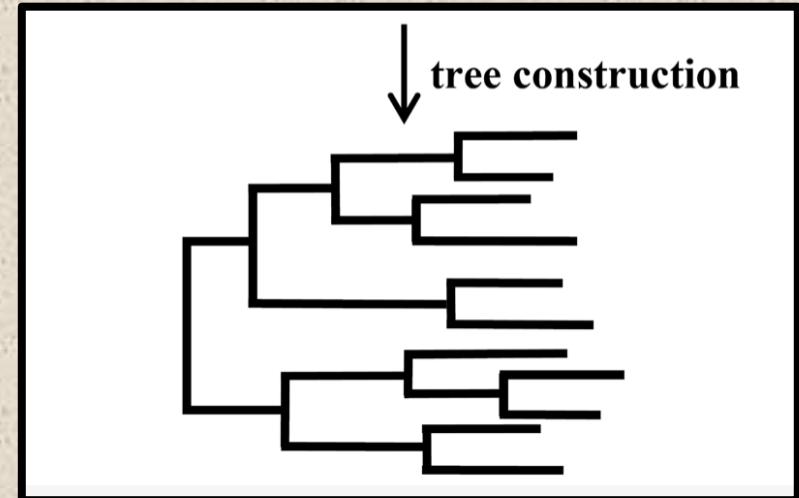
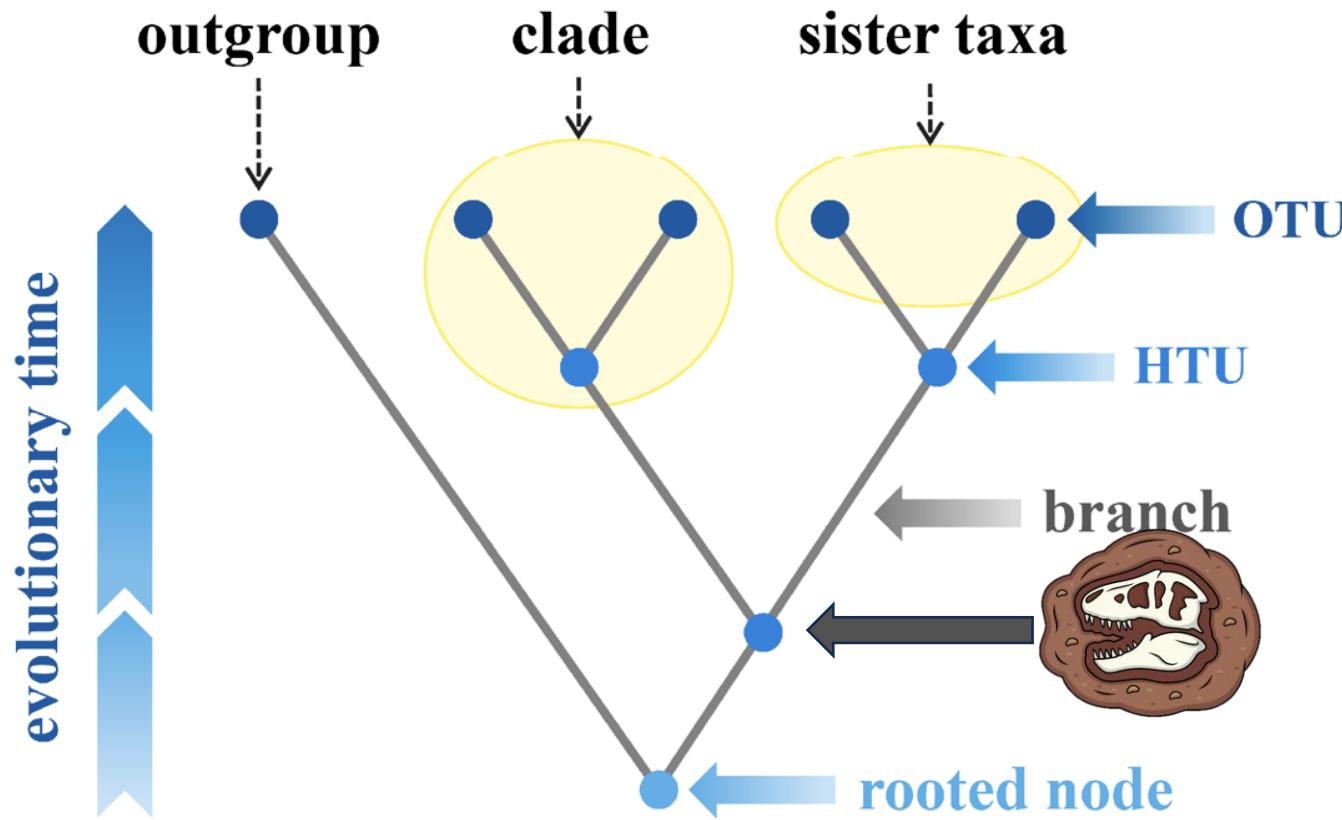


Figure 1. The general structure of a phylogenetic tree. The abbreviations in the figure are as follows: OTU, operational taxonomic unit; HTU, hypothetical taxonomic unit. All figures in this review were drawn by Yue Zou, using Microsoft PowerPoint 2010 and Adobe Illustrator 26.2.1.



Open Access Review

Common Methods for Phylogenetic Tree Construction and Their Implementation in R

by Yue Zou ^{1,†}   , Zixuan Zhang ^{1,†}   , Yujie Zeng ¹   , Hanyue Hu ¹   , Youjin Hao ¹   , Sheng Huang ^{2,*}  and Bo Li ^{1,*}  

¹ College of Life Sciences, Chongqing Normal University, Chongqing 401331, China

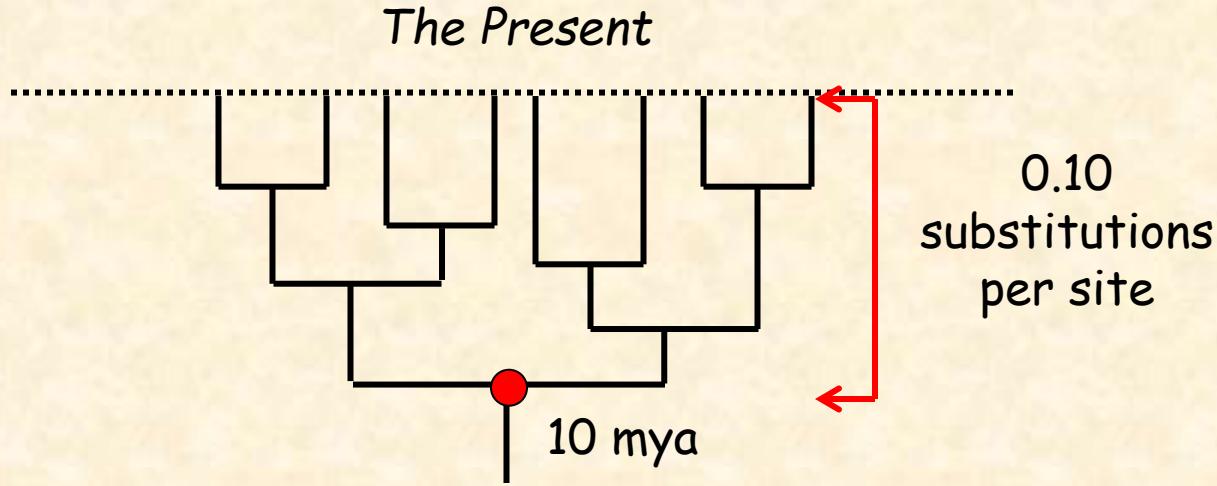
² Animal Nutrition Institute, Chongqing Academy of Animal Science, Chongqing 402460, China

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† These authors contributed equally to this work.

Bioengineering **2024**, *11*(5), 480; <https://doi.org/10.3390/bioengineering11050480>

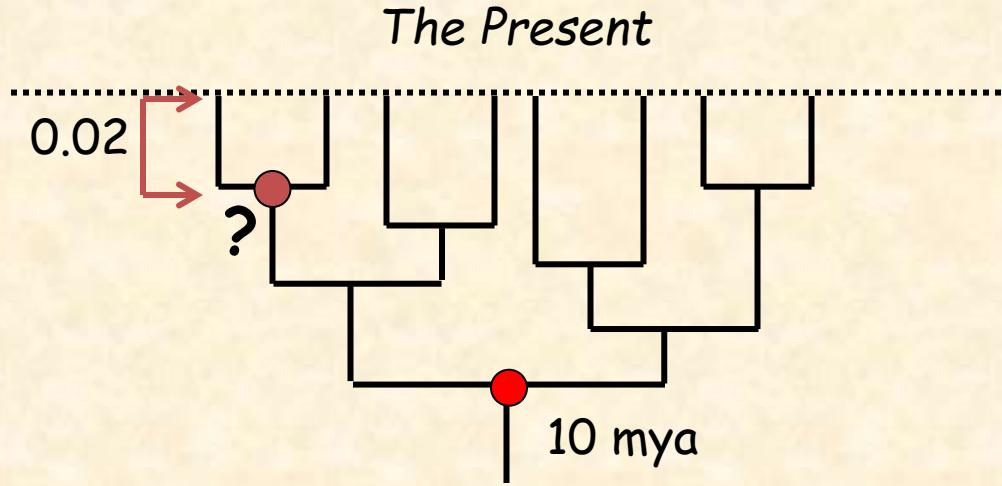
The Molecular Clock



Divide branch length by time, and get rate

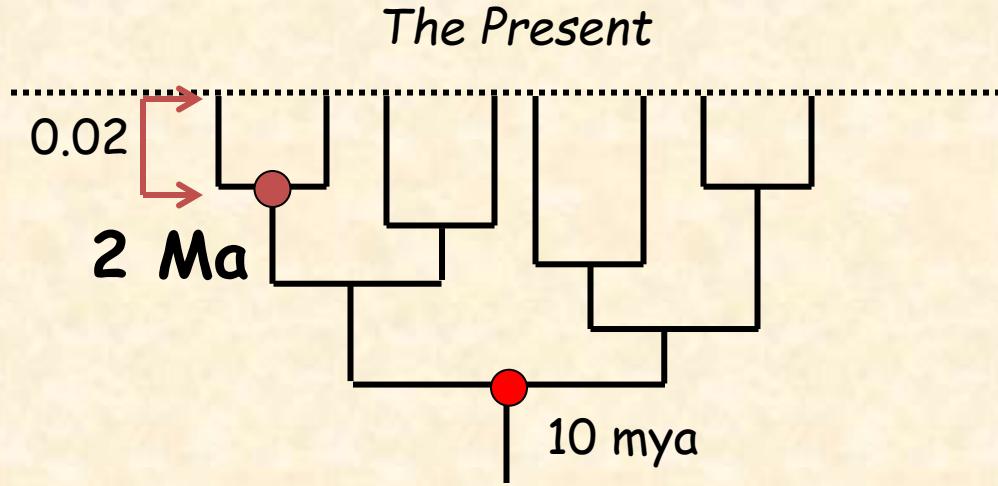
$$0.1/10 = 0.01 \text{ substitutions/site/my}$$

The Molecular Clock



$$0.1/10 = 0.01 \text{ substitutions/site/my}$$

The Molecular Clock



Divide branch length by rate, and get time

$$0.02/0.01 = 2 \text{ my}$$

ALL KINDS OF ASSUMPTIONS!!!

- Rates of evolution are constant (there's a "molecular clock")
- Your "species tree" is correct
- Your model assumptions are correct
- You have placed the fossil in the correct part of the tree
- The age of the fossil is correct
- Shall I go on????

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DOI:10.1093/sysbio/syy001

Advance Access publication January 12, 2018

Using Phylogenomic Data to Explore the Effects of Relaxed Clocks and Calibration Strategies on Divergence Time Estimation: Primates as a Test Case

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²*Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK;*

³*Division of Fossil Primates, Duke University Lemur Center, Durham, 1013 Broad Street, NC 27705, USA;*

⁴*Department of Anthropology, The Ohio State University, Columbus, OH 43210, USA; and*

⁵*Department of Biology, Duke University, Durham, NC 27708, USA*

[†]*Deceased.*

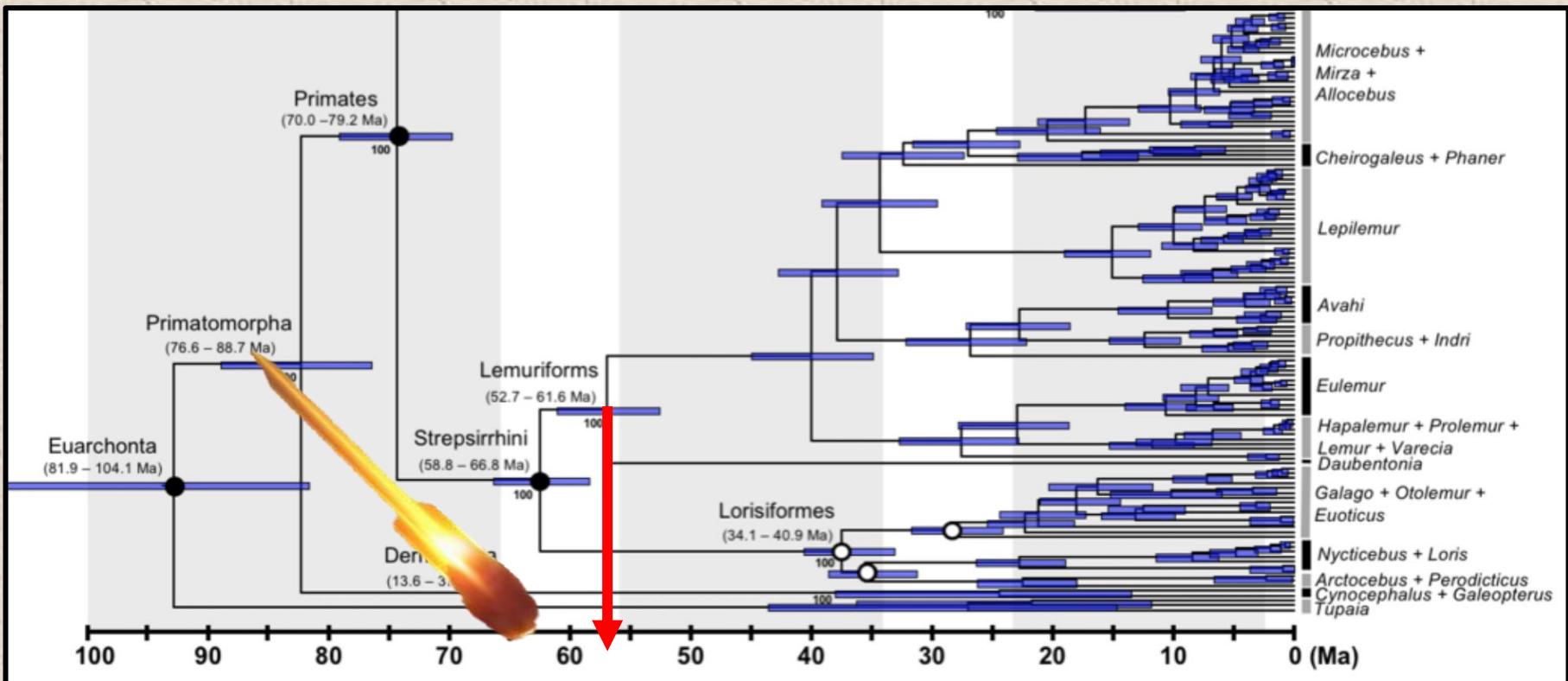
*Correspondence to be sent to: School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, UK;
Email: m.dosreisbarros@qmul.ac.uk.

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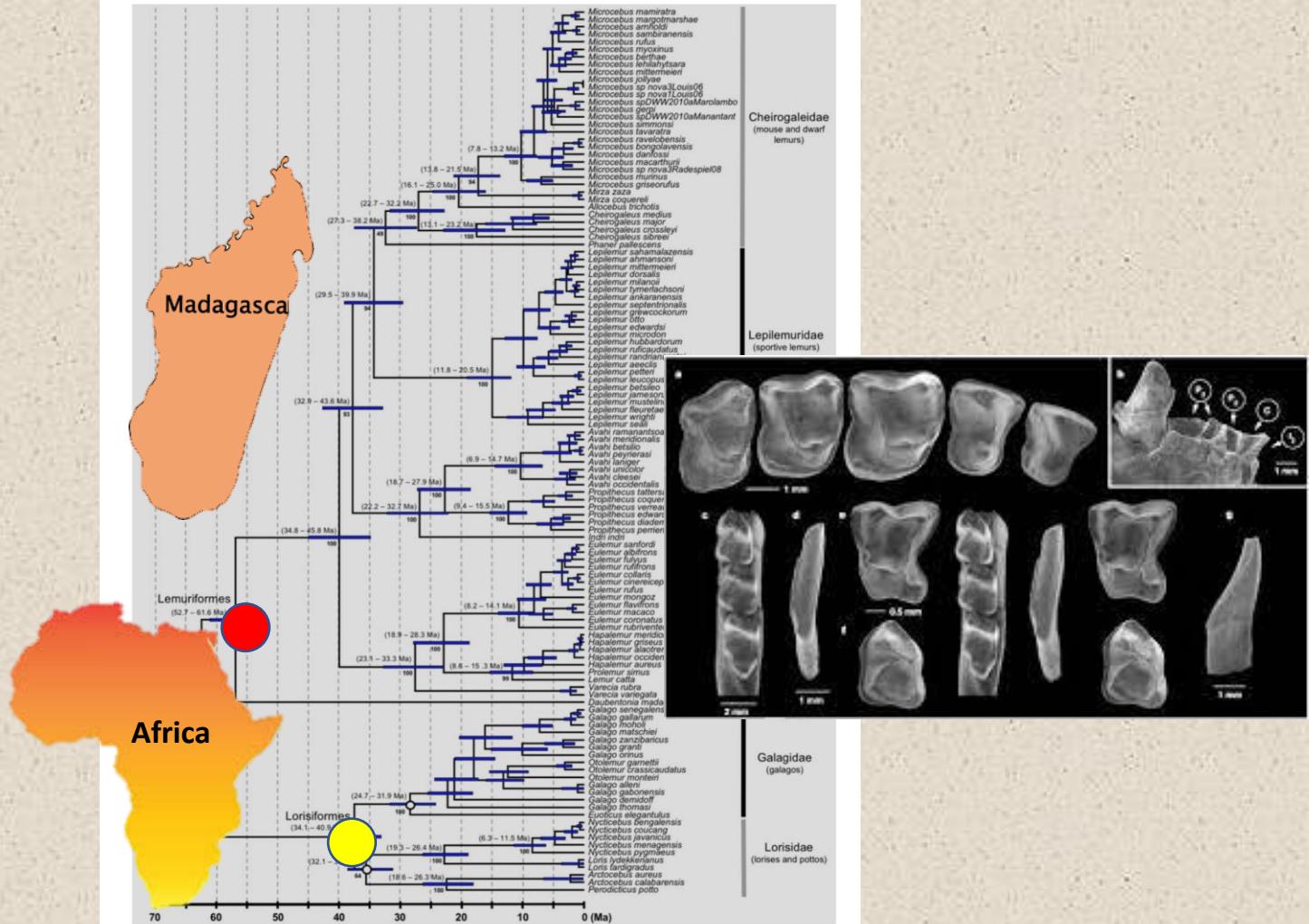
Associate Editor: Simon Ho

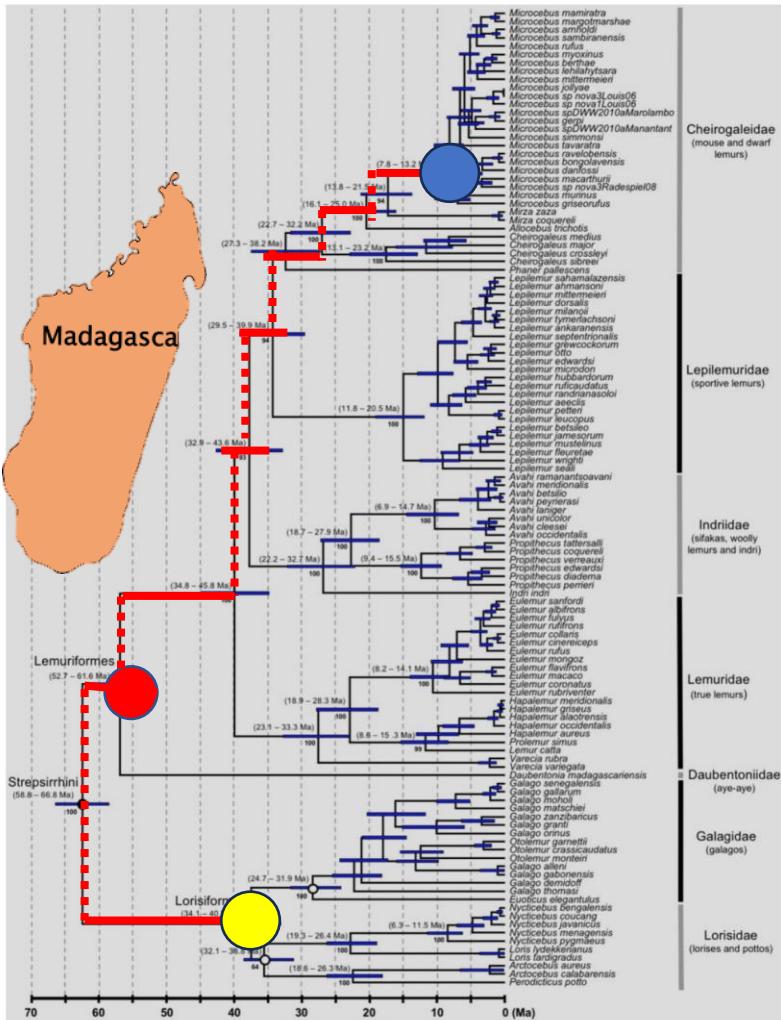
2018

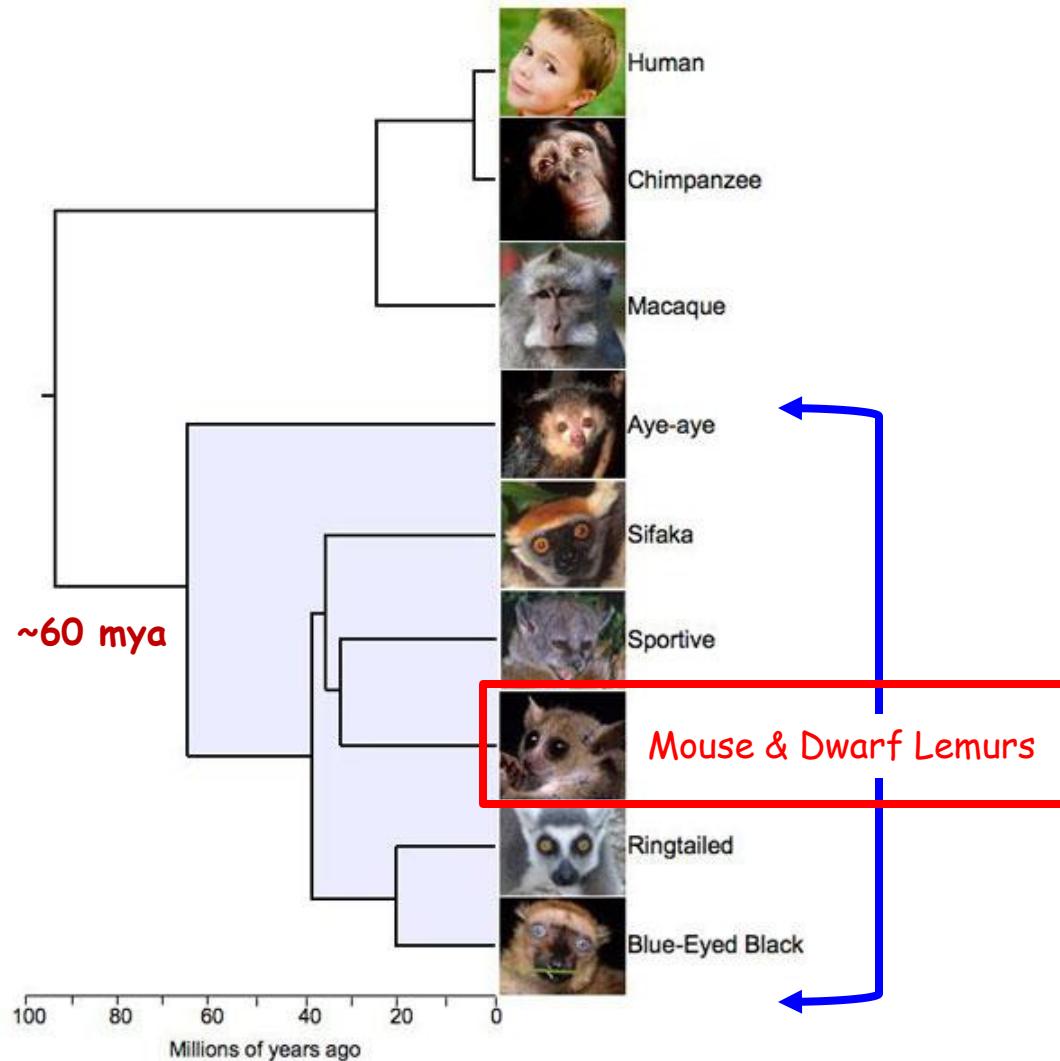
CONCATENATION!!!!



No fossil record!!!







My Addiction Story



Joerg Ganzhorn

Steve Goodman

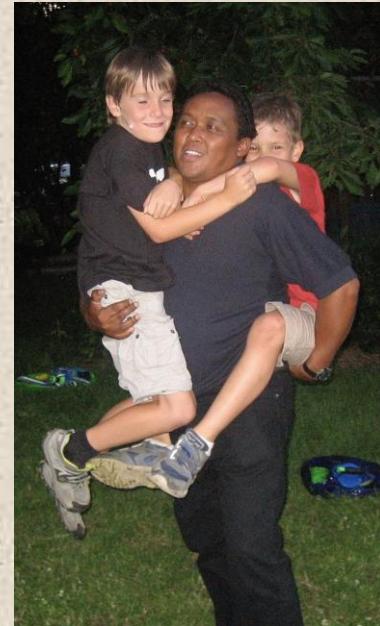


Dear Anne,

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

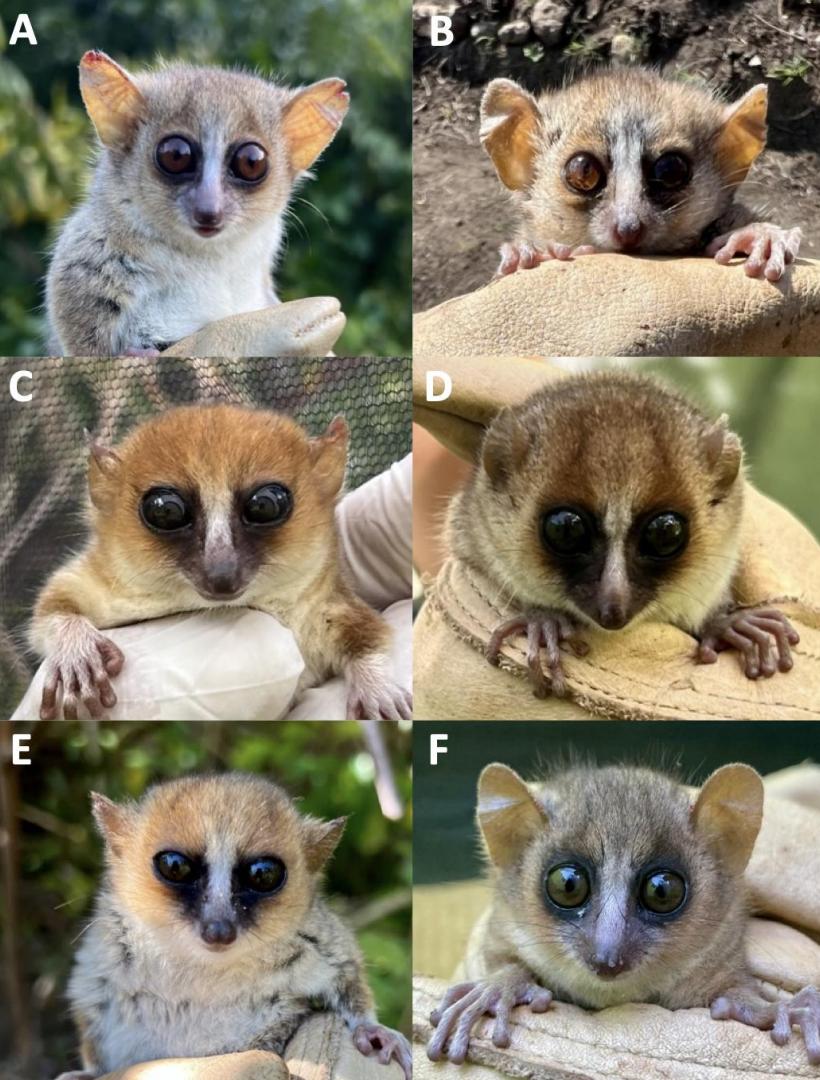
January 27, 1997



Rodin Rasoloarison

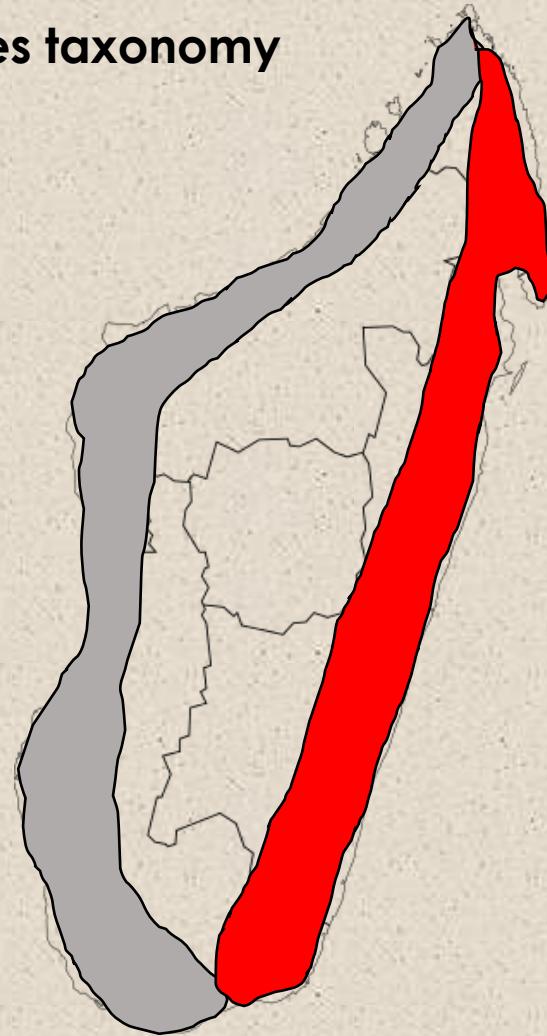
Mouse lemurs are morphologically cryptic and very difficult to study!

- World's smallest primates (60 – 80 grams)
- Nocturnal
- Many do NOT like Sherman traps



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.

1999: D-loop sequences – barely alignable!

Project of "D-loopGDE.nxs" Character Matrix

Matrix Alter Select Display Analysis:Matrix Window

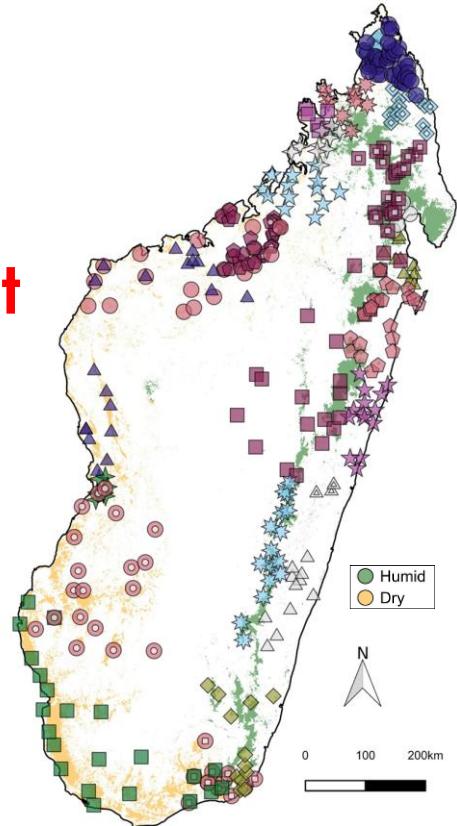
Taxon \ Character

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Phylogeography

Mouse lemurs (*Microcebus* spp.)

- Distributed across all forest habitats of Madagascar



- *M. rufus*
- *M. berthae*
- *M. myoxinus*
- *M. lehilahytsara*
- *M. mittermeieri*
- *M. tanosi*
- *M. mamaratra*
- *M. margotmarshae*
- *M. sambiranensis*
- *M. arnoldi*
- *M. sp. 1*
- *M. tavaratra*
- *M. boraha*
- *M. simonensi*
- *M. jollyae*
- *M. marohita*
- *M. gerpi*
- *M. macarthurii*
- *M. jonahi*
- *M. manitatra*
- *M. ganzhorni*
- *M. murinus* (central)
- *M. murinus* (north)
- *M. griseorufus*
- *M. ravelobensis*
- *M. bongolavensis*
- *M. danfossi*



M. gerpi
© D. Schübler



M. gerpi
Radespiel et al. 2012

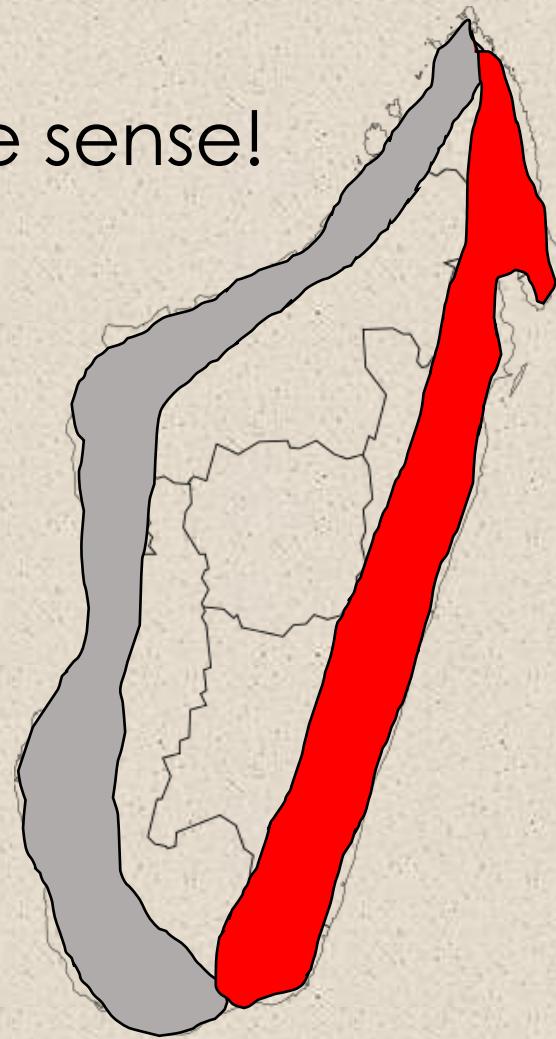


M. jollyae
© E. Louis, Jr.



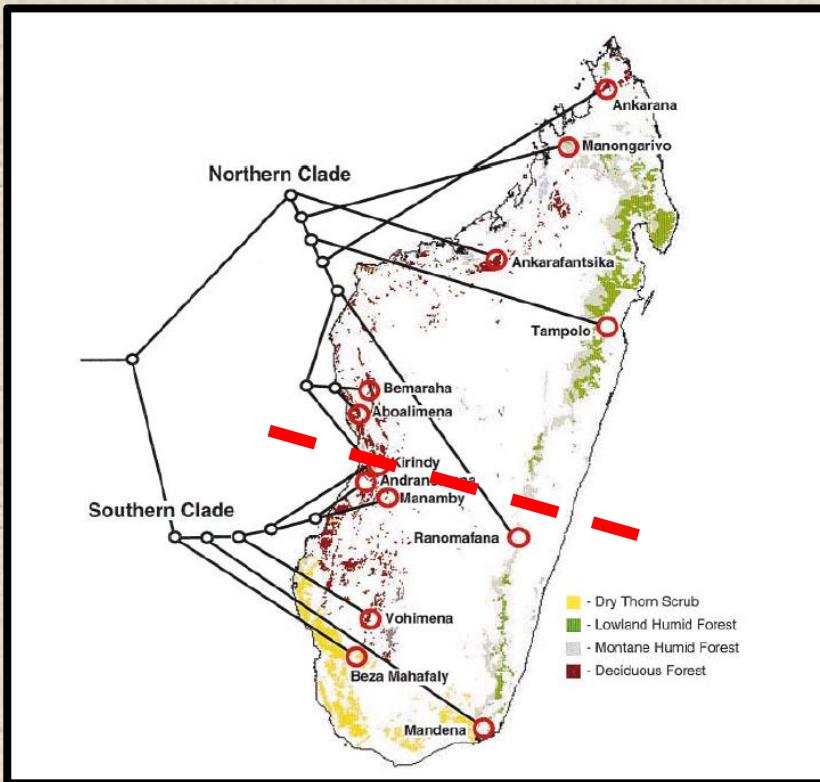
ENVIRONMENTAL HETEROGENEITY!

So this really made sense!



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

Anne D. Yoder*^{†‡}, Rodin M. Rasoloarison[§], Steven M. Goodman^{†¶}, Jodi A. Irwin*^{||}, Sylvia Atsalis*, Matthew J. Ravosa*[†], and Jörg U. Ganzhorn**



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



Two decades+ later:
recognized species
diversity has gone from
two species to ~ 25 species

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 Madagascar¹ (Table 1). Today the equivalent figures are \$7.40, \$95, and 83 lemur species² (Table 1).

Species Delimitation



Portugal
(Oeiras)



Lounès Chikhi



USA
(Durham)

France
(Toulouse)



Jordi Salmona



Ute Radespiel



Germany
(Hannover)

RADseq Consortium



N > 1000
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 Ankafoibe Mlehi (4)

M. lehilahytsara from Ankafoibe

M. myoxinus from Aboalimena M. myoxinus from Aboalimena

M. myoxinus from Aboalimena M. myoxinus from Aboalimena

M. murinus from Andranomena

M. lehilahytsara 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

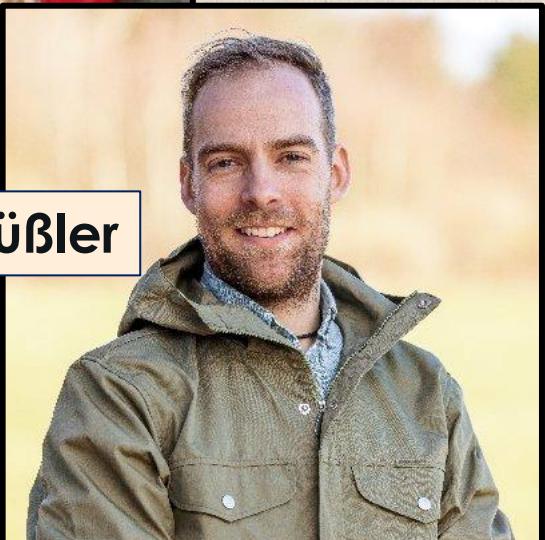
M. griseorufus from Mahavelo



Jordi Salmona



Tobias van Elst



Dominik Schüßler

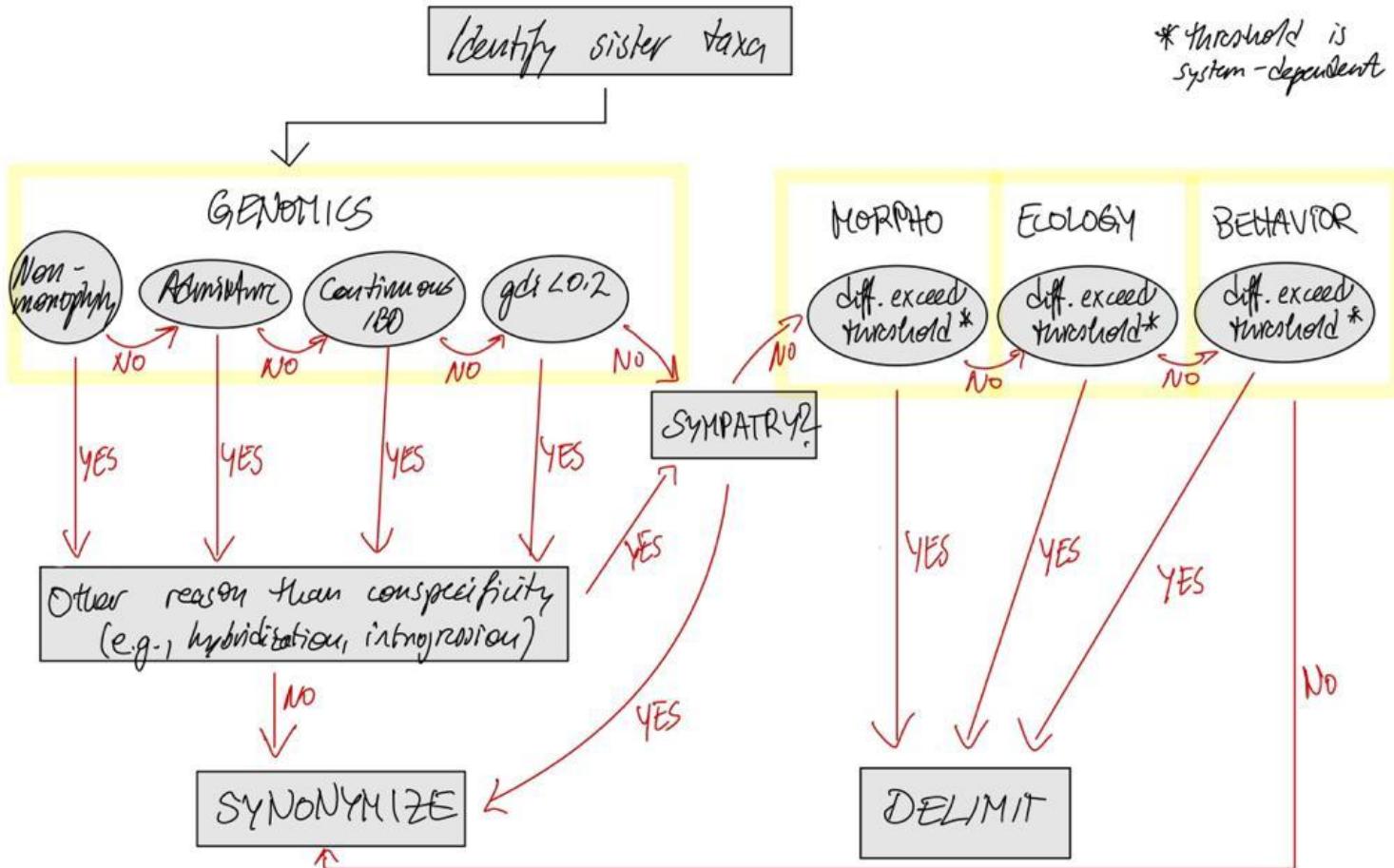


Gabriele Sgarlata

Motivating Questions:

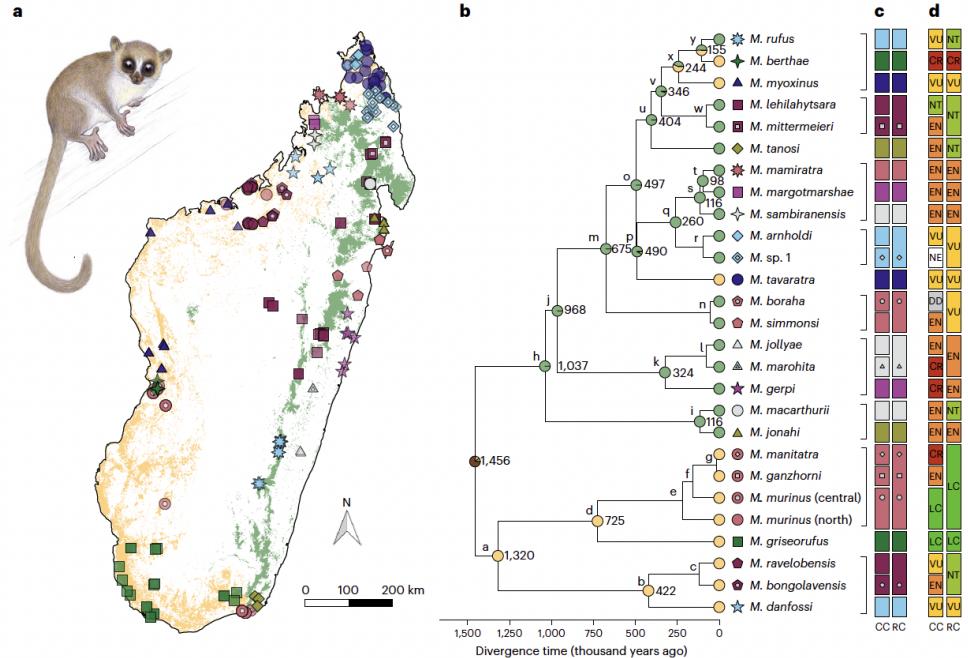
- Which of the 25 named species are “good”?
- What are their phylogenetic relationships?
- What has driven their diversification?
- What is maintaining species/phylogenetic boundaries?
- What explains morphological stasis in the face of genetic diversity? (e.g., crypsis)
- What impacts might a new understanding of species diversity have on conservation priorities?

Flowchart - delimitation framework



Article

<https://doi.org/10.1038/s41559-024-02547-w>



nature ecology & evolution

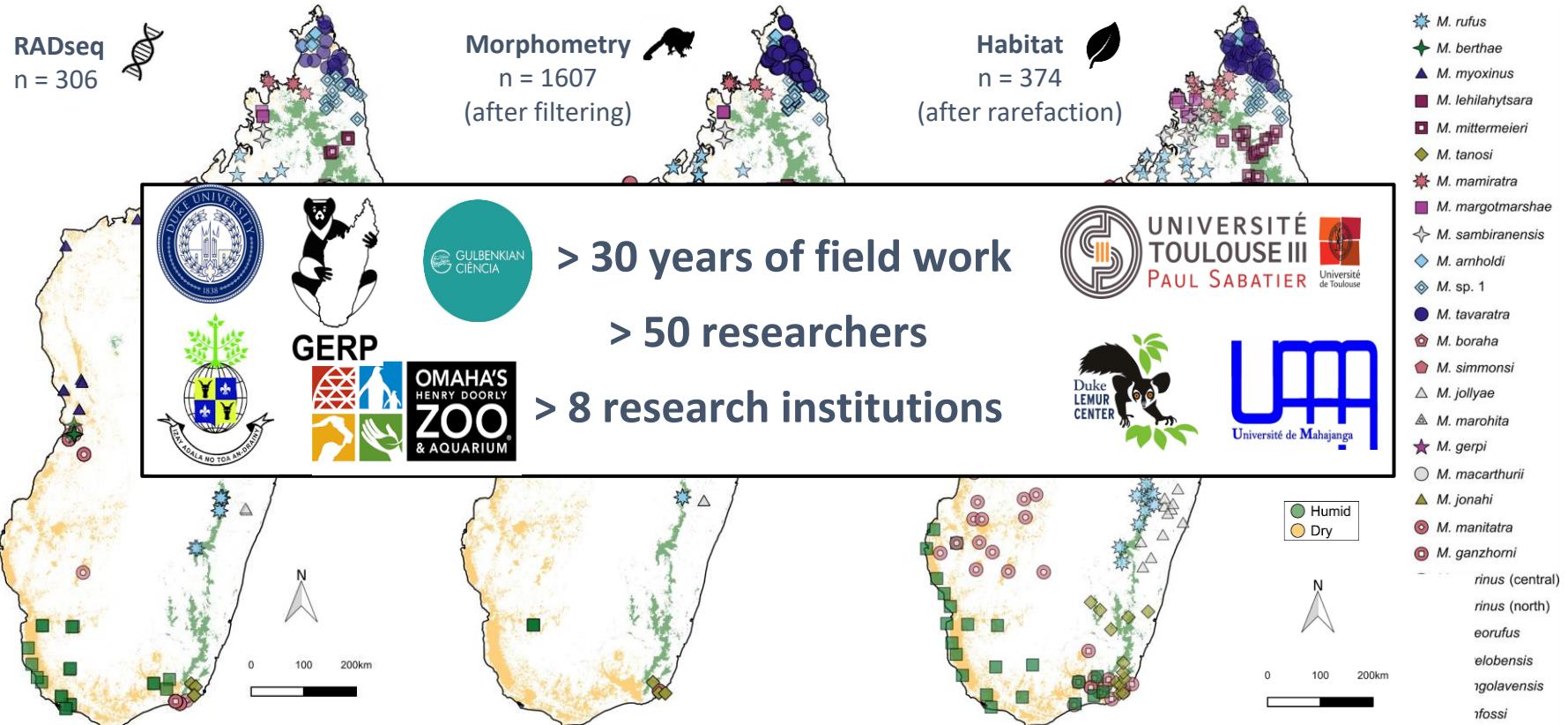
Article

<https://doi.org/10.1038/s41559-024-02547-w>

Integrative taxonomy clarifies the evolution of a cryptic primate clade

Tobias van Elst   Gabriele M. Sgarlata   Dominik Schüßler   George P. Tiley   , Jelle W. Poelstra   , Marina Schumann   , Marina B. Blanco   , Isma Al-Teiko-Poelstra   , Maria Rina Evasio , Jörg U. Ganzhorn , Steven M. Goodman , Alida F. Hasinthal , Daniel Hending , Paul A. Hohenlohe , Mohamed T. Ibourou , Amaia Iribar , Fabien Jan , Peter M. Kappeler , Barbara Le Pors , Sophie Manz , Gillian Oliviero , Ando N. Rakotonanahary , S. Jacques Rakotondranary , Romual Rakotondravony , José M. Ralston , J. Freddy Ranavalisoa , Blanchard Randrianambinana , Rodin M. Rasolosarison , Sofoniforina Rasoloharolahaina , Emmanuel Rasolondraibe , Helena Teixeira , J. Jorge Zepeda-Veloz , Edward E. Louis Jr. , Anna D. Yoder , Lounés Chikhi , Ute Radepsch , O. Jordi Salmona

Methods – Data



Conclusions:

- Species diversity has been overestimated; we “deflate” taxonomy from 25 to 19 species (~ 25% reduction)
- Mouse lemur crypsis is best explained by a model of **morphological stasis imposed by stabilizing selection** along with a neutral process of niche diversification
- Phylogenetic diversification was likely facilitated by climatic fluctuations and a species-pump effect of humid forests during the **Pleistocene**

(Last month in Science)

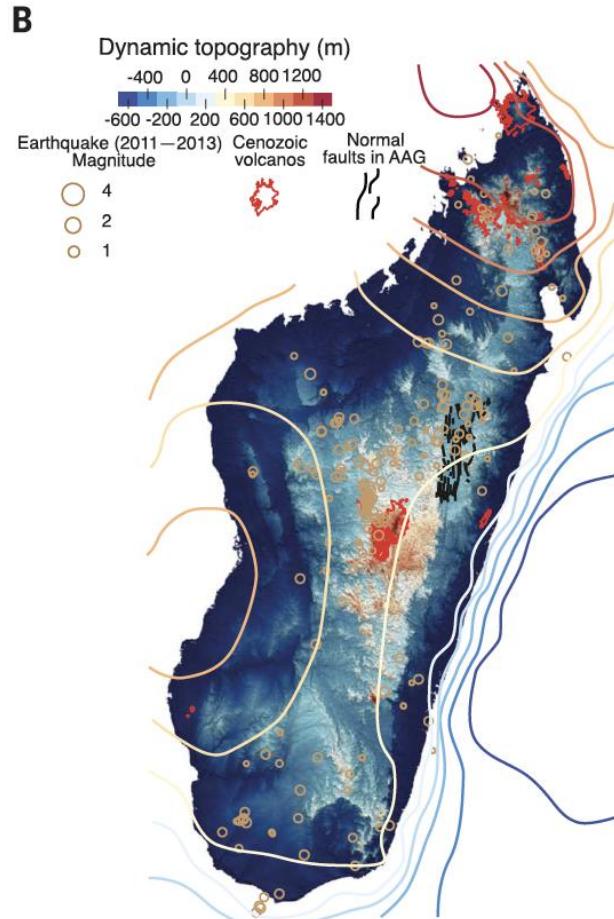
RESEARCH

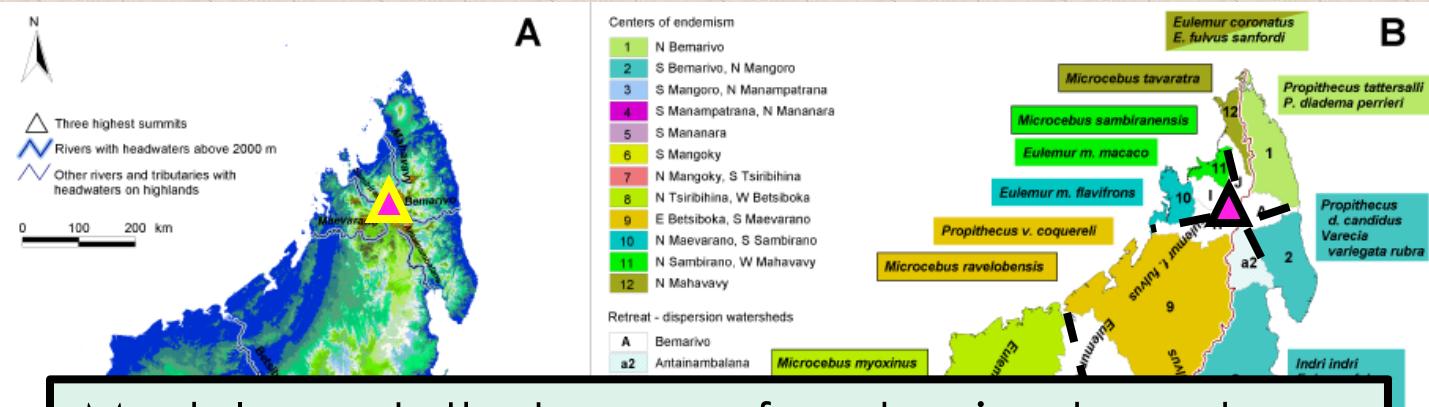
BIOGEOGRAPHY

Escarpe ment evolution drives the diversification of the Madagascar flora

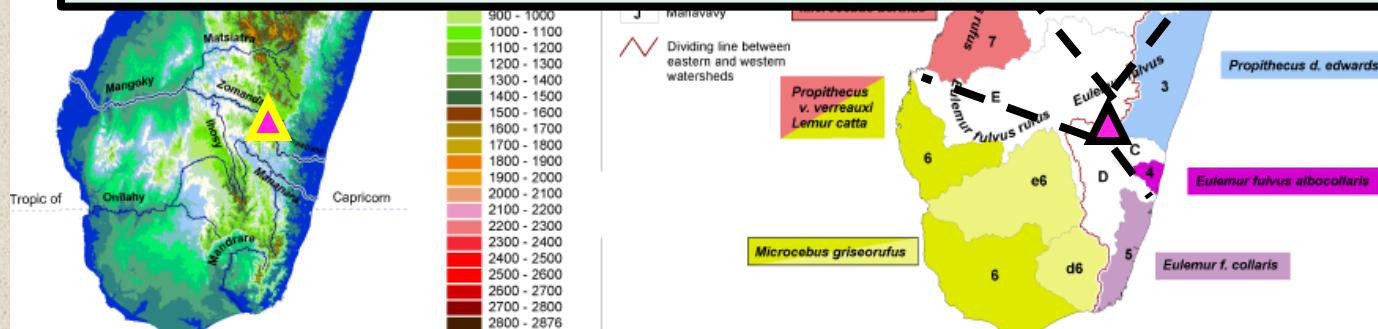
Yi Liu^{1,2*}†, Yanyan Wang^{3*}†, Sean D. Willett³, Niklaus E. Zimmermann^{1,2‡}, Loïc Pellissier^{1,2‡}

“Habitat isolation and reconnection on a million-year timescale serves as an allopatric speciation pump creating the observed biodiversity”



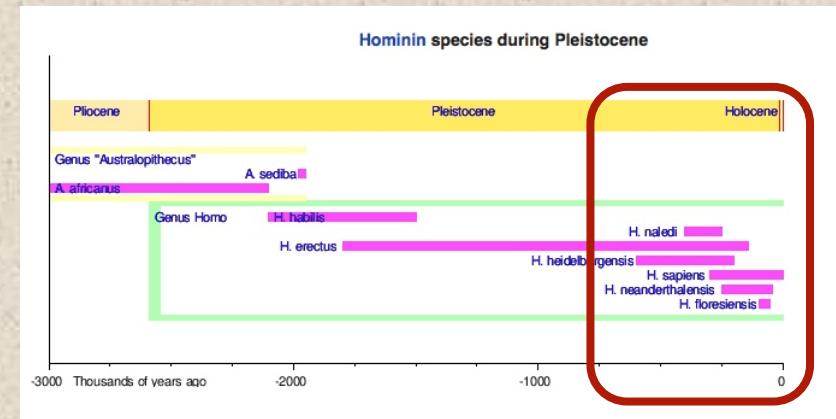
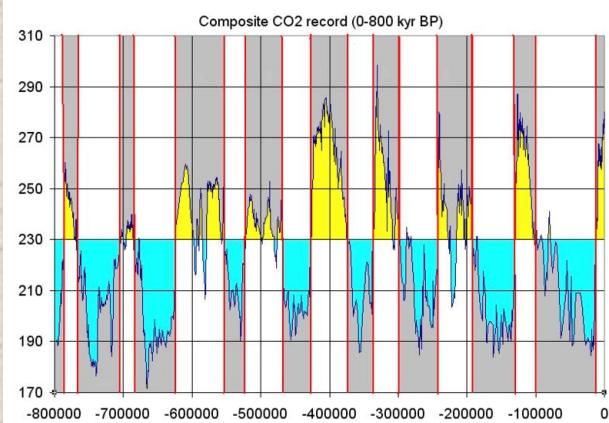


Model asserts that areas of endemism have been formed by watersheds and climatic fluctuations throughout Pleistocene to recent ...



Has climate change been a driver of speciation?

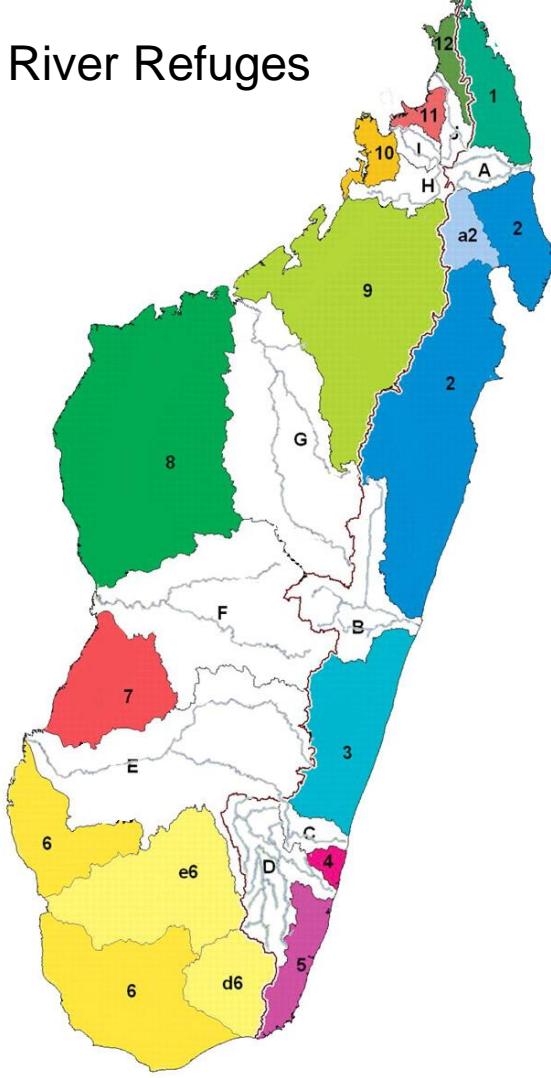
Climate Fluctuation during the Pleistocene



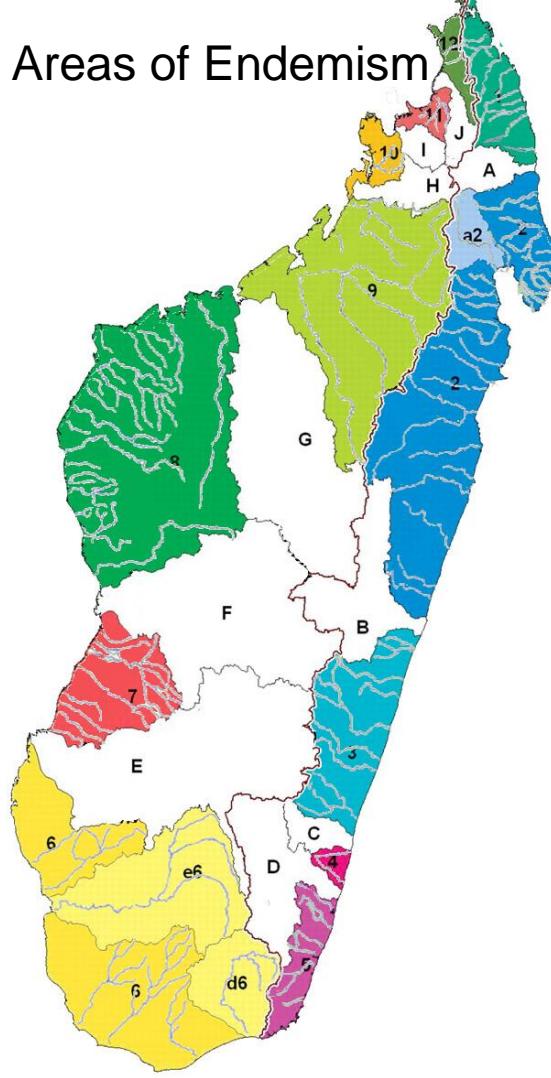
Lineage diversification
within hominins

~ 2.6 mya through ~ 12 kya

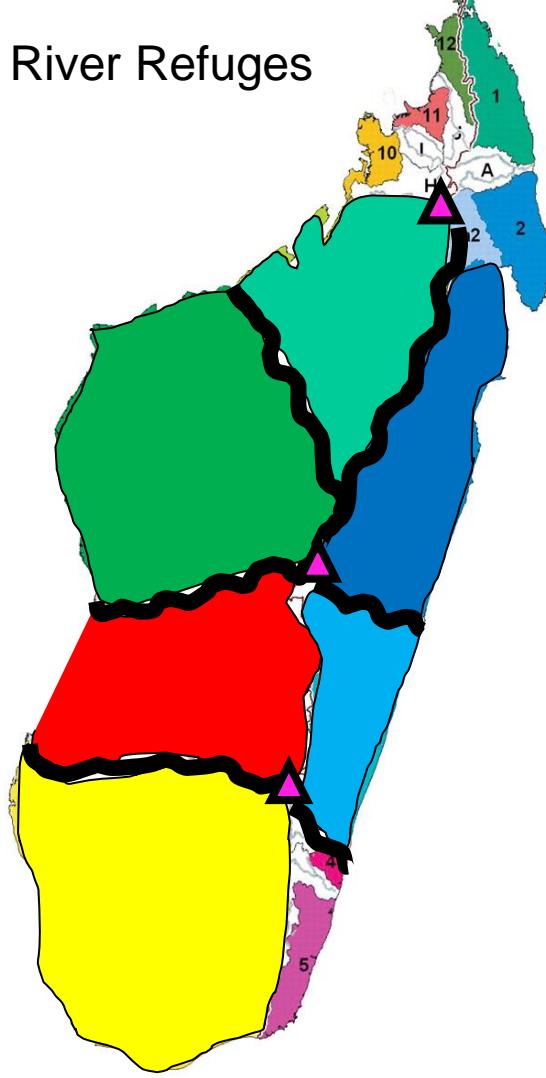
River Refuges



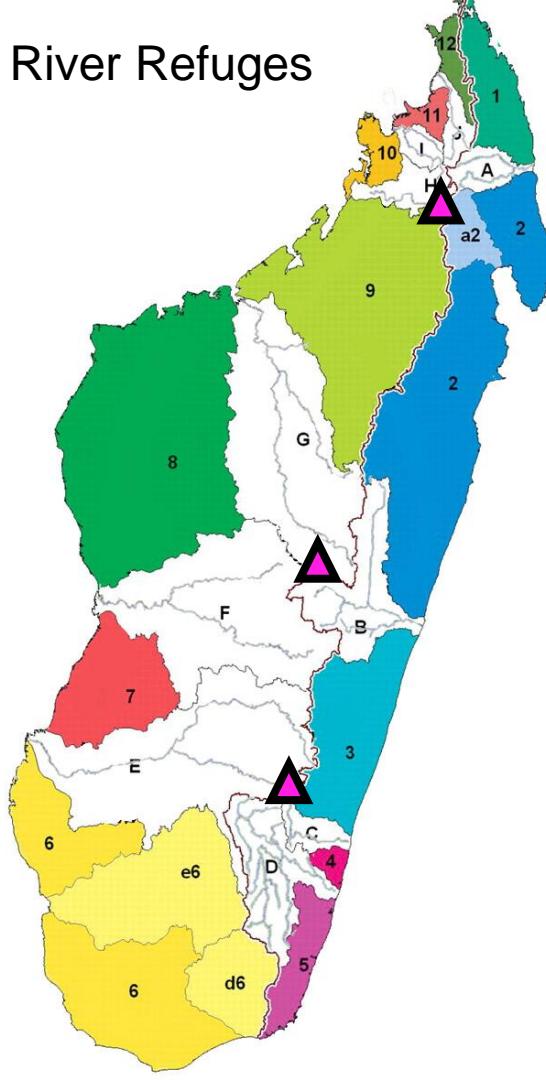
Areas of Endemism



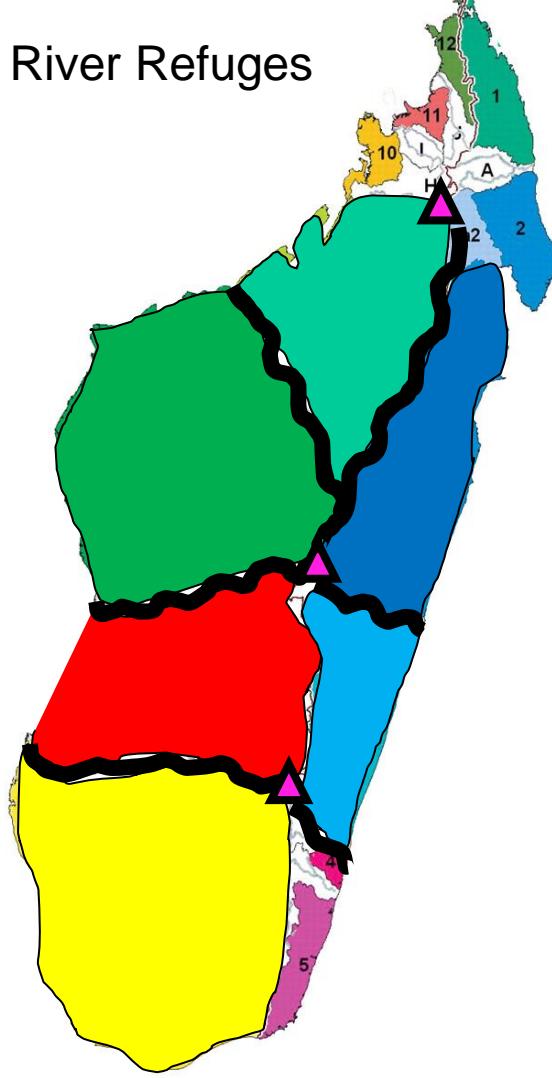
River Refuges



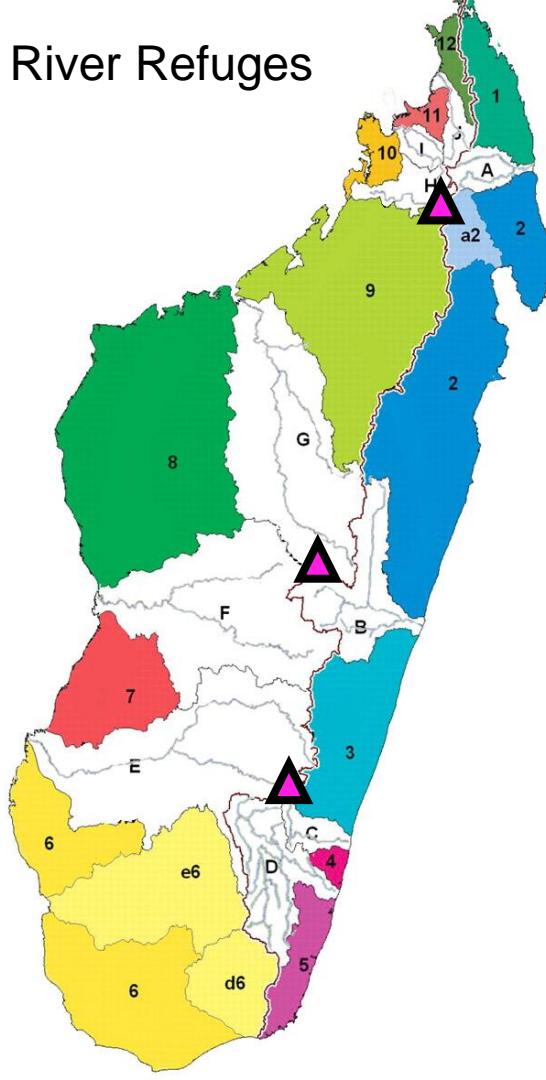
River Refuges



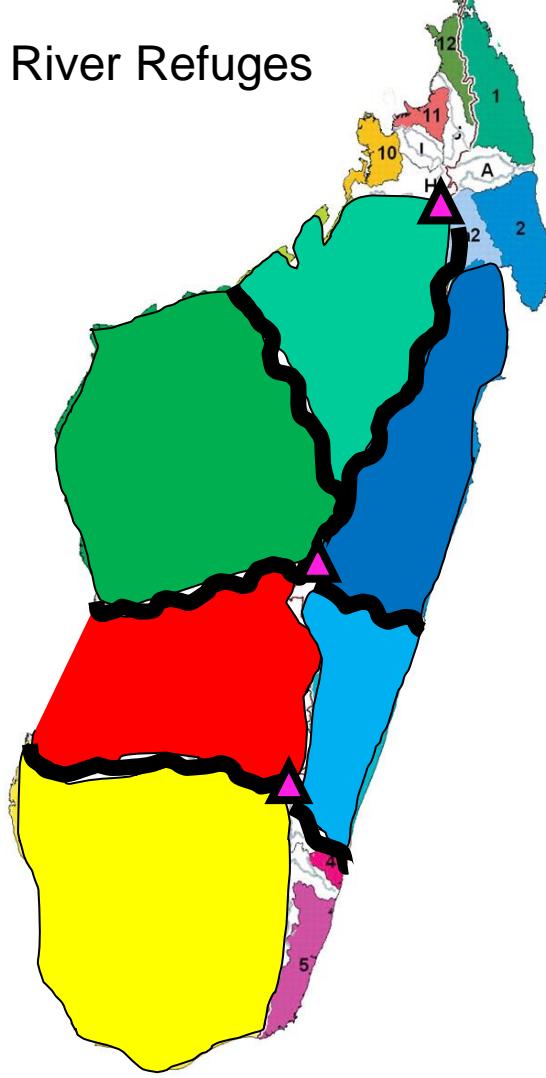
River Refuges



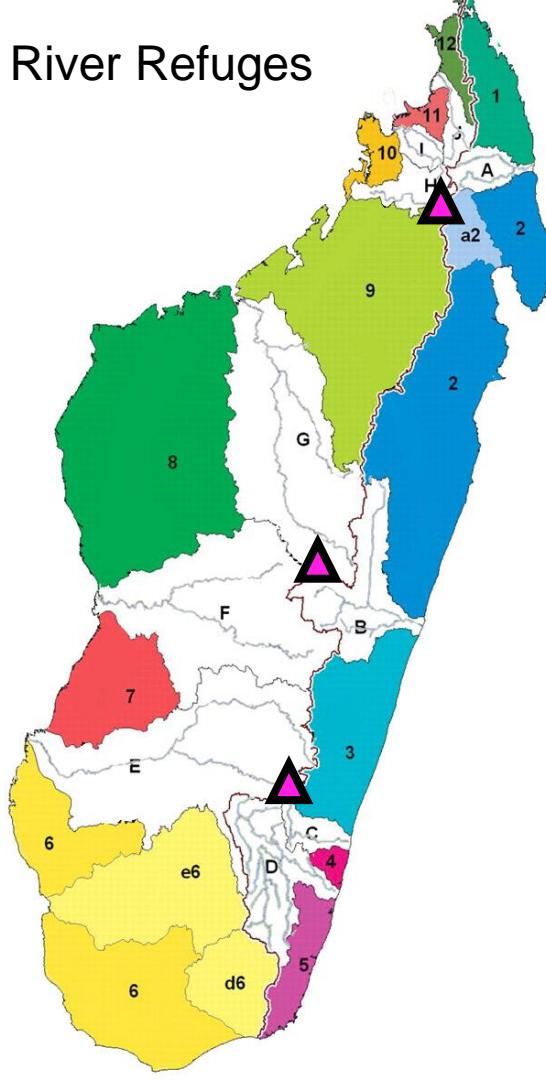
River Refuges



River Refuges

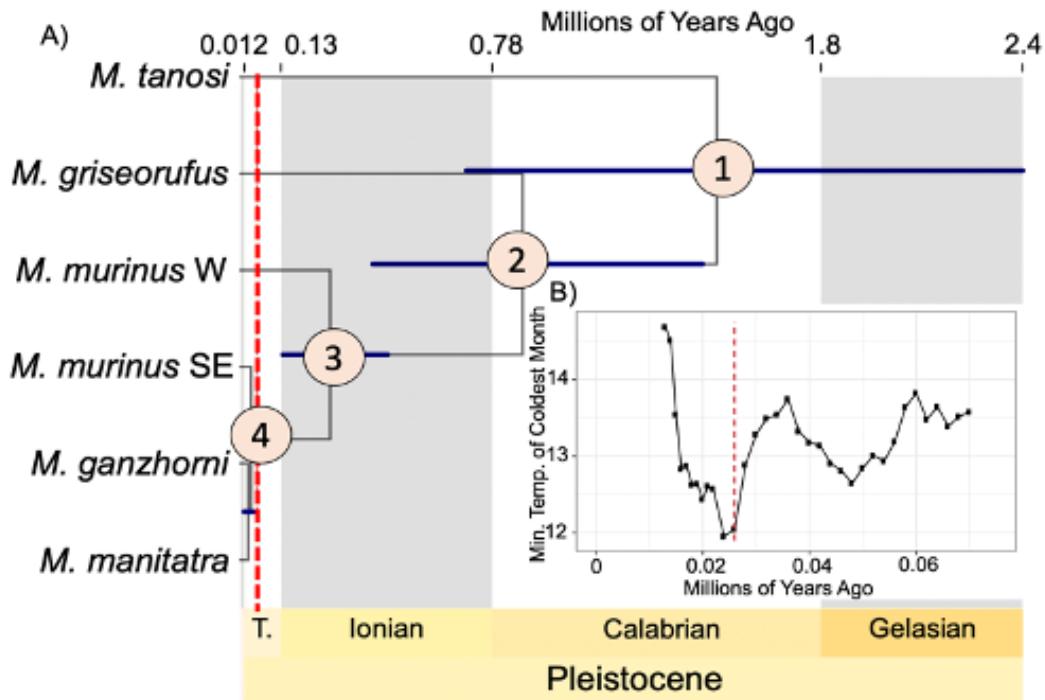


River Refuges



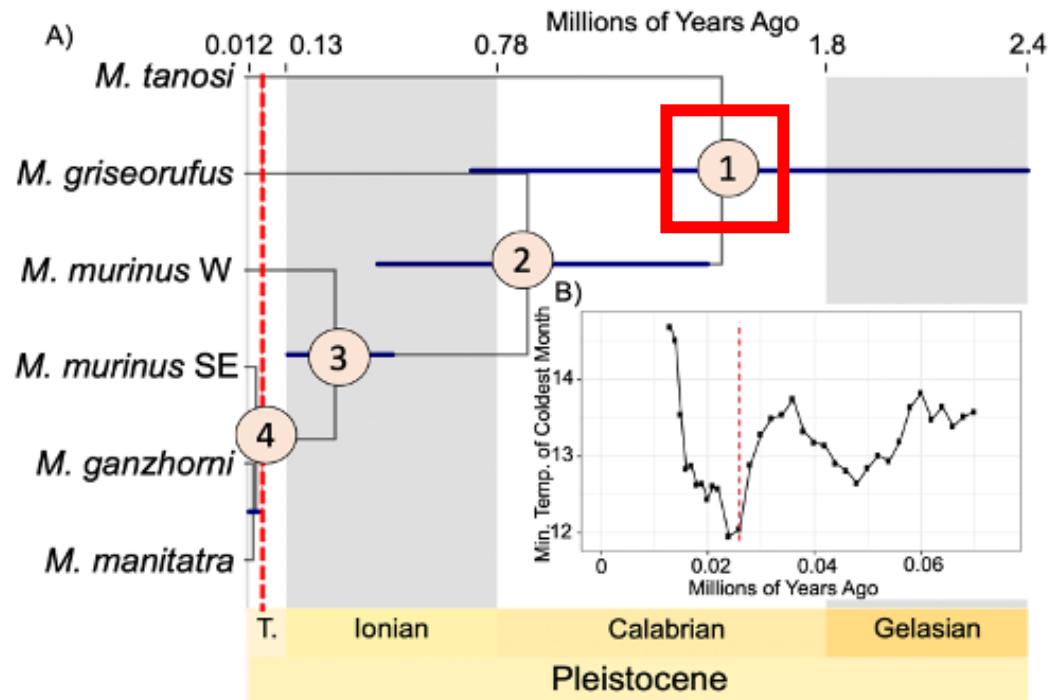
**If this model is “correct”
the age of the splits should
be of the appropriate age
(just for starters)**

Divergence Time Estimation



A “speciation continuum”

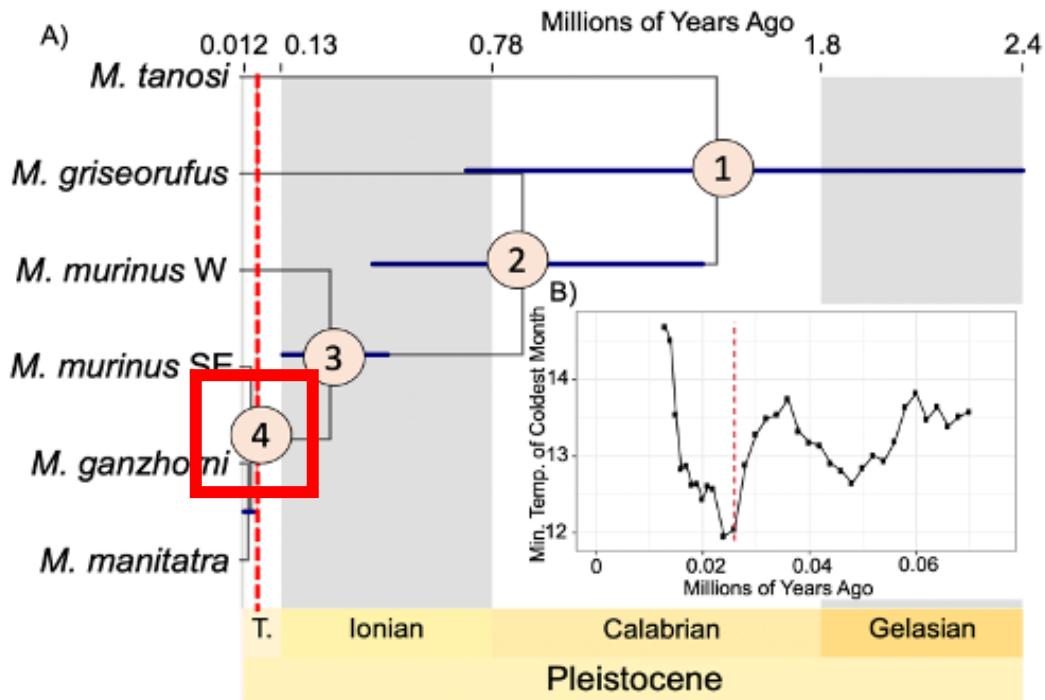
Figure 6: Speciation Continuum of Focal Lineages. **A)** The radiation among *M. murinus* (SE), *M. ganzhorni*, and *M. manitatra* (Node 4) is estimated to coincide precisely with the Last Glacial Maximum at 26,000 years ago (illustrated with dashed line). **B)** Paleoclimatic temperatures for SE Madagascar based on simulations under the HadCM3 circulation model (84).



~ 1.2 mya

A “speciation continuum”

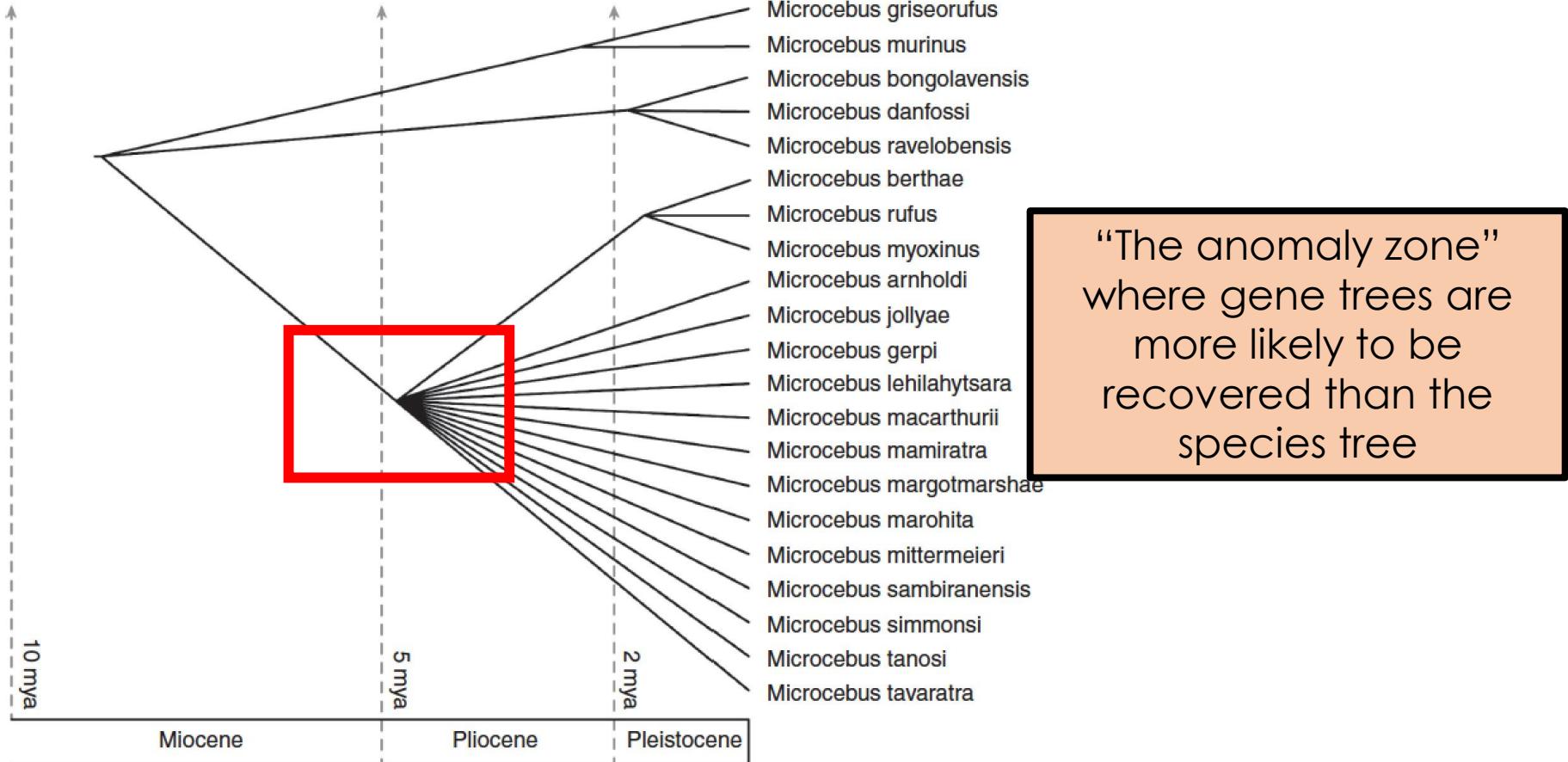
Figure 6: Speciation Continuum of Focal Lineages. **A)** The radiation among *M. murinus* (SE), *M. ganzhorni*, and *M. manitatra* (Node 4) is estimated to coincide precisely with the Last Glacial Maximum at 26,000 years ago (illustrated with dashed line). **B)** Paleoclimatic temperatures for SE Madagascar based on simulations under the HadCM3 circulation model (84).



~ 26K years ago
(last glacial maximum)

A “speciation continuum”

Figure 6: Speciation Continuum of Focal Lineages. **A)** The radiation among *M. murinus* (SE), *M. ganzhorni*, and *M. manitatra* (Node 4) is estimated to coincide precisely with the Last Glacial Maximum at 26,000 years ago (illustrated with dashed line). **B)** Paleoclimatic temperatures for SE Madagascar based on simulations under the HadCM3 circulation model (84).

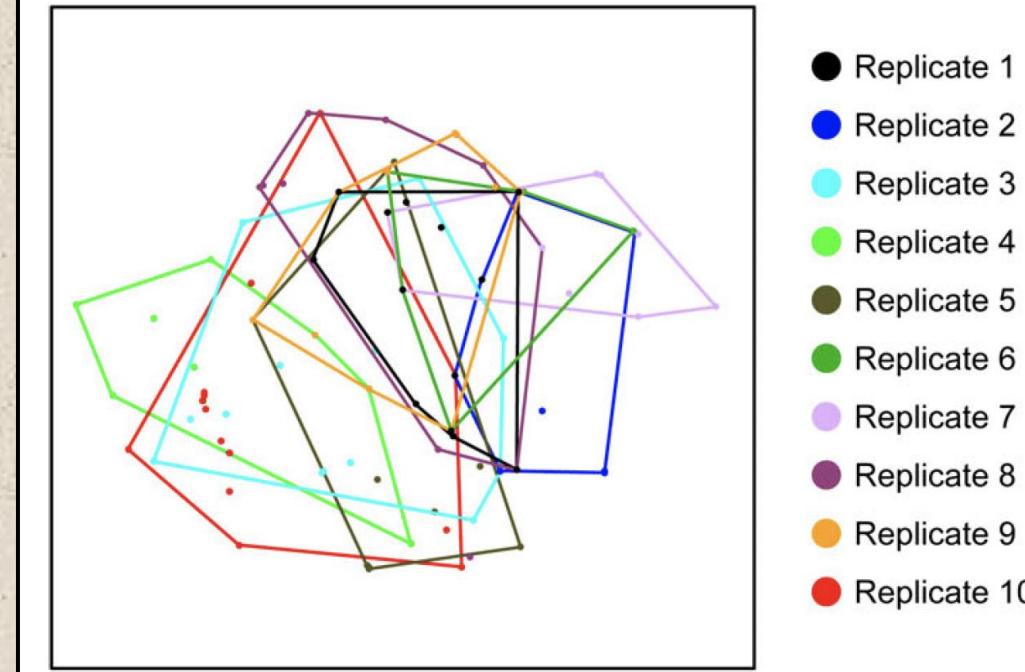


Phylogenetic resolution is highly sensitive to sample of chosen loci in concatenated phylogenetic analysis (in mouse lemurs)

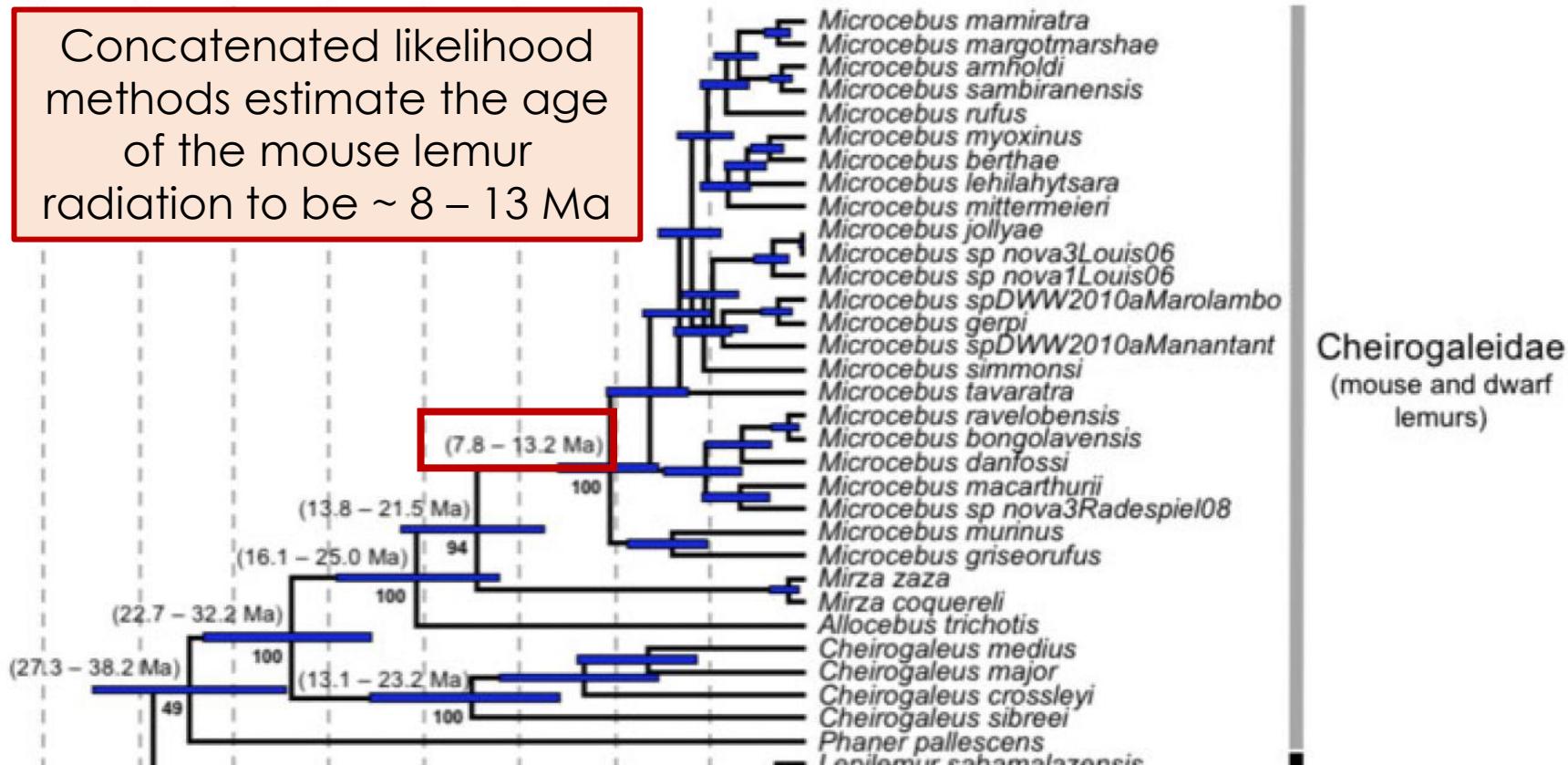


Dave Weisrock

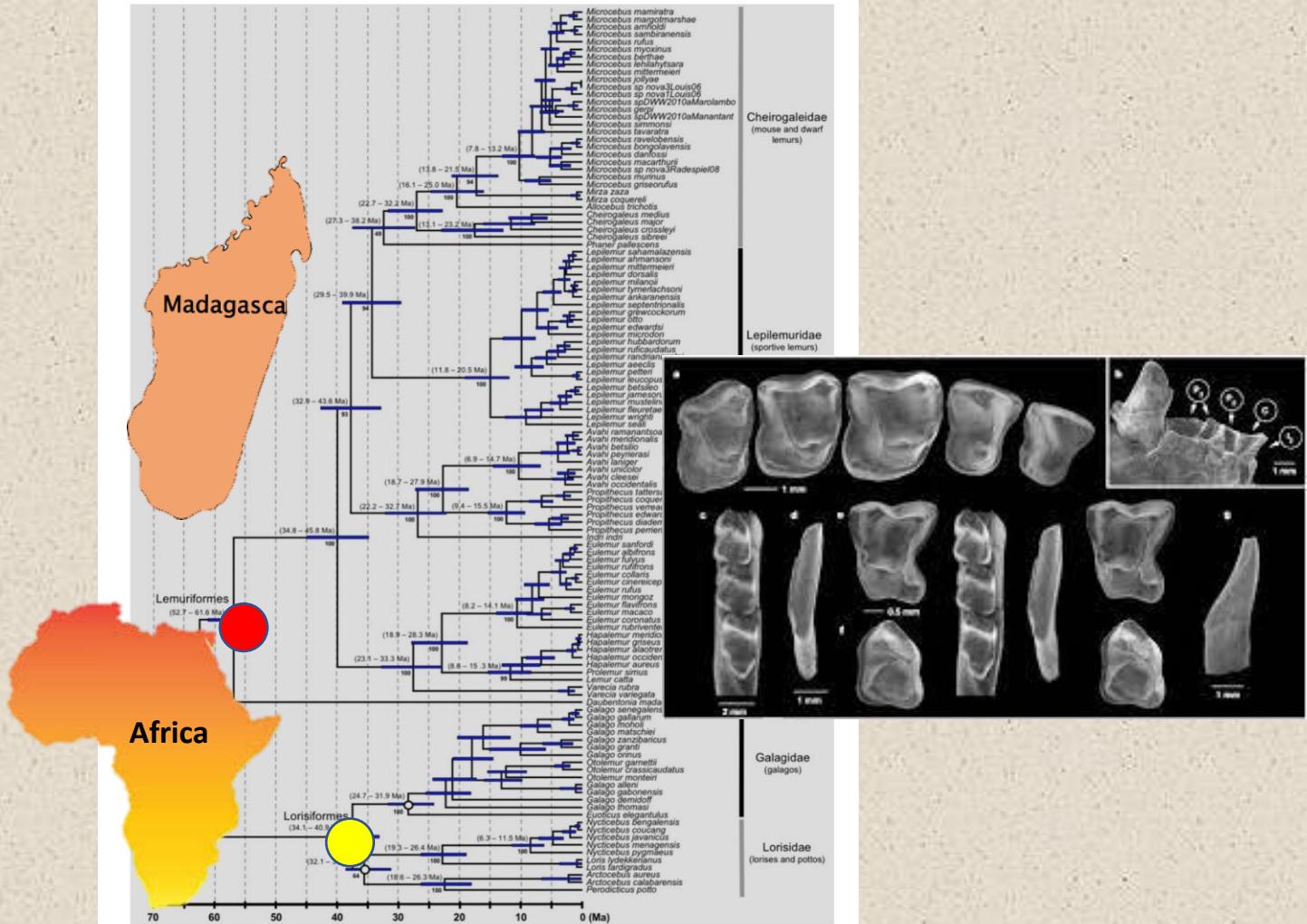
B) Concatenated nuclear and mitochondrial data



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



No fossil record!!!



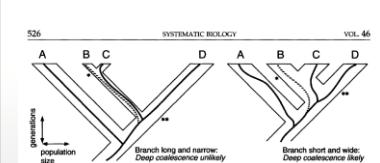
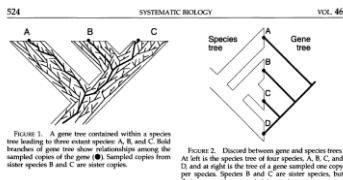
AKA, Coalescent Theory ...

Syst. Biol. 46(3):523–536, 1997

GENE TREES IN SPECIES TREES

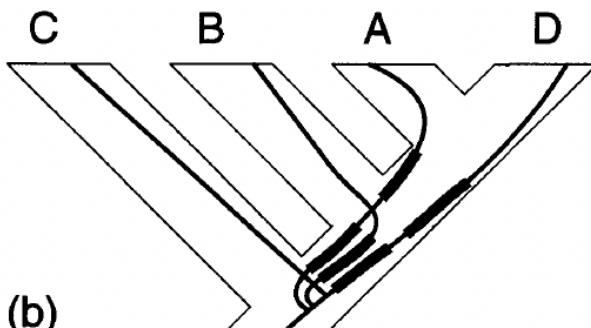
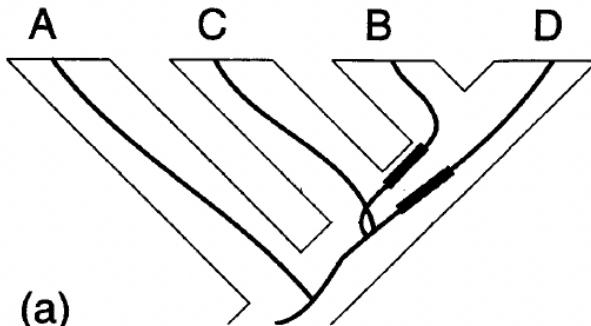
WAYNE P. MADDISON

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

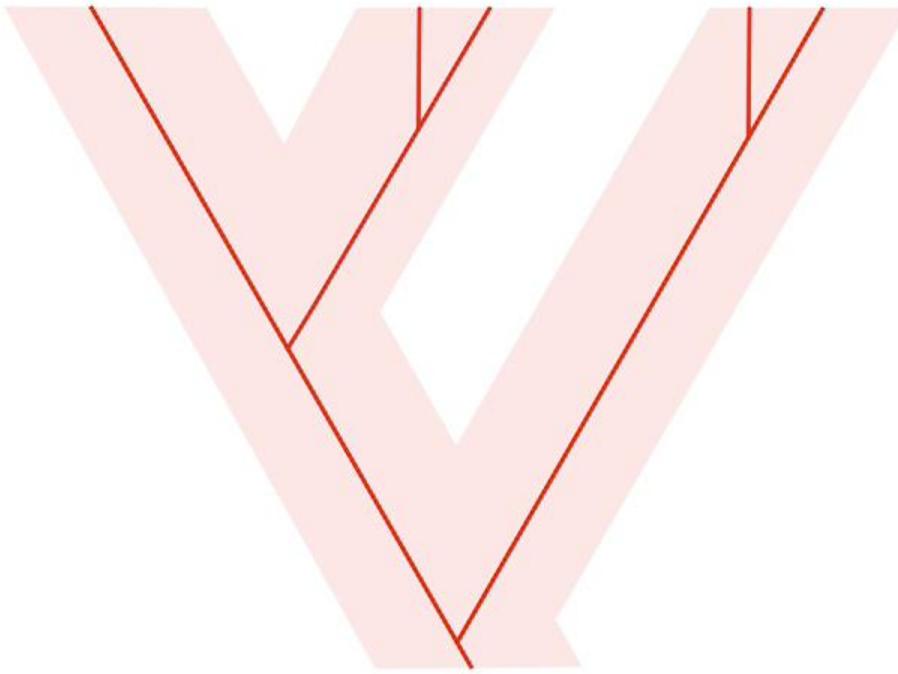


530

SYSTEMATI



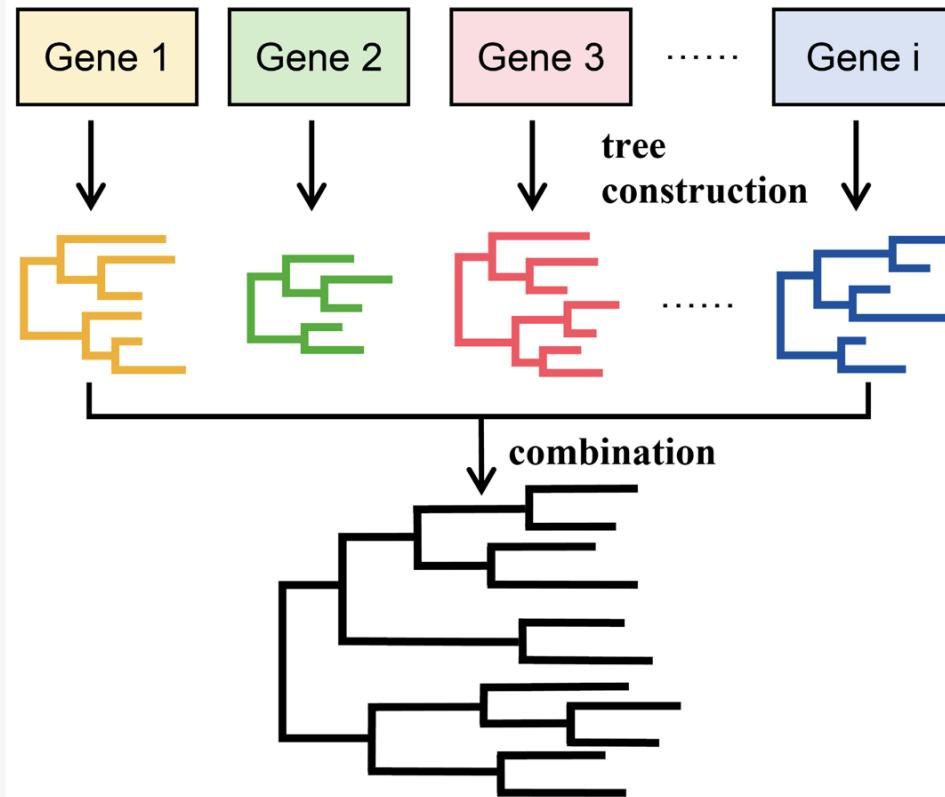
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AGCACTGACC CGGGATGCTACGATCGACATCGACGACACTAGCCGGGTTCATCGATCACGCAT GCGACT
AGCACTGACC CGGGATGCTACGATCGACATGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT
AGCACTGACC CGGGATGCTACGATCGACATCGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT
AGCACTGACC CGGGATGCAACGATCGACATGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT
AGCACTGACC CGGGATGCTACGATCGACATGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT



Animation courtesy of Kelsie Hunnicutt

Coalescent Approach (“it’s the gene trees, stupid”)

Figure 8. Strategies and principles of the coalescence phylogeny method for constructing phylogenetic trees.



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

Thank you, Peter, Laura, and Claudia!

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
- Increasingly clear that concatenation is inferior to MSC for estimating species tree
- Theoretically, coalescent times should be as old or older than speciation events

**So how old IS the mouse lemur
clade???**

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.

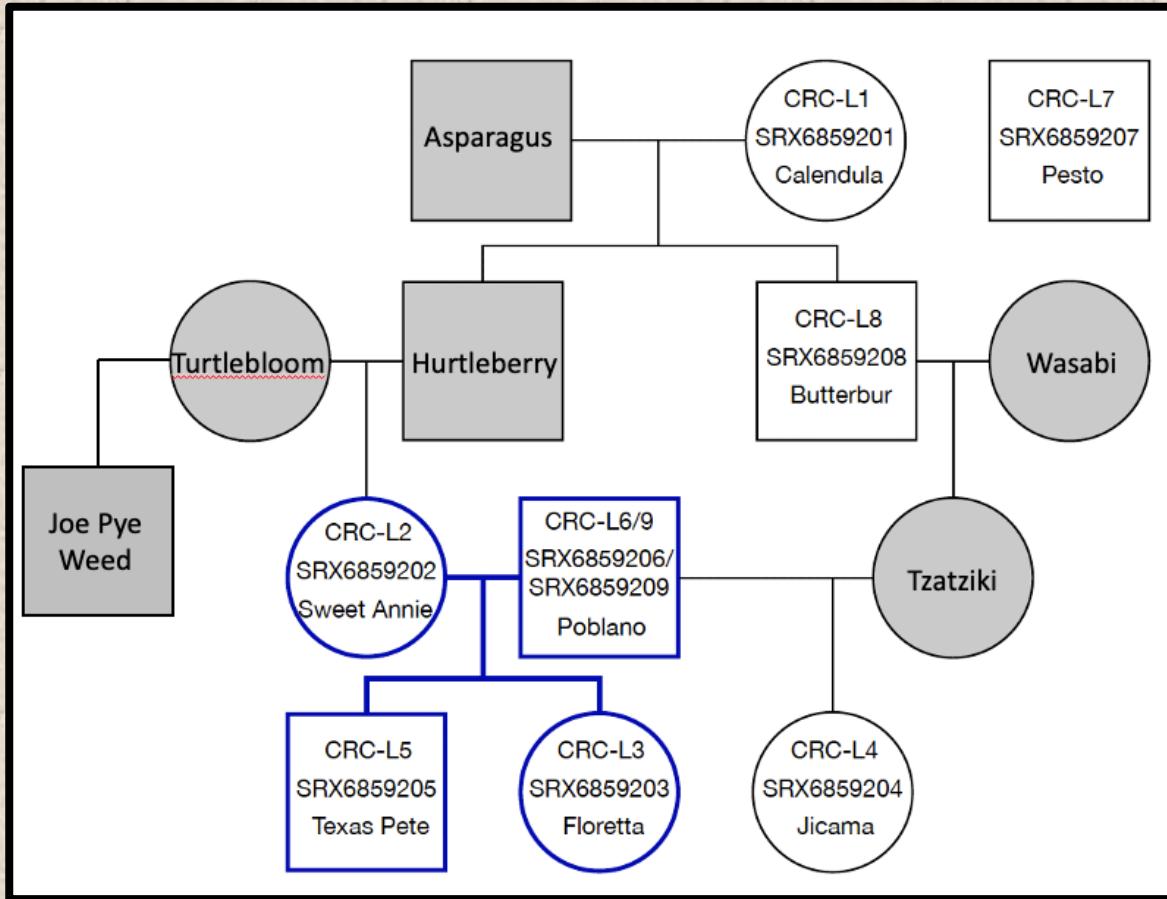
How to calibrate coalescent trees to geological time

Jul 3, 2016 • Mario dos Reis

There has been much interest recently in using the multi-species coalescent to estimate species phylogenies from molecular data. The advantage of the method is that incomplete lineage sorting (the discrepancy between gene trees and the species tree), and ancestral polymorphism are accounted for during phylogenetic inference. Bayesian implementations of the method are computationally expensive, and are best suited for inference among closely related species (or populations).

The figure below shows an example of a phylogeny of mouse lemurs (*Microcebus* spp.) estimated from RADseq (restriction site associated DNA sequencing) data using the program BPP (Yang, 2015), which implements the multi-species coalescent. Each node in the tree represents a speciation event, with the node ages given as numbers of substitutions per site. The blue bars represent the 95% credibility interval of the node age. For example, the molecular distance from the last common ancestor of *M. rufus* and *M. berthae* to the present is 1.29×10^{-4} substitutions per site. If we knew the molecular substitution rate per year for mouse lemurs, we could calibrate the tree to geological time, that is, we could convert the node ages from units of substitution per site to units of real time. I'll explain how to do so in this post.

Using pedigrees to estimate de novo mutation rates



Problems (just a few):

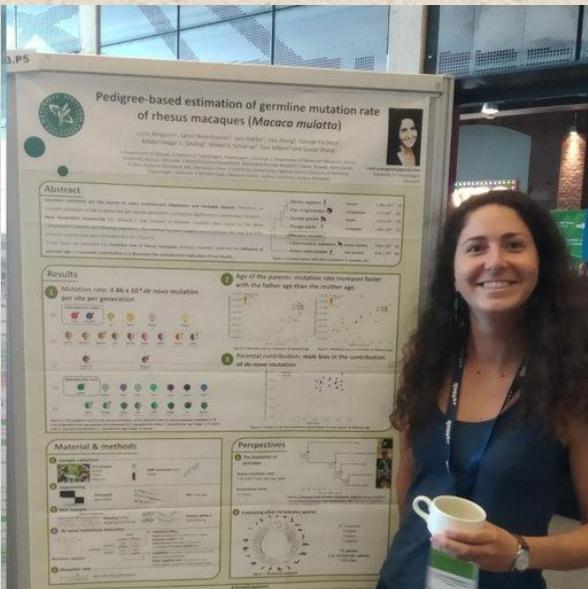
- Depth of coverage (some mutations will be called with more confidence than others)
- Genome assembly really, really matters (need long contigs to avoid, as much as possible, missing or misassembled regions due to highly repetitive regions)
- The number of “mutations” caused by sequencing error will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



**And you are looking for maybe
100 mutations in a single
generation!**

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

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



Lucie Bergeron

eLife

TOOLS AND RESOURCES



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

Lucie A Bergeron^{1*}, Søren Besenbacher², Tychele Turner³, Cyril J Versoza⁴, Richard J Wang⁵, Alivia Lee Price¹, Ellie Armstrong⁶, Meritxell Riera⁷, Jedidiah Carlson⁸, Hwei-yan Chen¹, Matthew W Hahn⁵, Kelley Harris⁸, April Snøfrid Kleppe², Elora H López-Nandam⁹, Priya Moorjani¹⁰, Susanne P Pfeifer¹¹, George P Tiley¹², Anne D Yoder¹², Guojie Zhang¹, Mikkel H Schierup^{7*}

2022

“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.”

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



the
geneticssociety

www.nature.com/hdy

ARTICLE

Check for updates

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

C. Ryan Campbell^{1,2,8}, George P. Tiley^{1,8}, Jelmer W. Poelstra¹, Kelsie E. Hunnicutt^{1,6}, Peter A. Larsen^{1,7}, Hui-Jie Lee^{1,3}, Jeffrey L. Thome⁴, Mario dos Reis^{1,5} and Anne D. Yoder^{1,8}

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Low CpG rates

C.R. Campbell et al.

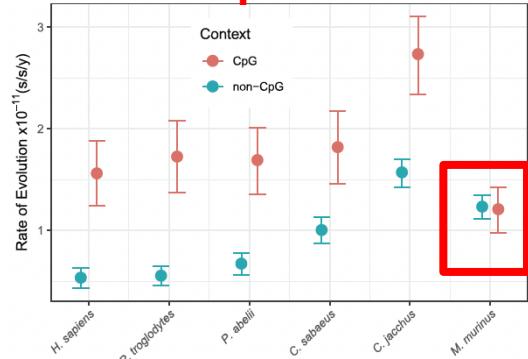


Fig. 4 Context-dependent relaxed clock analysis shows low rates of C-T substitution rates at CpG sites in the gray mouse lemur. C-T substitution-rate estimates at non-CpG versus CpG sites are compared for six species of primate, including the gray mouse lemur (*M. maurus*). Note that with the exception of *M. maurus*, all primates examined show significantly higher CpG rates than non-CpG rates. The C-T substitution rates at non-CpG and CpG sites are nearly identical in *M. maurus*. Error bars represent 95% highest posterior densities.

the
geneticssociety

www.nature.com/hdy

ARTICLE

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

C. Ryan Campbell^{1,2*}, George P. Tiley^{1,3}, Jelmer W. Poelstra¹, Kelsie E. Hunnicutt^{1,6}, Peter A. Larsen^{1,7}, Hui-Jie Lee^{1,8}, Jeffrey L. Thorne⁴, Mario dos Reis^{1,9} and Anne D. Yoder^{1,5}

* The Author(s), under exclusive licence to The Genetics Society 2021

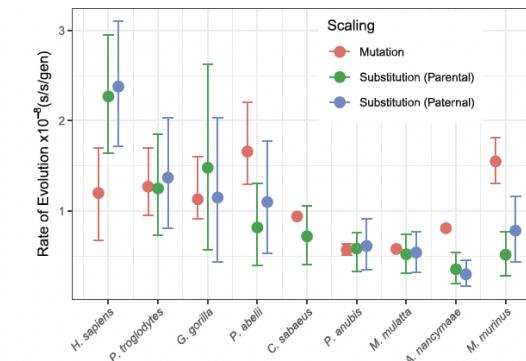


Fig. 5 Difference between mutation and substitution rates among primates. Error bars around substitution rates are 95% highest posterior density intervals from a Bayesian relaxed-clock analysis. Credible intervals are given for mutation rates where available from published data. Substitution rates are scaled from per-year to per-generation based on the average parent (parental) age at the time of conception, except for *C. sabaeus* where data were not available and the generation time assumed from external information. Where age information on parents was available, substitution rates were also scaled by the average father (paternal) age. Data are given in Table S3.



- We estimated the **mutation rate to be among the highest calculated for a mammal** at $1.52 \text{ Å} \sim 10^{-8}$ (95% credible interval: $1.28 \text{ Å} \sim 10^{-8}$ – $1.78 \text{ Å} \sim 10^{-8}$) mutations/site/generation. Further, we found an **unexpectedly low count of paternal mutations**, and only a modest overrepresentation of mutations at CpG sites.



 OPEN ACCESS  PEER-REVIEWED

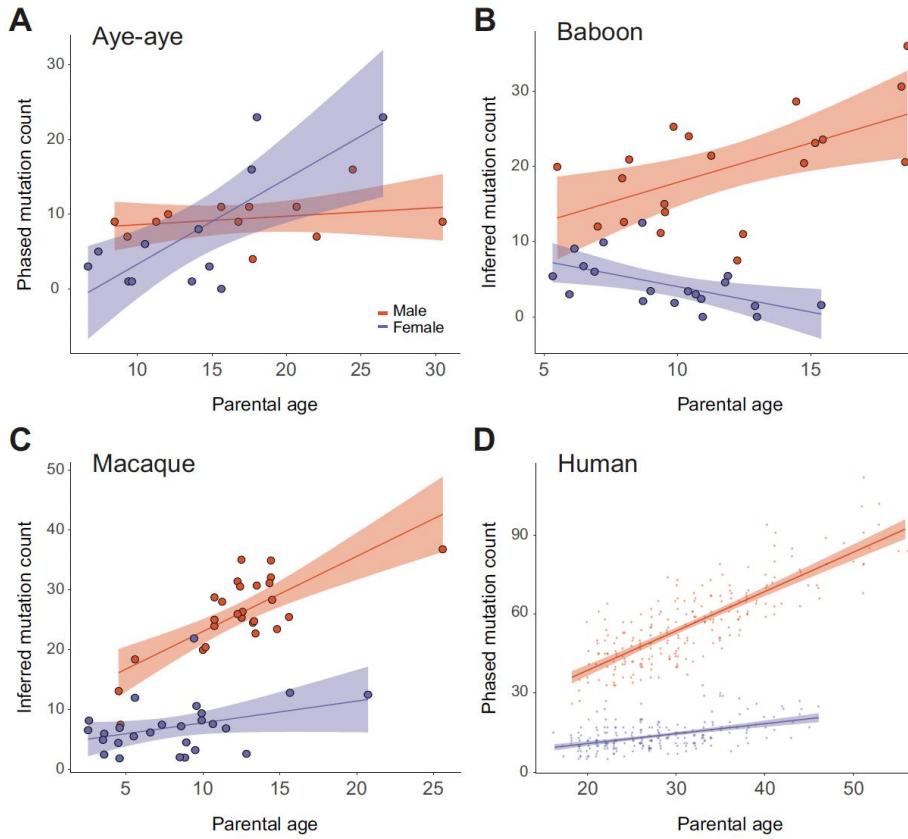
DISCOVERY REPORT

Unprecedented female mutation bias in the aye-aye, a highly unusual lemur from Madagascar

Richard J. Wang, Yadira Peña-García, Muthuswamy Raveendran, R. Alan Harris, Thuy-Trang Nguyen, Marie-Claude Gingras, Yifan Wu, Lesette Perez, Anne D. Yoder, Joe H. Simmons, Jeffrey Rogers, Matthew W. Hahn 

Version 2

Published: February 7, 2025 • <https://doi.org/10.1371/journal.pbio.3003015>



**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)
- Repproductively mature at age = 1 year
- Only one litter per year (two – three offspring)



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)





Trends in Genetics

CellPress
REVIEWS

Review

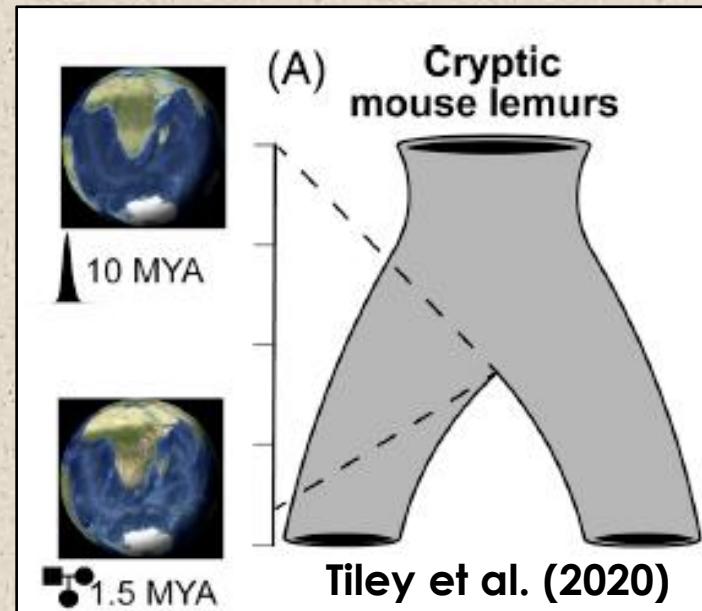
Molecular Clocks without Rocks: New Solutions for Old Problems

George P. Tiley ,¹ Jelmer W. Poelstra,¹ Mario dos Reis,² Zheng Yang ,³ and Anne D. Yoder^{1,*}



MRCA = 8 – 10 Ma
(concatenation)

MRCA ~ 1.5 Ma
(MSC)



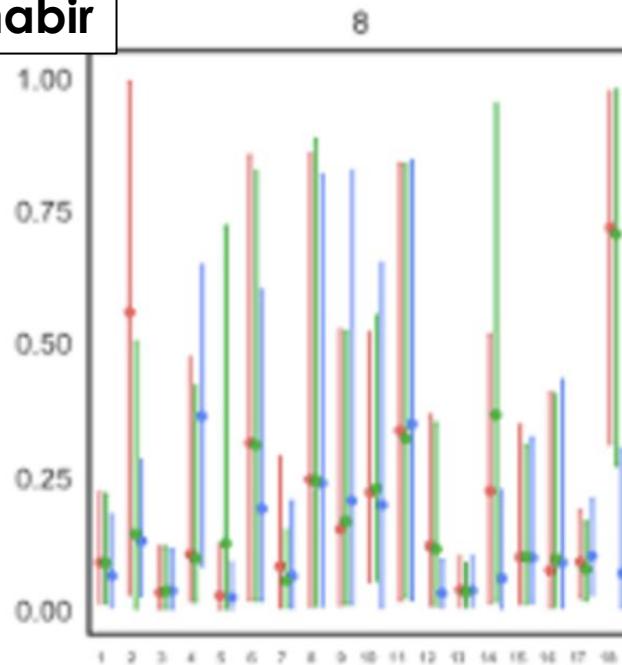
But why???



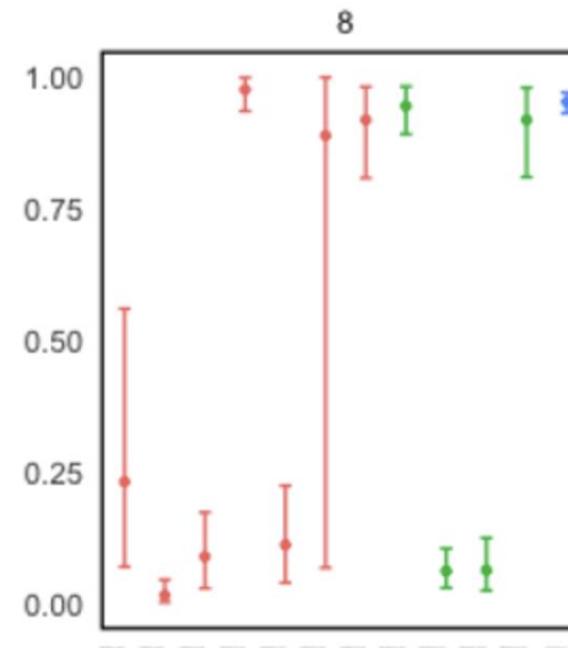
Comparison of Chromosome 8

Jason Mohabir

200 loci [3 runs]



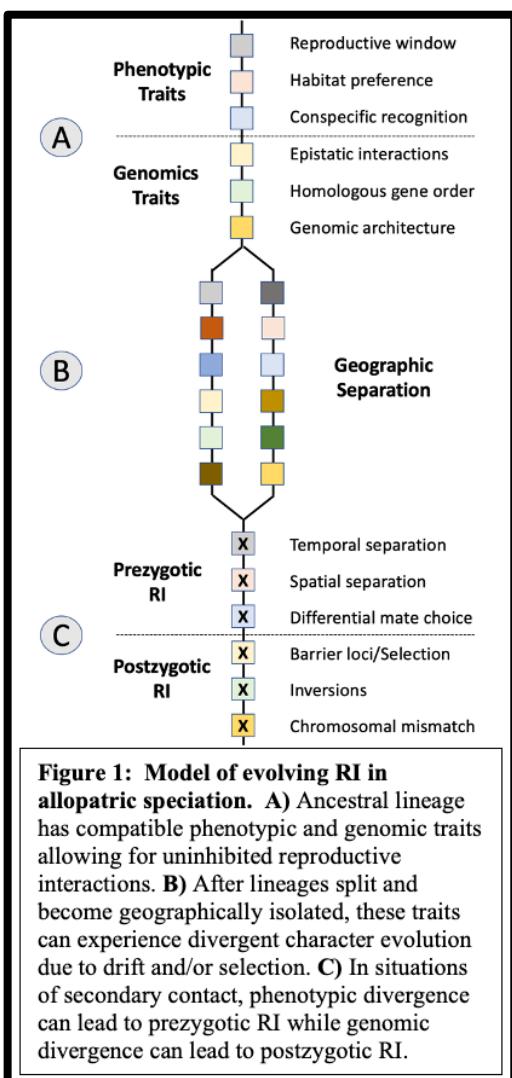
500 loci, 1000 loci, full chromosome



Break, anyone?

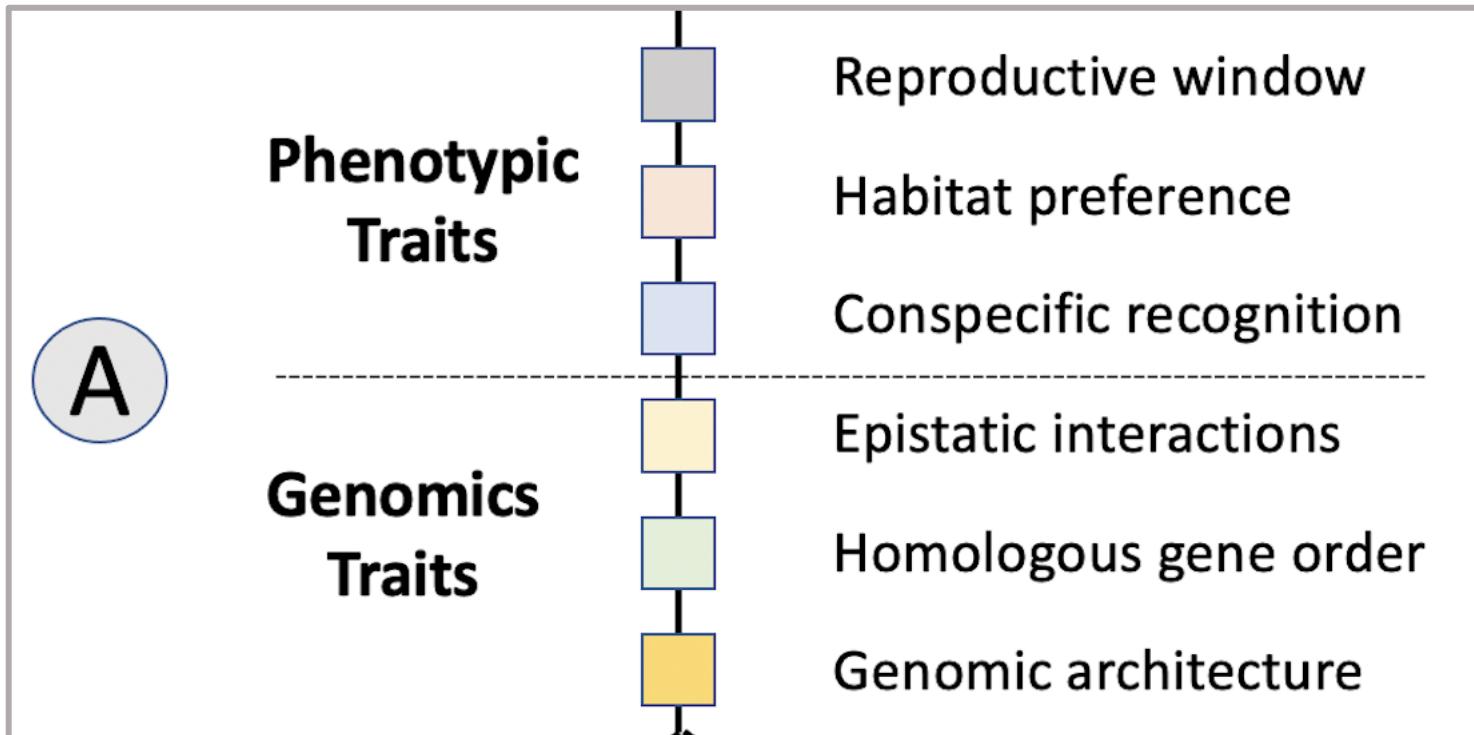
We've talked about patterns,
how about process???

SPECIATION!!!!

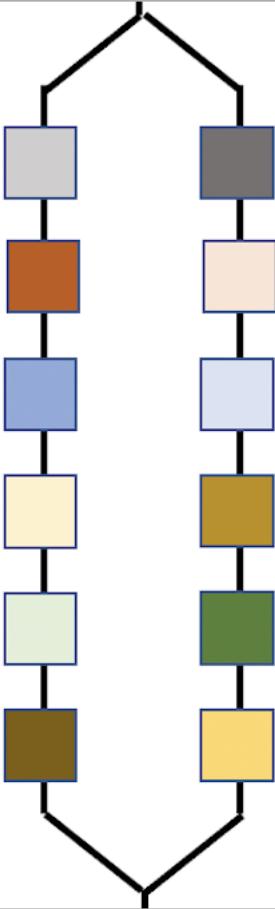


An expanded view of the DMI model

Ancestral Lineage

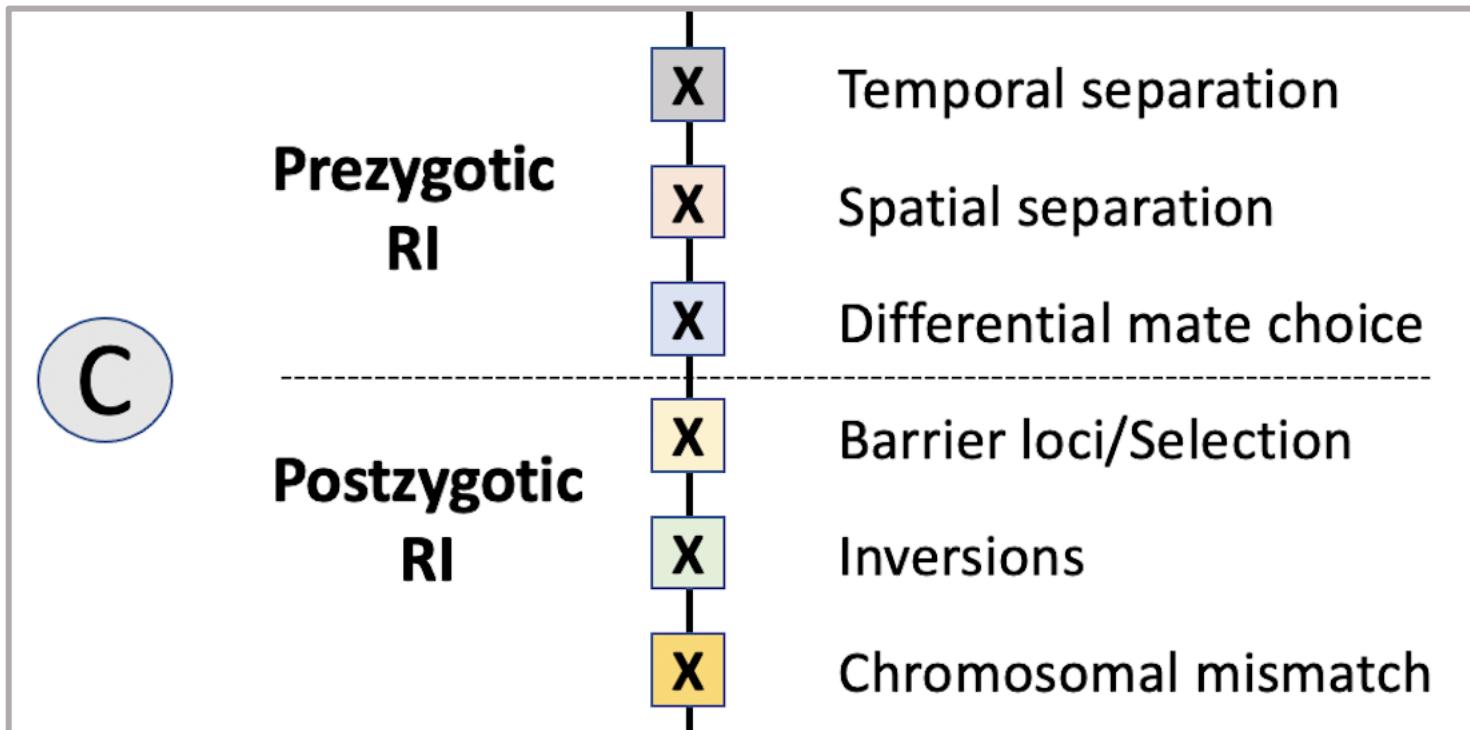


B



**Geographic
Separation**

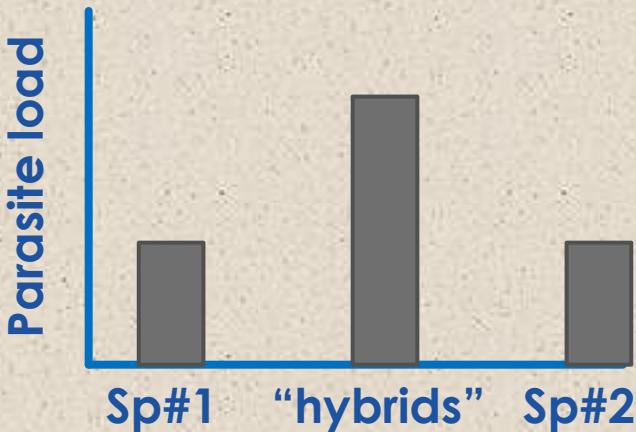
Secondary Contact





Mouse lemur speciation project (ongoing!)

Combination of microsatellite data, subtle morphological distinctions, and parasite loads have suggested that there is an active hybrid zone in the southeast



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

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

Gligor et al. 2009, Mol Ecol

Hybridization of mouse lemurs: different patterns under different ecological conditions

Andreas Hapke^{1*}, Mark Gligor^{1,2}, S Jacques Rakotondranary³, David Rosenkranz¹ and Oliver Zupke^{1,4}

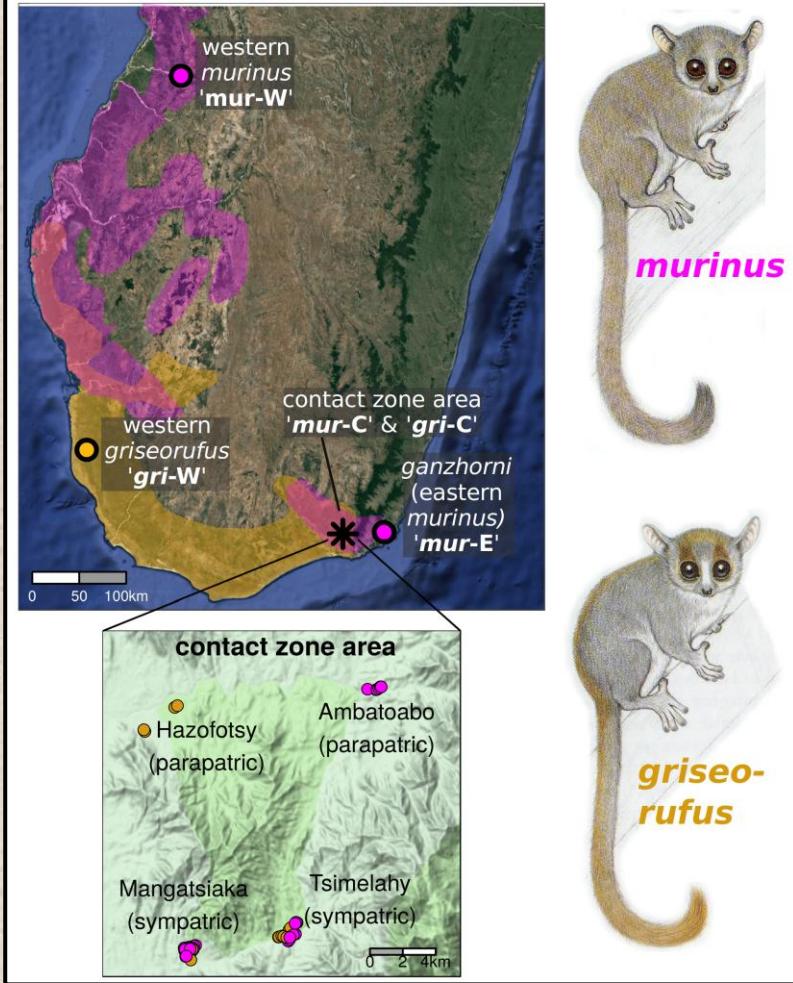
Hapke et al. 2011, BMC Ev Biol



Jelmer Poelstra

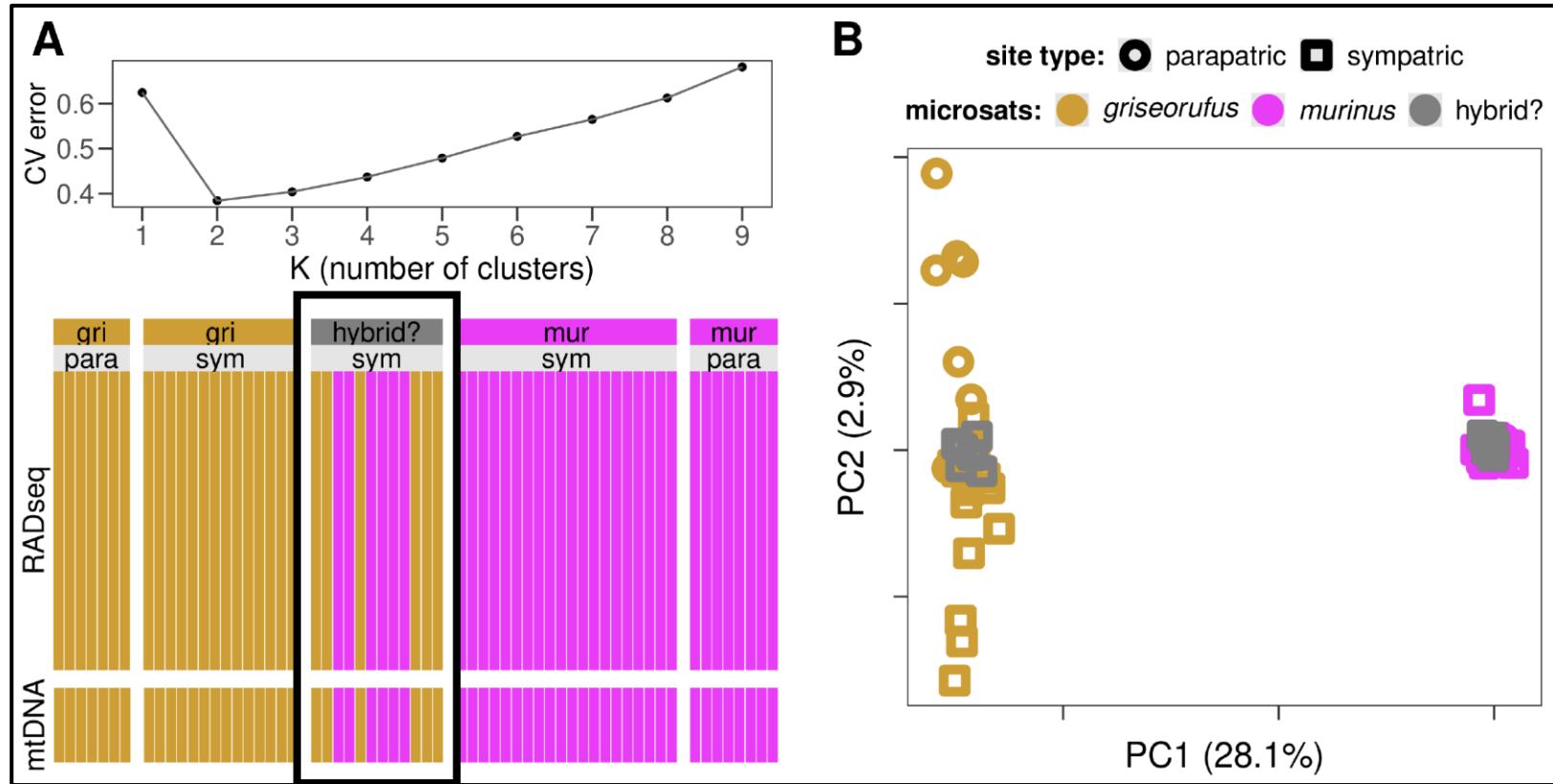


Jörg Ganzhorn



- 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*)

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





Slide courtesy of Jörg Ganzhorn

2022

PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research



Cite this article: Poelstra JW, Montero BK, Lüdemann J, Yang Z, Rakotondranary SJ, Hohenlohe P, Stetter N, Ganzhorn JU, Yoder AD. 2022 RADseq data reveal a lack of admixture in a mouse lemur contact zone contrary to previous microsatellite results.

Proc. R. Soc. B **289**: 20220596.

<https://doi.org/10.1098/rspb.2022.0596>

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

Jelmer W. Poelstra^{1,2}, B. Karina Montero³, Jan Lüdemann³, Ziheng Yang⁴, S. Jacques Rakotondranary^{3,5}, Paul Hohenlohe⁶, Nadine Stetter^{3,7}, Jörg U. Ganzhorn^{3,†} and Anne D. Yoder^{1,†}

¹Department of Biology, Duke University, Durham, NC 27708, USA

²Molecular and Cellular Imaging Center, Ohio State University, Wooster, OH 44691, USA

³Institute of Zoology, Department of Animal Ecology and Conservation, Universität Hamburg, Hamburg, 20146, Germany

⁴Department of Genetics, Evolution and Environment, University College London, London, UK

⁵Anthropobiologie et Développement Durable, Faculté des Sciences, Université d'Antananarivo, PO Box 906, Antananarivo 101, Madagascar

⁶Institute for Bioinformatics and Evolutionary Studies, Department of Biological Sciences, University of Idaho, Moscow, ID 83844, USA

⁷Bernhard Nocht Institute for Tropical Medicine, 20359 Hamburg, Germany



Five (possibly six)
diverging lineages in
the southeast ---
occurring in patterns
of both allopatry and
(near?) sympatry

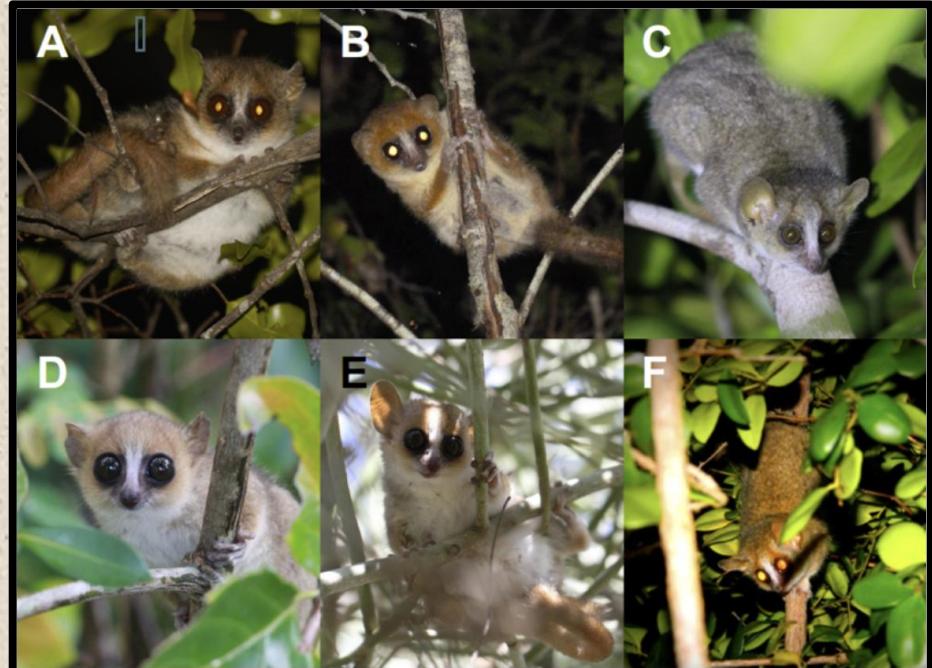


Figure 2 – The *Microcebus* spp. of Southeast Madagascar. Figure illustrates cryptic morphology of diverged lineages currently recognized as named species. **A)** *M. manitatra*, **B)** *M. tanosi*, **C)** *M. ganzhorni*, **D)** *M. tanosi* (pale variant), **E)** *M. griseorufus*, **F)** *Microcebus*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



Hannah Kania
**(genomics; TE
annotation)**



Paschalia Kapli
**(computational
genomics)**



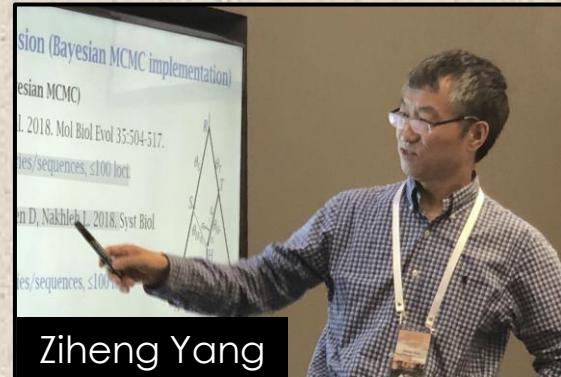
Carolina Segami
**(speciation
genomics)**



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



me



Ziheng Yang

Five (possibly six)
diverging lineages in
the southeast ---
occurring in patterns
of both allopatry and
(near?) sympatry

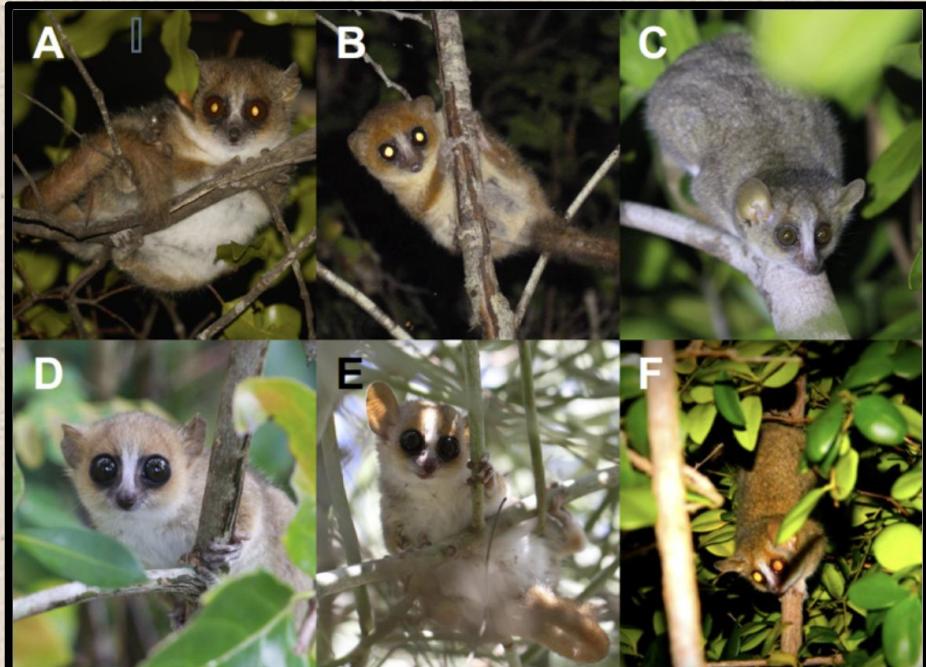
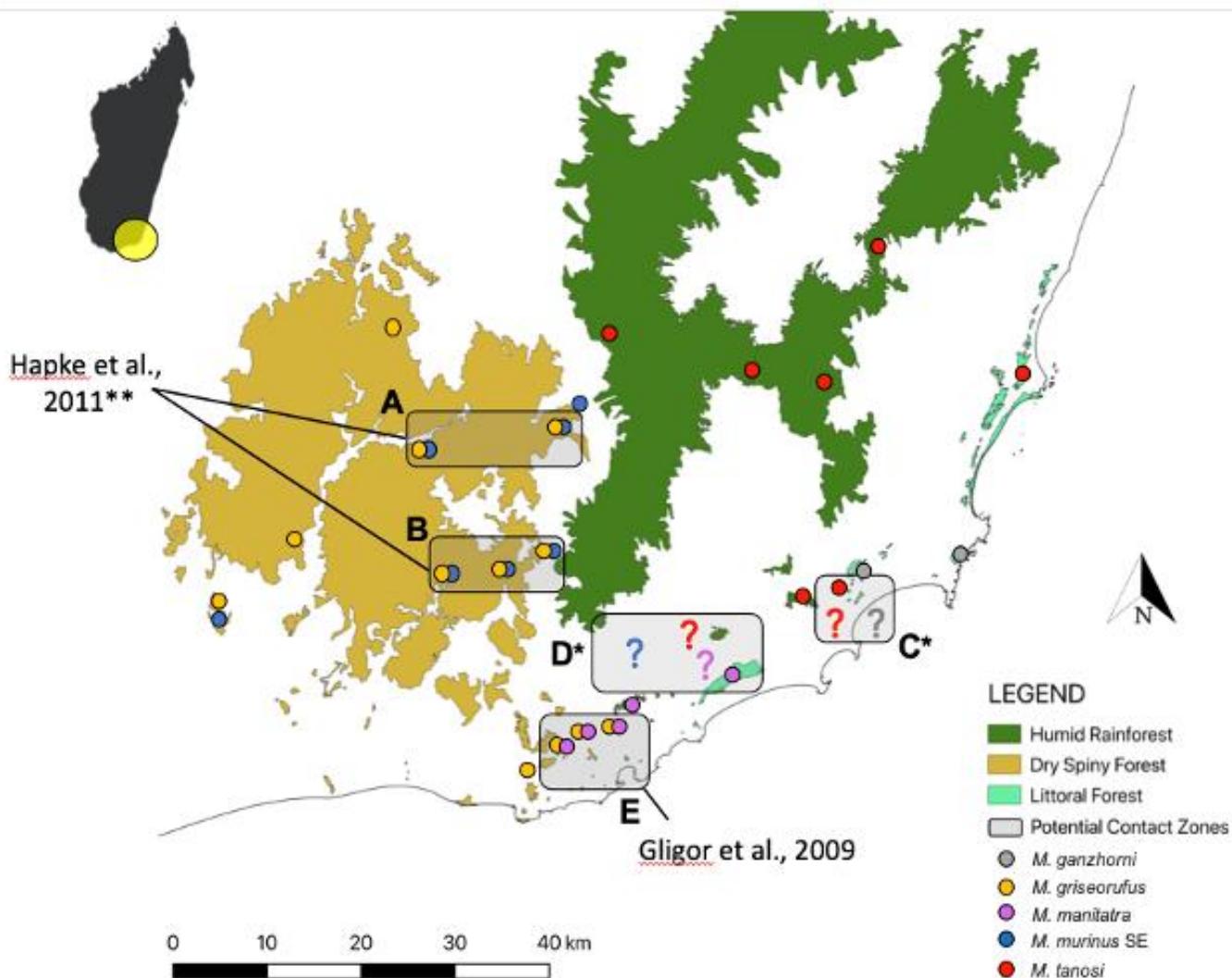


Figure 2 – The *Microcebus* spp. of Southeast Madagascar. Figure illustrates cryptic morphology of diverged lineages currently recognized as named species. **A)** *M. manitatra*, **B)** *M. tanosi*, **C)** *M. ganzhorni*, **D)** *M. tanosi* (pale variant), **E)** *M. griseorufus*, **F)** *Microcebus*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



Litoral forest



Spiny forest



Transition forest

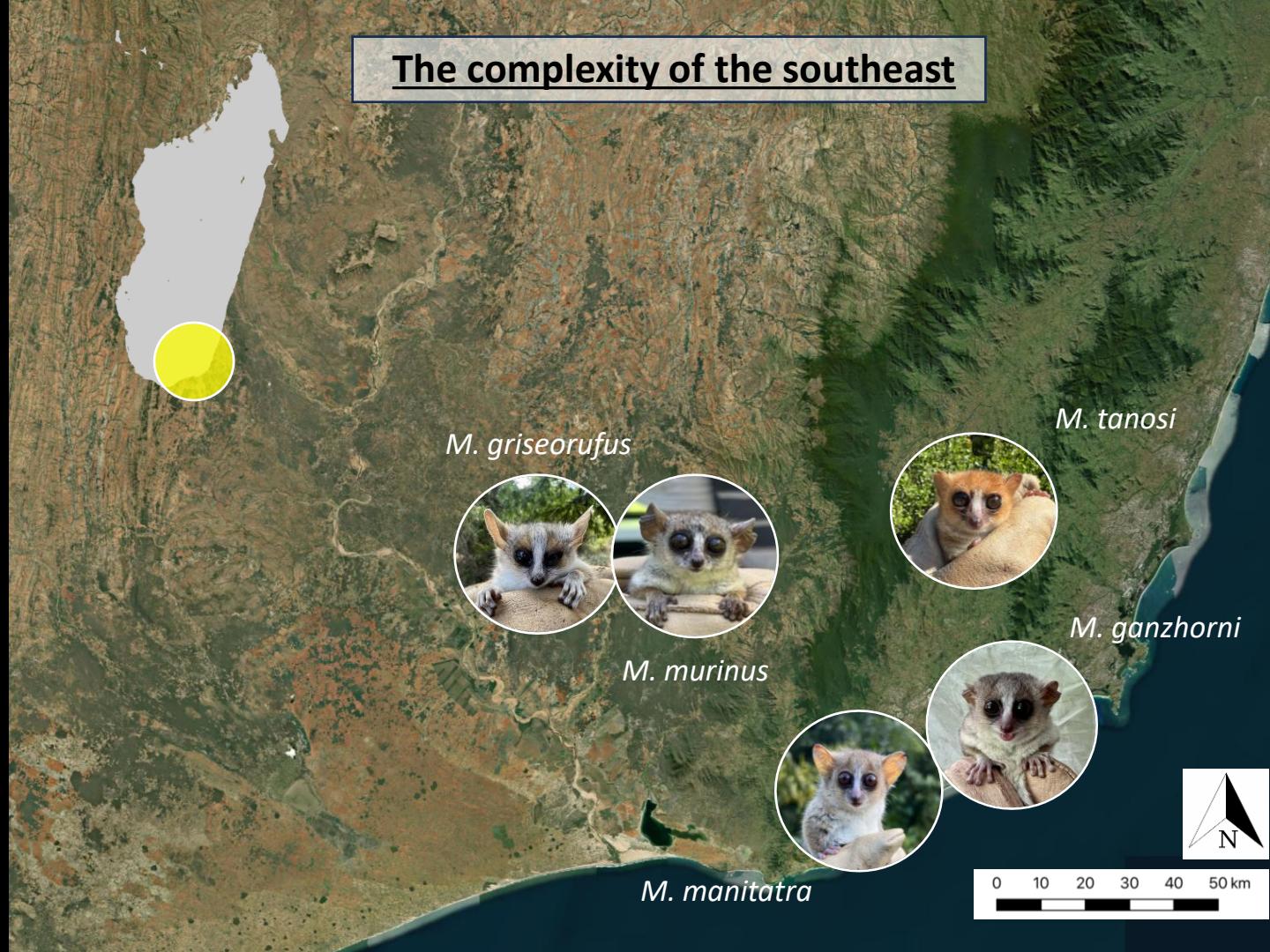


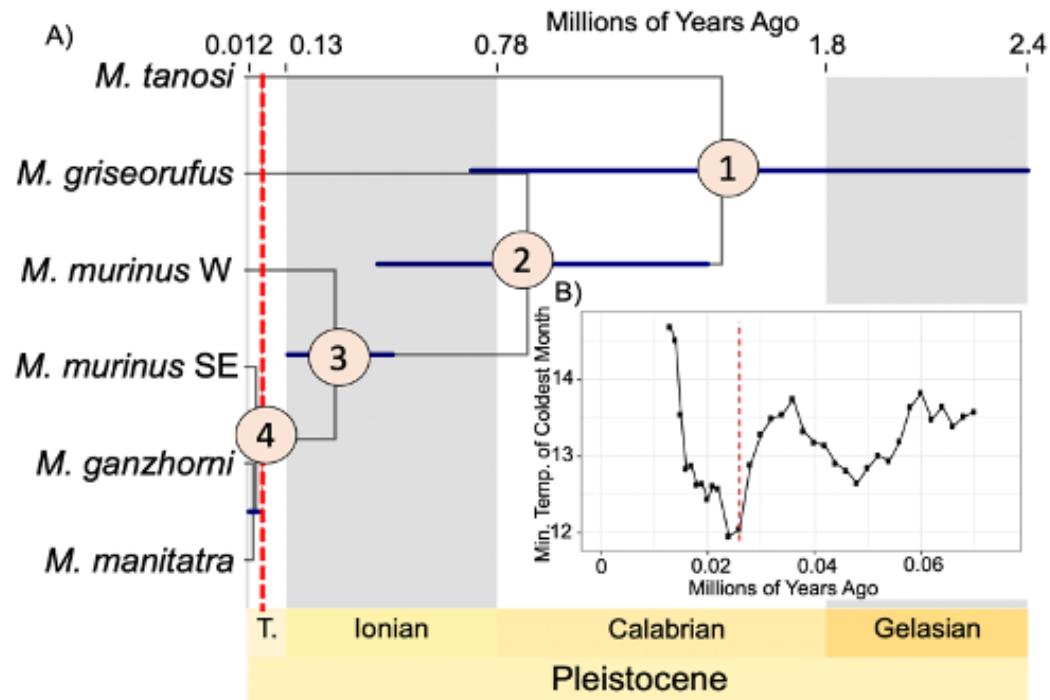


Grassland

Rain forest

The complexity of the southeast





A “speciation continuum”

Figure 6: Speciation Continuum of Focal Lineages. A) The radiation among *M. murinus* (SE), *M. ganzhorni*, and *M. manitatra* (Node 4) is estimated to coincide precisely with the Last Glacial Maximum at 26,000 years ago (illustrated with dashed line). B) Paleoclimatic temperatures for SE Madagascar based on simulations under the HadCM3 circulation model (84).





M. griseorufus



M. murinus

Proposed Aims:

1) Develop Multispecies Coalescent (MSC) methods to model gene flow among diverging lineages.

1.3 Explore the sensitivity of BPP/MSC to **recombination rates**.

2) Investigate phenotypic mechanisms of prezygotic RI:

2.2 Test for conspecific mate choice

- Acoustic communication
- Semiochemical communication

3) Identify genomic signatures of postzygotic RI

3.1 Generate chromosome-level reference genomes for each named lineage

3.2 Whole genome resequencing across contact zones

3.3 Characterize the recombination landscape for named lineages

3.4 Employ MSC methods developed in Aim 1 to measure frequency, magnitude, timing, and direction of gene flow among lineages.



Prezygotic Isolation:

- Wrong place, wrong time? (ecological preferences)
- Different metabolic regimes? (topor vs. not torpor)
- They're just not into each other? (chemosensory; auditory)



Madagascar Expedition 2023

(and 2024, and 2025 ...)



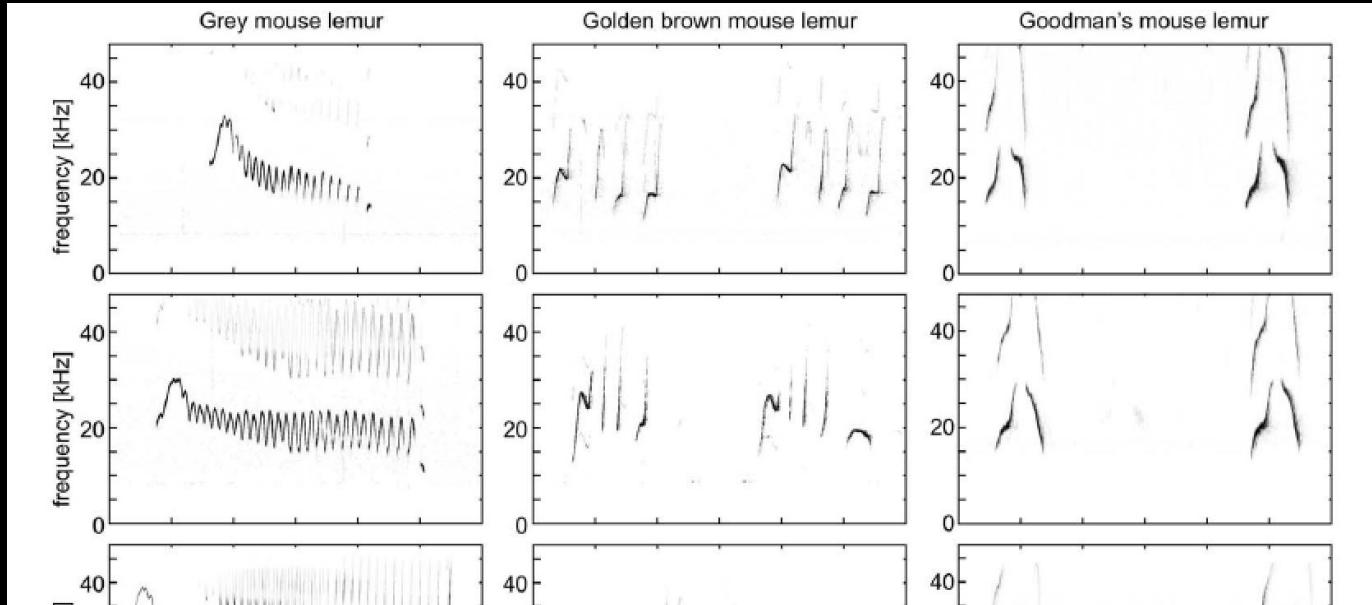
Slide courtesy of Sam Hyde Roberts



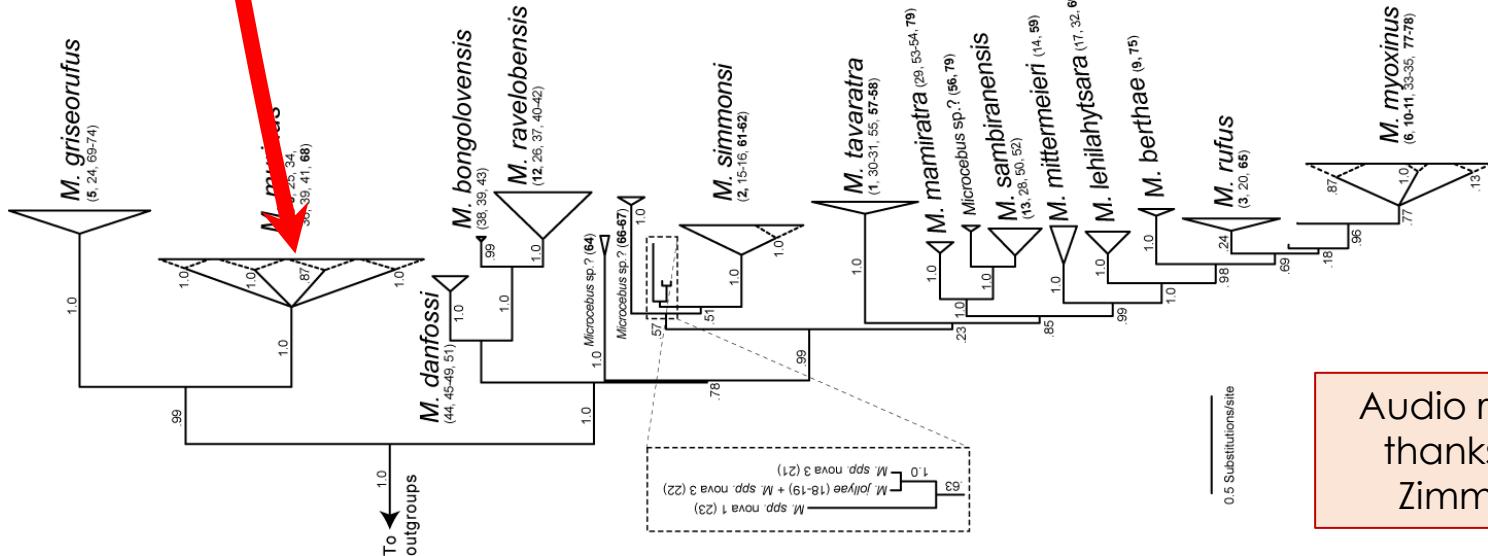
Prezygotic Isolation:

- Wrong place, wrong time? (ecological preferences)
- Different metabolic regimes? (topor vs. not torpor)
- **They're just not into each other? (chemosensory; auditory)**

Male advertisement calls for three species



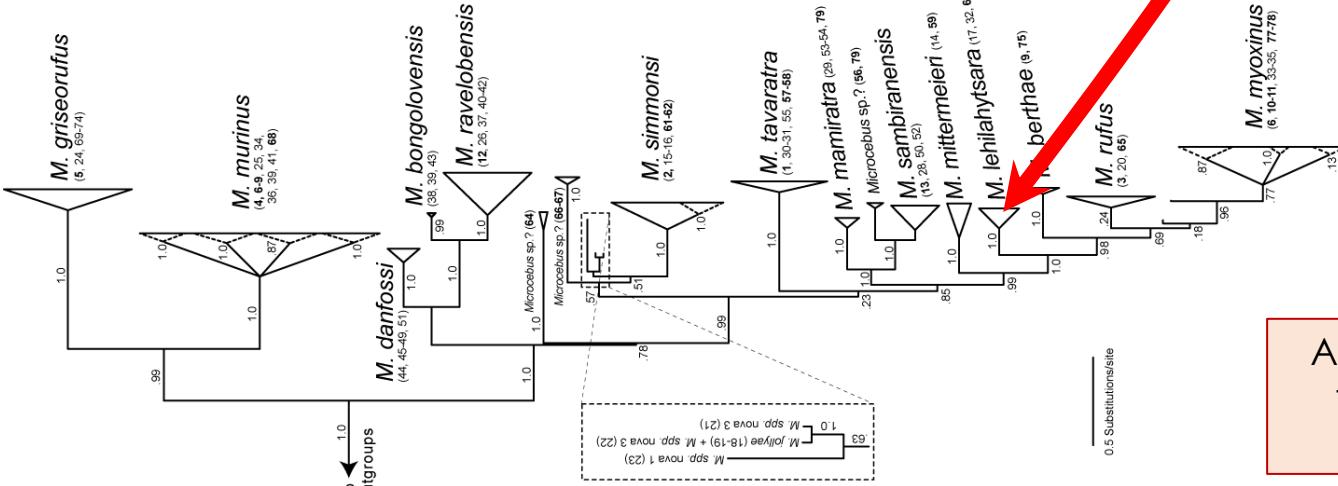
Lineage-specific acoustic
signatures



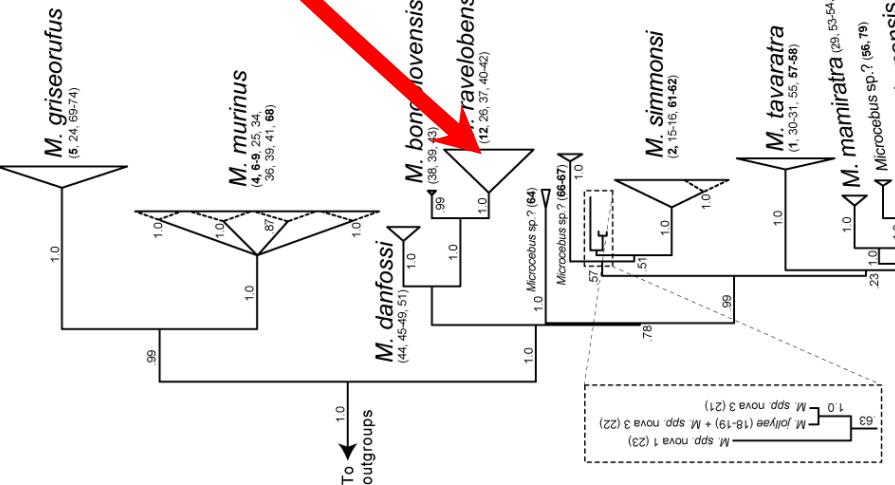
Audio recording
thanks to Elke
Zimmerman



© Jörn Köhler



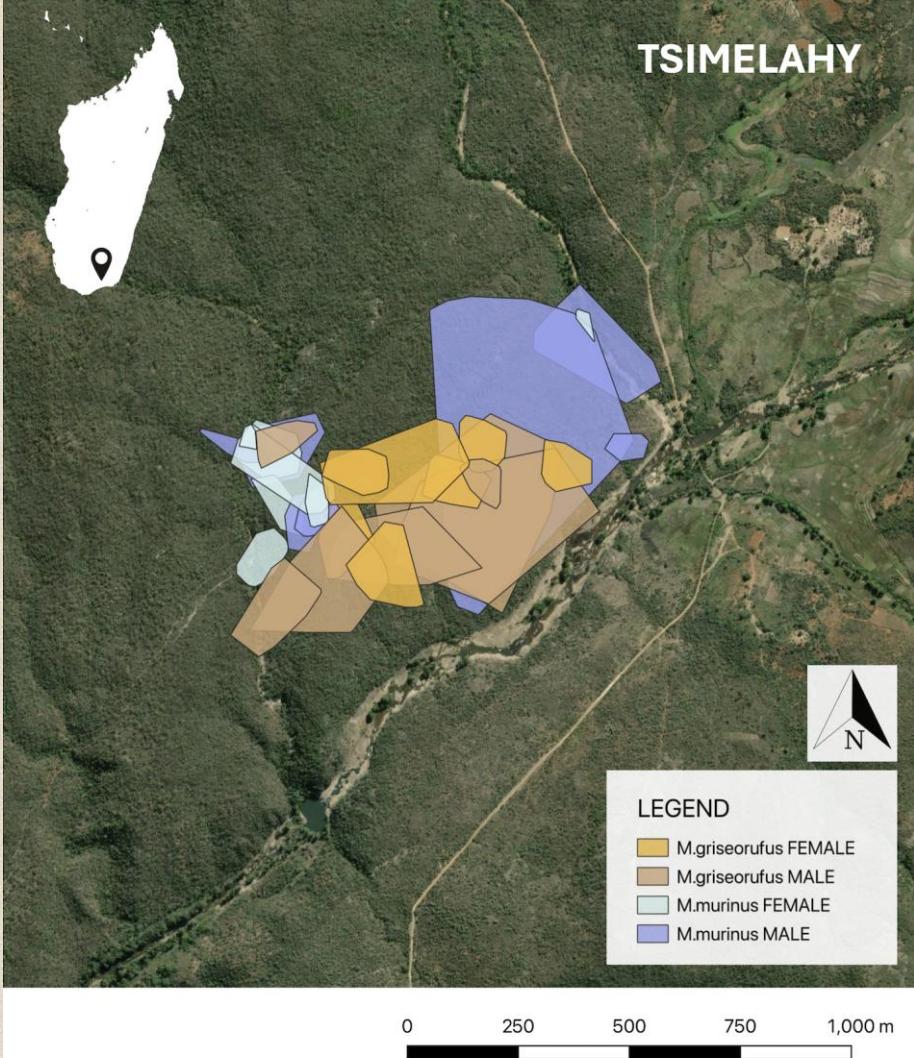
Audio recording
thanks to Elke
Zimmerman



Audio recording
thanks to Elke
Zimmerman

Prezygotic Isolation:

- **Wrong place, wrong time? (ecological preferences)**
- Different metabolic regimes? (topor vs. not torpor)
- They're just not into each other? (chemosensory; auditory)

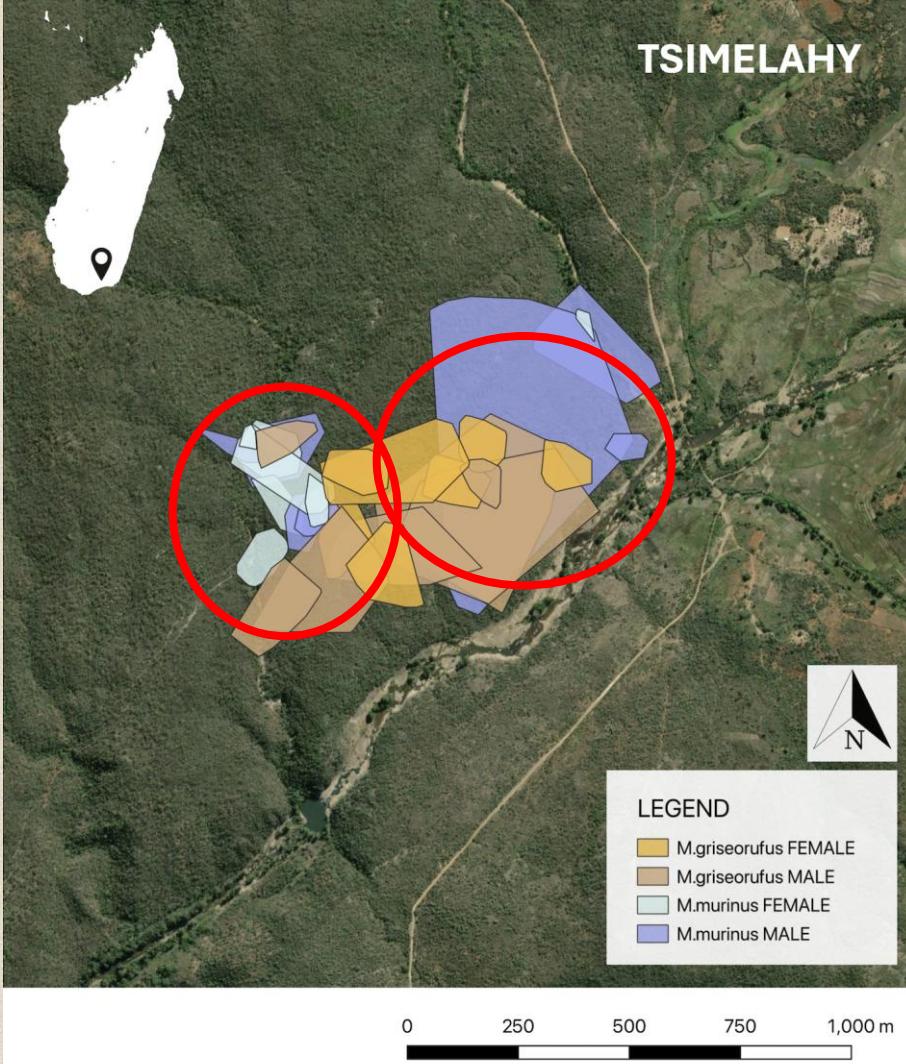


- 56 animals (two species) were hand-captured and fitted with ATS M1420 radio collar transmitters
- Animal follows 3X/week for 12 hours (dusk > dawn)
- **TOTAL = 2,464 hours of observation**

LEGEND

- | | | |
|---|---|----------------------|
| ♀ | ■ | M.griseorufus FEMALE |
| ♂ | ■ | M.griseorufus MALE |
| ♀ | ■ | M.murinus FEMALE |
| ♂ | ■ | M.murinus MALE |

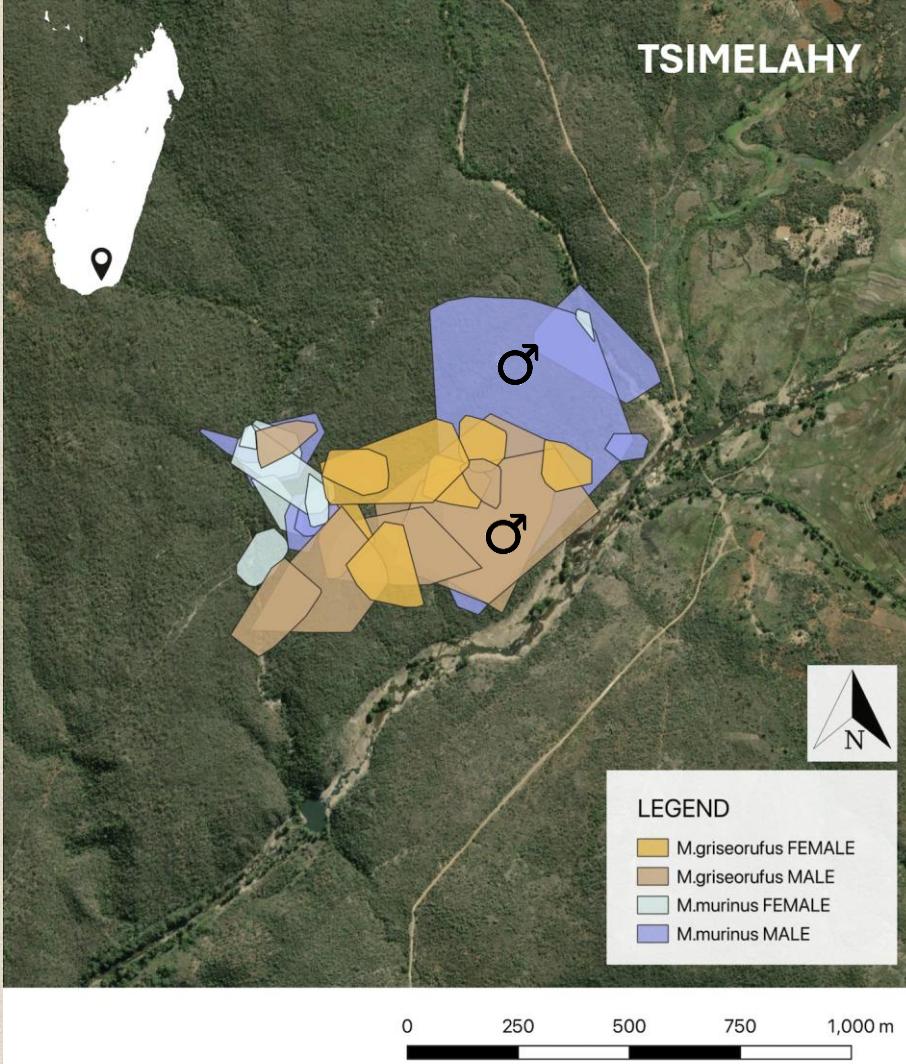
- Each polygon represents total data for one animal
- **Blue = *M. murinus*; Tan = *M. griseorufus***
- Light colors = females; dark = males



**Extensive overlap
between the two species
in wetter habitats**

LEGEND

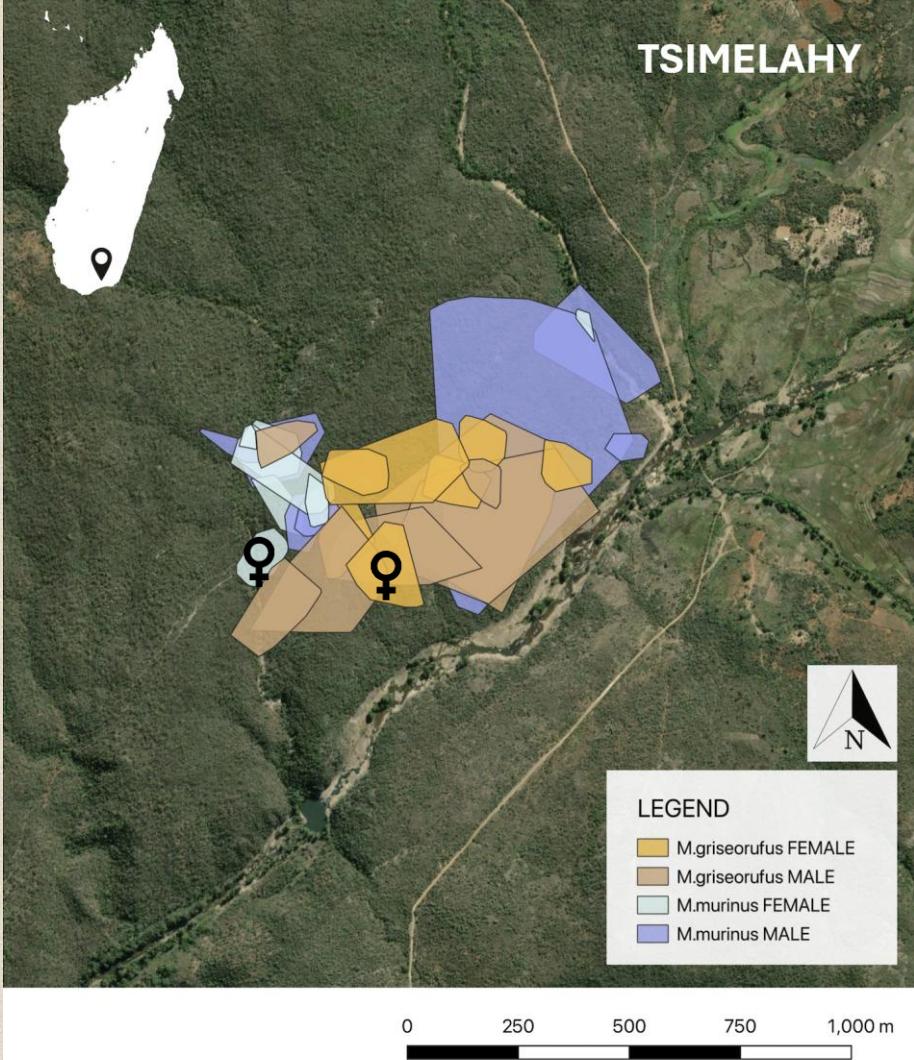
- ♀ M.griseorufus FEMALE
- ♂ M.griseorufus MALE
- ♀ M.murinus FEMALE
- ♂ M.murinus MALE



Male home ranges are
HUGE

LEGEND

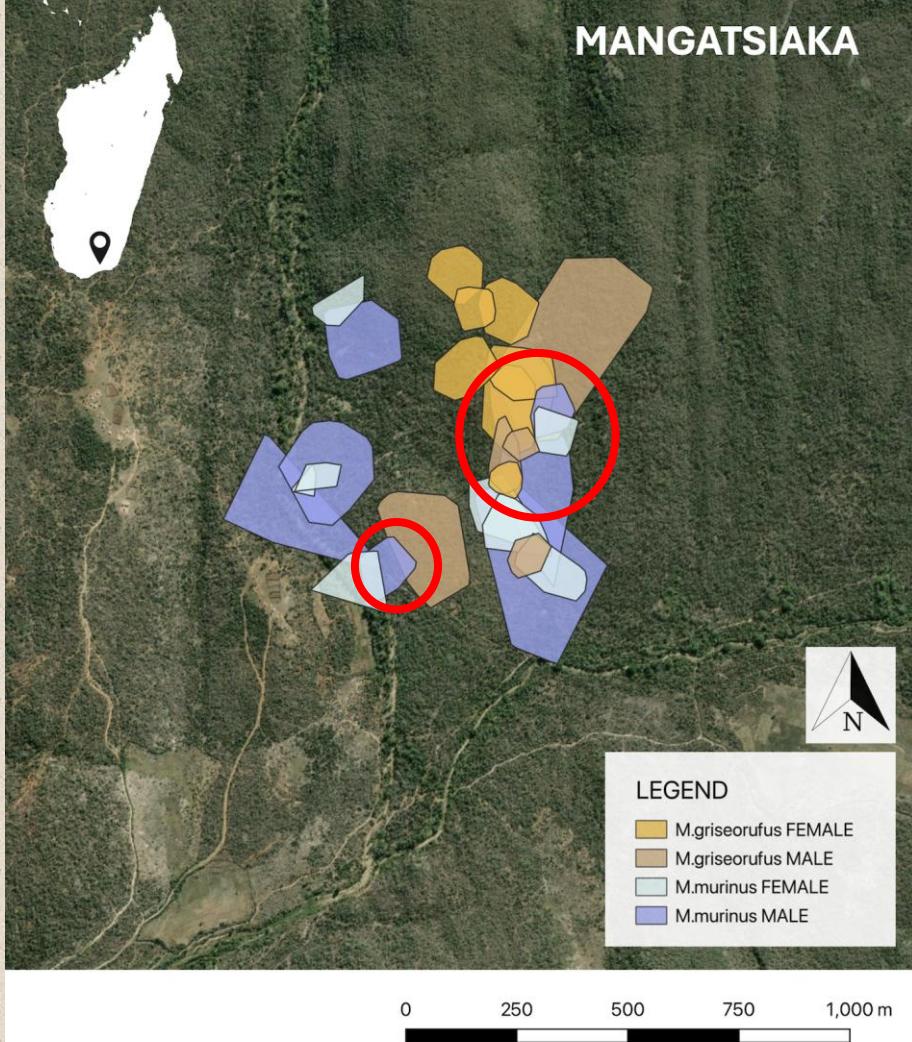
- ♀ M.griseorufus FEMALE
- ♂ M.griseorufus MALE
- ♀ M.murinus FEMALE
- ♂ M.murinus MALE



Female home ranges MUCH smaller

LEGEND

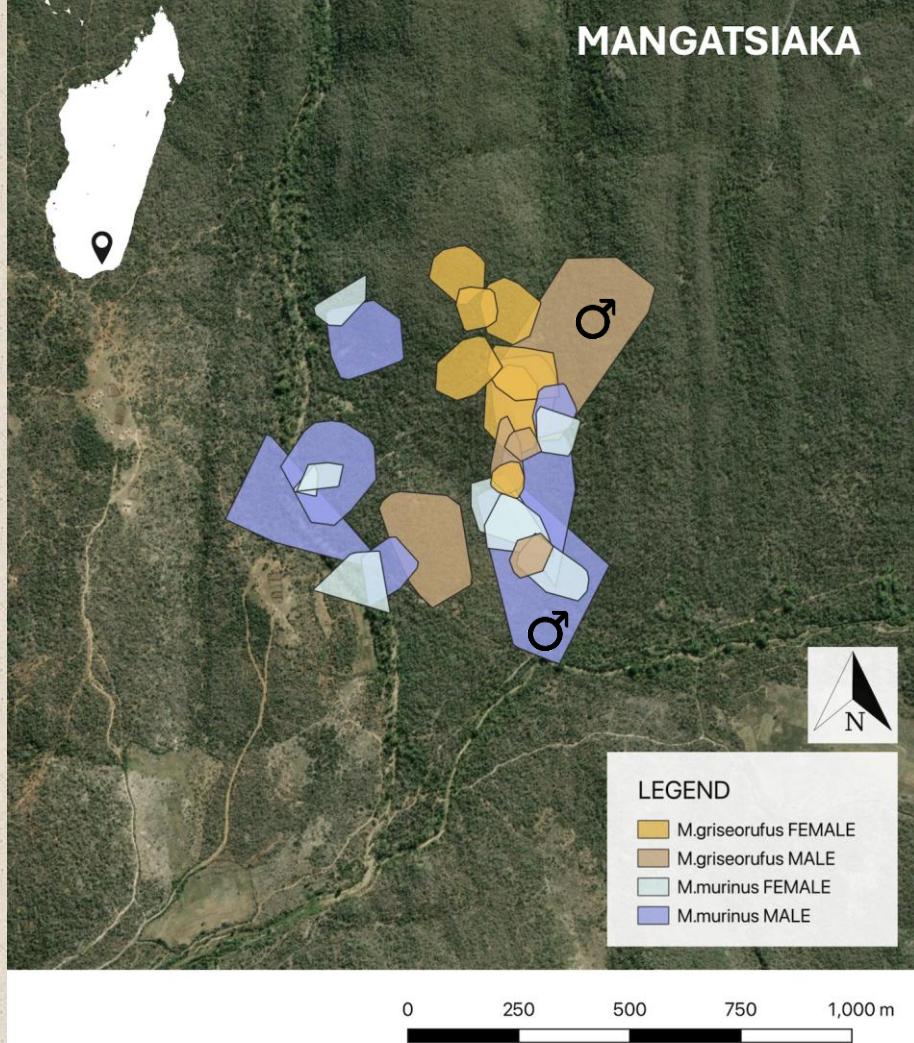
- ♀ M.griseorufus FEMALE
- ♂ M.griseorufus MALE
- ♀ M.murinus FEMALE
- ♂ M.murinus MALE



**Much less overlap
between the two species
in dry habitats**

LEGEND

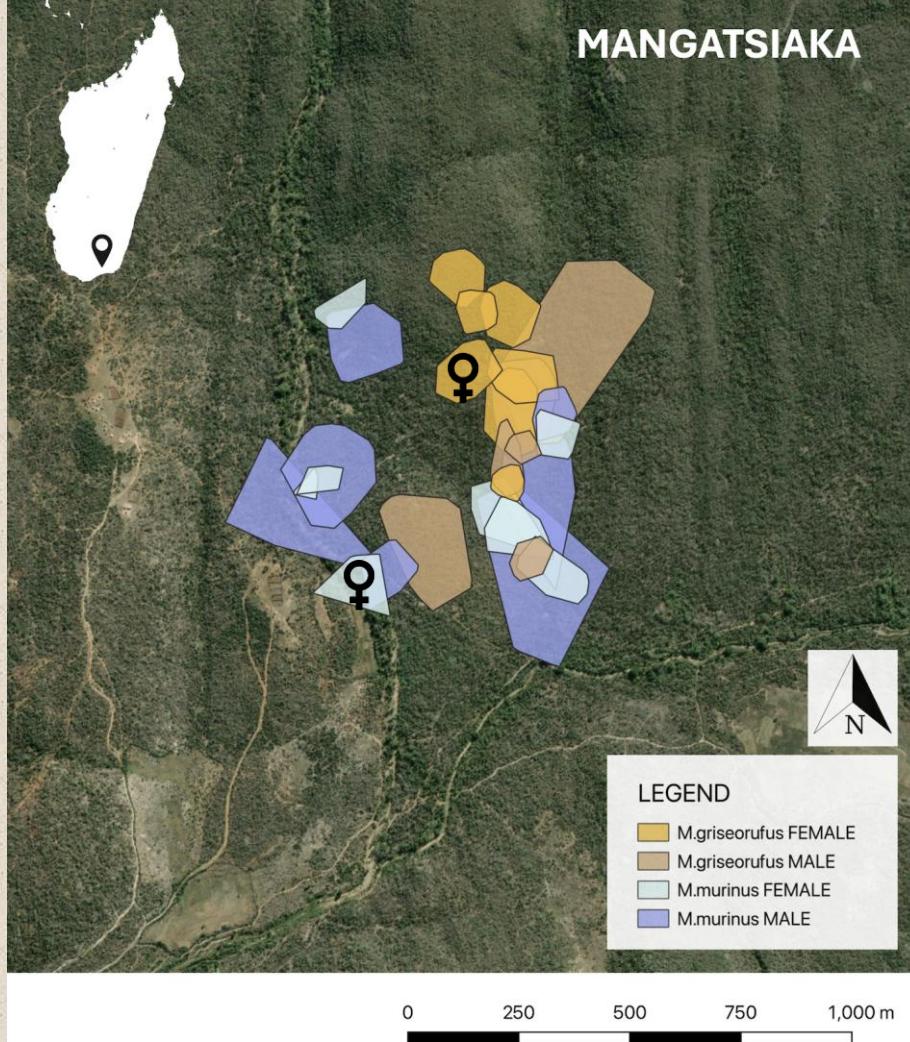
- ♀ M.griseorufus FEMALE
- ♂ M.griseorufus MALE
- ♀ M.murinus FEMALE
- ♂ M.murinus MALE



Male home ranges are larger than females' (again)

LEGEND

- ♀ M.griseorufus FEMALE
- ♂ M.griseorufus MALE
- ♀ M.murinus FEMALE
- ♂ M.murinus MALE



MANGATSIAKA

Female home ranges much smaller (TINY)

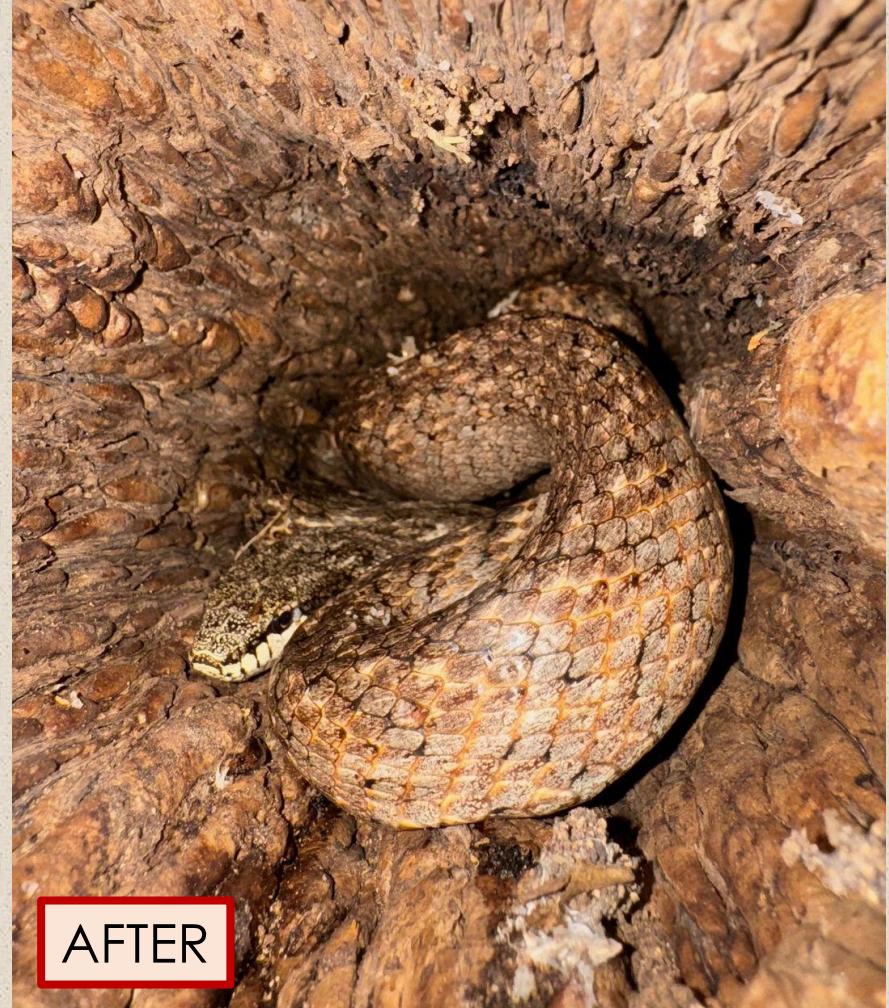
LEGEND

- ♀ M.griseorufus FEMALE
- ♂ M.griseorufus MALE
- ♀ M.murinus FEMALE
- ♂ M.murinus MALE



BEFORE

PREDATION



AFTER

Prezygotic Isolation:

- Wrong place, wrong time? (ecological preferences)
- **Different metabolic regimes? (topor vs. not torpor)**
- They're just not into each other? (chemosensory; auditory)



Can undergo a “**metabolic switch**” that leads to fattening and prolonged torpor/hibernation ... seems to be highly flexible by species and environment.







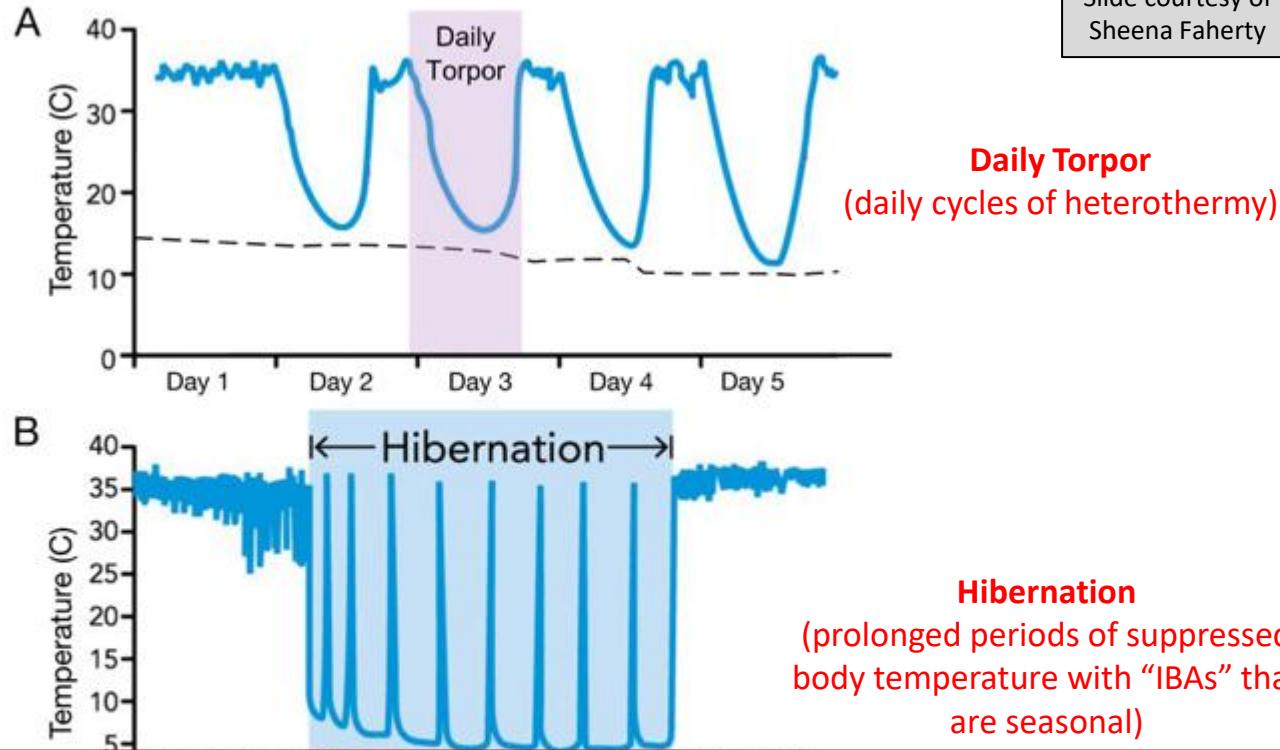
Sleeping



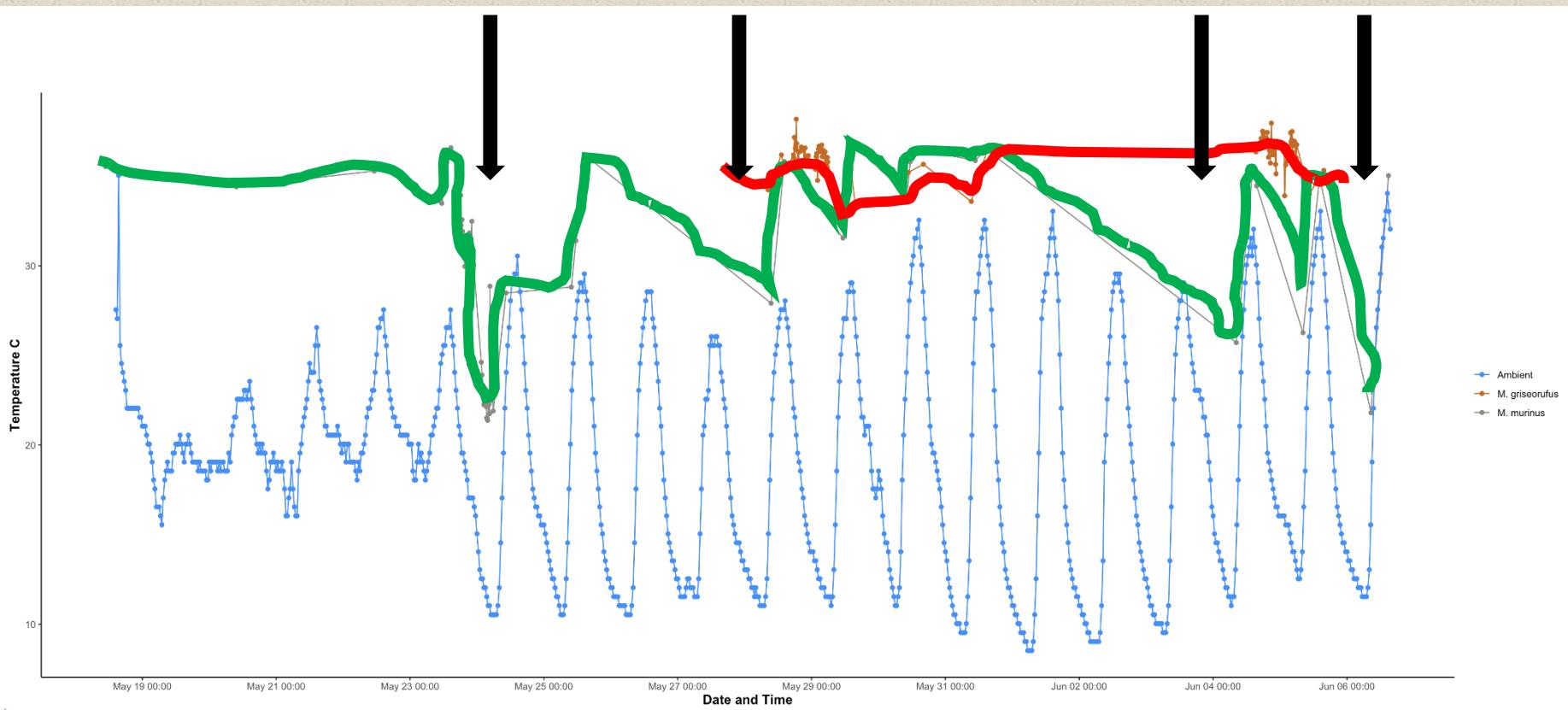
SOLO



WITH FRIENDS



Asynchronous reproductive windows



Ambient temperature in blue; *M. murinus* in green; *M. griseorufus* in red tracked over 28 days



-
- *M. griseorufus*:
 - More slender/no tail fattening
 - Tend to be solitary/sleep alone
 - Found on smaller more delicate trees, typically with thorny antipredator bracts
 - Sleep “in the open” on small branches
 - (Apparently) does not torpor
 - Have larger home ranges/more exploratory (even found underground!)



-
- *M. murinus*:
 - Robust/tail fattening
 - More gregarious (sleeps in groups)
 - Found in taller & larger trees with deep thermally protective holes
 - Experience seasonal torpor
 - Bolder/more aggressive
 - Ecological generalist --- comfortable in degraded habitat

Bottom line: the two species are living right on top of each other but are living completely different lives!

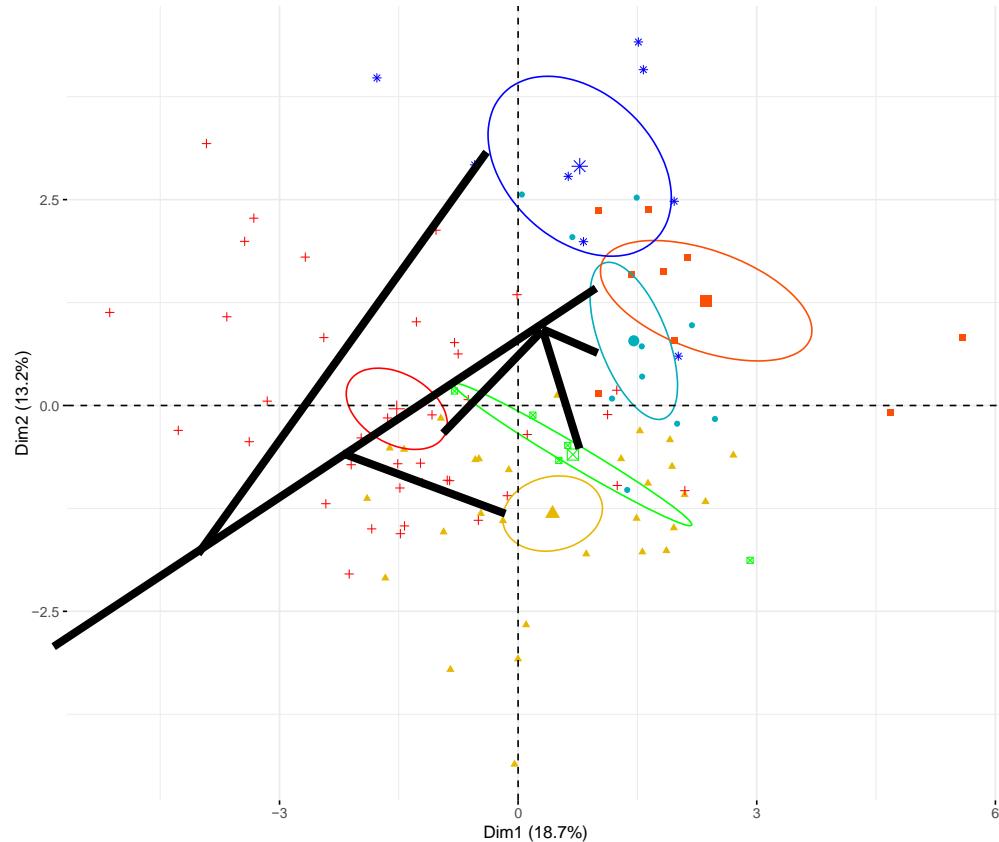
COMPLETE RI IN < 800 KY
(we think)



Sampling Process

- Morphometric measurements
- Ear biopsy (x1 or x2)
- 300 ul of blood from saphenous vein.

Individuals - PCA

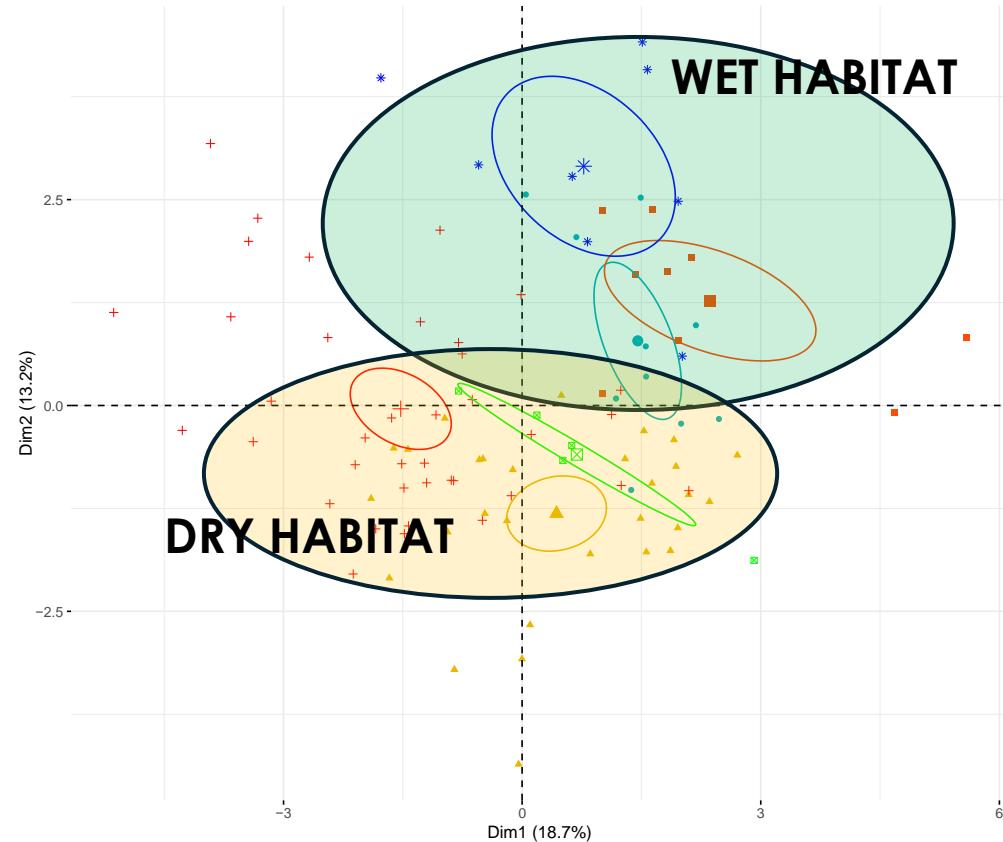


Species

- Microcebus ganzhorni
- Microcebus griseorufus
- Microcebus manitatra
- Microcebus murinus
- Microcebus sp
- Microcebus tanosi



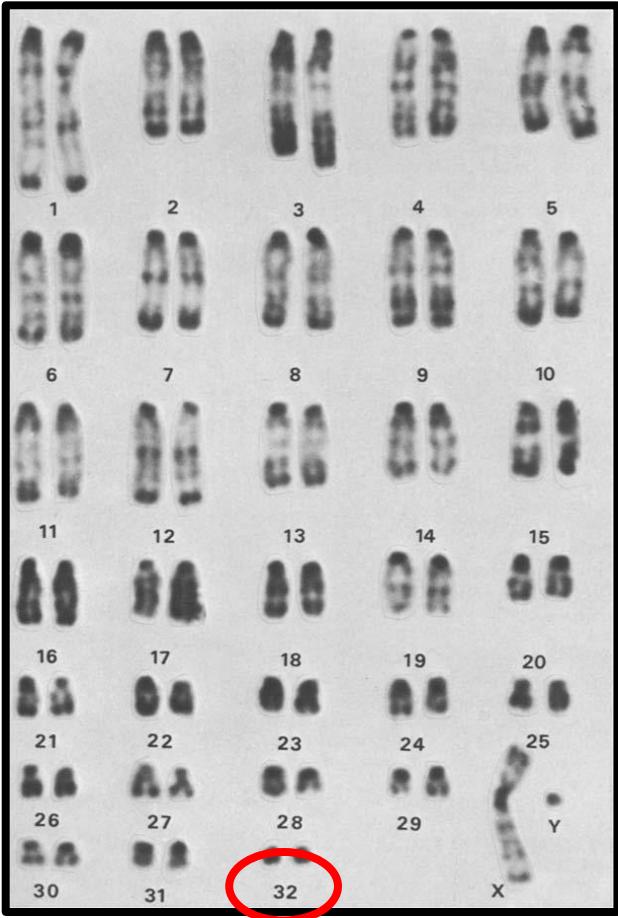
Individuals - PCA



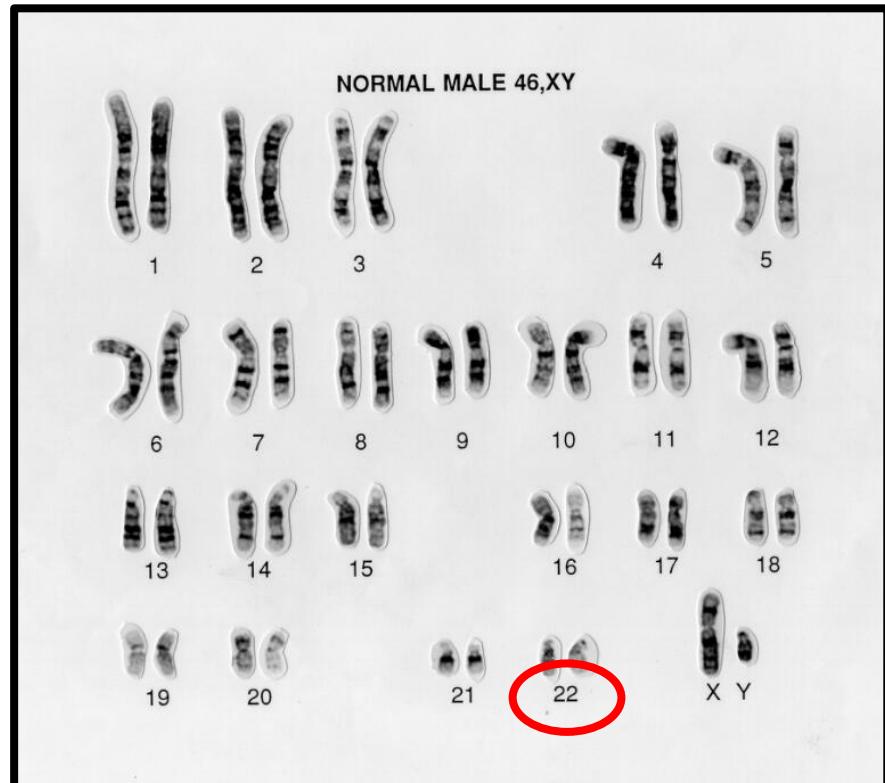
Postzygotic Isolation:

- Different karyotypes? (lemurs have crazy chromosomes!)
- Different recombination landscape/genomic architecture?
- Barrier loci???

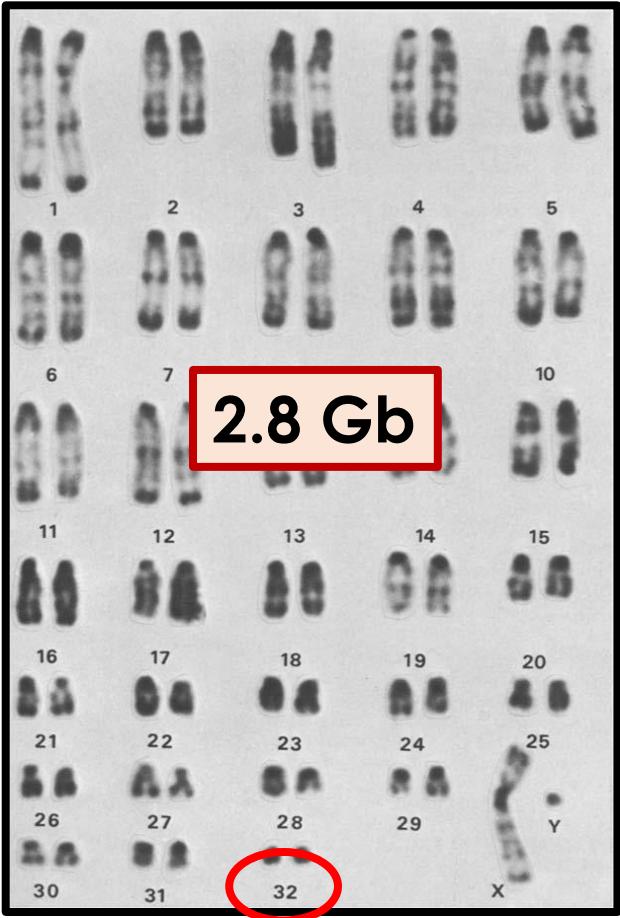
Mouse lemur karyotype (male)



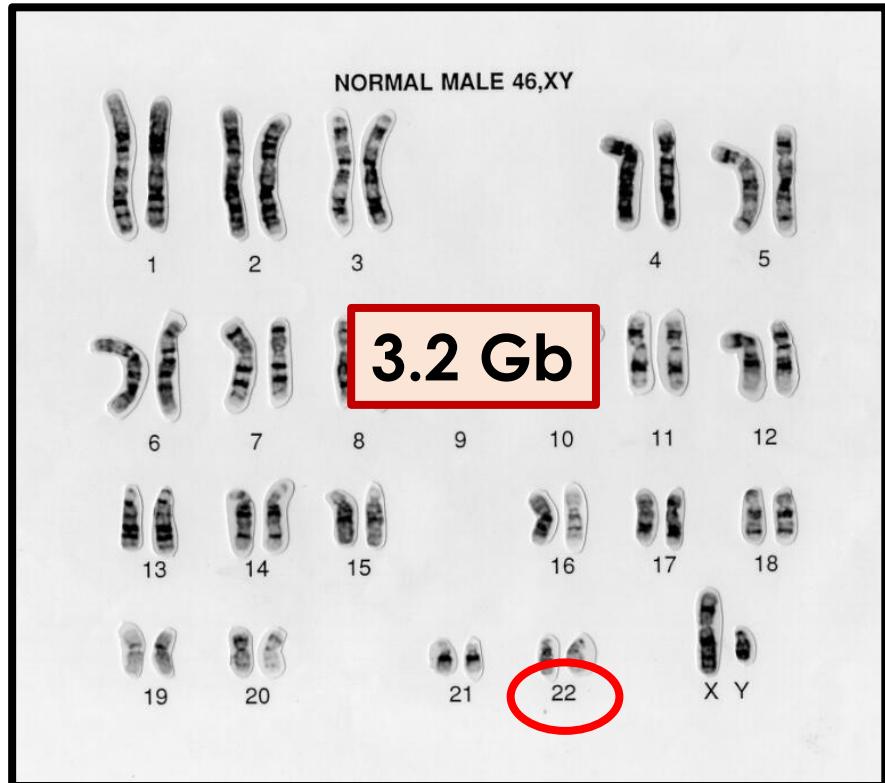
Human karyotype (male)

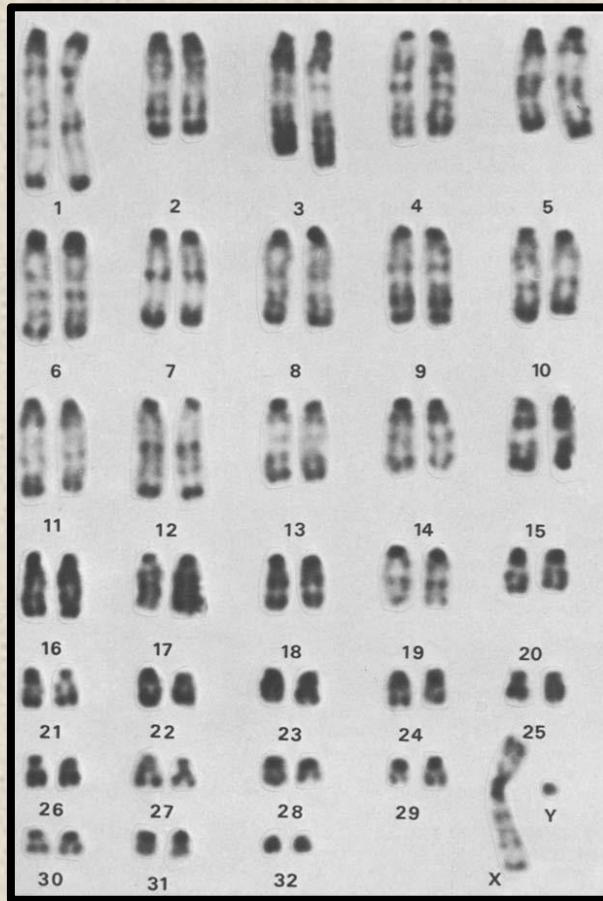


Mouse lemur karyotype (male)

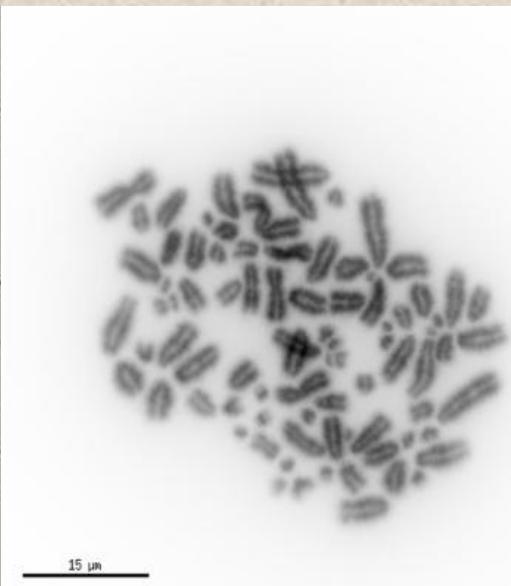
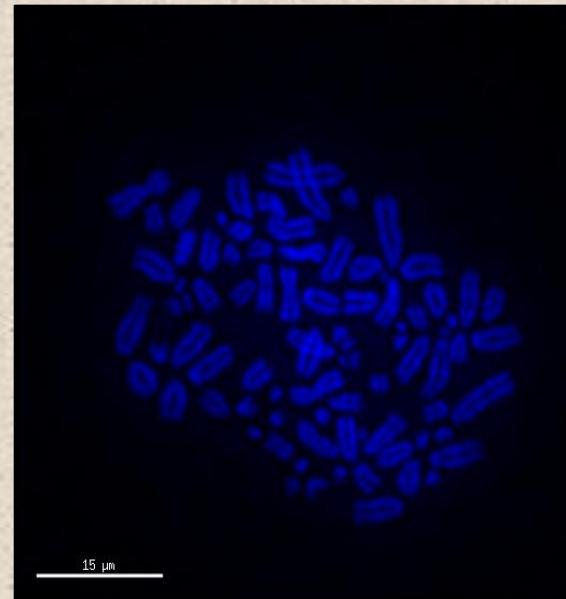


Human karyotype (male)

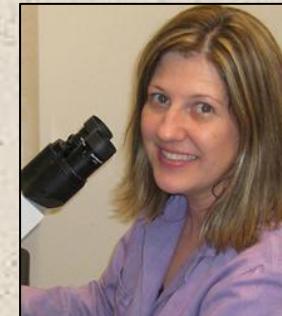




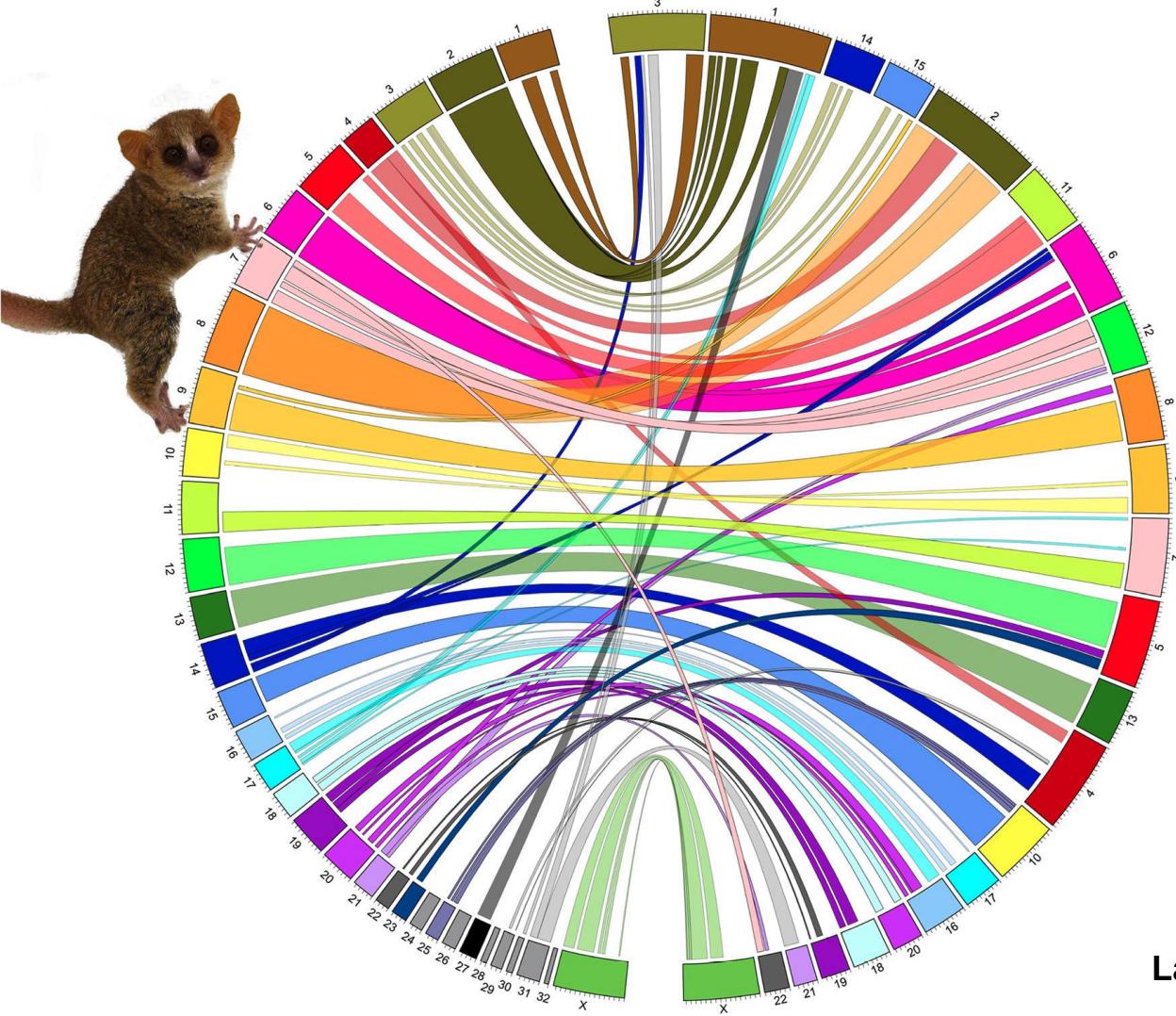
Dutrillaux (1979)
Human Genetics



66 chromosomes (acrocentric, except for X)



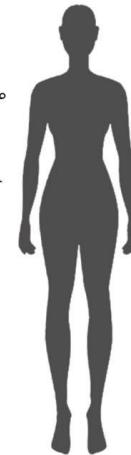
Beth Sullivan



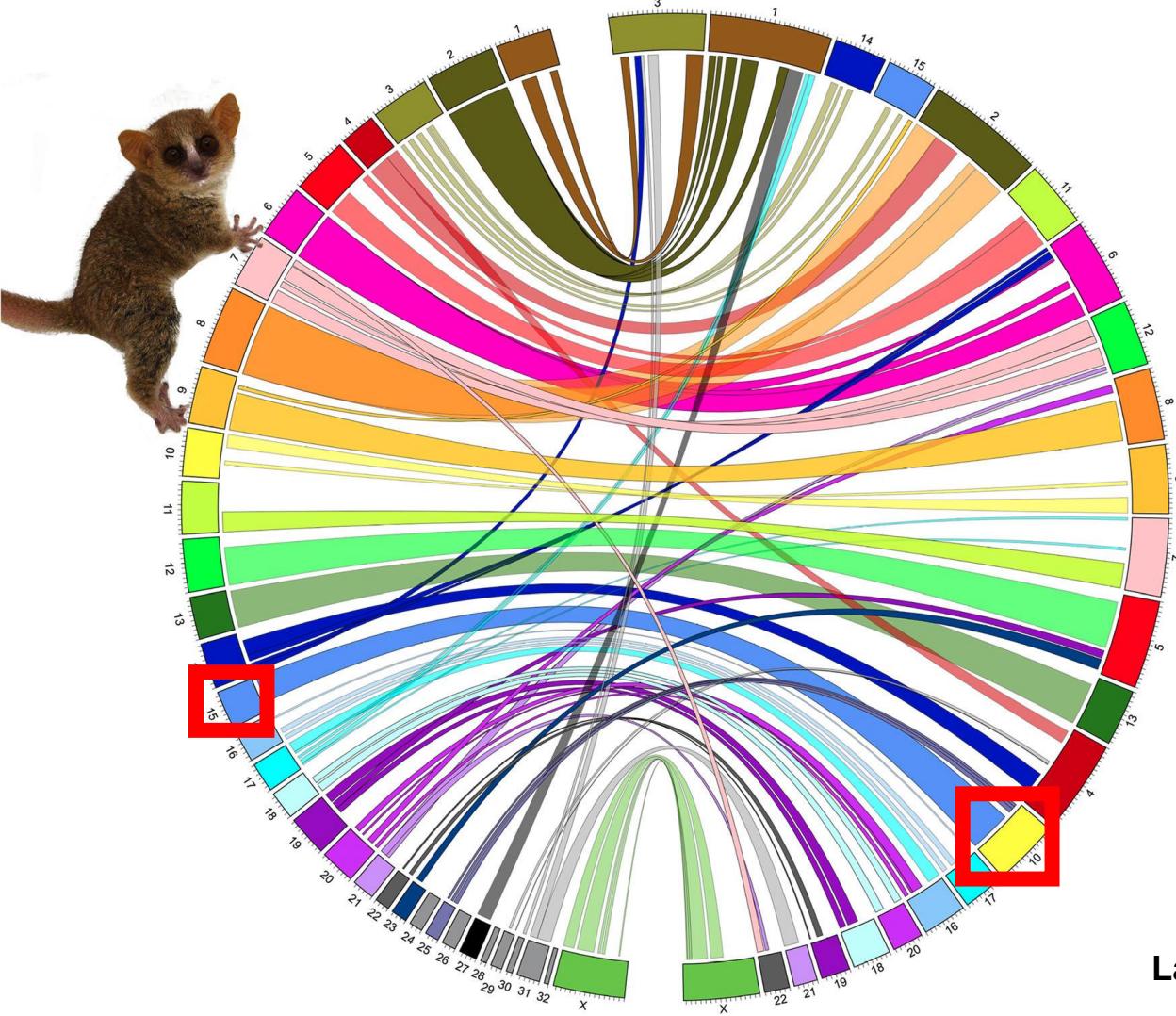
Circos Plot

<http://circos.ca>

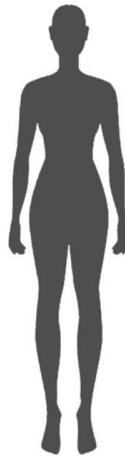
(note: synteny was determined
bioinformatically, not with
chromosome painting)

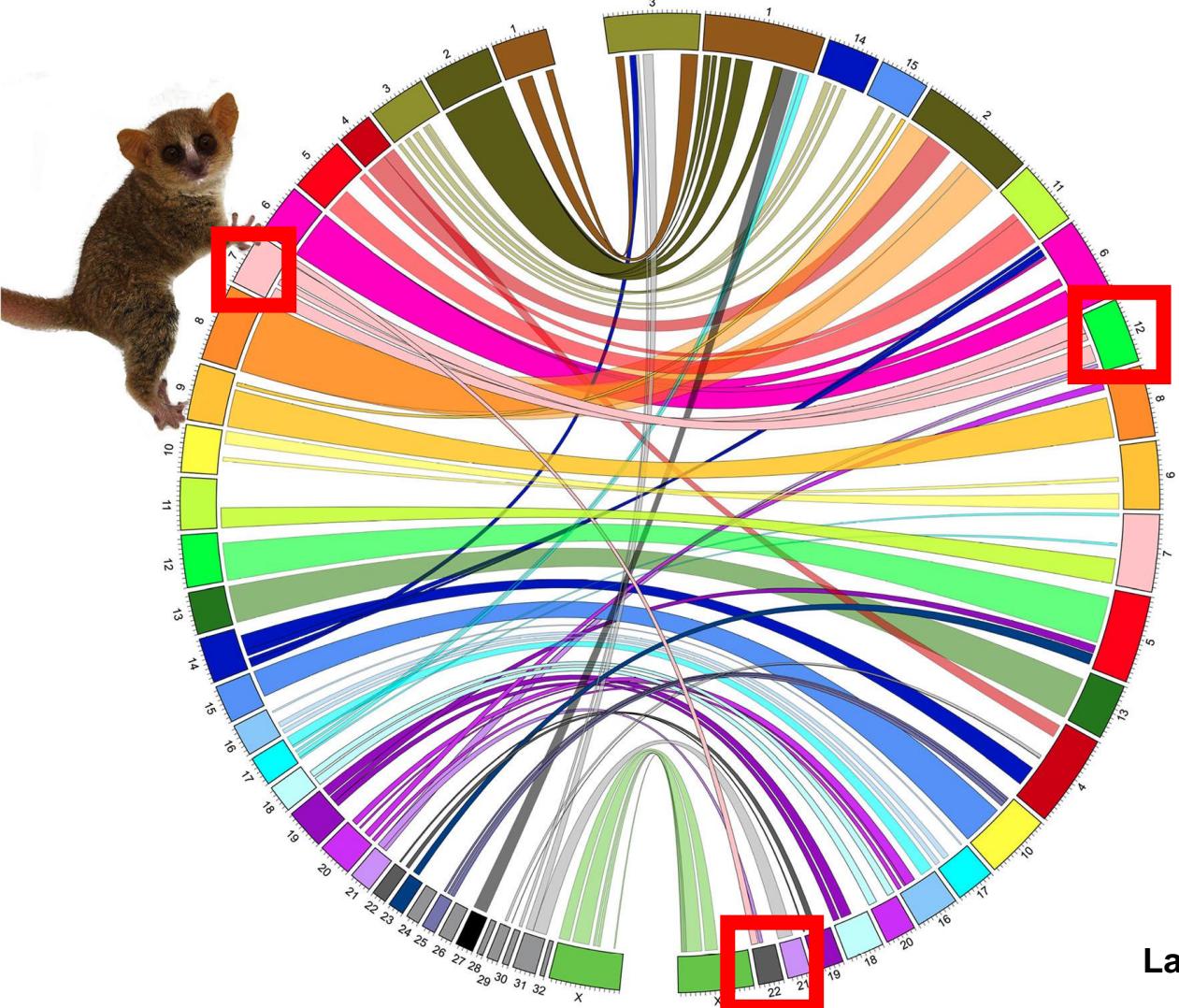


Larsen PA et al. [2017](#). *BMC Biol* 15: 110.

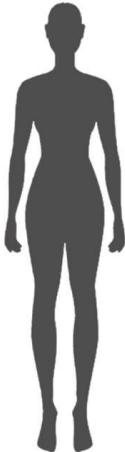


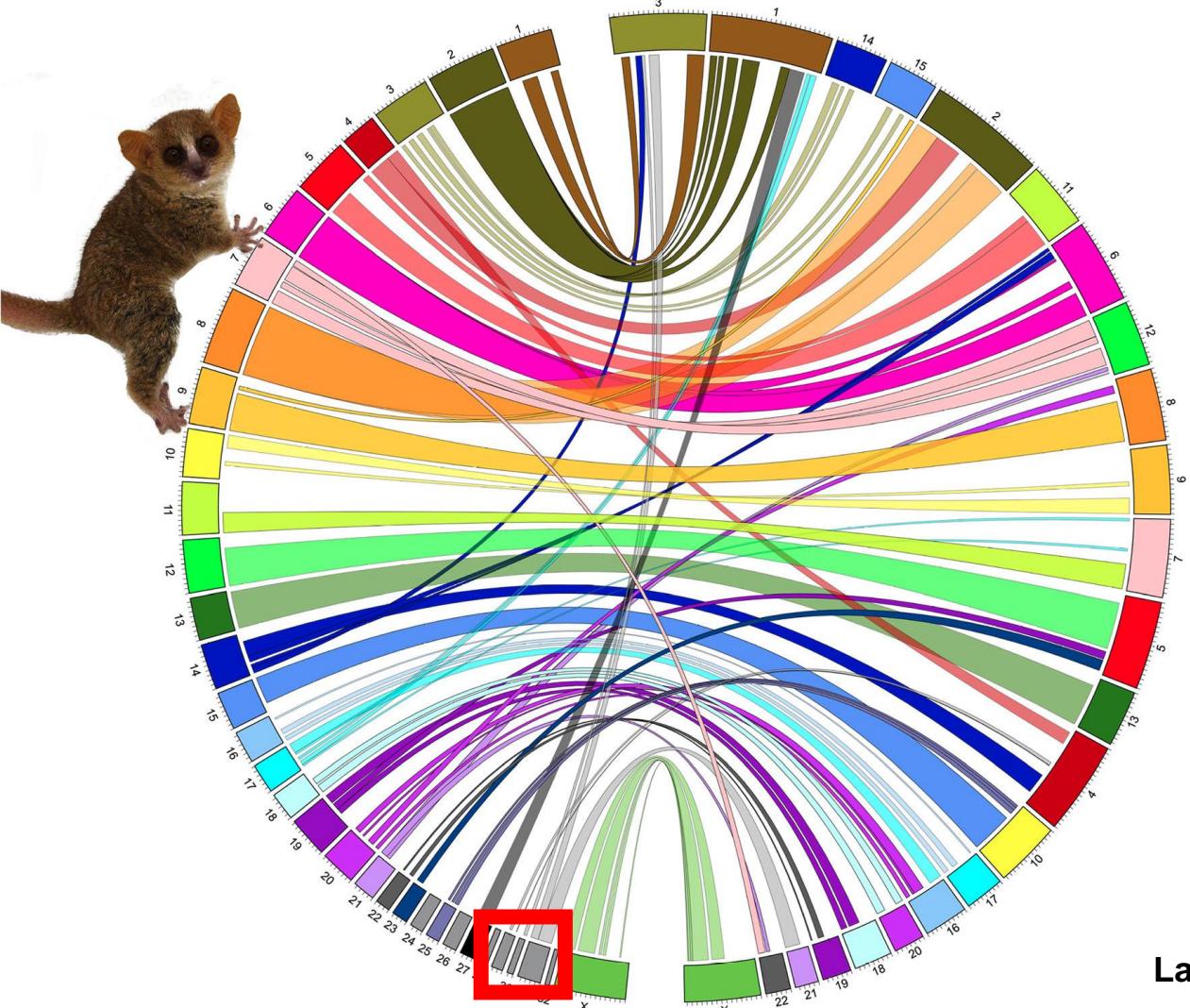
Chromosome #15 in mouse lemur appears to have near perfect homology with human chromosome #10 (in yellow)



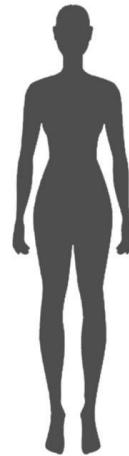


Chromosome #7 in mouse lemur appears to have homology with human chromosome #12 (in green) AND with human chromosome #22 (in brown)





Microchromosomes in mouse lemur (in grey) appear to be all over the place!



Telomere-to-Telomere (T2T)

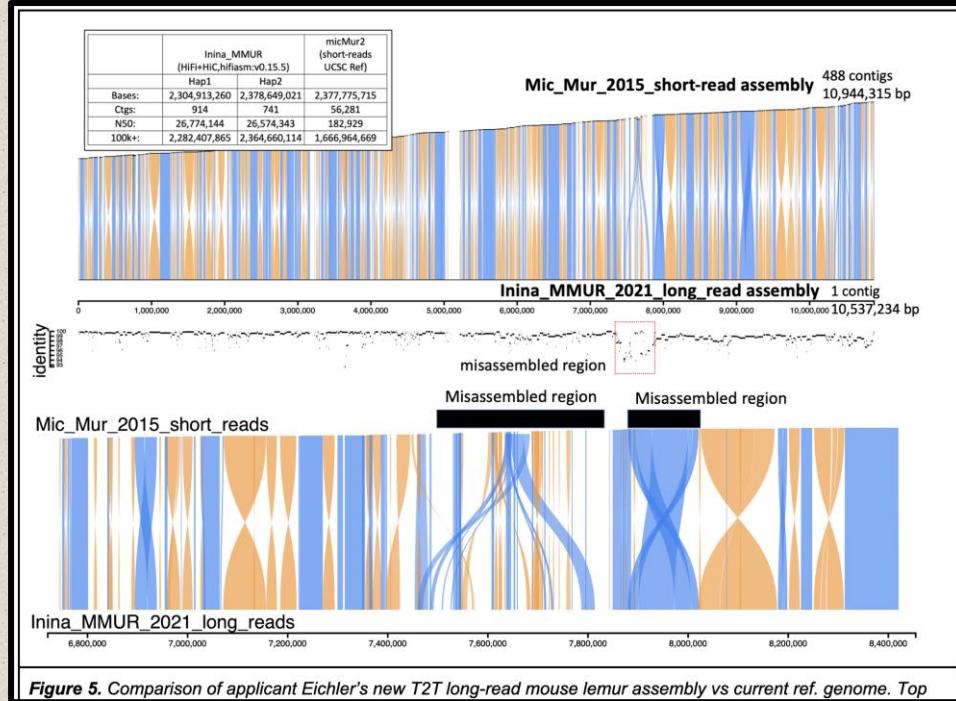


Figure 5. Comparison of applicant Eichler's new T2T long-read mouse lemur assembly vs current ref. genome. Top



Evan Eichler
University of Washington

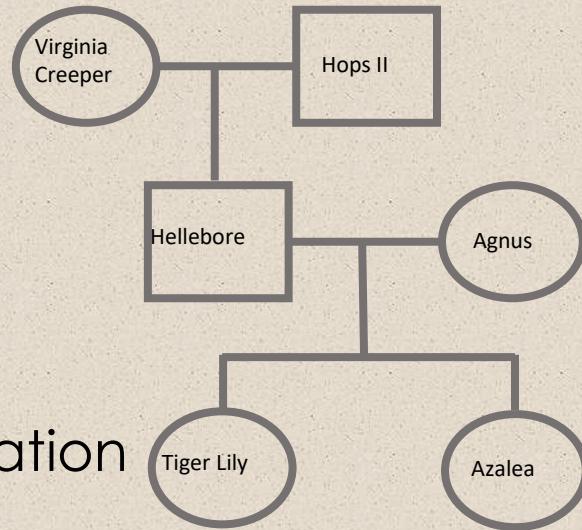
T2T genomes

DLC pedigrees

- Recombination map / mutation rates

Wild genomes

- Better quality reference genomes – population resequencing
- Structural variation (gene copy variation, inversions, translocations, insertions, deletions, etc.)
- TE composition and possible role in speciation



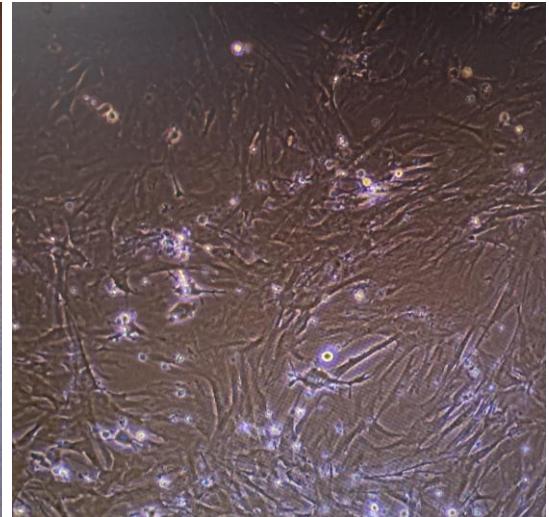
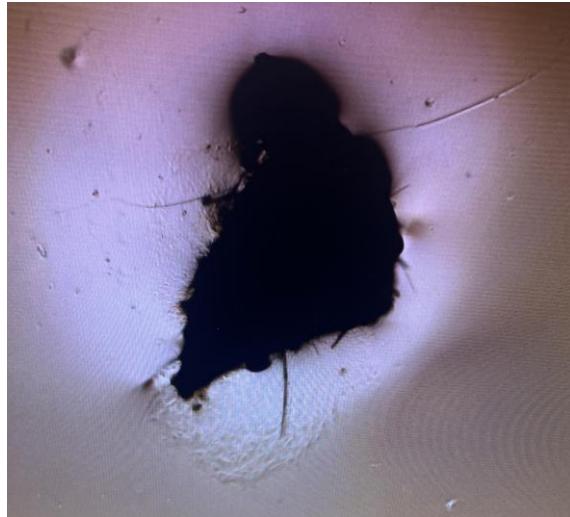




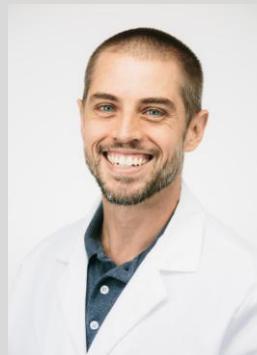


Early Preliminary Results

- Blood, skin biopsy, urine, and feces collected from 100 individuals sampled evenly from all five phylogenetic lineages!!!
- 30X PacBio HiFi from 200 ul. of blood!
- Second set of historical samples sequenced (short reads) at 10X coverage --- AGAIN, no hybrids detected!
- Transposable element mapping is showing crazy variation across lineages!

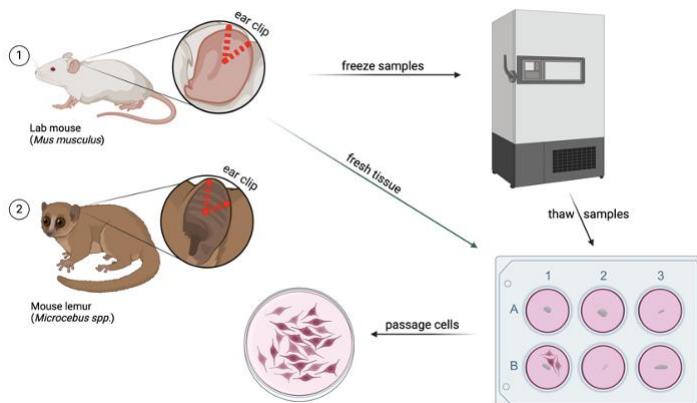


Cell cultures – Somarelli lab

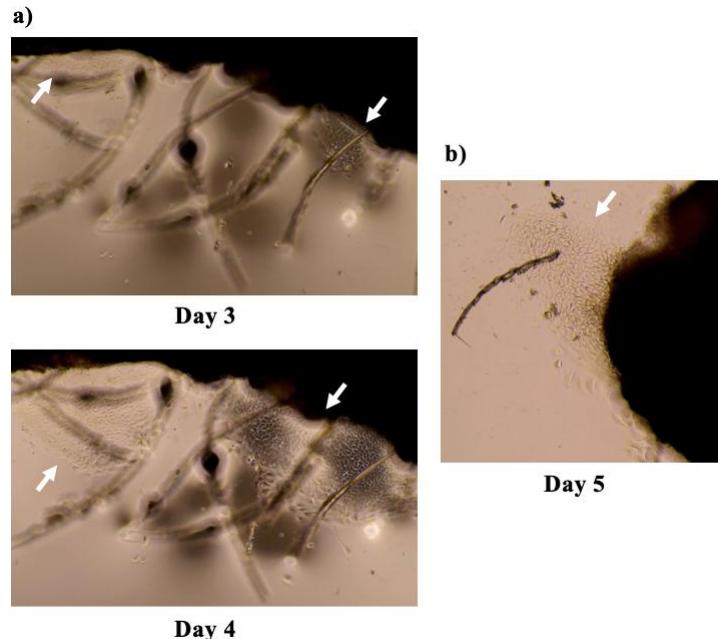


Fibroblast cell cultures of mouse and mouse lemurs under simulated field conditions

We tested protocols on mouse tissue before mouse lemur tissue.



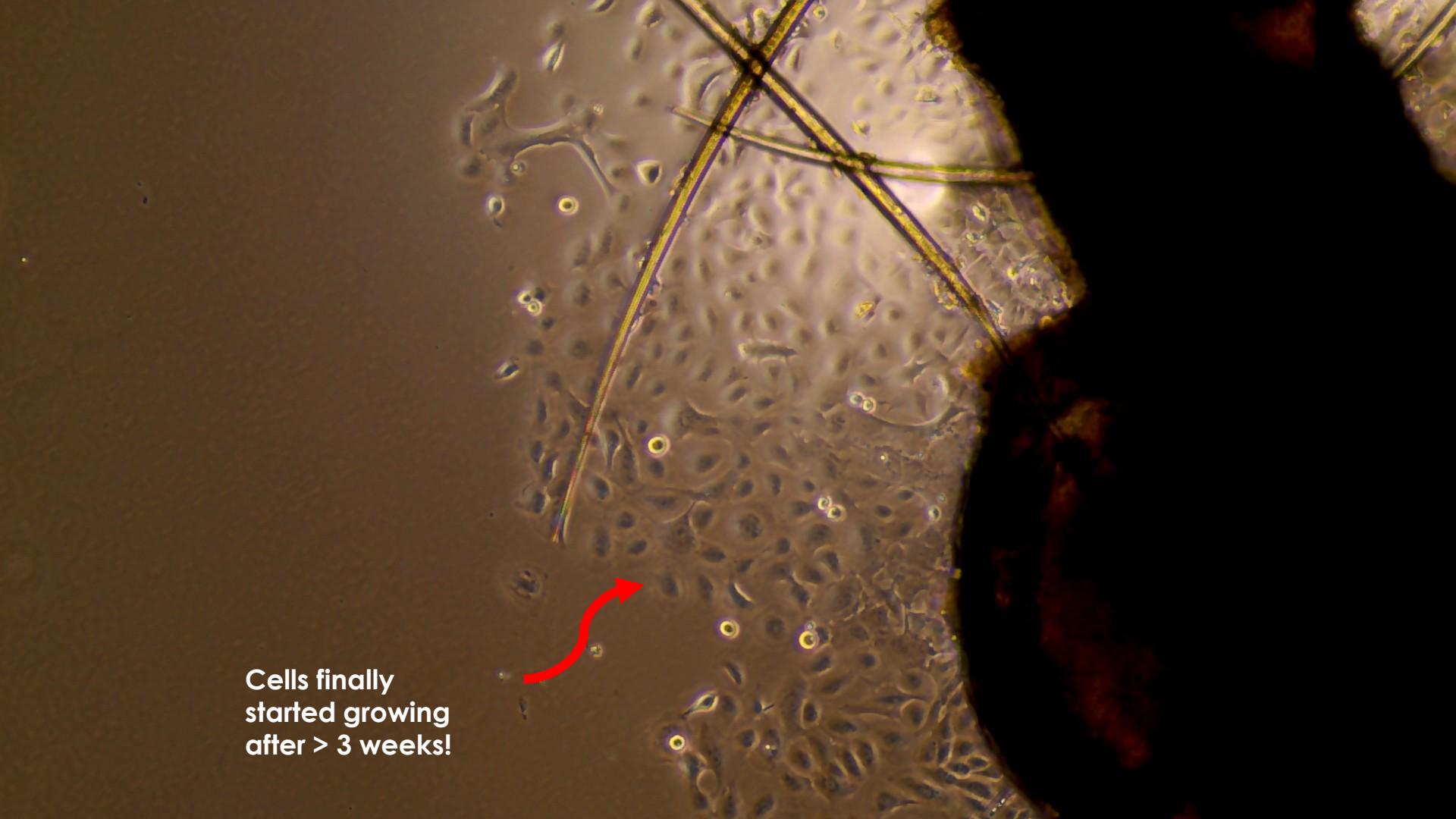
Simplified protocol³



(a) Mouse lemur tissue previously frozen in 10% DMSO freezing medium (b) Mouse lemur tissue previously frozen in Cryostor medium.

Keys to make it work in the field





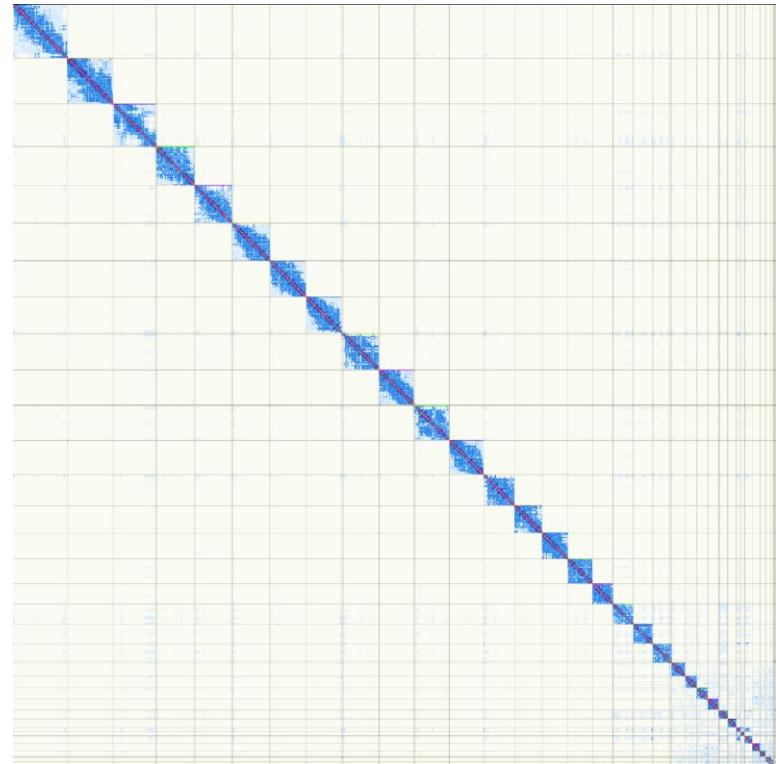
A microscopic image of a petri dish containing a sparse layer of cells. The cells are small and rounded, with some having distinct yellowish-green fluorescent spots. A large, irregularly shaped cluster of cells is visible in the upper right quadrant. A red arrow points from the bottom left towards this cluster, indicating its location. The background is dark, suggesting a low-light environment or a different focal plane.

Cells finally
started growing
after > 3 weeks!

M. ganzhorni



HiC contact map

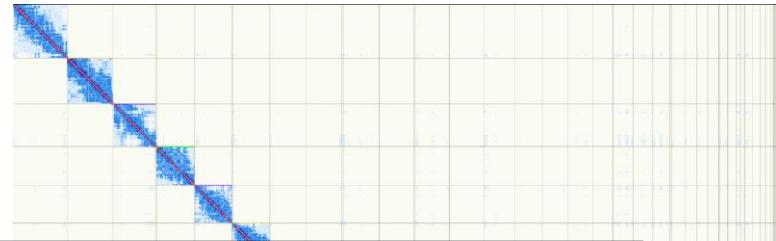


HiFi reads from ~300ul of blood
HiC reads from Ear tissue

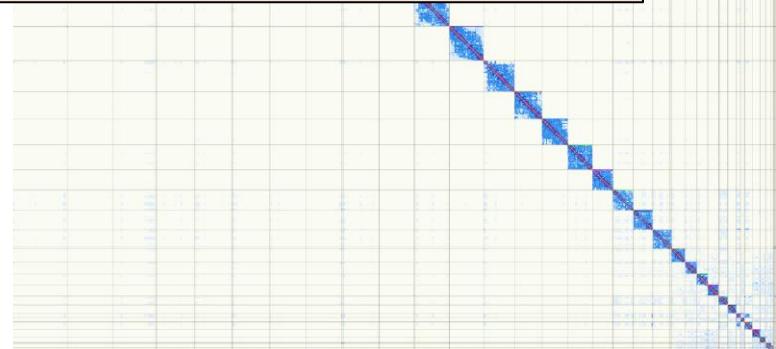
M. ganzhorni



HiC contact map



Chromosome-level de novo assembly from
 $< \frac{1}{2}$ ml blood and a tiny ear clip

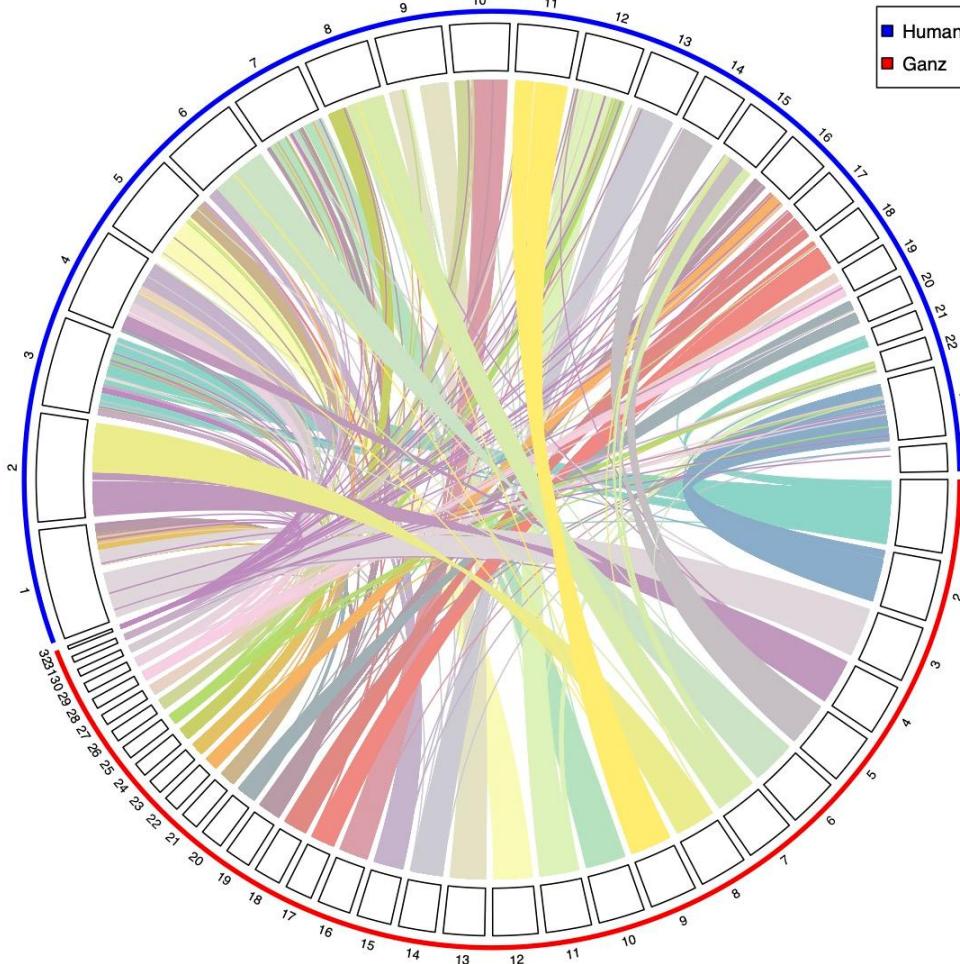


HiFi reads from ~300ul of blood
HiC reads from Ear tissue



Hannah Kania
**(genomics; TE
annotation)**

Ganz Top 1000 Alignments per Scaff

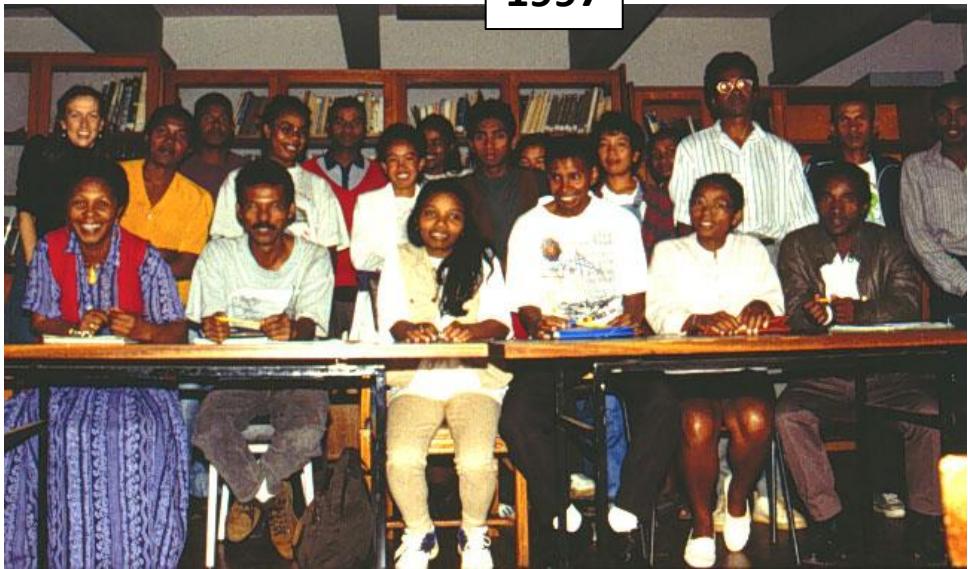


THANK YOU, MADAGASCAR!!!



2024

1997





2004

2002



2024



2004



2024



2024



Thanks for listening!





Thanks to The Village!

RADseq Consortium:

- Lounes Chikhi
- Paul Hohenlohe
- Ed Louis
- Ute Radespiel
- Jordi Salmona
- Elke Zimmerman

Yoder Lab (past & present):

- Caro Segami
- Sam Hyde Roberts
- Hannah Kania
- Jelmer Poelstra
- George Tiley
- Rachel Williams

Field collaborators:

- Jean B. Andriambeloson
- Marina Blanco
- Jörg Ganzhorn
- José M. Ralison
- Blanchard Randrianambinina
- Rodin M. Rasoloarison
- David W. Rasolofoson
- Jonah Ratsimbazafy



John Simon
Guggenheim
Memorial Foundation

