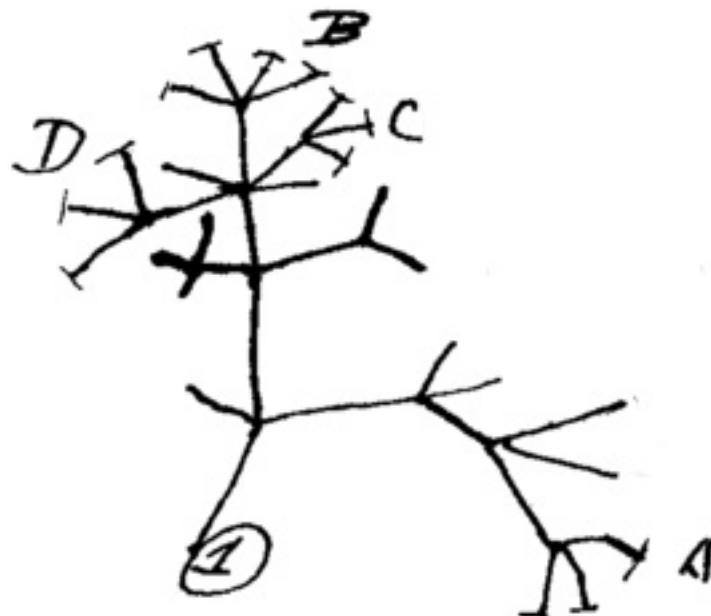


“What are we even talking about when we say “species tree’?”

I think



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

Themes from yesterday ... (thank you Peter, Laura, and Claudia!)

In no particular order ...

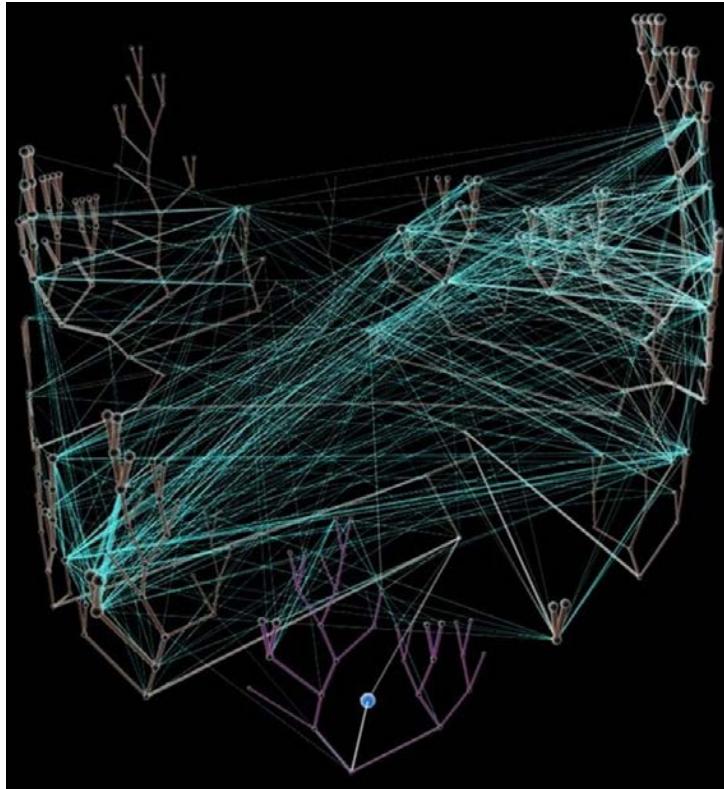
- The coalescent is a powerful tool for understanding historical demography (e.g., ancestral Ne and divergence times)
- Branch lengths can have an enormous impact on recovering the species tree from gene trees
- Rapid radiations with short internal branches can really F* U up!
- Ancestral population 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 (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

Themes from yesterday ... (thank you Peter, Laura,
and Claudia!)

BOTTOM LINE:

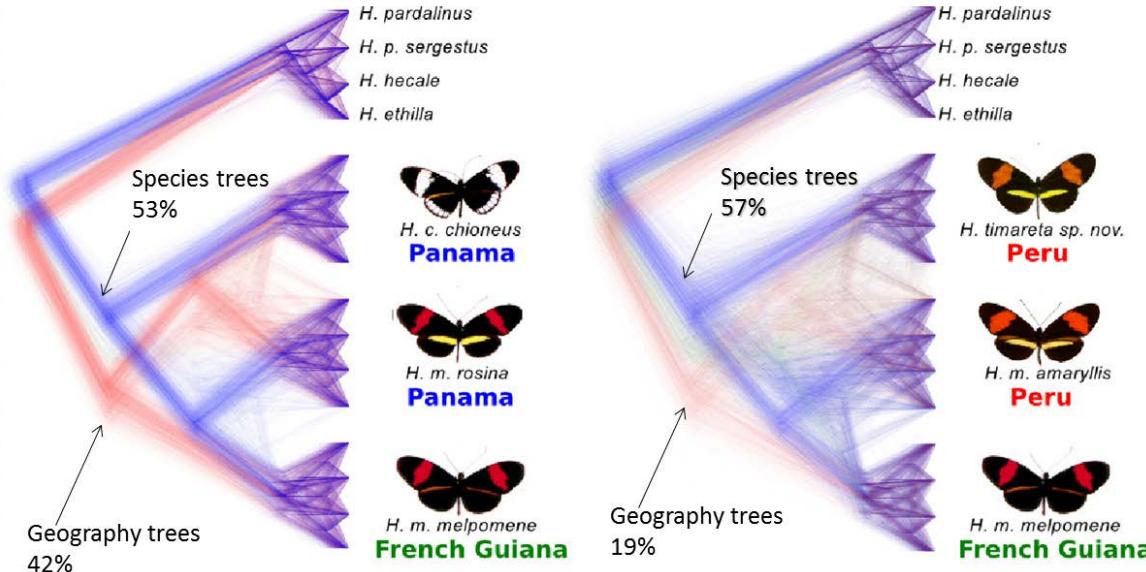
IT'S BLOODY COMPLICATED!!!

Gene flow only makes matters worse!



Lateral Gene Transfer

Gene flow only makes matters worse!



Jiggins, Mallet, and colleagues (over the years)

Ongoing gene flow during speciation

So ... do we give up???

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. ■

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?



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



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

Onja 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.

RIJASOLD/RNA PRESS

Why mouse lemurs???

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}



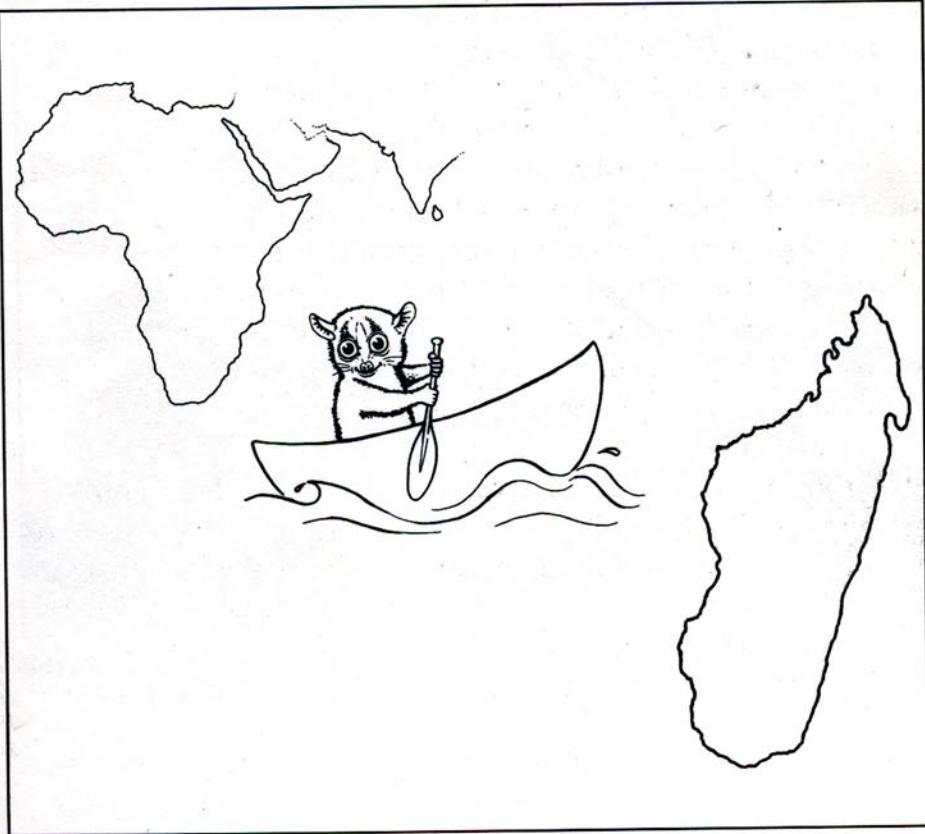


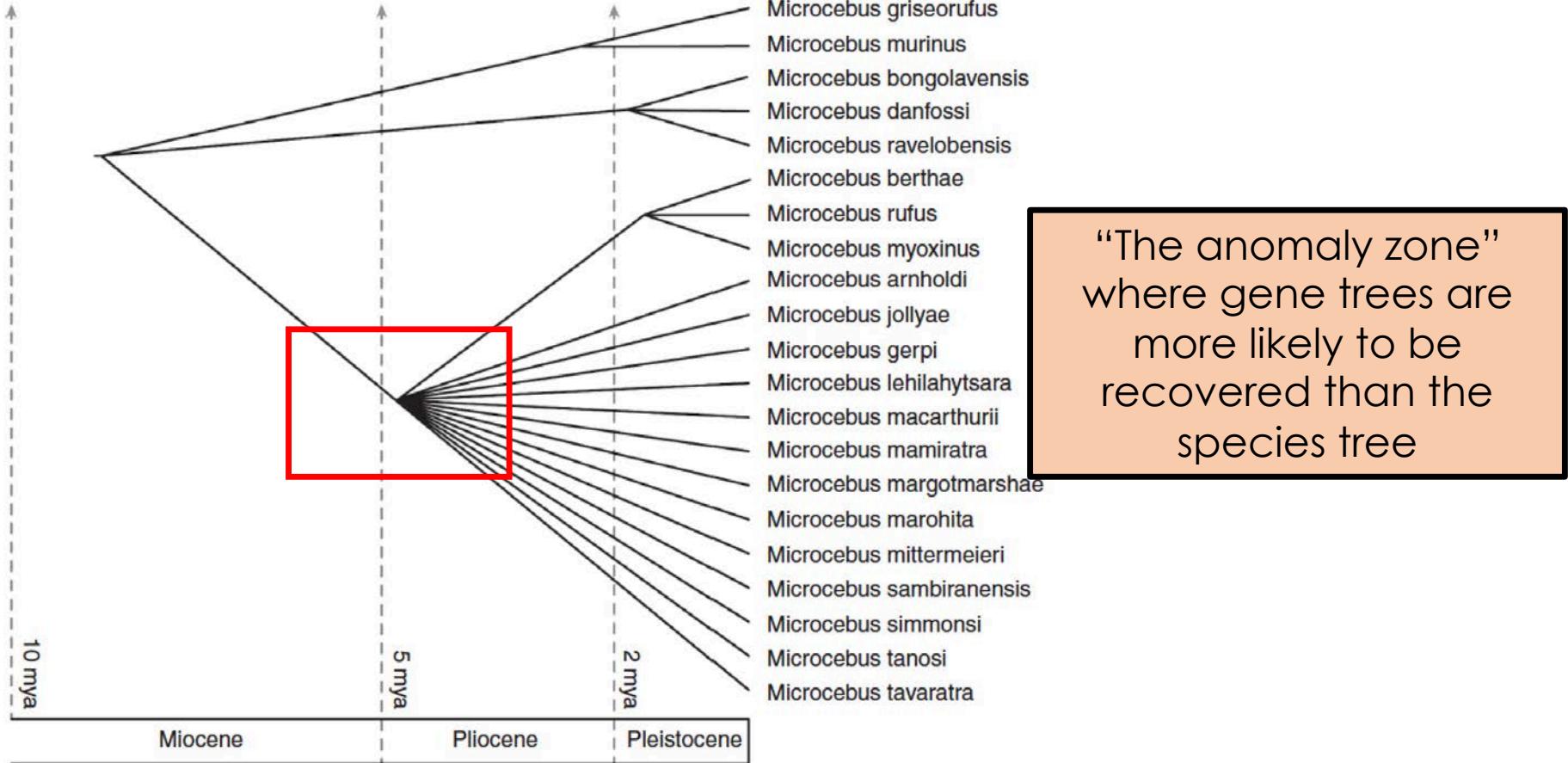
ILLUSTRATION BY STEPHEN D. NASH

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

~ 24 species described at present



MITTERMEIER RA ET AL. 2010. LEMURS OF MADAGASCAR. 3RD ED.
ILLUSTRATED BY SD NASH.



2018

Trends in Ecology & Evolution

CellPress
REVIEWS

Opinion

Finding Evolutionary Processes Hidden in Cryptic Species

Torsten H. Struck,^{1,*} Jeffrey L. Feder,² Mika Bendiksby,^{1,3} Siri Birkeland,¹ José Cerca,¹ Vladimir I. Gusarov,¹ Sonja Kistenich,¹ Karl-Henrik Larsson,¹ Lee Hsiang Liow,^{1,4} Michael D. Nowak,¹ Brita Stedje,¹ Lutz Bachmann,¹ and Dimitar Dimitrov^{1,5}



M. ravelobensis



M. murinus



M. griseorufus

Can you tell them apart???



M. tavaratra



M. berthae



M. rufus

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

D. L. Hull

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

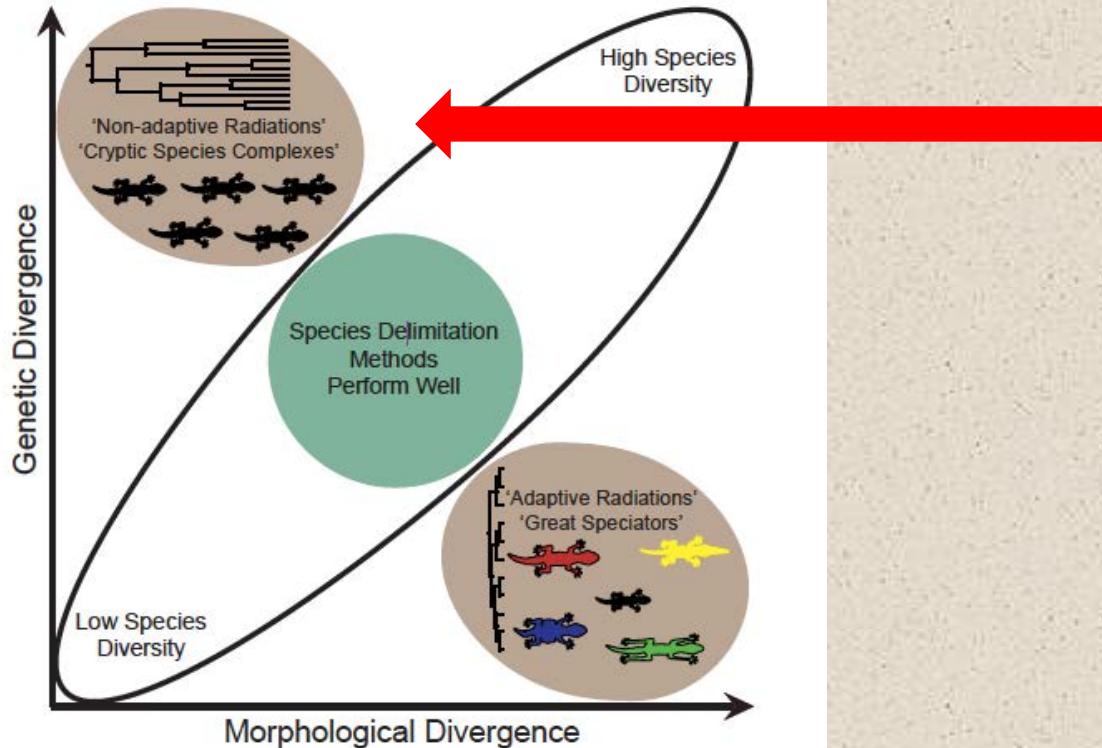
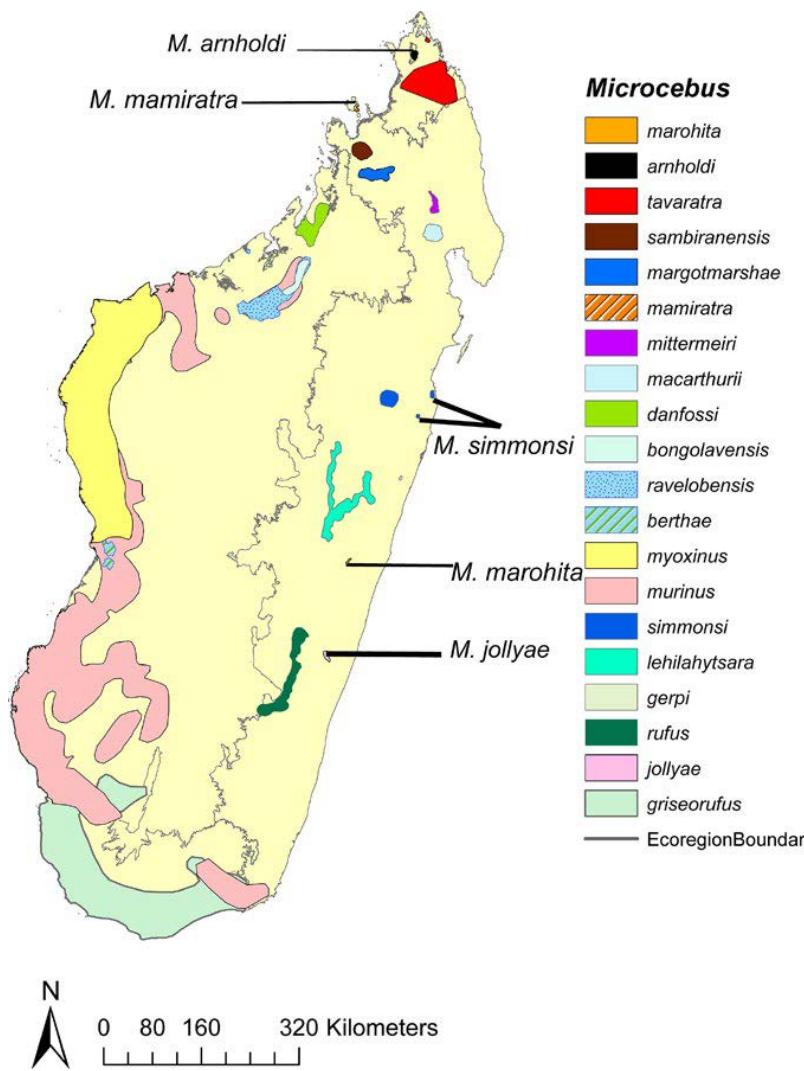


Figure 1. Hypothetical axes of morphological and genetic diversity within a species group. Area circumscribed by black ellipse represents systems where species delimitation is usually simple.

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



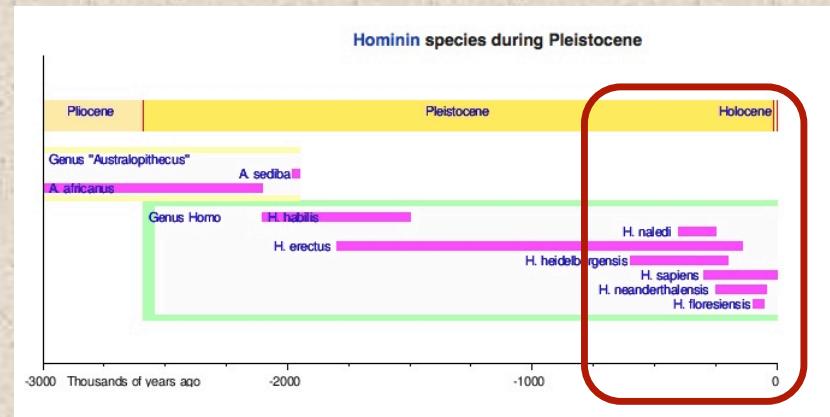
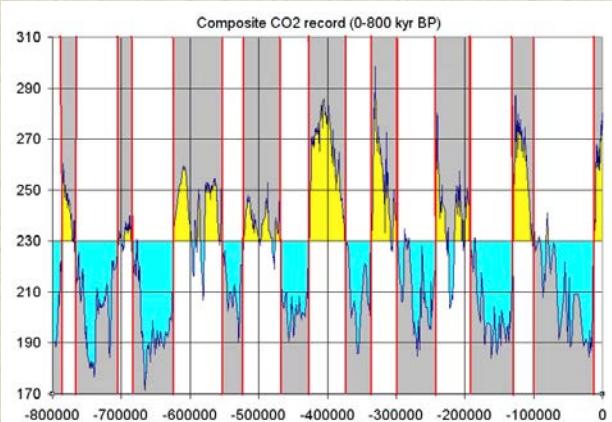
The majority of named species appear to be ***microendemics***, though others have much broader geographic distributions ...





Has climate change been a driver of speciation?

Climate Fluctuation during the Pleistocene



Lineage diversification
within hominins

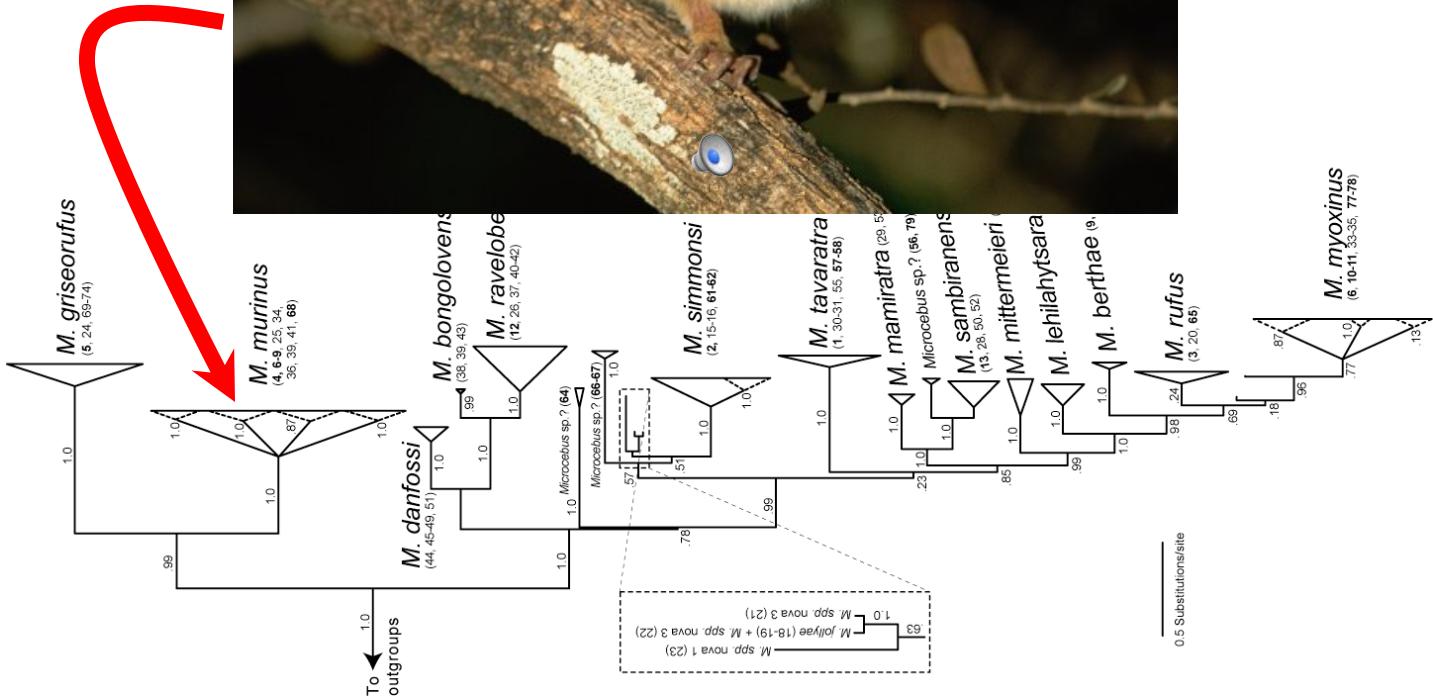
~ 2.6 mya through ~ 12 kya

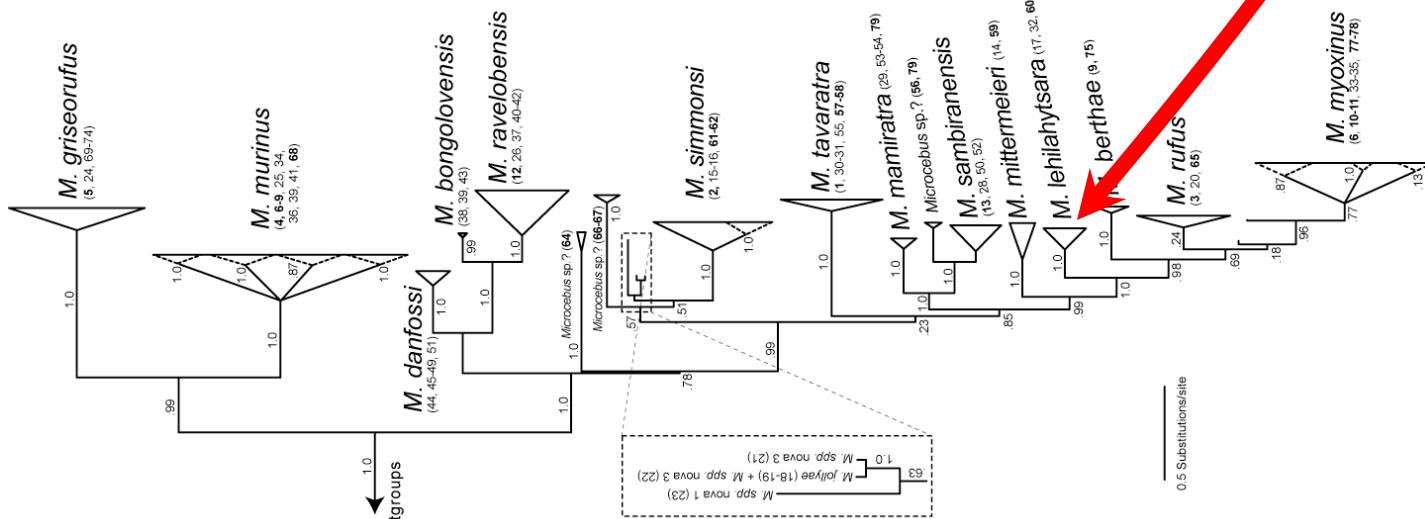


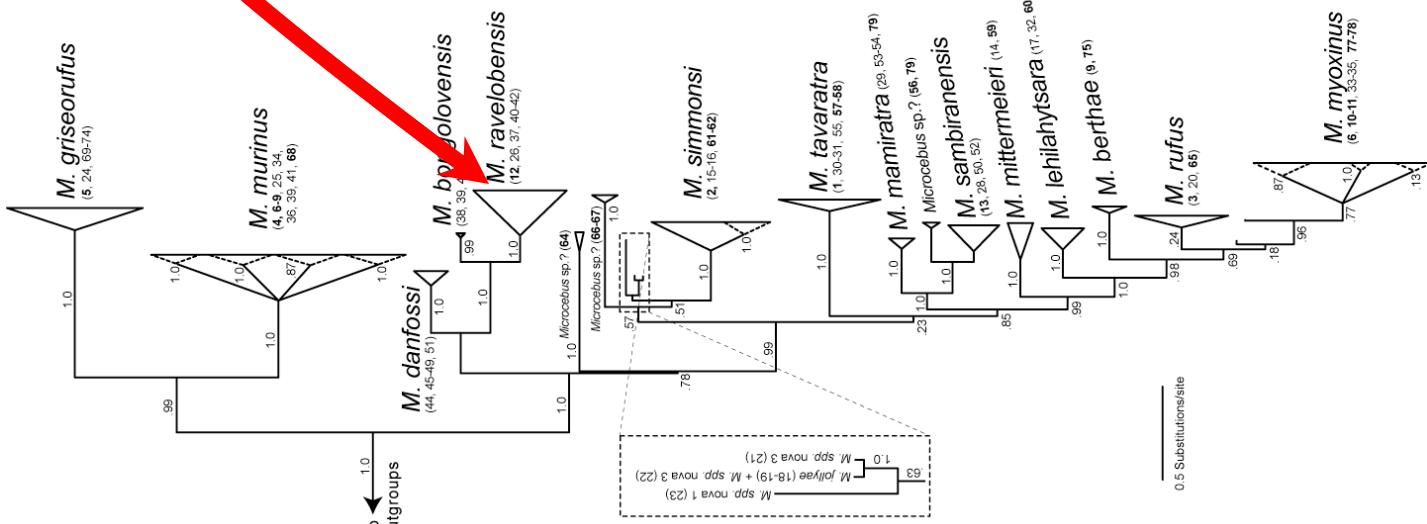


Sound

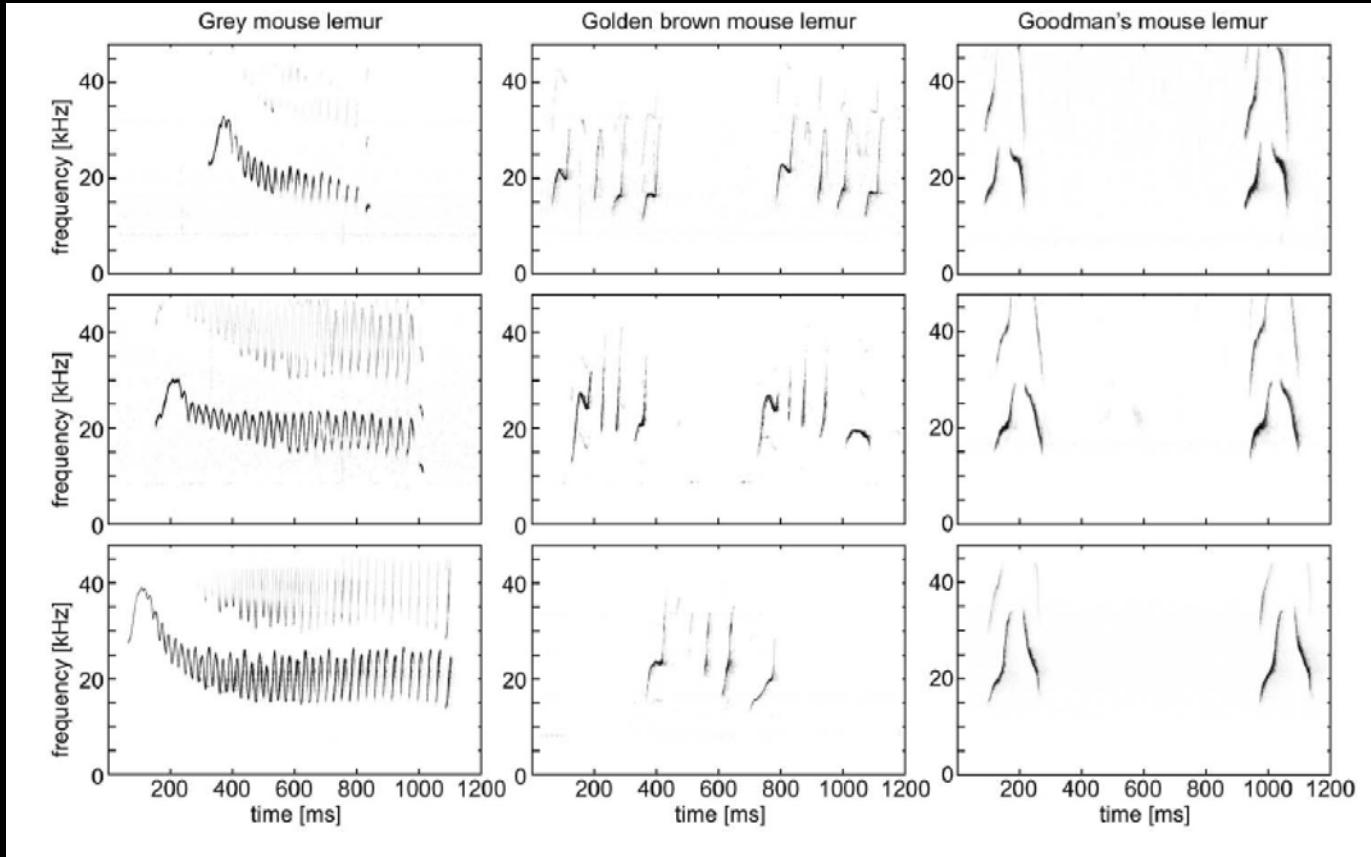
Smell





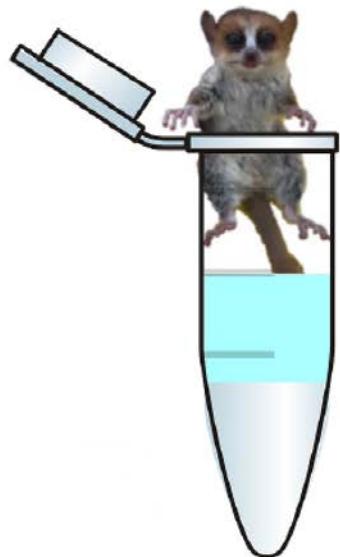


Male advertisement calls for three species





Mouse lemur sampling (TCI grant)



5 field sites
29 mouse lemurs
7 dwarf lemurs
4 flights
4200Km by road



(or, “It takes a village to study mouse lemurs”)





RADseq Consortium

N = 283 individuals from > 20 species







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^{1,*}, Lauren M. Chan^{1,†}, Mario dos Reis^{2,†}, Peter A. Larsen^{1,†}, C. Ryan Campbell¹, Rodin Rasoloarison^{3,4}, Meredith Barrett⁵, Christian Roos⁴, Peter Kappeler⁶, Joseph Bielawski⁷, and Ziheng Yang²

¹Department of Biology, Duke University

²Department of Genetics, Evolution and Environment, University College London, London, United Kingdom

³Département de Biologie Animale, Université d'Antananarivo, Antananarivo, Madagascar

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

⁵UCSF Center for Health & Community

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

The screenshot shows a bioRxiv preprint page. At the top left is the CSHL logo with the text "Cold Spring Harbor Laboratory". The bioRxiv logo is prominently displayed in the center. Below it, the text "THE PREPRINT SERVER FOR BIOLOGY" is visible. On the right side, there are links for "HOME" and "ABOUT", and a search bar. The main title of the preprint is "Elaborate expansion of syntenic VIR hotspots correlates with high species diversity in nocturnal mouse and dwarf lemurs". Below the title, the authors are listed as Kelsie E. Hunnicutt, George P. Tiley, Rachel C. Williams, Peter A. Larsen, Marina B. Blanco, Rodin M. Rasolosarison, C. Ryan Campbell, Kevin Zhu, David W. Weisrock, Hiroaki Matsunami, and Anne D. Yoder. The DOI is provided as [doi: https://doi.org/10.1101/637348](https://doi.org/10.1101/637348). A note at the bottom states, "This article is a preprint and has not been peer-reviewed [what does this mean?]."

Sensory drive speciation and patterns of variation at selectively neutral genes

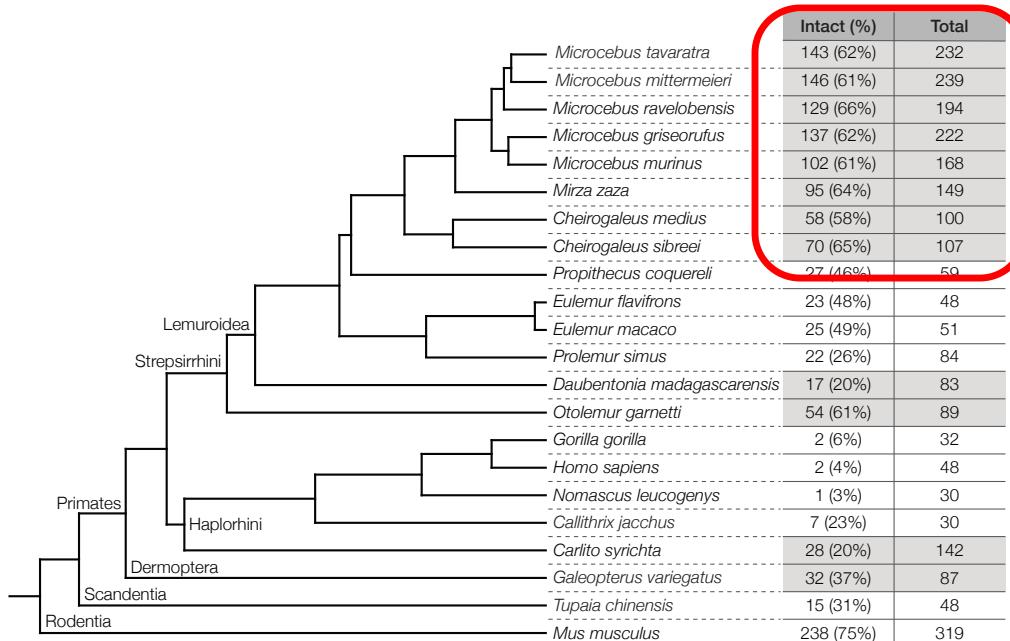
Authors

[Authors and affiliations](#)

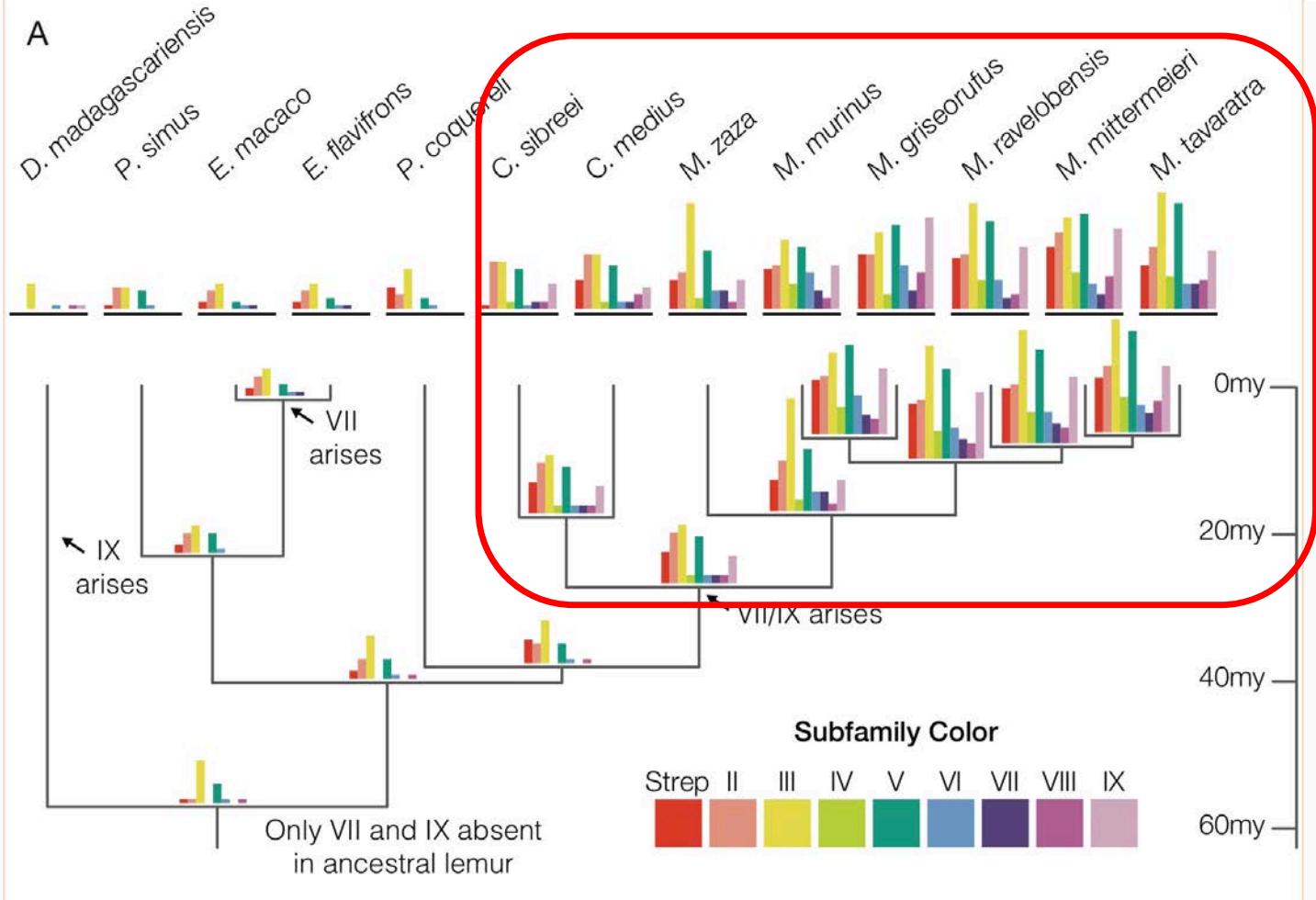
Tomotaka Matsumoto , Yohey Terai, Norihiro Okada, Hidenori Tachida

“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 et al. (in revision)



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.

A

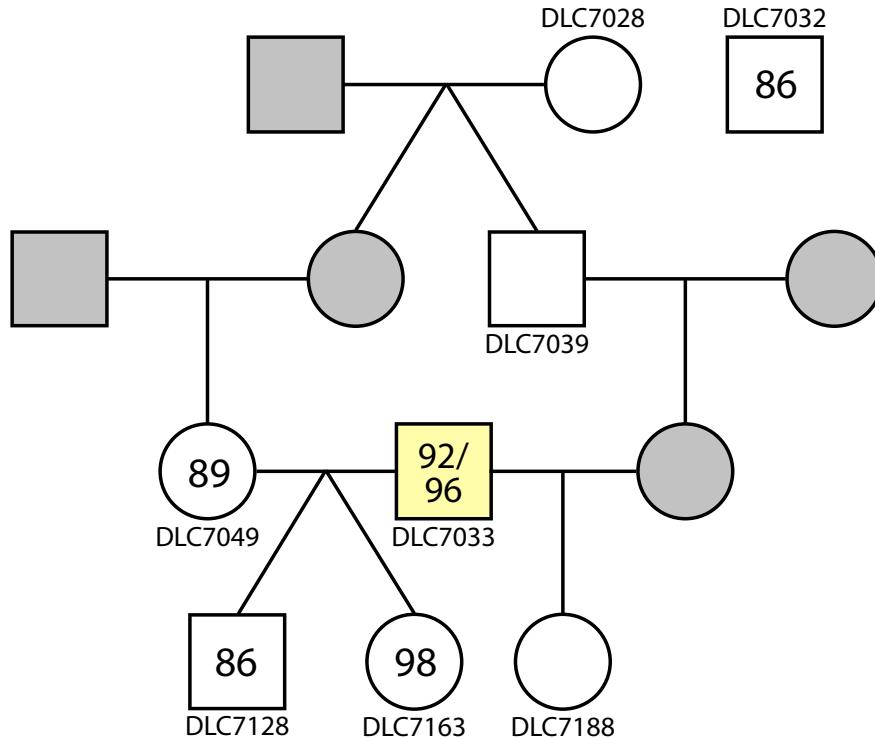
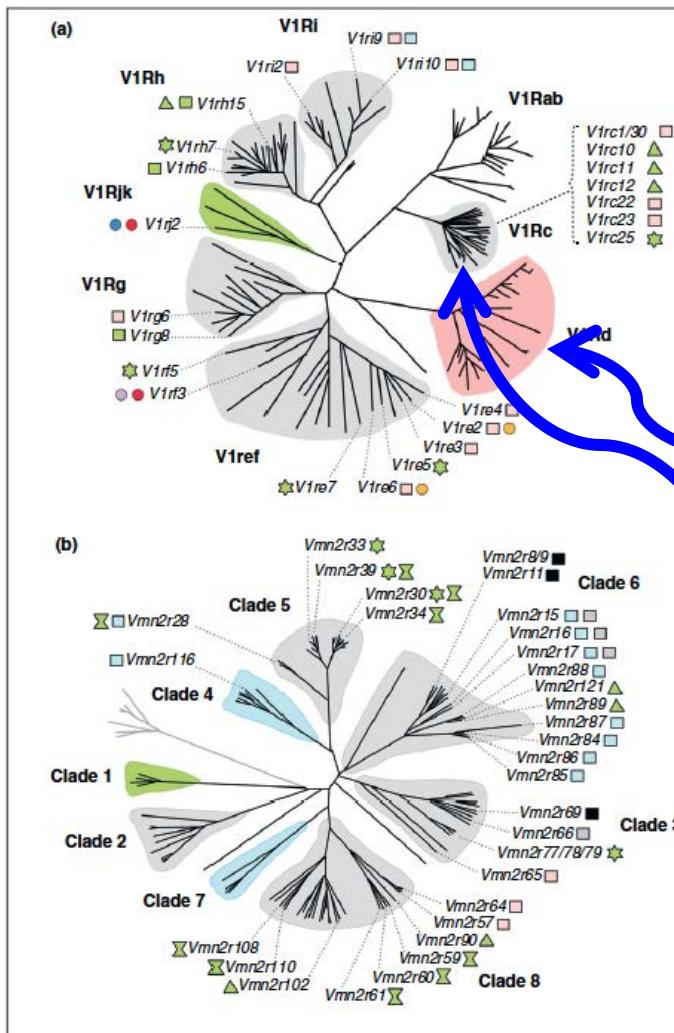


Figure 5 Intraspecific *Microcebus murinus* V1R repertoire size diversity

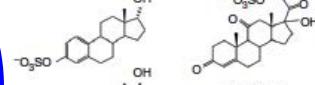


We can know precisely which stimuli effect the evolution of which genes (*in mice*):

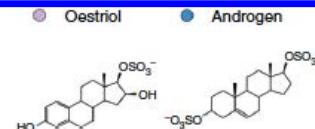
Heterospecific cues:

- Snake bedding
- Ferret bedding
- Owl bedding

For example, this V1R subfamily is receptive only to the stimuli from female mouse bedding

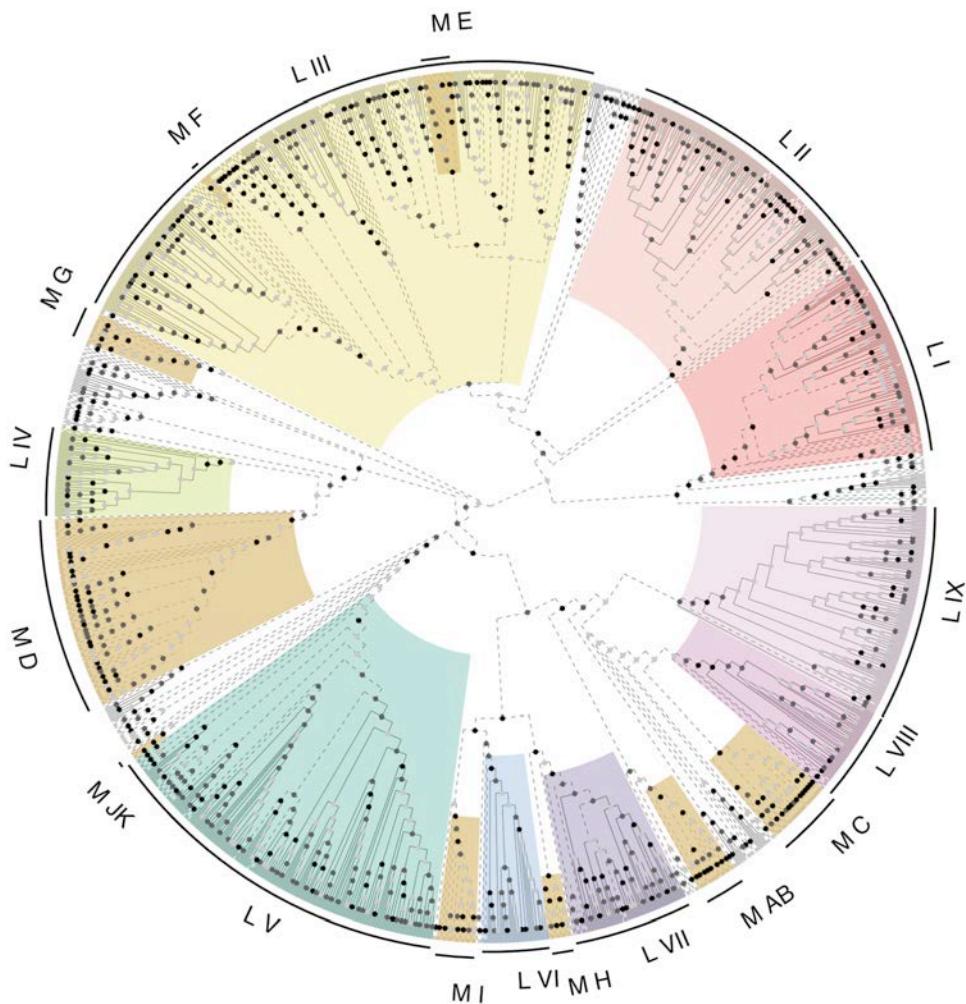


On the other hand, this subfamily responds to female mouse bedding, snake bedding, and ferret bedding



TRENDS in Neurosciences

Figure 3. Linking vomeronasal receptors and sensory cues. Schematic map of *Egr1* colocalization with either (a) V1R or (b) V2R probes performed by dual-color RNA *in situ*



In conclusion (re: are they using different sensory signals, perhaps driving and/or maintaining species boundaries?):

YES!!!

GENE TREES IN SPECIES TREES

WAYNE P. MADDISON

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

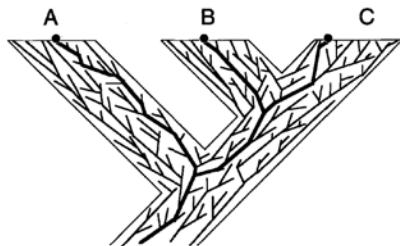


FIGURE 1. A gene tree contained within a species tree leading to three extant species: A, B, and C. Bold branches of gene tree show relationships among the sampled copies of the gene (●). Sampled copies from sister species B and C are sister copies.

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

Gene trees often don't match the species tree

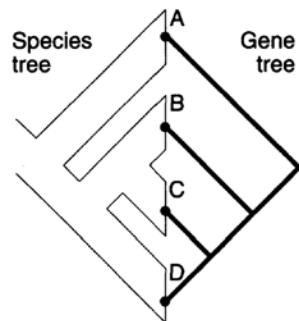
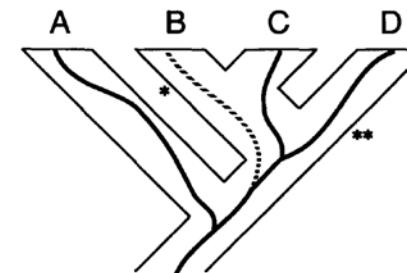


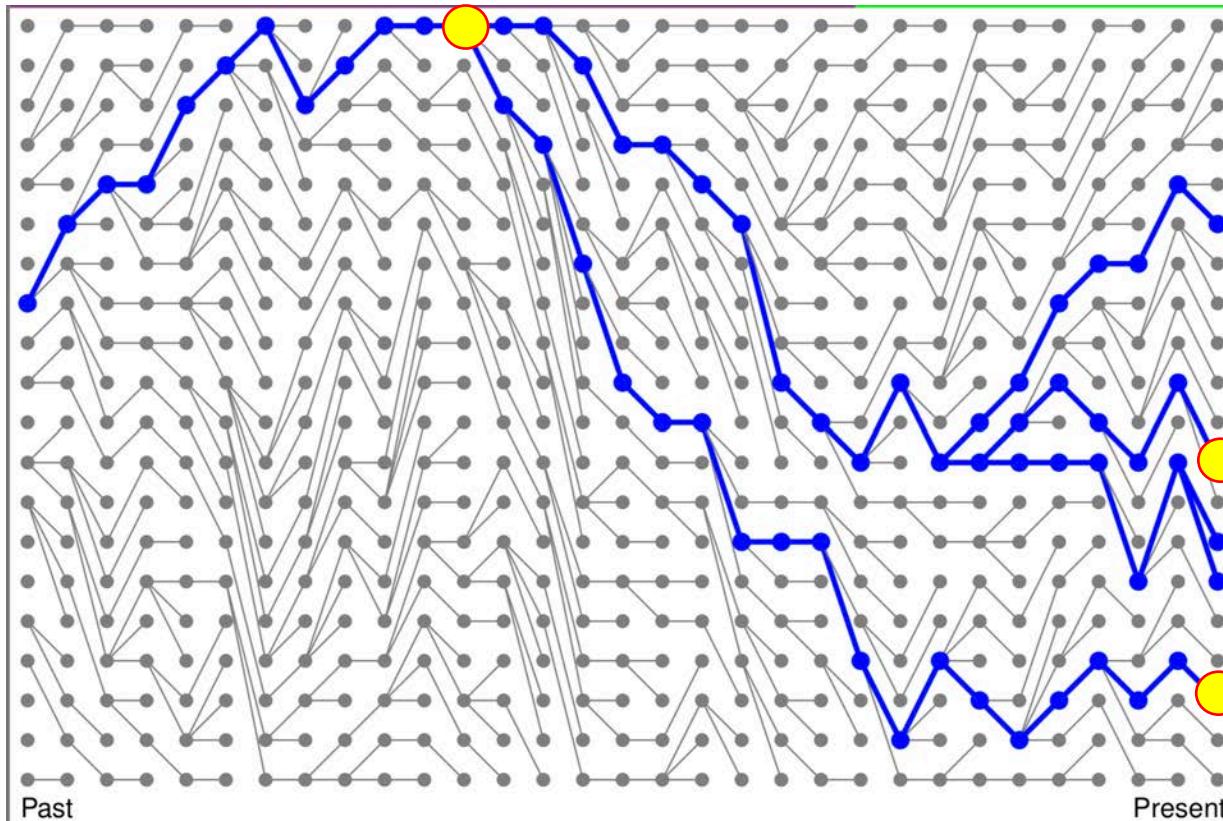
FIGURE 2. Discord between gene and species trees. At left is the species tree of four species, A, B, C, and D, and at right is the tree of a gene sampled one copy per species. Species B and C are sister species, but their gene copies are not sister copies.



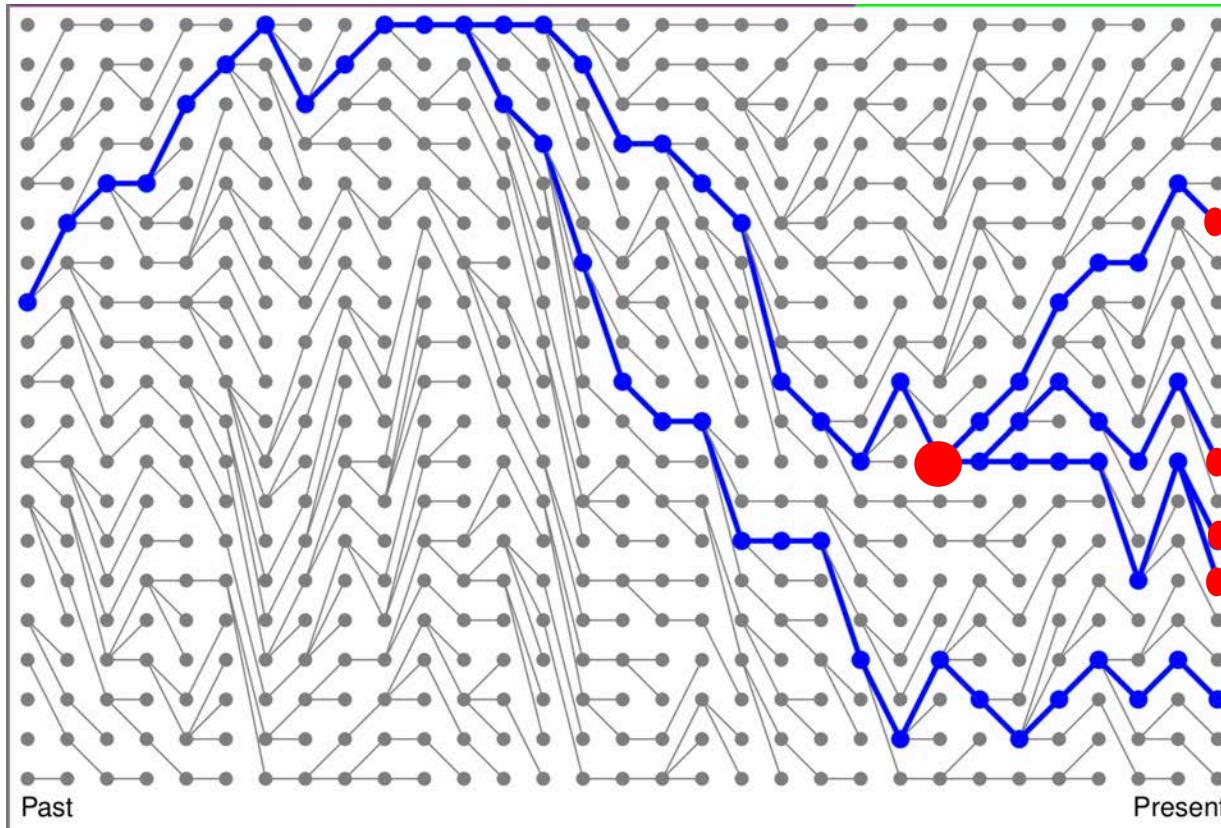
Branch short and wide:
Deep coalescence likely

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

Even small sample sizes (in terms of individuals) can yield powerful results

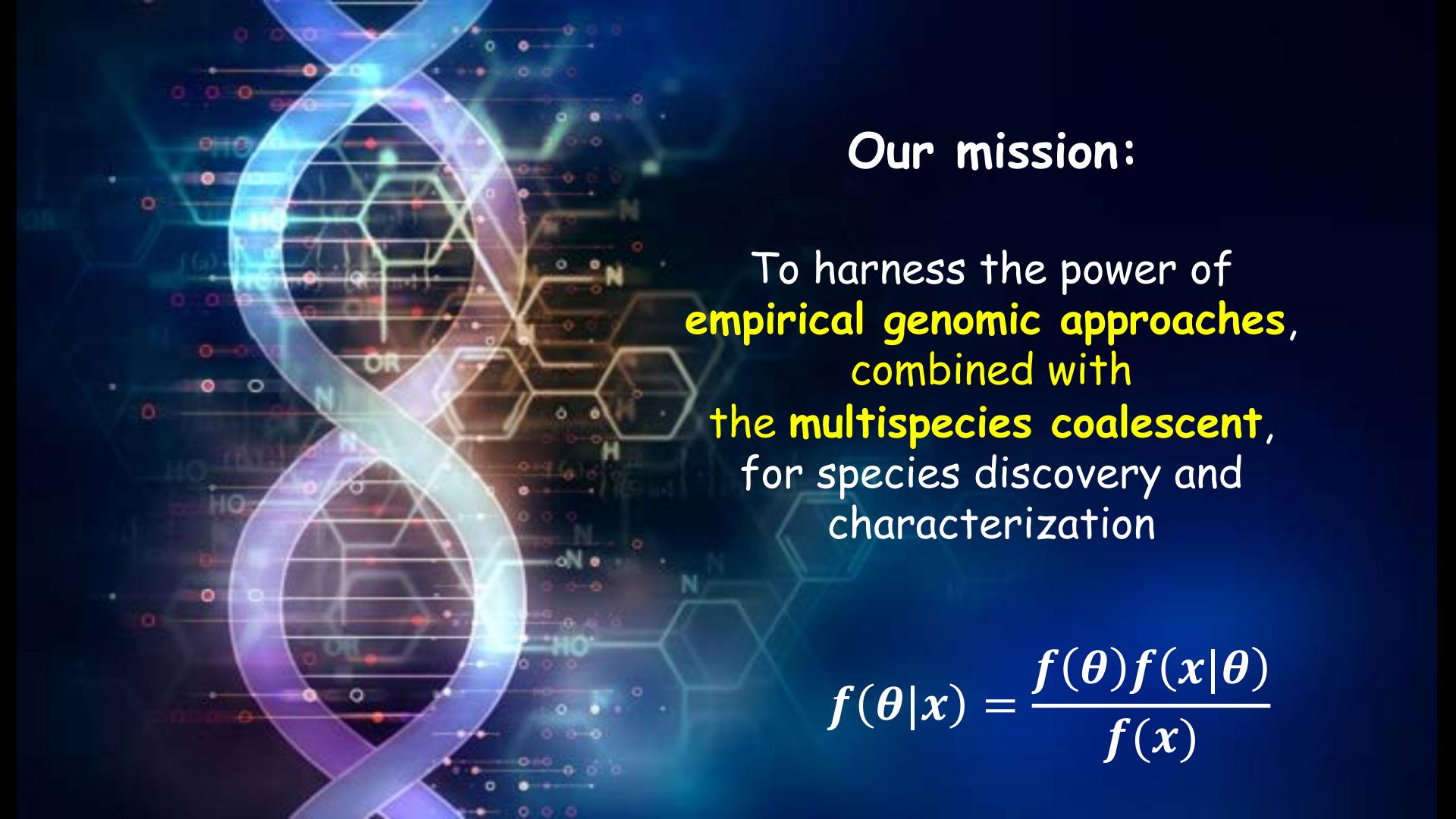


**Though sampling strategy must be carefully considered!
(remember the frogs in a single pond)**



The Multispecies Coalescent is a powerful toolkit for estimating :

- The species tree (i.e., phylogeny)
- Effective Population Size (N_e) through time
- Divergence ages
- Patterns of gene flow (both rate and direction)



Our mission:

To harness the power of
empirical genomic approaches,
combined with
the multispecies coalescent,
for species discovery and
characterization

$$f(\theta|x) = \frac{f(\theta)f(x|\theta)}{f(x)}$$

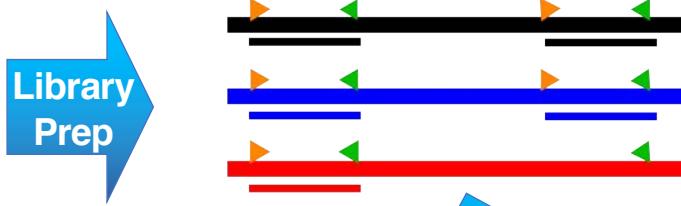
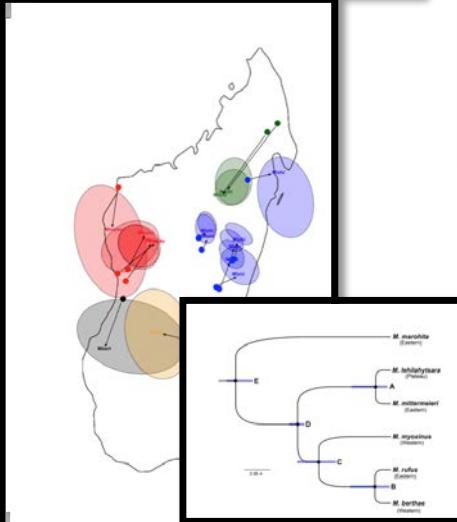
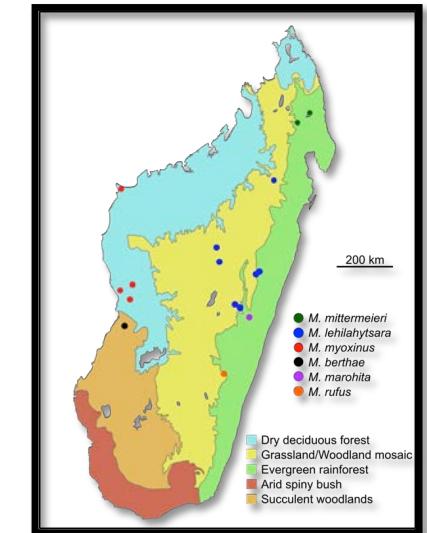
Recall from yesterday:

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

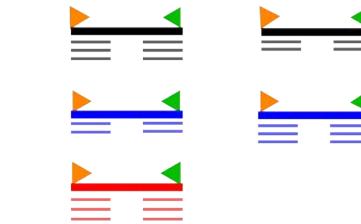
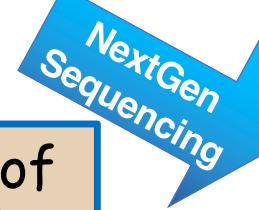
The entire field of population genetics will benefit from increased exchange between coalescents and phylogeography ... [and] the future availability of multilocus genetic data will serve as a bridge between these two approaches."

"Intralocus recombination is quite problematic for inferred gene-tree approaches since the genealogy is no longer a bifurcating tree (Hein, 1993)."

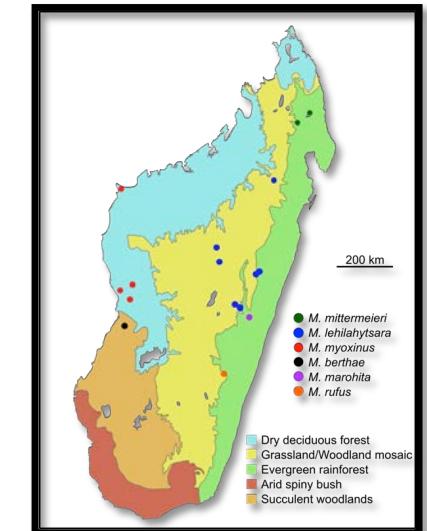
Wakeley, 2003



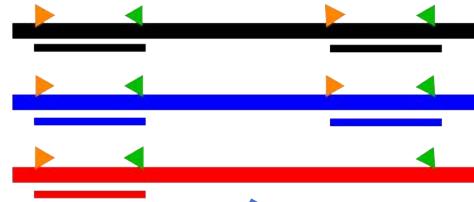
RADseq: Tens of thousands of unlinked SNPs from across the genome!



Slide courtesy of C. Ryan Campbell

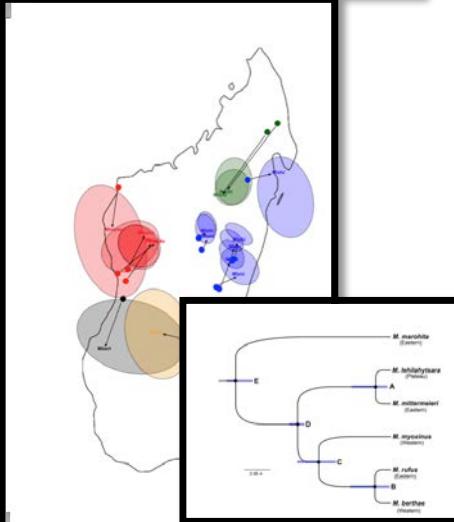


Library Prep

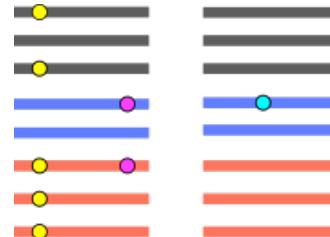


NextGen
Sequencing

ddRADseq Data Workflow



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.
- Nr of invariant loci: 1,822.

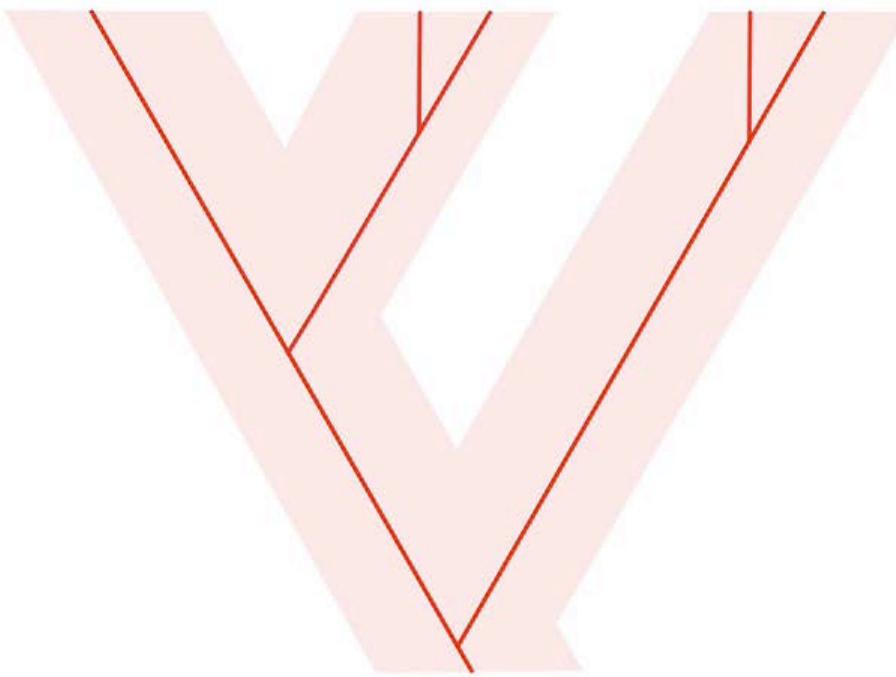
Mean locus length: 231 bp.

Mean nr of variable sites: 4.7.

Mean nr of parsimony-informative sites: 3.29.

Mean % of missing data (Ns): 5.53.

AGCACTGACC CGGGATGC TACGATCGACATCGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT
AGCACTGACC CGGGATGC TACGATCGACATCGACGACACTAGCCGGGT TACATCGATCACGCAT GCGACT
AGCACTGACC CGGGATGC TACGATCGACATGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT
AGCACTGAC CGGGATGC TACGATCGACATCGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT
AGCACTGAC CGGGATGC ACGATCGACATCGACGACACTAGCCGGGTACATCGATCACGCAT GCGACT
AGCACTGAC CGGGATGC TACGATCGACATCGACGACACTAGCCGGGTACATCGATCACGCA ACGACT



Remember Peter's whales ...

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



C. Ryan Campbell

The image shows a screenshot of the bioRxiv preprint server interface. At the top left is the CSHL logo with the text "Cold Spring Harbor Laboratory". Next to it is the bioRxiv logo with the tagline "THE PREPRINT SERVER FOR BIOLOGY". To the right are links for "HOME" and "ABOUT", and a search bar. Below the header, there are two buttons: "New Results" on the left and "Comment on this paper" on the right. The main content area features a bold title: "Pedigree-based measurement of the de novo mutation rate in the gray mouse lemur reveals a high mutation rate, few mutations in CpG sites, and a weak sex bias". Below the title is a list of authors: C. Ryan Campbell, George P. Tiley, Jelmer W. Poelstra, Kelsie E. Hunnicutt, Peter A. Larsen, Mario dos Reis, and Anne D. Yoder. A "doi:" link is provided with the value <https://doi.org/10.1101/724880>. At the bottom, a note states: "This article is a preprint and has not been peer-reviewed [what does this mean?]."

New Results

Comment on this paper

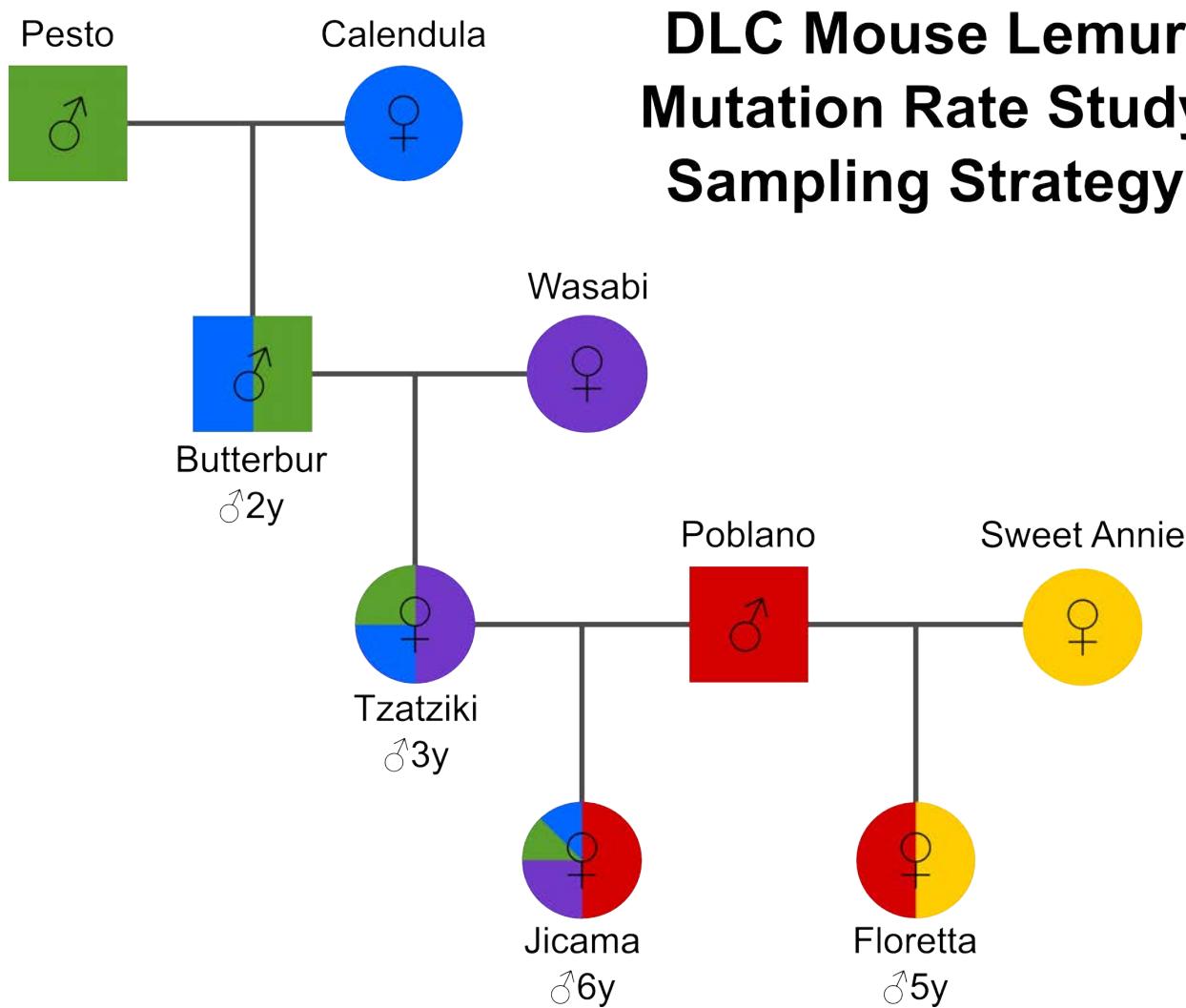
Pedigree-based measurement of the de novo mutation rate in the gray mouse lemur reveals a high mutation rate, few mutations in CpG sites, and a weak sex bias

C. Ryan Campbell, George P. Tiley, Jelmer W. Poelstra, Kelsie E. Hunnicutt, Peter A. Larsen, Mario dos Reis, Anne D. Yoder

doi: <https://doi.org/10.1101/724880>

This article is a preprint and has not been peer-reviewed [what does this mean?].

DLC Mouse Lemur Mutation Rate Study Sampling Strategy



Measuring de novo mutation rate:

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

Easy, right?

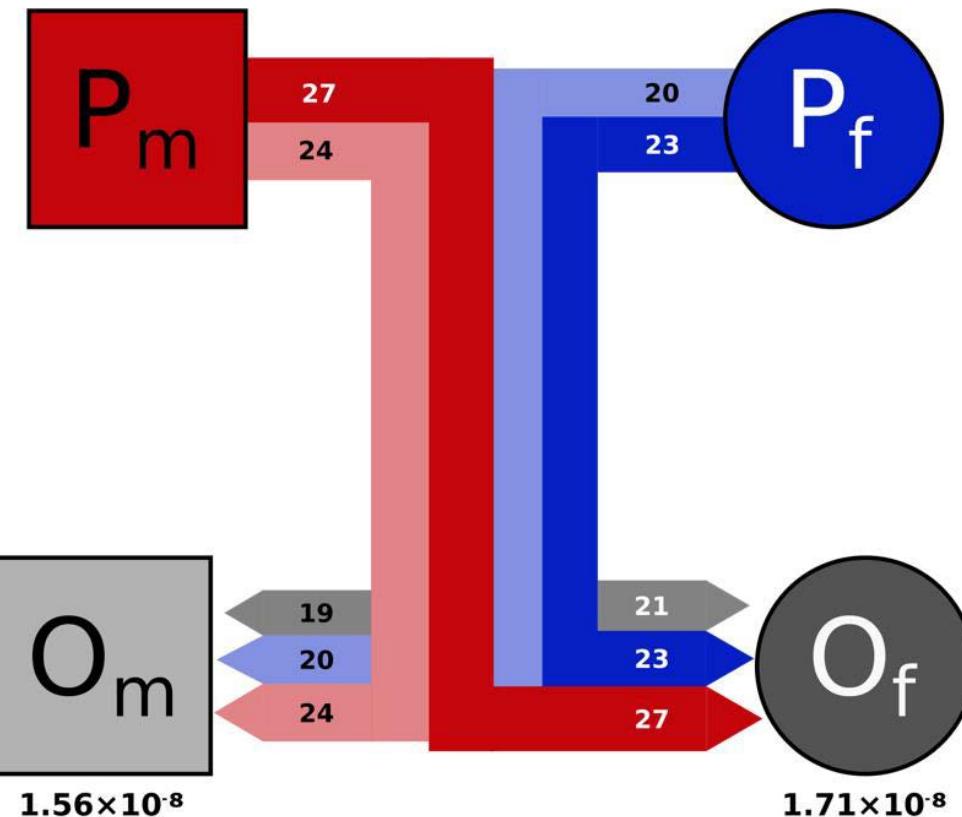
Problems (just a few):

- 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

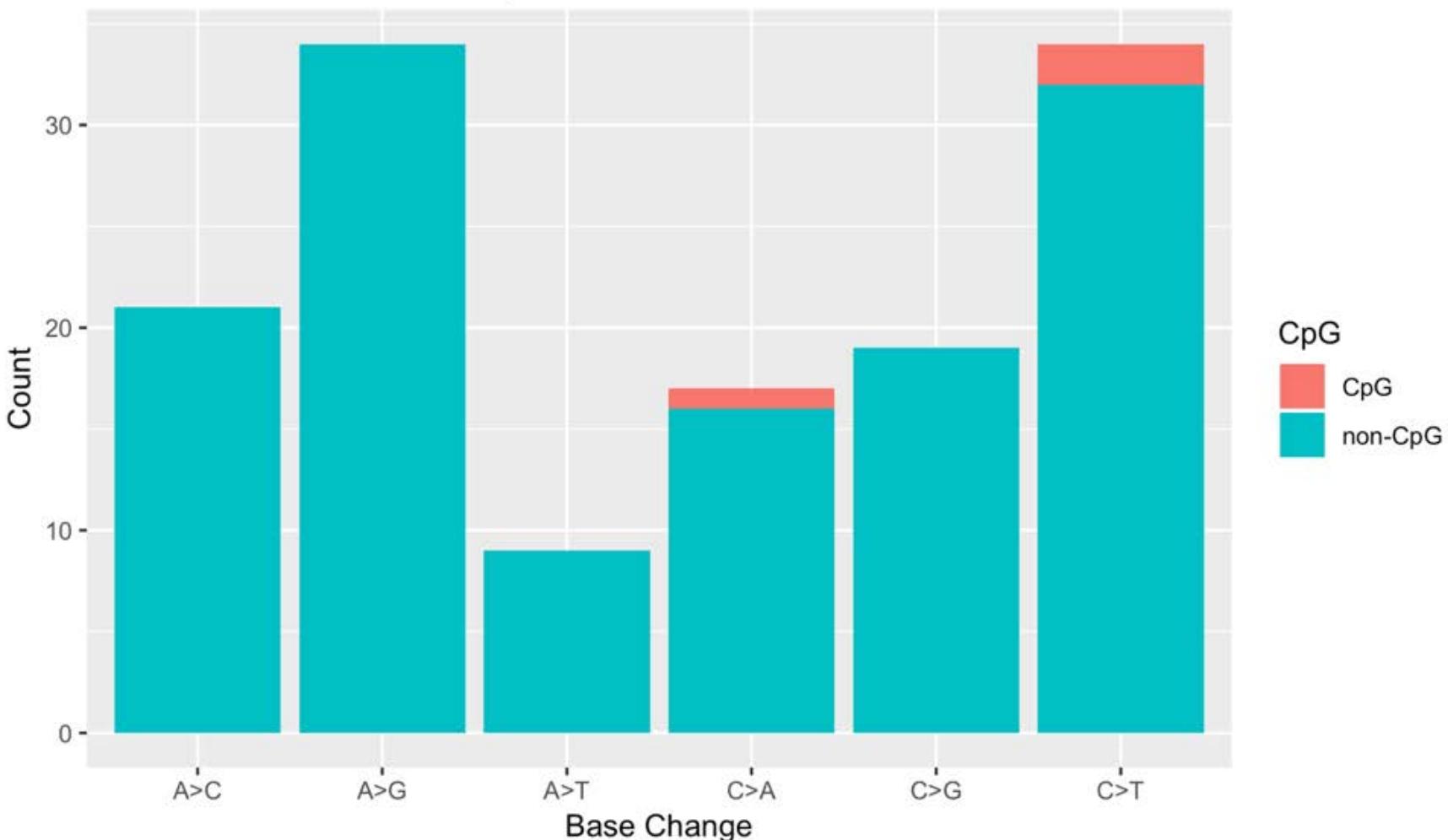
Estimating generation times:

Key parameters

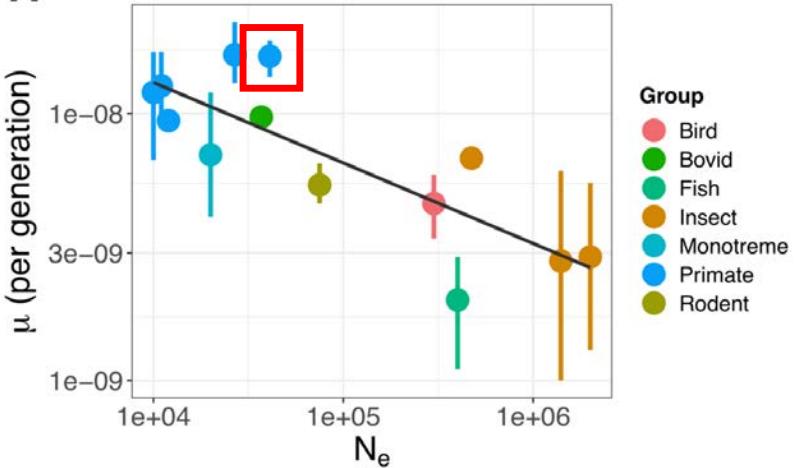
- Age at onset of first offspring to last reproductive event
- Number of offspring produced from first to last reproduction
- Probability of surviving to age of last reproduction



Mouse Lemur Mutation Spectrum

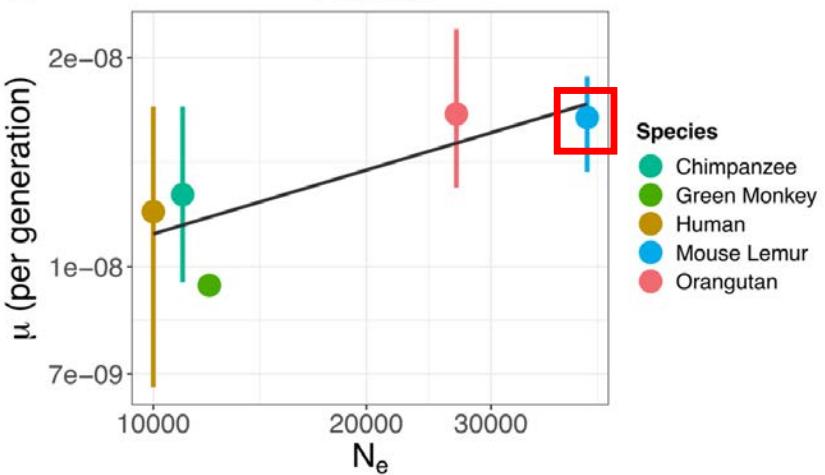


A All animals



1.64×10^{-8}

B Primates



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

Four case studies (all in prep):

1. Genomic homogeneity in the face of ecological divergence
2. Intraspecific divergence driven by natural climate change
(incipient?)
3. Lineage diversification in close geographic proximity **(ongoing?)**
4. Reproductive isolation in sympatry **(complete?)**

Break, anyone???

Case #1: “False alarm!” Investigating the genomic signal underlying (apparent) incipient speciation



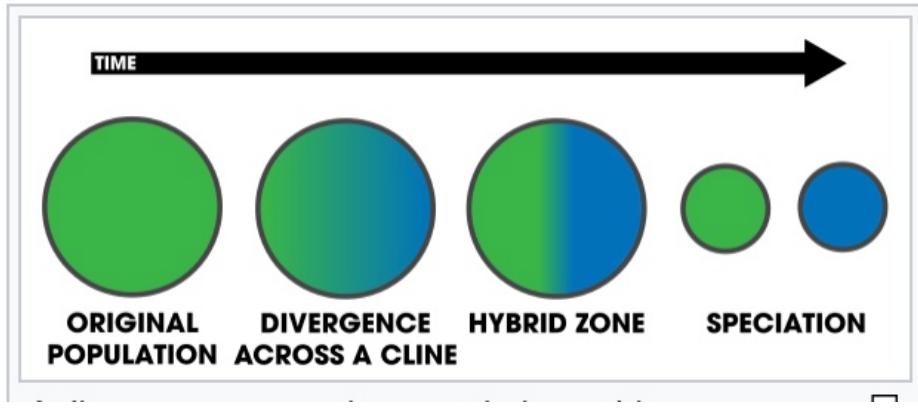
Jelmer Poelstra

Genomic homogeneity in the face of morphological and ecological diversification:
detecting hidden functional loci via genome scans in the grey-brown mouse
lemur: *Microcebus griseorufus*

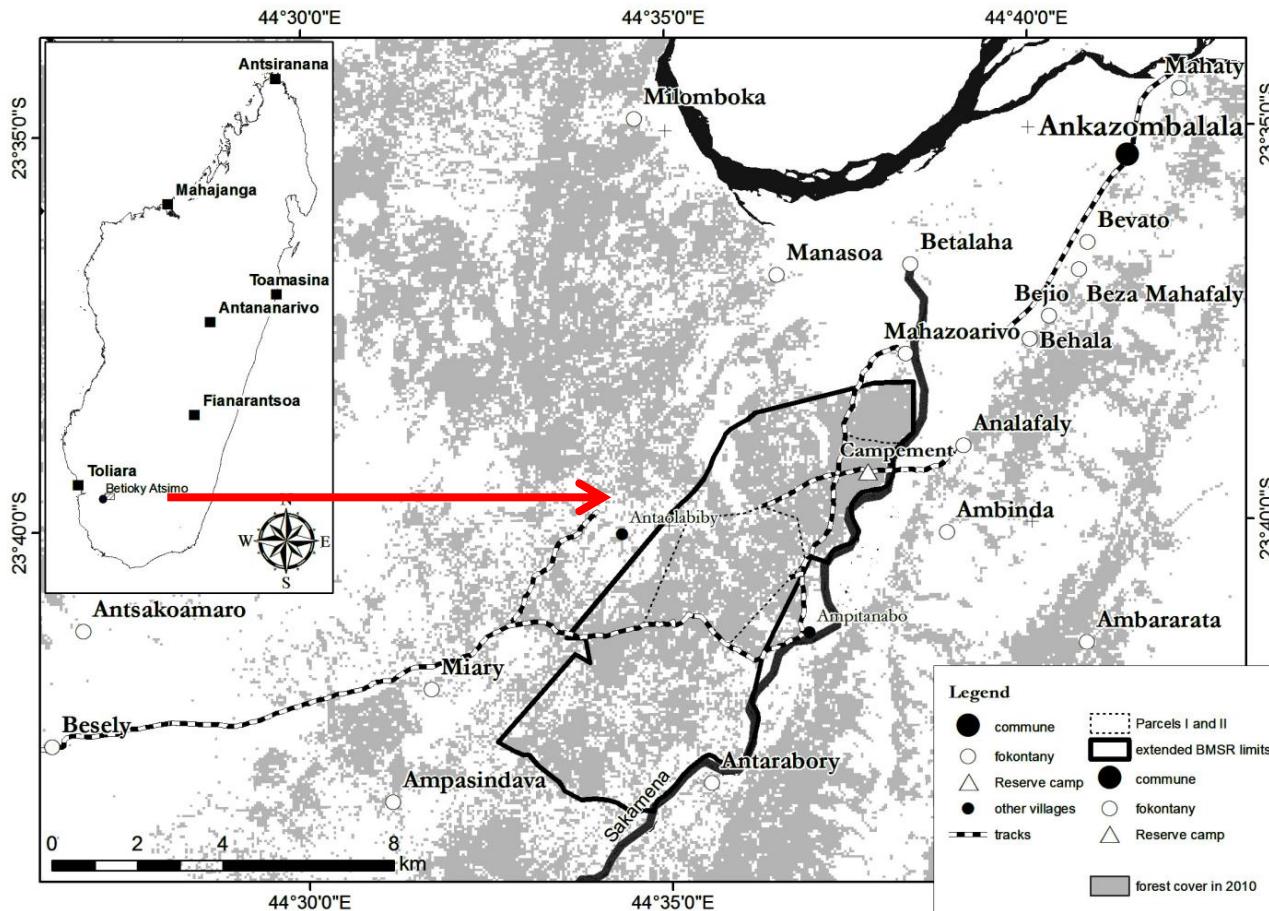
Jelmer Poelstra, Emilienne Rasoazanabary, Gina-Marie Agostini, Alison Richard, Laurie Godfrey,
and Anne D. Yoder

[in prep]

Parapatric Speciation



(at or to one side
of; beside; side by
side)



Beza Mahafaly Special Reserve ($\sim 6 \text{ km}^2$)

“Dissertation in a Box”: 256 samples

- Gallery = 92
- Ihazoara = 70
- Spiny = 94



Gallery forest



Spiny forest

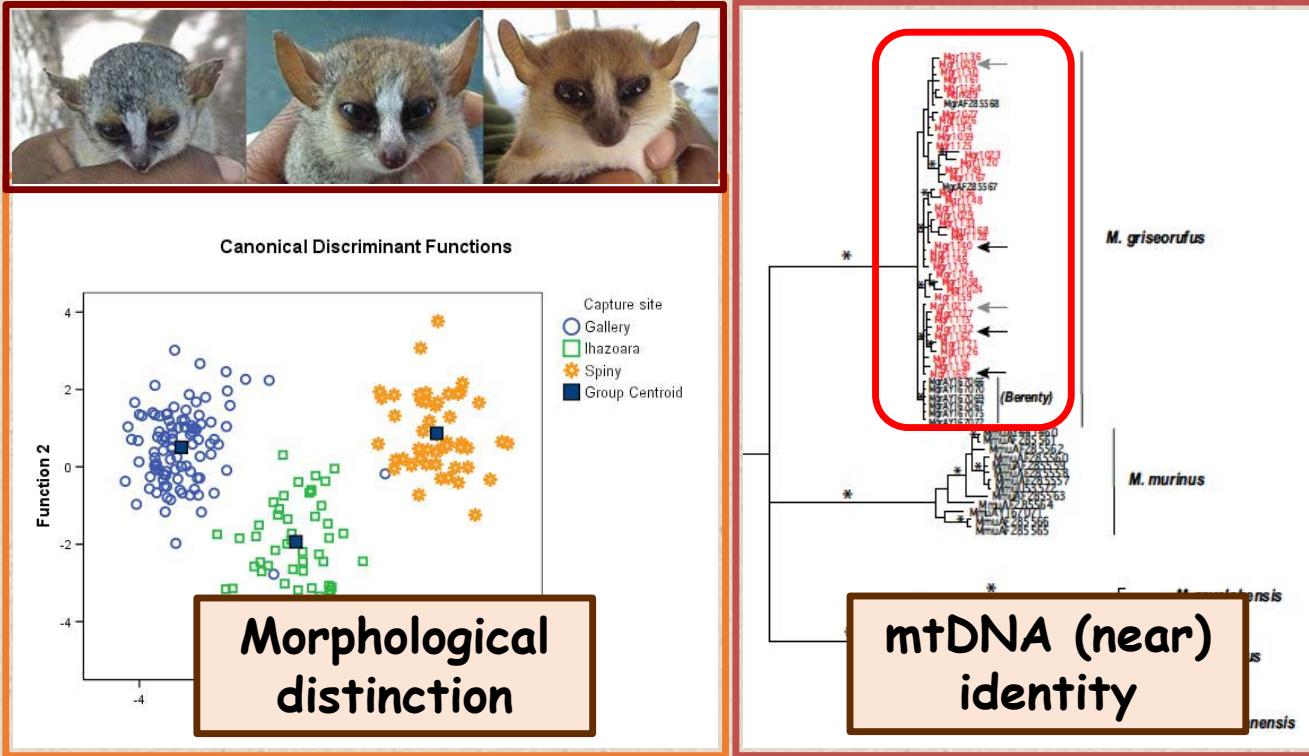


Ihazoara forest

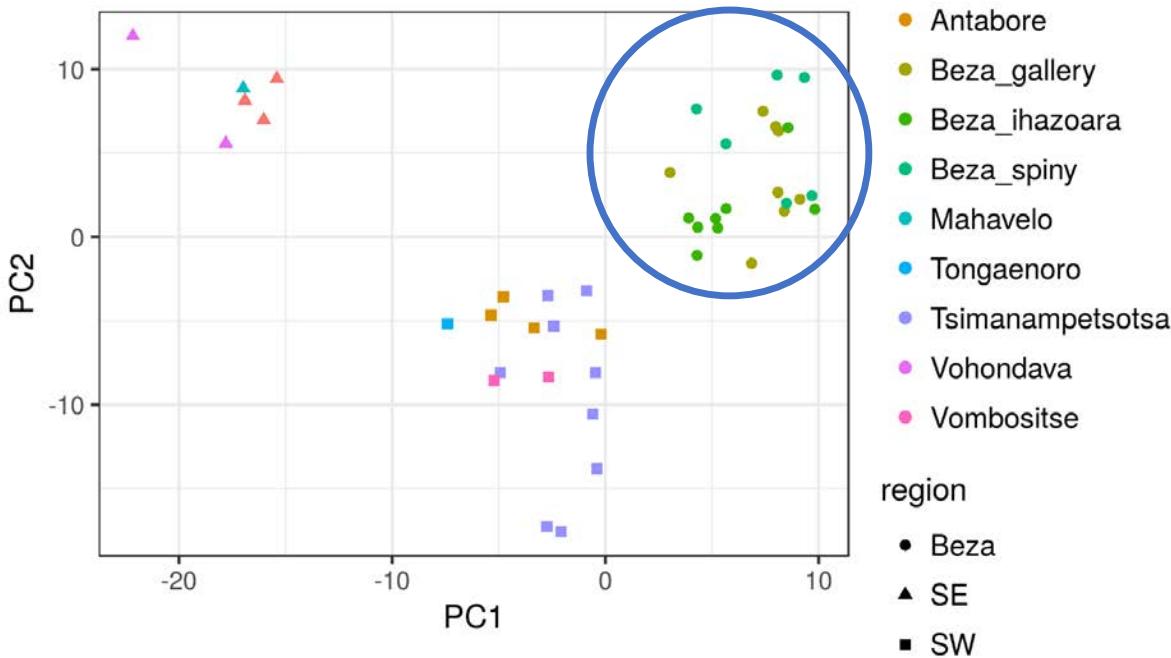
Three Distinct Color Morphs



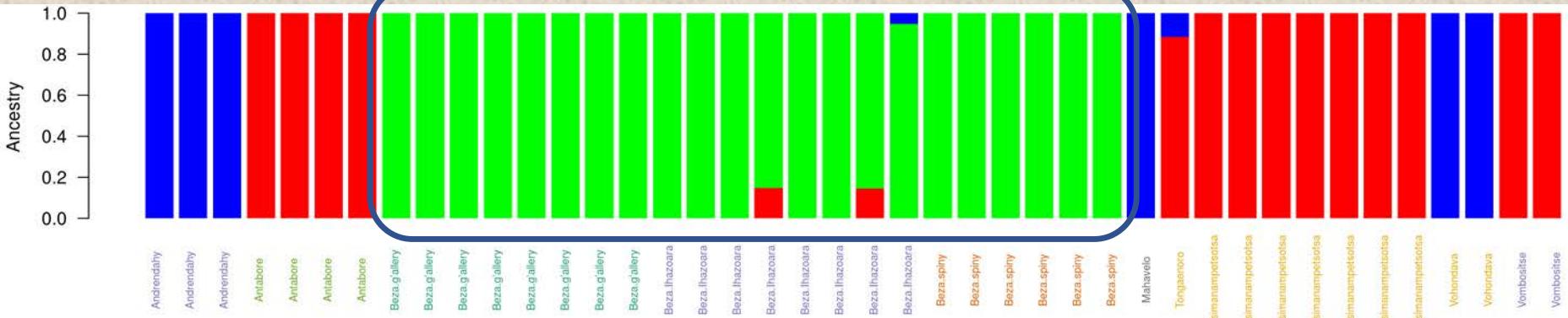
EARLIEST STAGES OF SPECIATION?



Beza samples (RADseq)



ADMIXTURE Plot



As with mtDNA analysis, complete lack of genetic structure within Beza Mahafaly

Conclusions (Case #1):

- Despite strong (for mouse lemurs!) indication of morphological and ecological divergence, this occurs on a background of seeming genomic homogeneity
- Fine-scale analysis of whole genome scans will be needed to fully explore this apparent anomaly

Case #2: Intraspecific divergence driven by natural climate change?

(Goodman's mouse lemur, *M. lehilahytsara*)



George Tiley

An expanded geographic perspective of Goodman's mouse lemur reveals the effects of Pleistocene forest fragmentation in Madagascar's Central Highland Savannah with early symptoms of lineage diversification

George P. Tiley, Marina B. Blanco, Rodin M. Rasoloarison, José M. Ralison, Paul A. Hohenlohe, and Anne D. Yoder

[In progress ...]

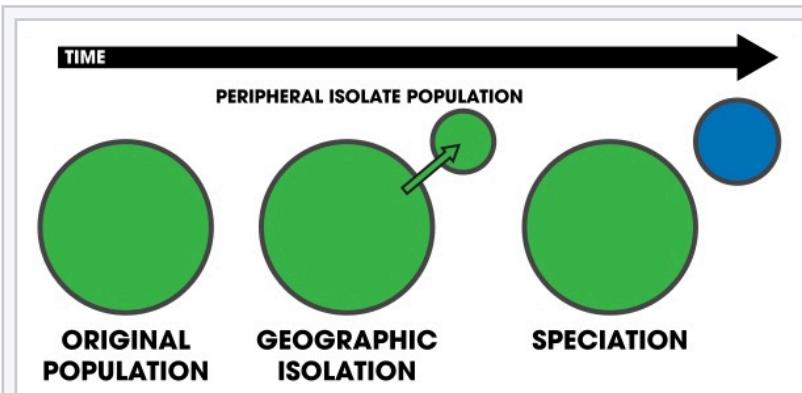


Figure 1: Peripatric speciation

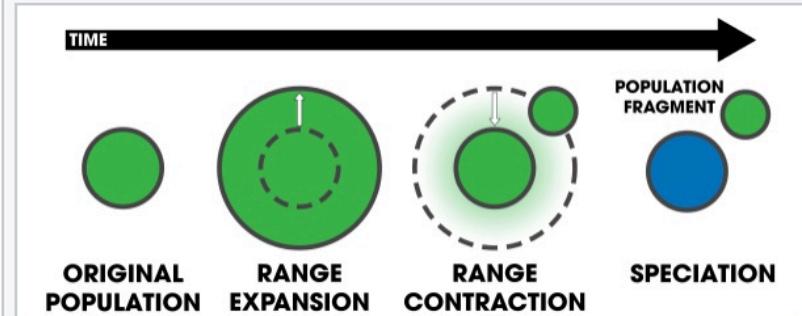


Figure 2: Centrifugal speciation

Peripatric Speciation

(all around; near ---
think “periphery”)

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

Anne D. Yoder^{a,b,1}, C. Ryan Campbell^a, Marina B. Blanco^b, Mario dos Reis^c, Jörg U. Ganzhorn^d, Steven M. Goodman^{e,f}, Kelsie E. Hunnicutt^a, Peter A. Larsen^a, Peter M. Kappeler^g, Rodin M. Rasoloarison^{g,h}, José M. Ralison^h, David L. Swoffordⁱ, and David W. Weisrockⁱ

^aDepartment of Biology, Duke University, Durham, NC 27708; ^bDuke Lemur Center, Duke University, Durham, NC 27705; ^cSchool of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, United Kingdom; ^dTierökologie und Naturschutz, Universität Hamburg, 20146 Hamburg, Germany; ^eField Museum of Natural History, Chicago, IL 60605; ^fAssociation Vahatra, BP 3972, Antananarivo 101, Madagascar; ^gBehavioral Ecology and Sociobiology Unit, German Primate Centre, 37077 Goettingen, Germany; ^hDépartement de Biologie Animale, Université d'Antananarivo, BP 906, Antananarivo 101, Madagascar; and ⁱDepartment of Biology, University of Kentucky, Lexington, KY 40506





370 mi

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

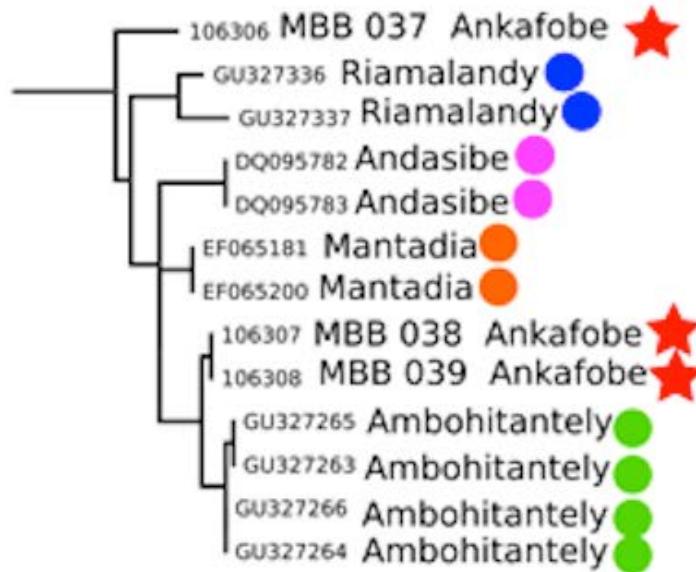
Google earth



~ 1 Km

Ankafobe

M. lehilahysara



Intra Genetic Distance

Ankafobe: 1.1%
Riamalandy: 0.8%
Andasibe: 0%
Mantadia: 0%
Ambohitantely: 0.1%

Ankafobe forest patch has an area
of ~0.05 square miles

★ There appears to be higher genetic diversity within this tiny patch of relictual forest than can be seen at any other locality (thus far sampled).

Previous locality information appeared to indicate a rainforest edge specialist

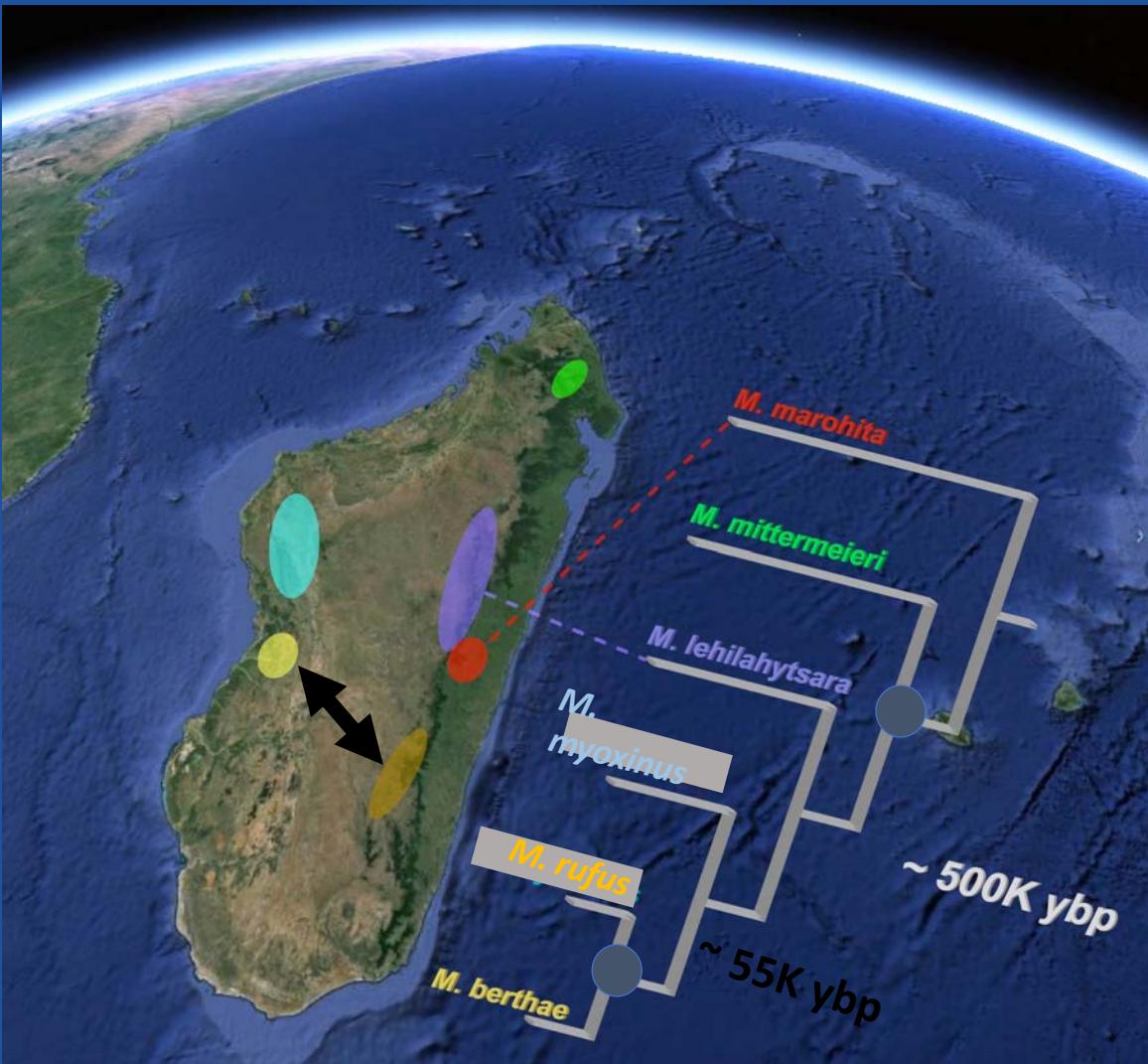


Have now extended its range by ~150
km to the west



Have revised our view from edge specialist to plateau specialist







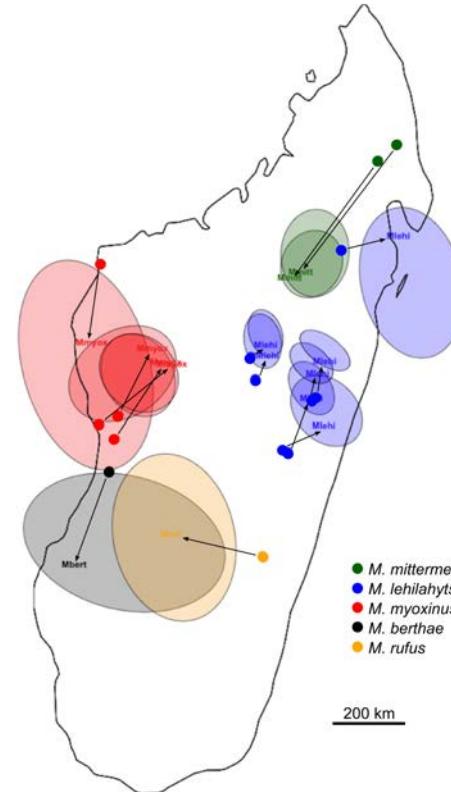
Microcebus berthae

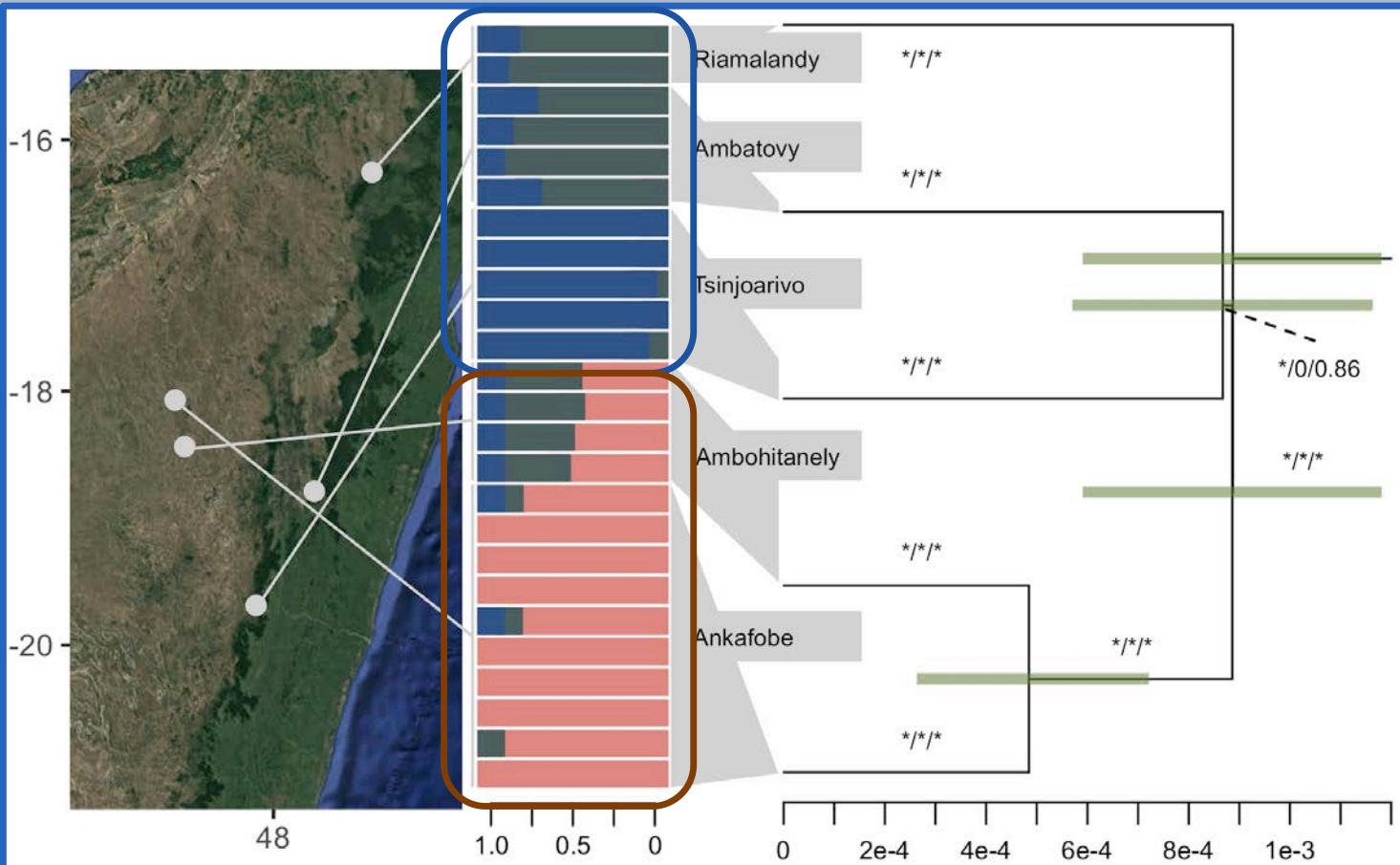


Microcebus rufus

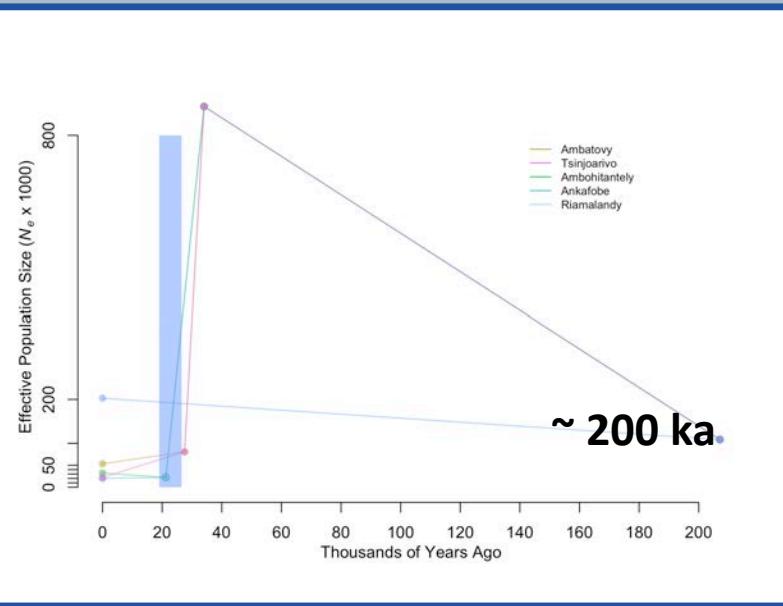
"Geogenetic patterns"
indicate that there
were forest "bridges"
across Madagascar that
must have entirely
disappeared by ~ 50K
ybp

SpaceMix
(Bradburd et al., 2016)

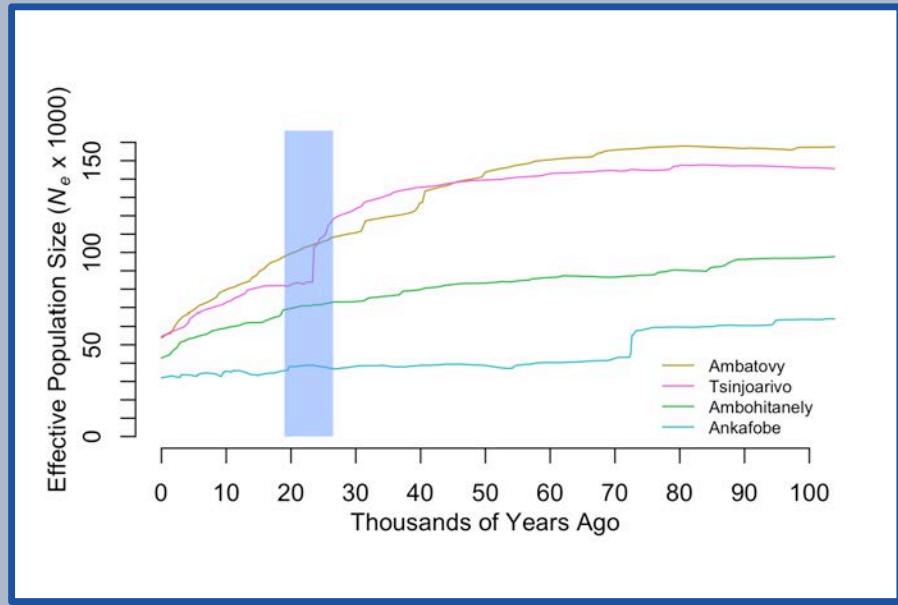




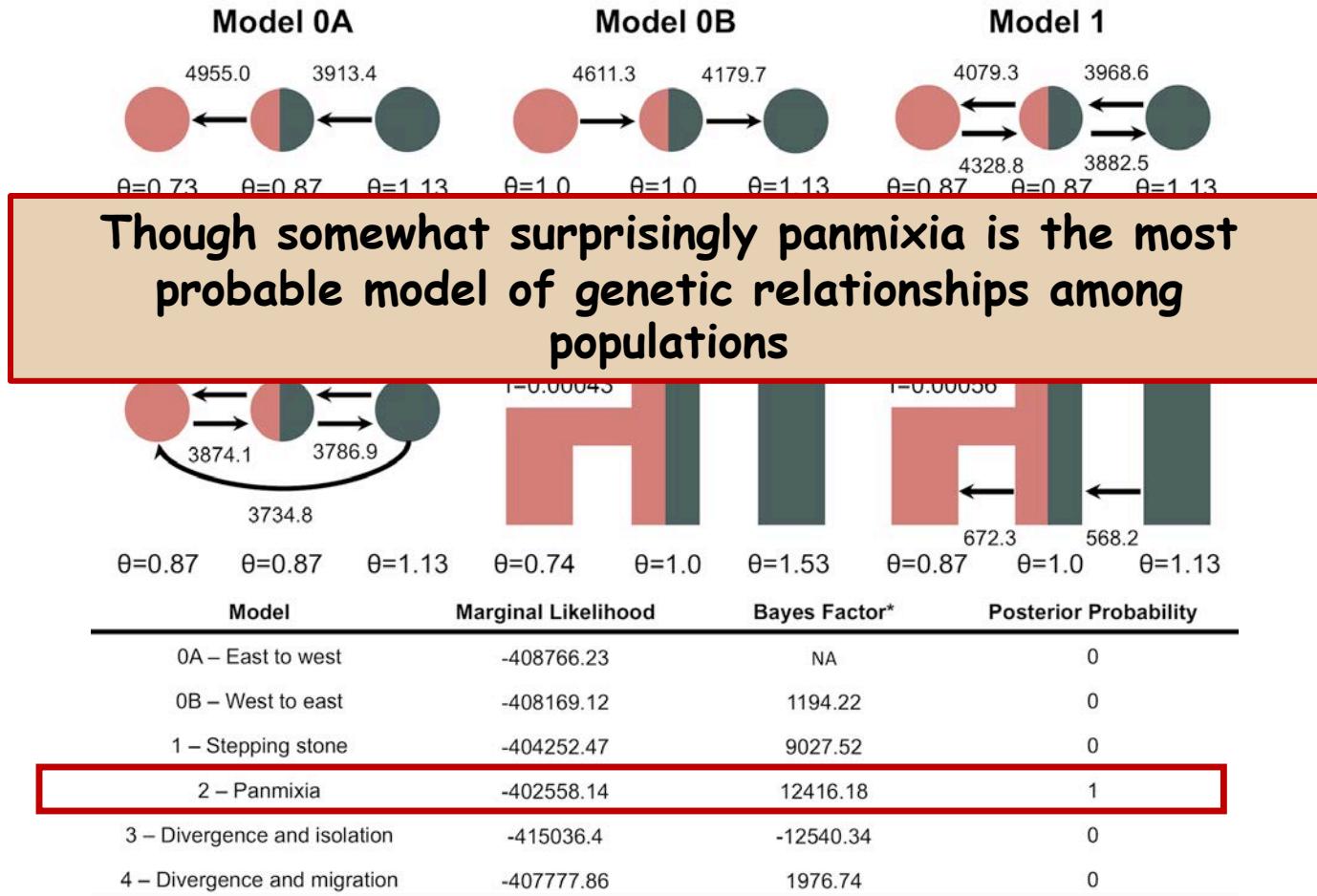
Bayesian Phylogenetics and Phylogeography (BPP)



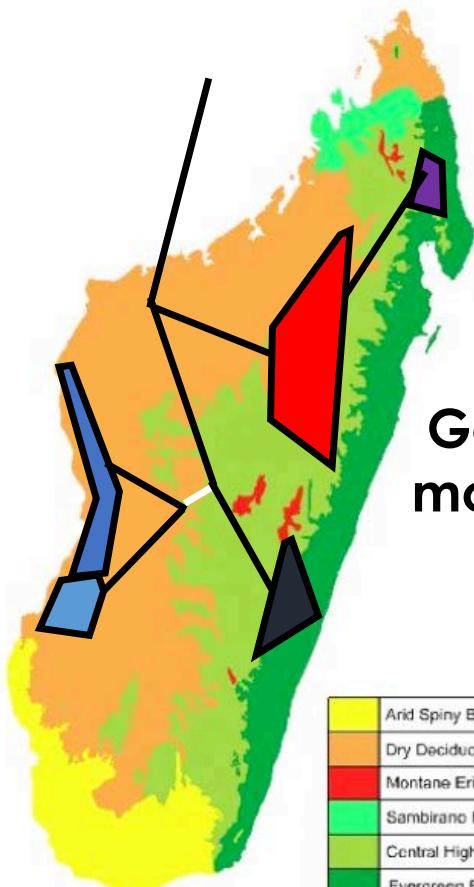
Extended Bayesian Skyline Plots (EBSP)



Both analyses show a marked decline in N_e coincident with the LGM



* Bayes factors computed with natural logarithms of marginal likelihoods with respect to model 0A as $2 * (\ln L_i - \ln L_{0A})$
 † θ values are presented as 10^{-3}



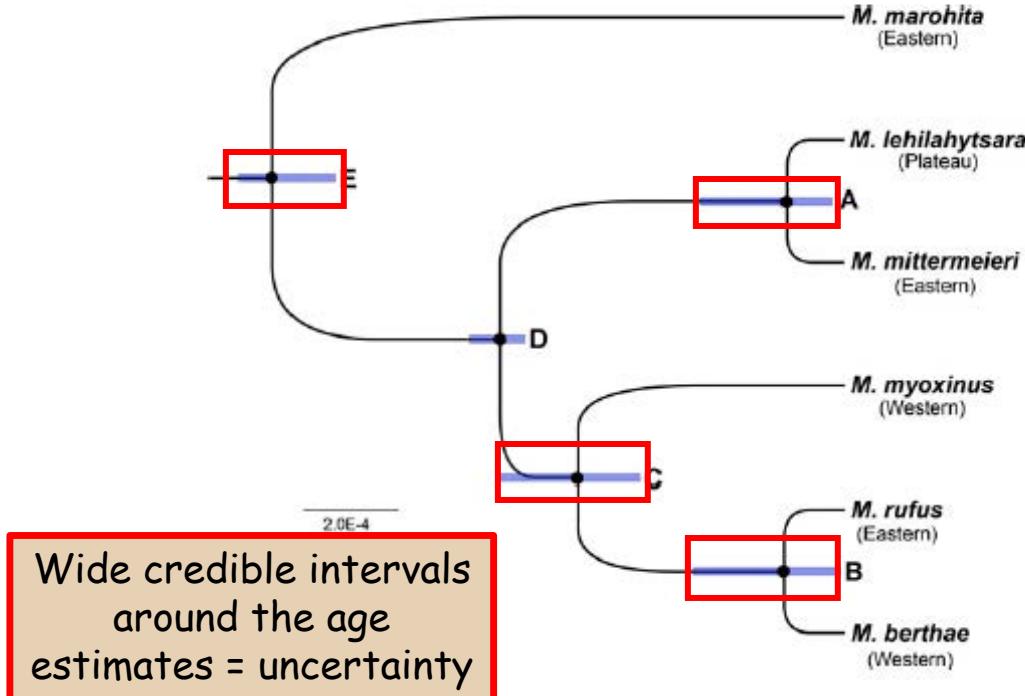
Mittermeier's
mouse lemur

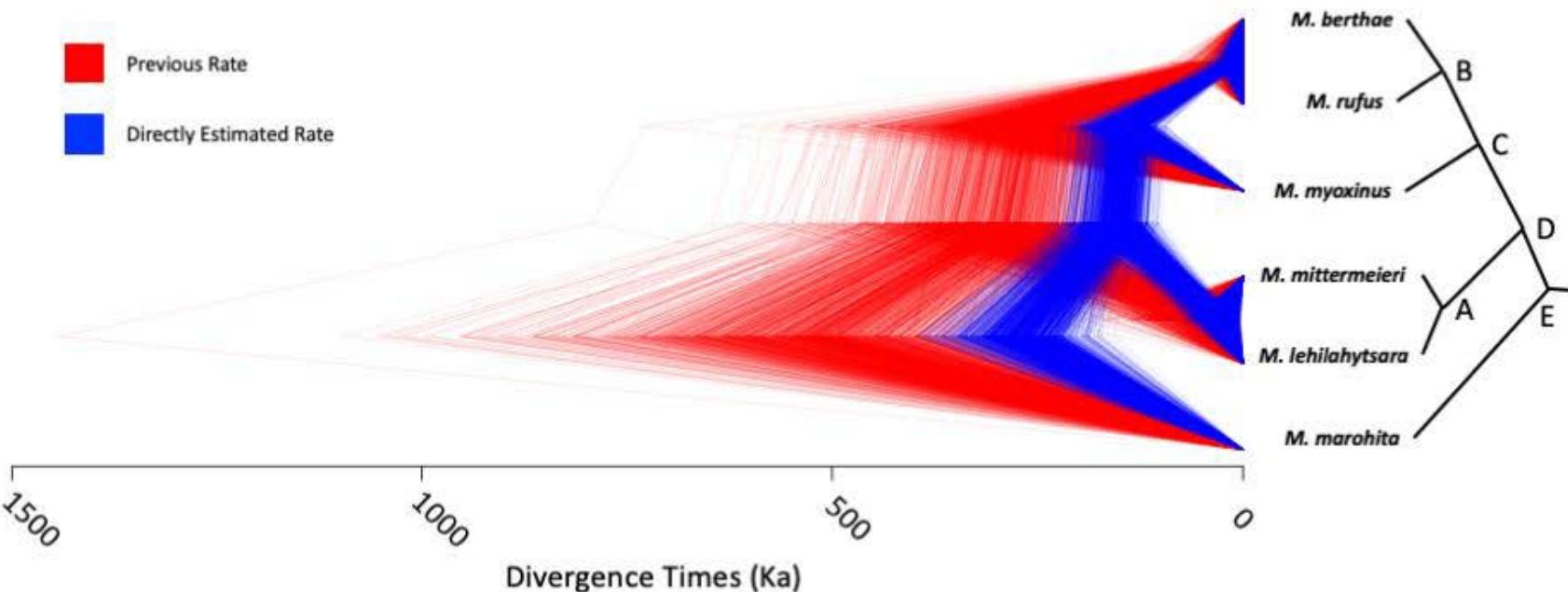
Goodman's
mouse lemur

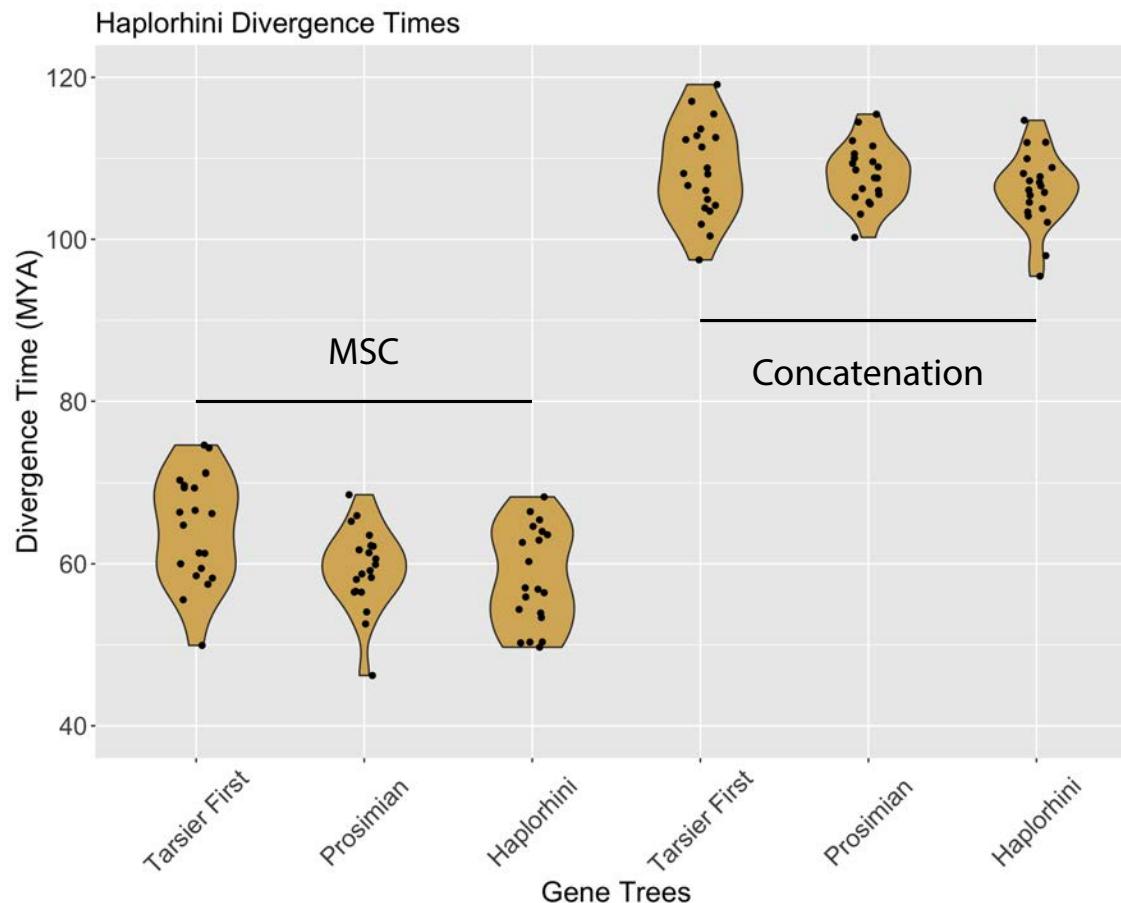
(peripatric
speciation?)

Yellow	Arid Spiny Bush
Orange	Dry Deciduous Forest
Red	Montane Ericoid Thicket
Green	Sambrano Rainforest
Light Green	Central Highlands
Dark Green	Evergreen Rainforest

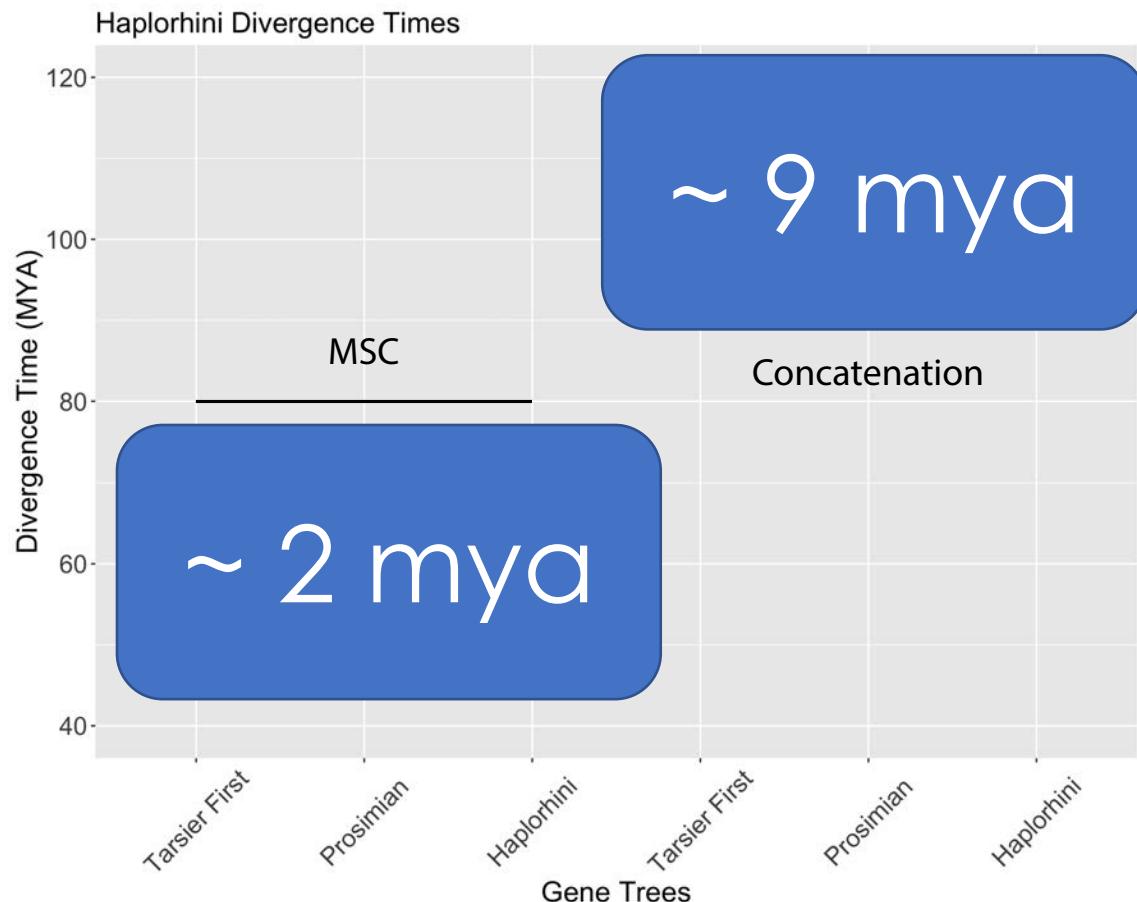
Geogenetic patterns in mouse lemurs (genus *Microcebus*) reveal the ghosts of Madagascar's forests past







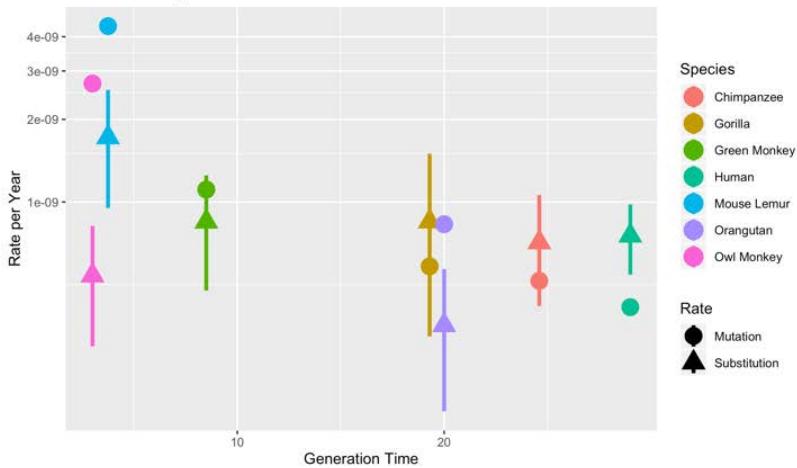
Comparison of divergence times between MSC and concatenated uncorrelated molecular clocks.



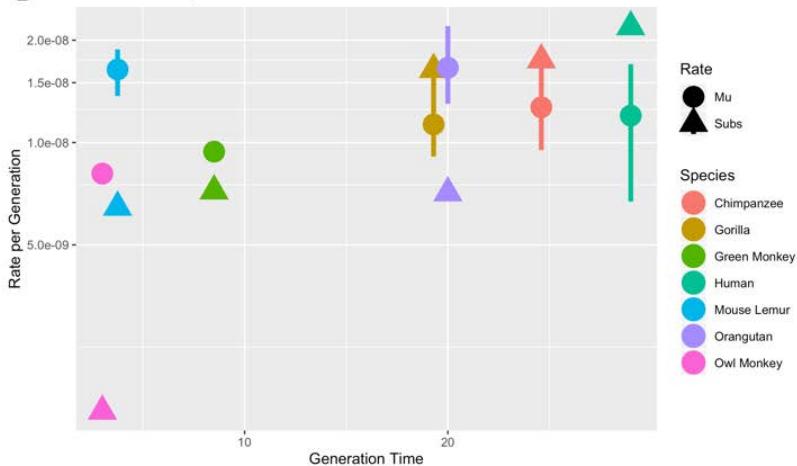
Comparison of divergence times between MSC and concatenated uncorrelated molecular clocks.

A

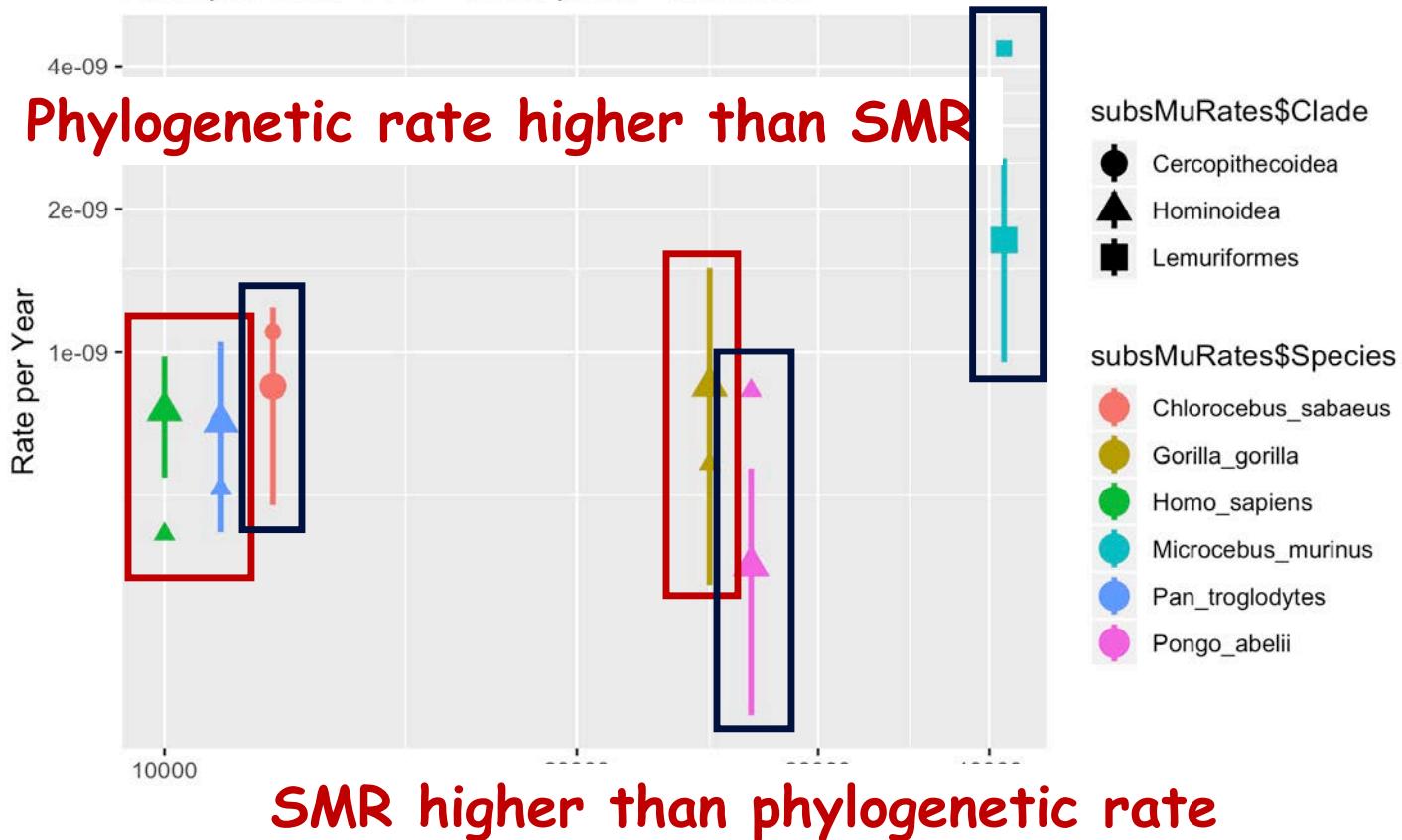
Primate Rate per Year v Generation Time

**B**

Primate Rate per Generation v Generation Time



Rate per Year v Ne - small point = Mu Rate



Conclusions (Case #2):

- Forest fragmentation occurred rapidly and affected both the eastern rainforests and Central Highlands during a period of decreased precipitation near the last glacial maximum (LGM)
- Population substructure is evident but with a high degree of connectivity still detectable between the eastern rainforests and the Central Highland Savannah
- Origin of the Central Highland Savannah predates human arrival, but also shows that continued habitat loss from deforestation will likely endanger Goodman's mouse lemur
- We're very worried/intrigued by the apparent conflict between different methods for estimating divergence times

Case #3: Lineage diversification in close geographic proximity (ecological speciation?)

Complex processes of cryptic speciation in mouse lemurs from a micro-endemism hotspot in Madagascar

Dominik Schüßler^{1#}, Jordi Salmona^{2#}, Marina B. Blanco^{3,4#}, Jelmer Poelstra^{4#}, George P. Tiley^{4#},

Jean B. Andriambeloson⁵, Guillaume Besnard², Olivier Bouchez⁶, C. Ryan Campbell^{4†}, Paul D.

Etter⁷, Amaia Iribar Pelozuelo², Paul A. Hohenlohe⁸, Kelsie E. Hunnicutt^{4@}, Eric A. Johnson⁷,

Peter A. Larsen^{4&}, Jasmin Mantilla-Contreras¹, Sophie Manzi², Alexandra Miller⁹, Blanchard

Randrianambinina^{10,11}, David W. Rasolofoson¹⁰, Amanda R. Stahlke⁸, David Weisrock¹², Rachel

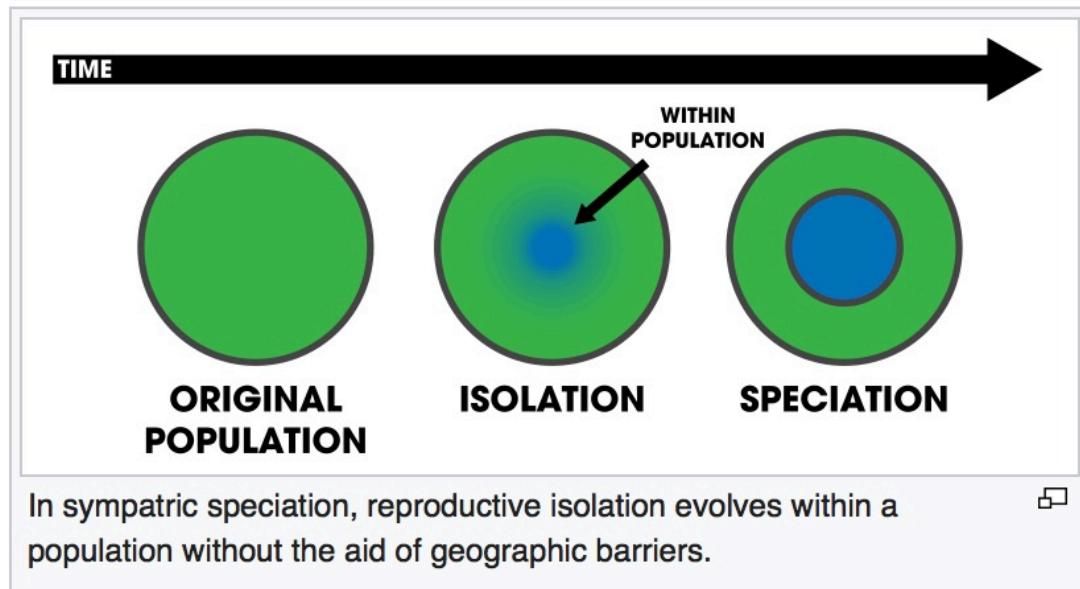
C. Williams^{3,4}, Lounès Chikhi^{2,9}, Edward E Louis Jr.¹³, Anne D. Yoder^{4*}, Ute Radespiel^{14*}

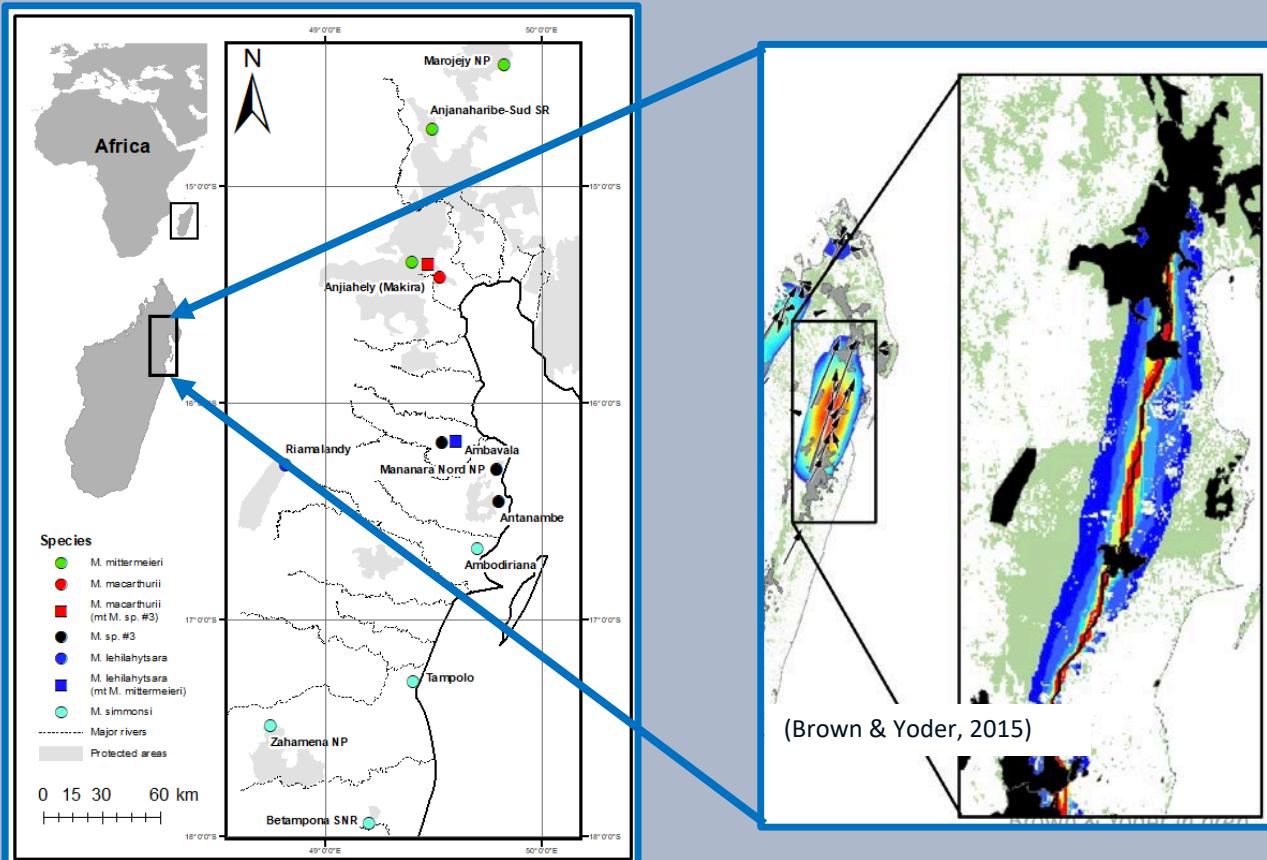


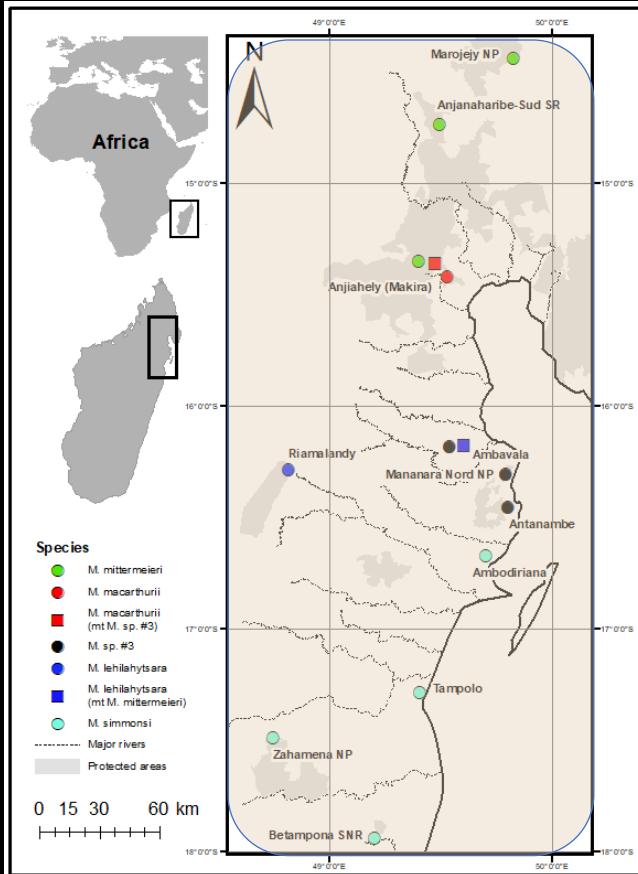
Dominik Schüßler*

*(with Salmona, Blanco,
Poelstra & Tiley)

Sympatric Speciation

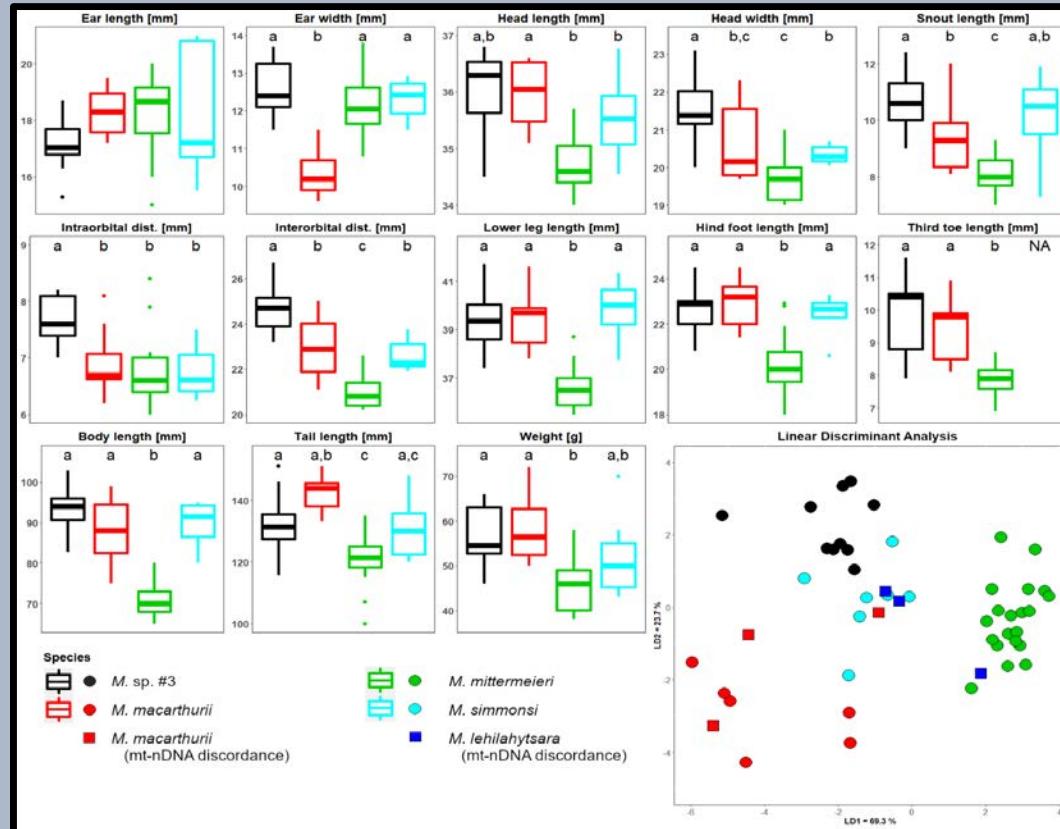




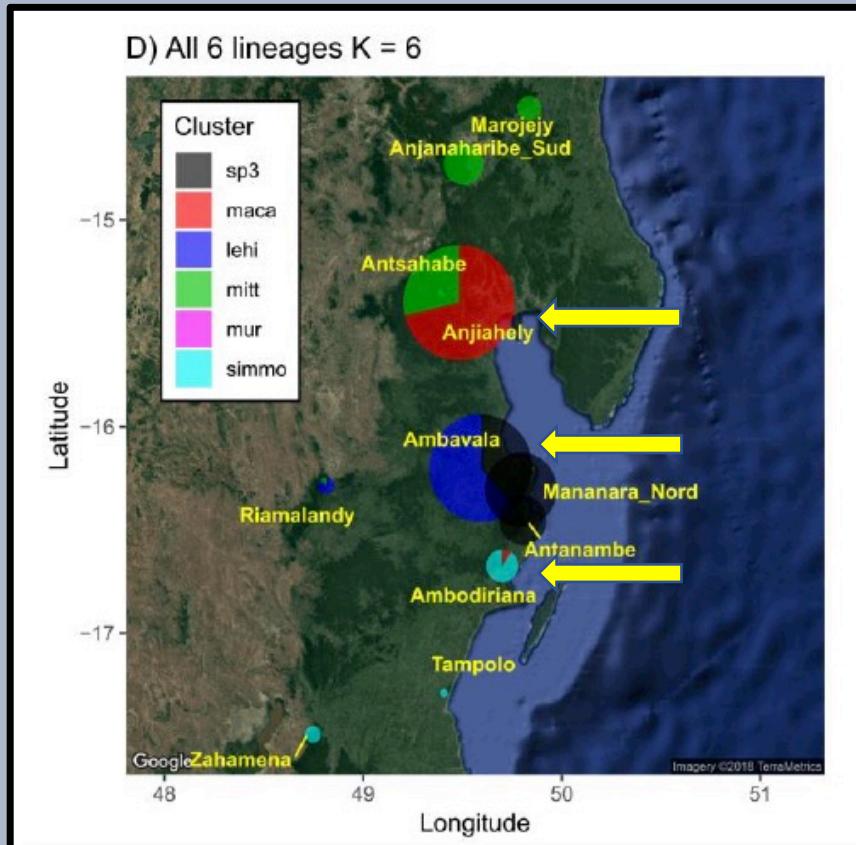


Putatively five
species of mouse
lemur in this one
corner of
Madagascar

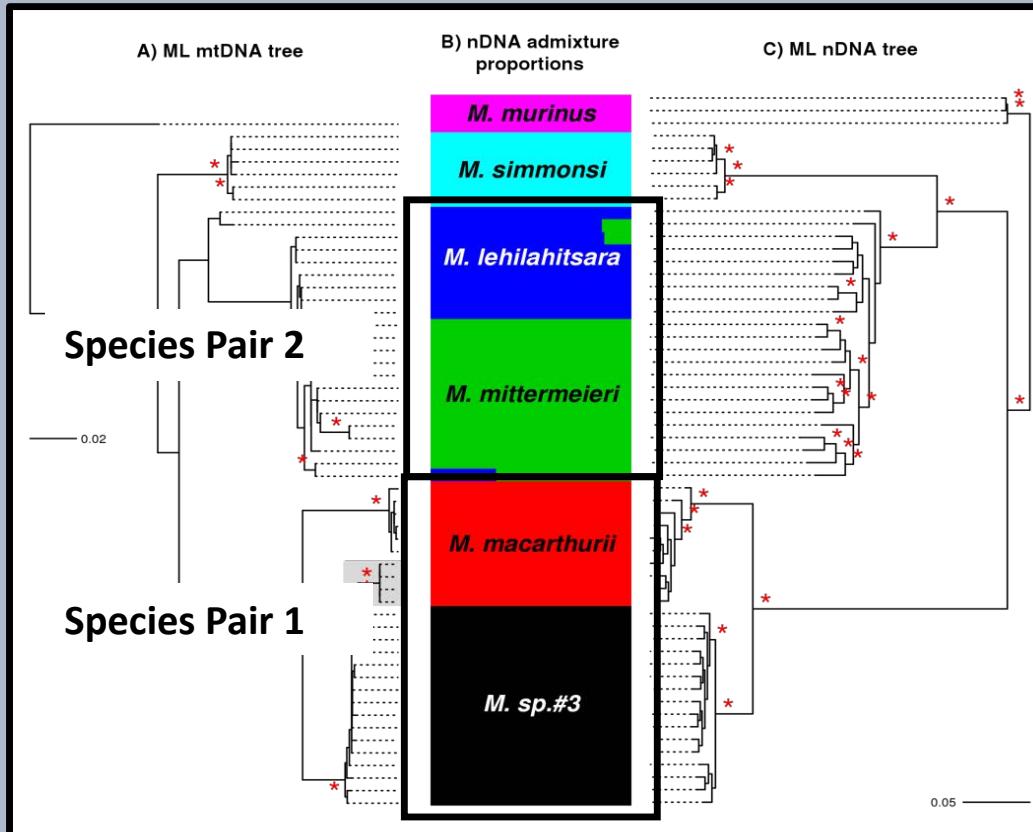
Morphological distinctions are cryptic and/or overlapping



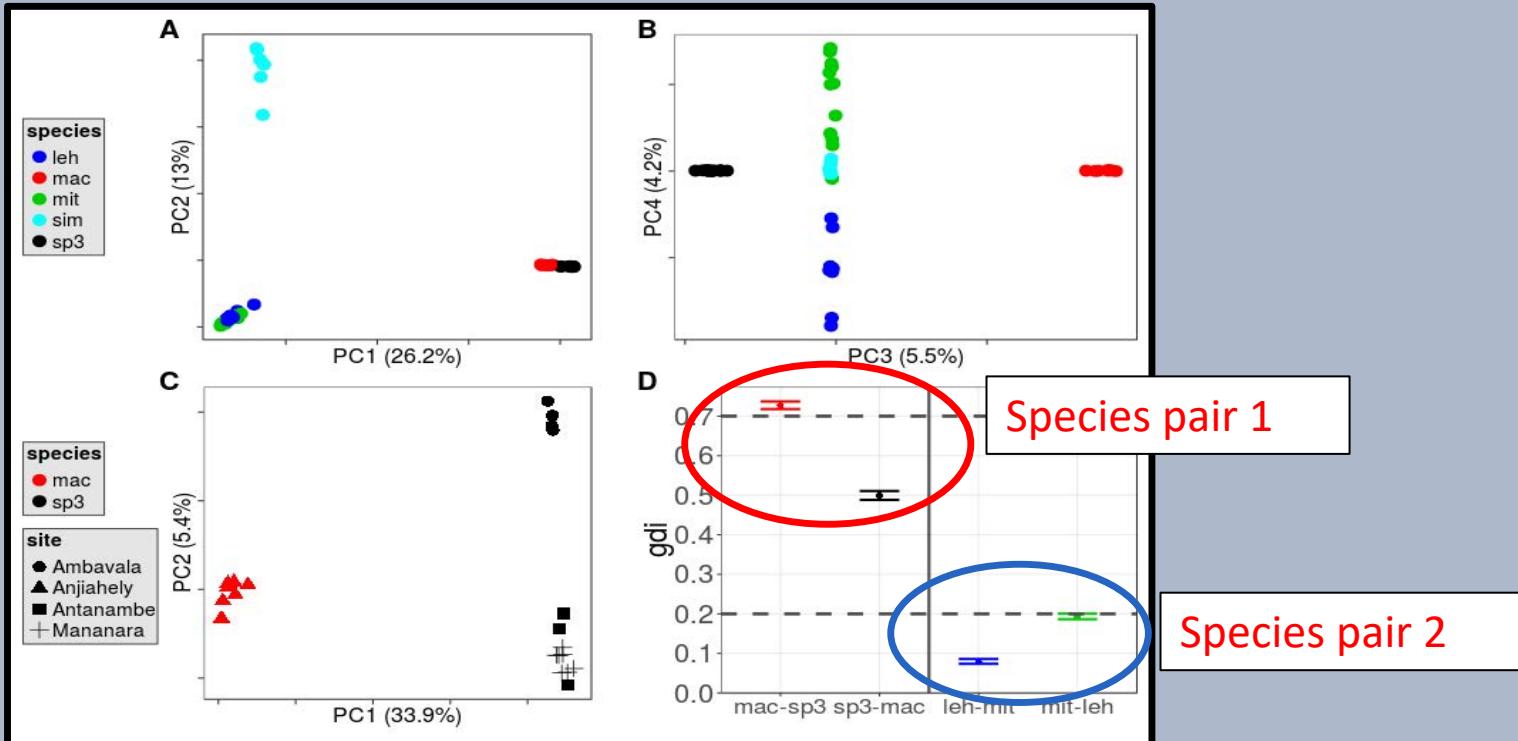
At least three cases of independent evolution in sympatry



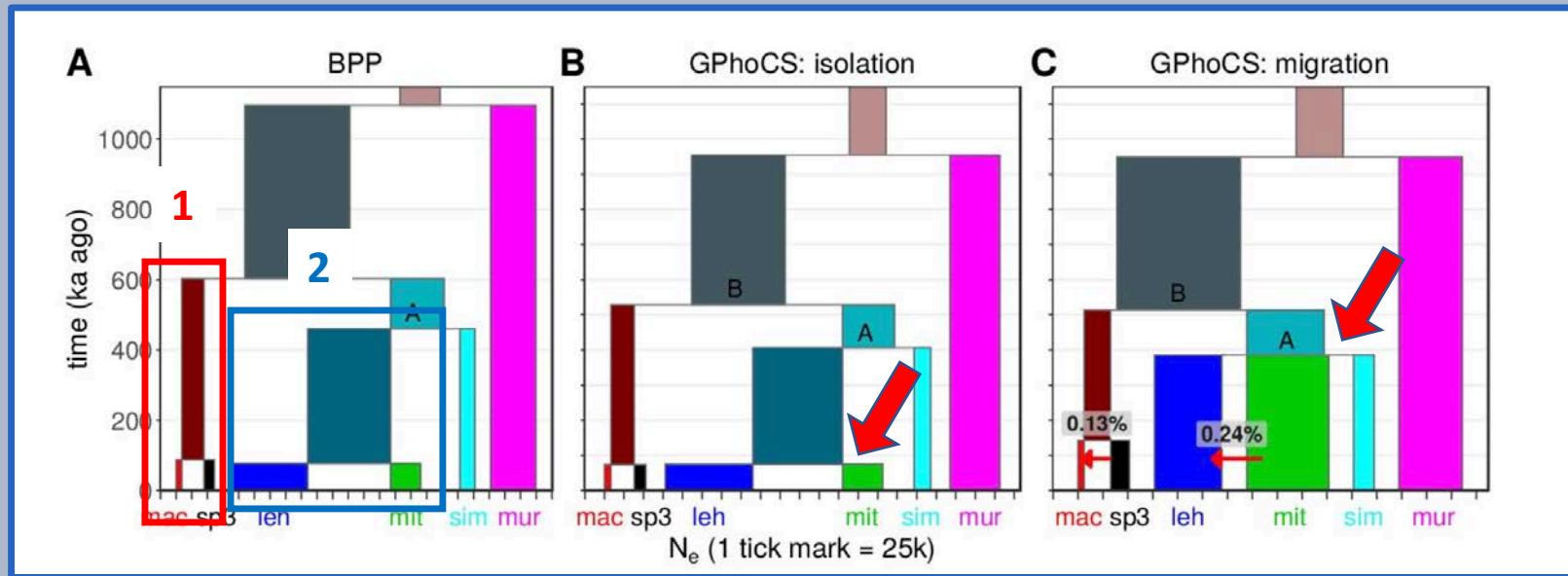
One case of mitonuclear discordance and another of paraphyletic “species”



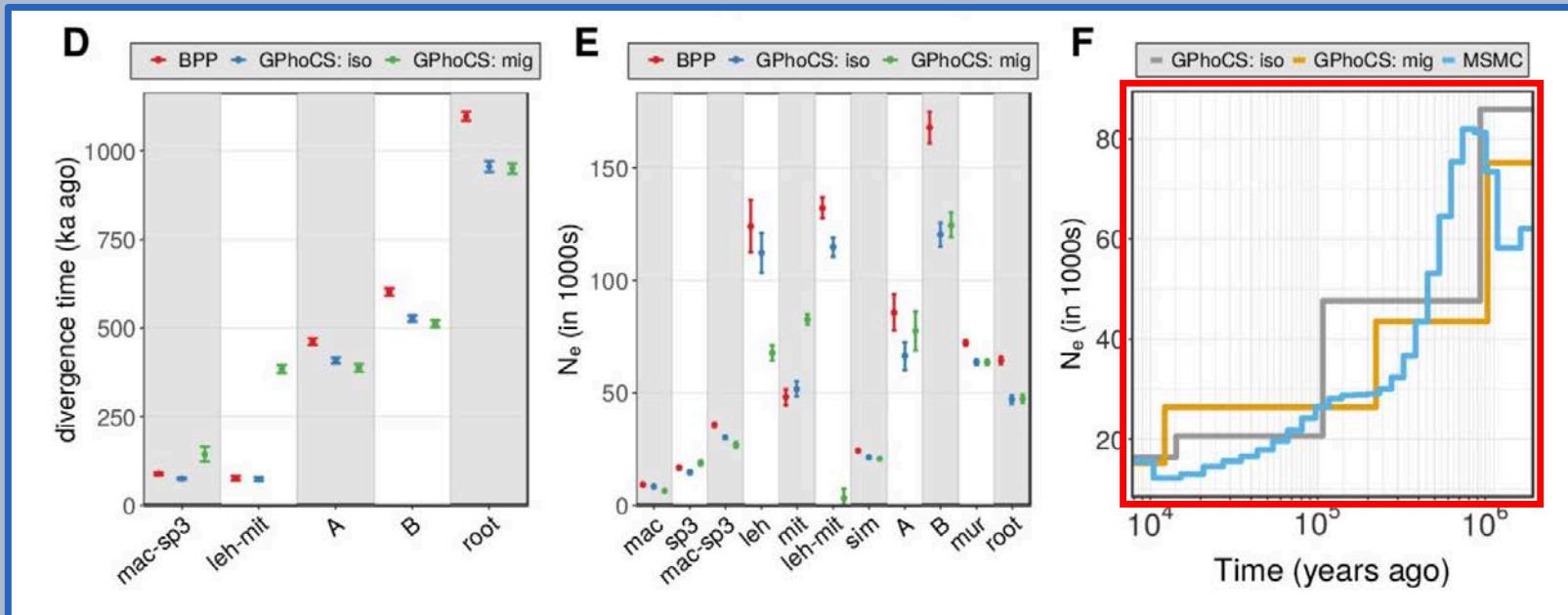
Genealogical Discordance Index (gdi) delimits one but not both sister-species pairs



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



(Presently) undescribed species shown to be in precipitous decline with both
RADseq (GPhoCS) and whole genome data (MSMC)
--- making it even more vulnerable to human impacts



Conclusions (Case #3):

- Evolutionary divergence is ongoing for five metapopulations lineages in a geographically constrained region of Madagascar of acute conservation concern
- There are at least two instances of sympatric overlap of diverging lineages
- It is presently unclear what is driving diversification
- There appears to be a correlation between N_e and ability to delimit species: cause or effect?
- Presently undescribed species has been in steep decline for tens of thousands of years (reflecting natural climate change?)

Case #4: Reproductive isolation in allopatry with secondary contact?



Jelmer Poelstra

RADseq reveals lack of evidence for recent gene flow
across previously characterized mouse lemur hybrid zone

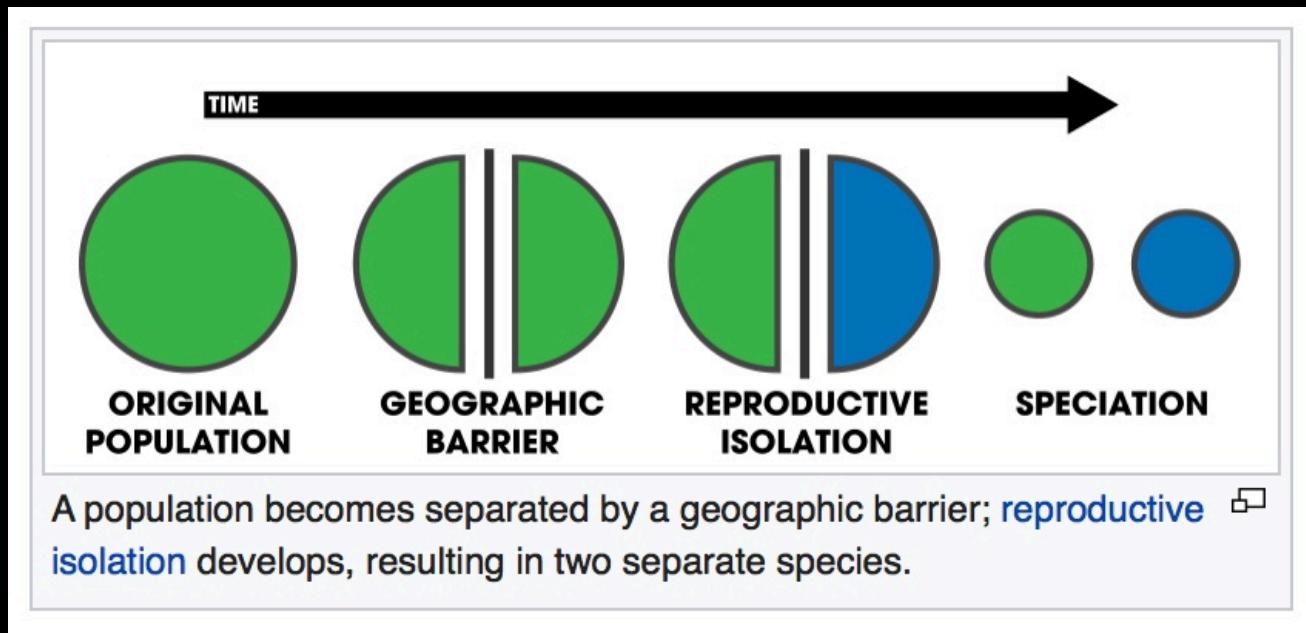
Jelmer Poelstra, Jan Lüdemann, Karina Montero, Jacques Rakotondranary, Tobias Lenz, Andreas Hapke,
Paul Hohenlohe, Jordi Salmona, Lounès Chikhi, Edward E Louis Jr., Ute Radespiel, Simone Sommer,
Jörg Ganzhorn, Anne D. Yoder

[In progress ...]

Jörg Ganzhorn



Allopatric Speciation



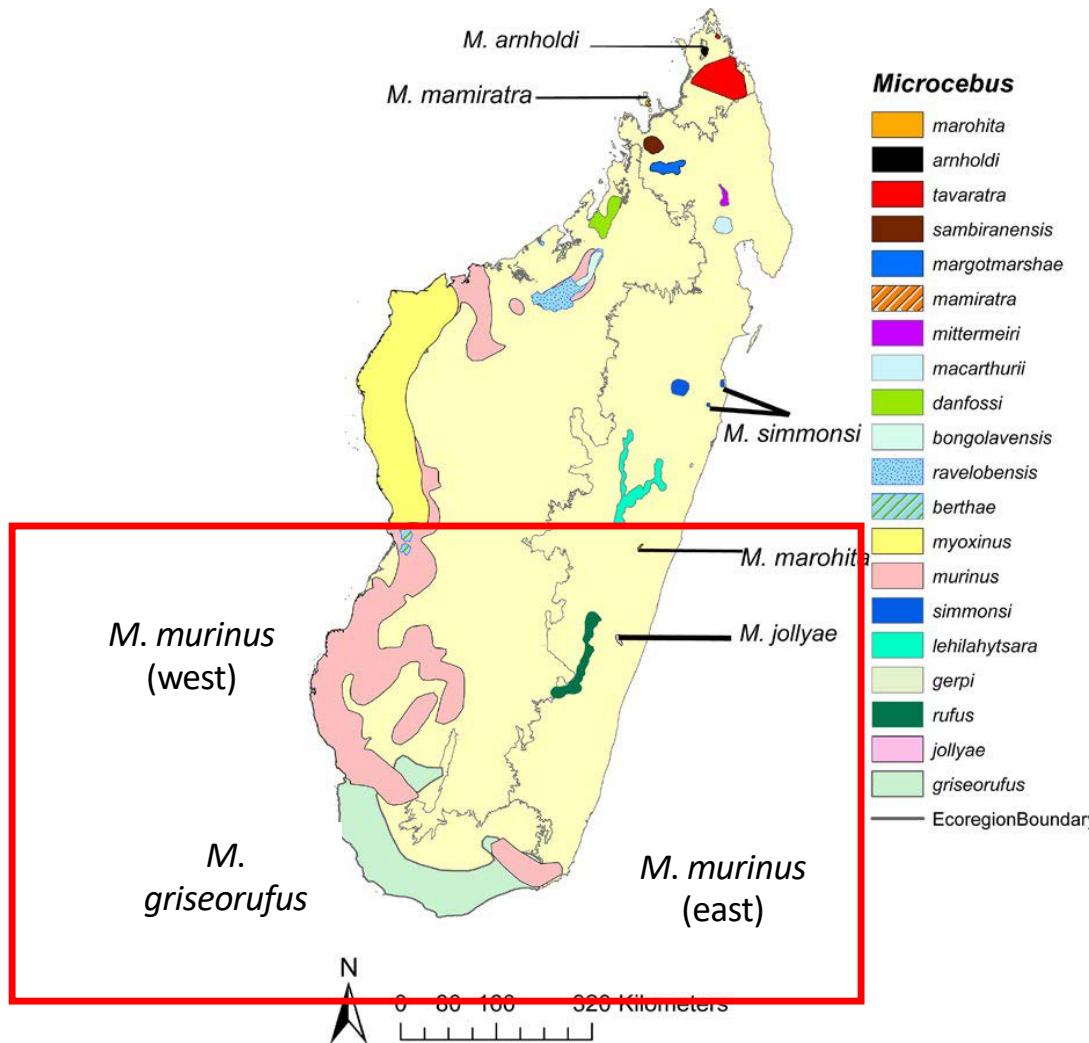
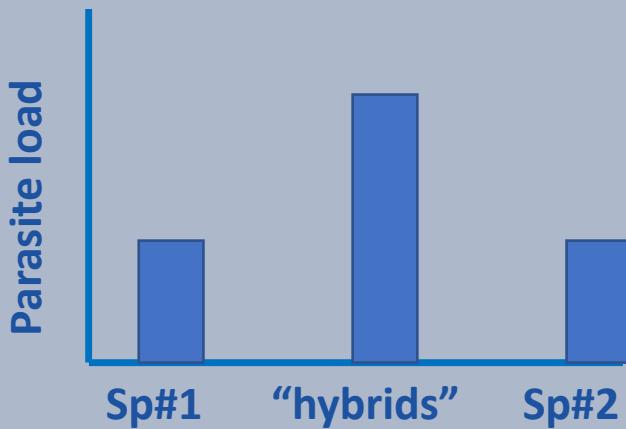


Figure from Setash et al., 2017



Figure from Setash et al., 2017

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

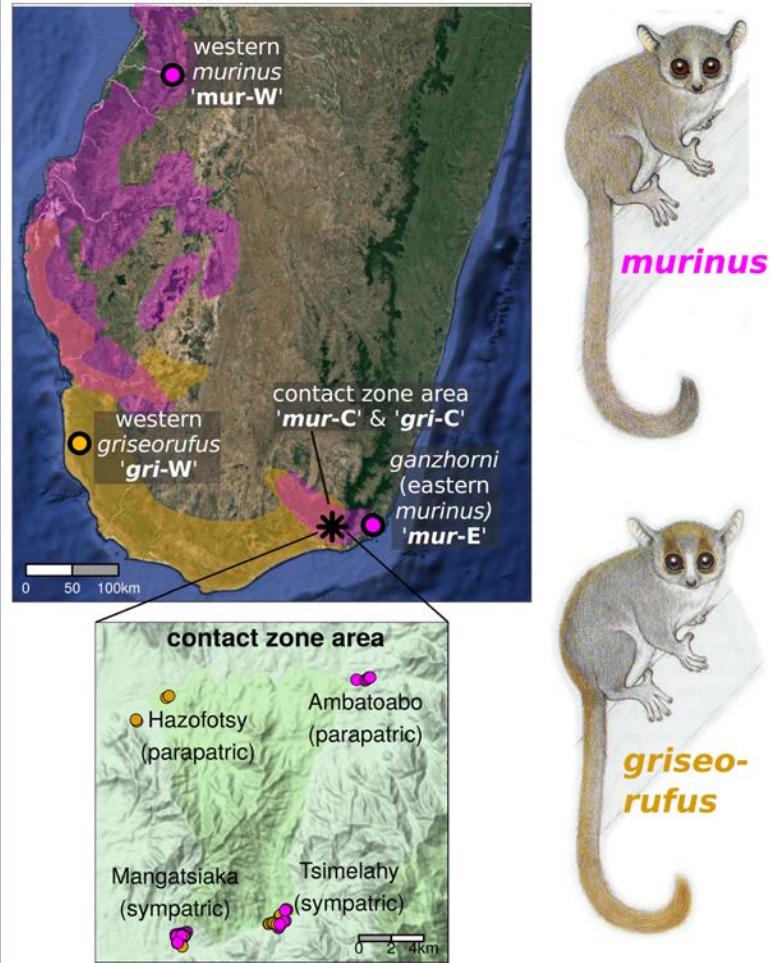
M. GLIGOR,* J. U. GANZHORN,† D. RAKOTONDRAVONY,‡ O. R. RAMILIJAONA,‡
E. RAZAFIMAHATRATRA,‡ H. ZISCHLER* and A. HAPKE*

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}

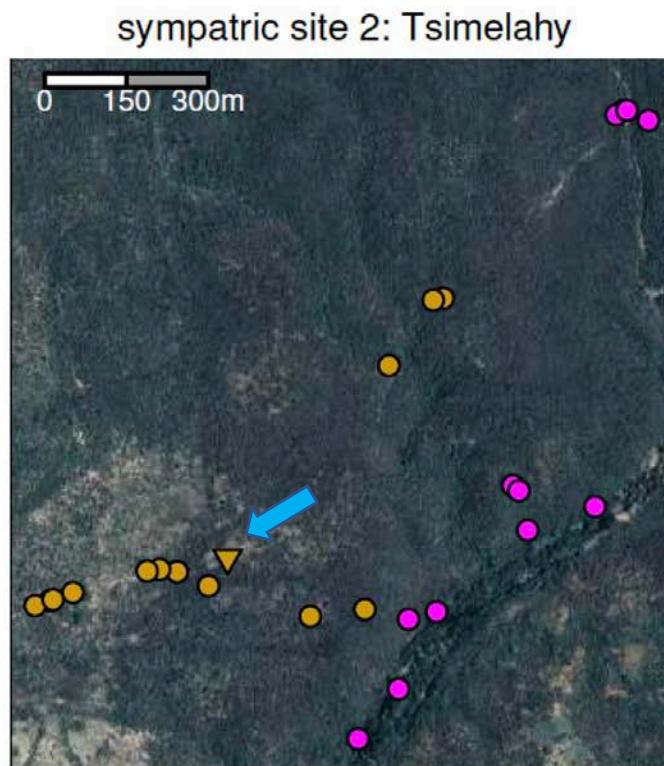
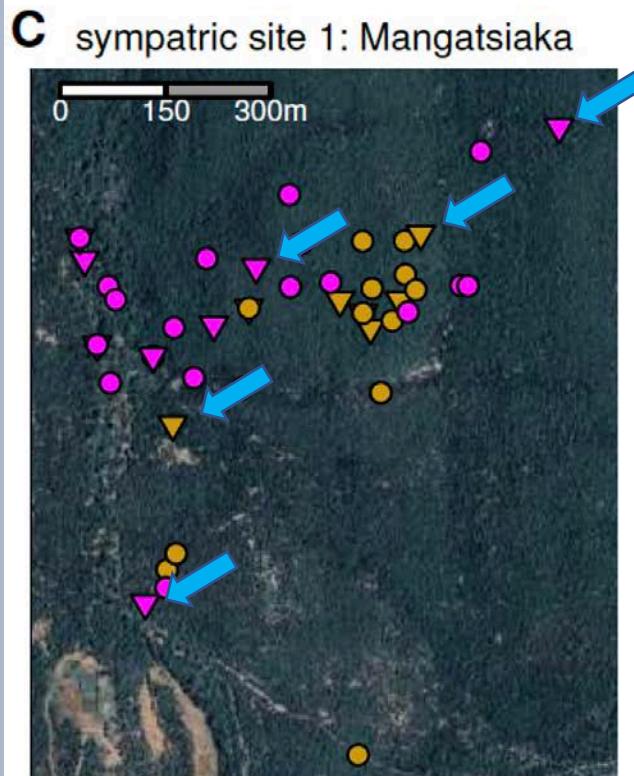
Hapke et al. 2011, BMC Ev Biol



- 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”



**microsat
assignment:**

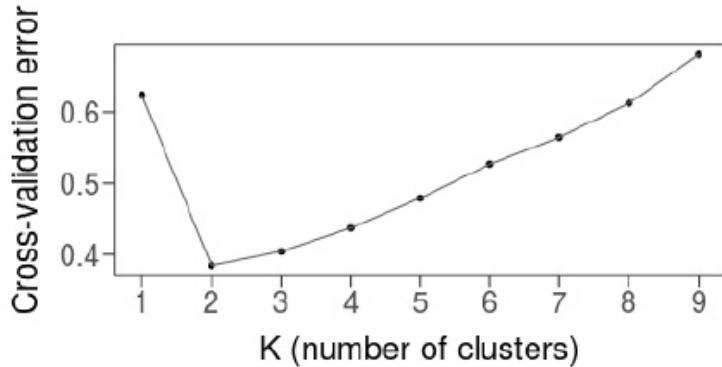
▽ hybrid
○ non-hybrid

**RADseq
assignment:**

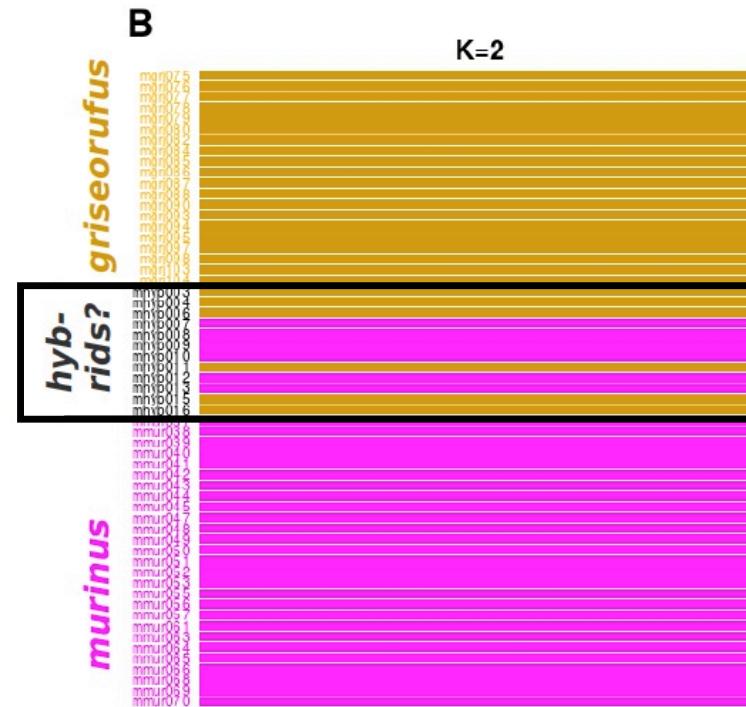
● *griseorufus*
● *murinus*

ADMIXTURE shows no evidence of admixed individuals

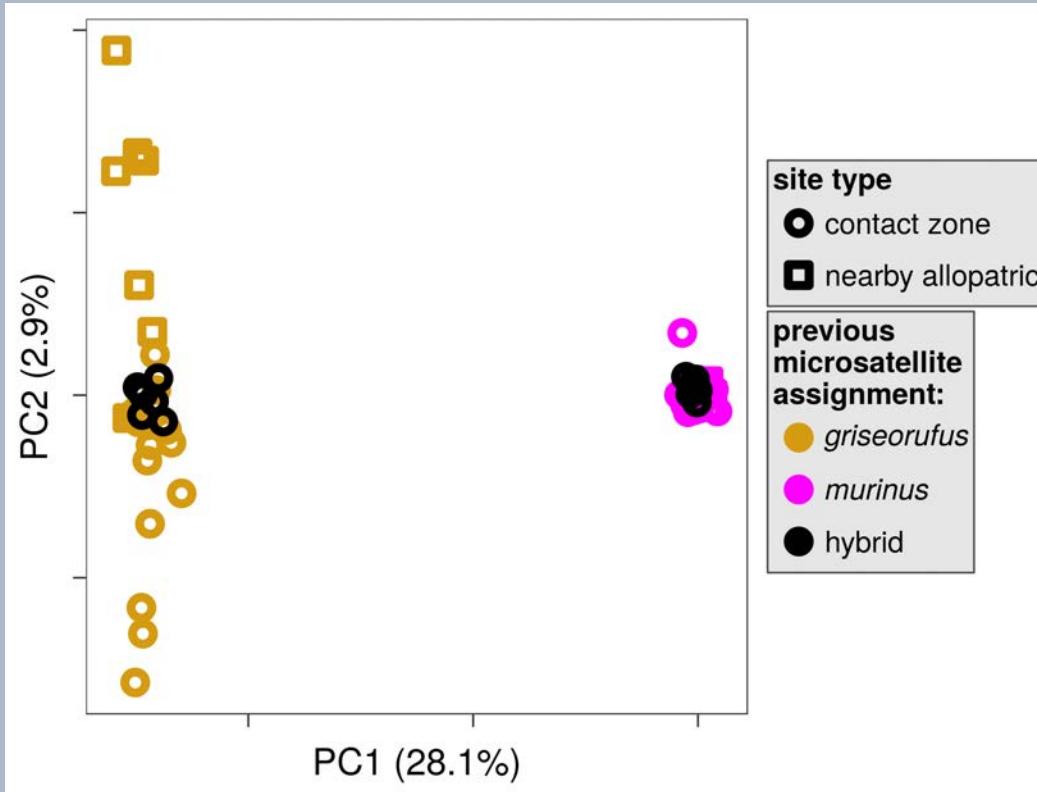
A



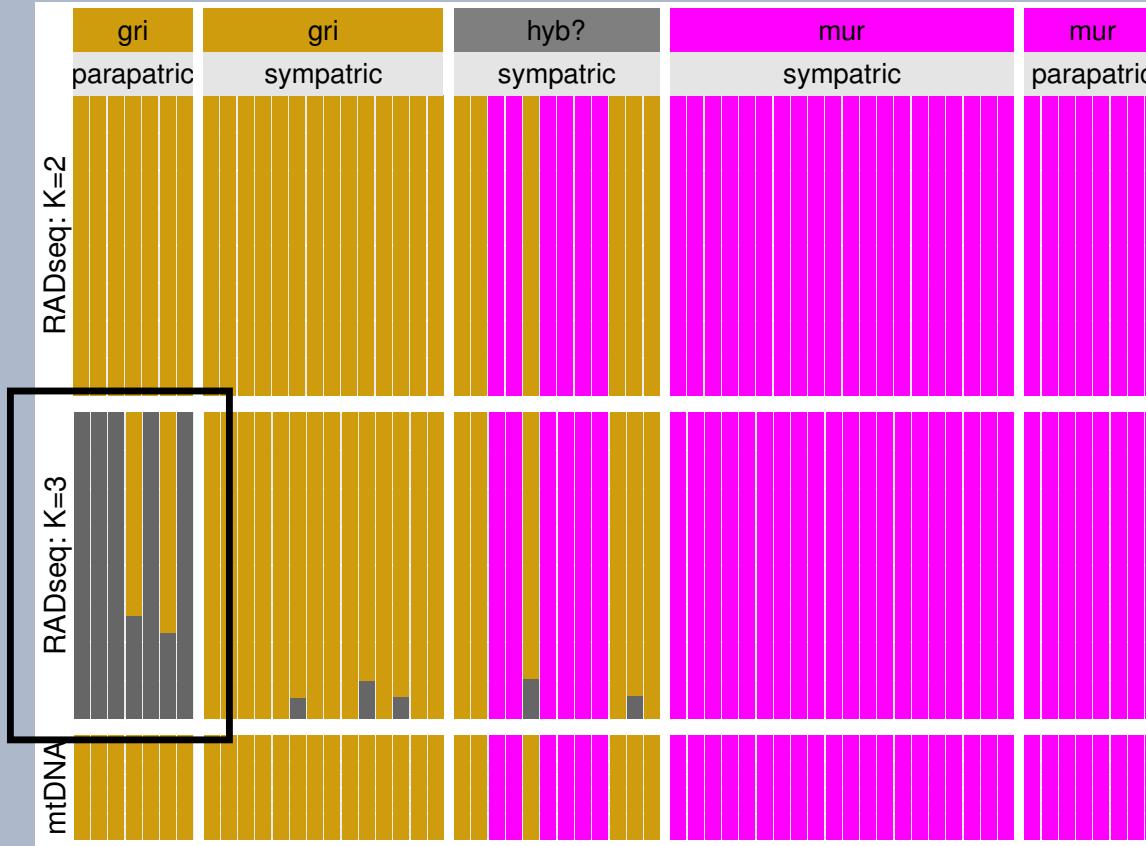
B



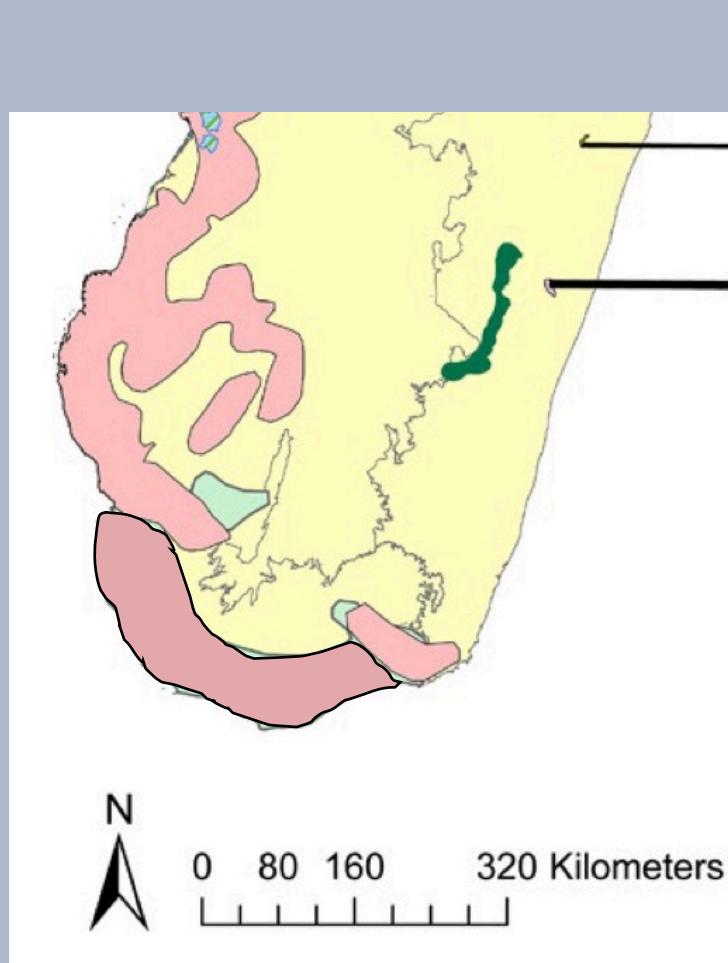
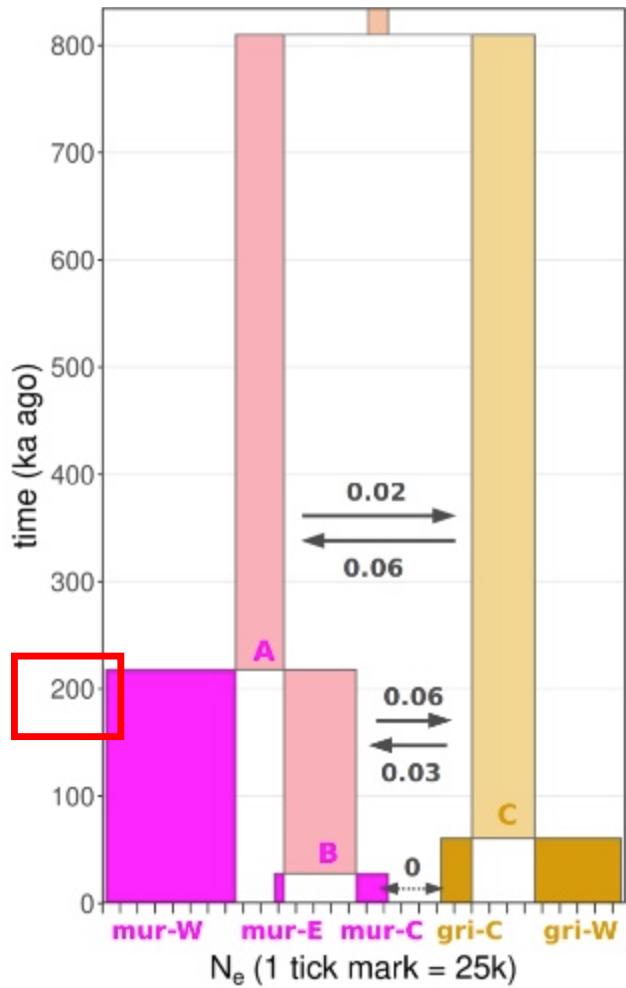
PCA shows no evidence of admixed individuals



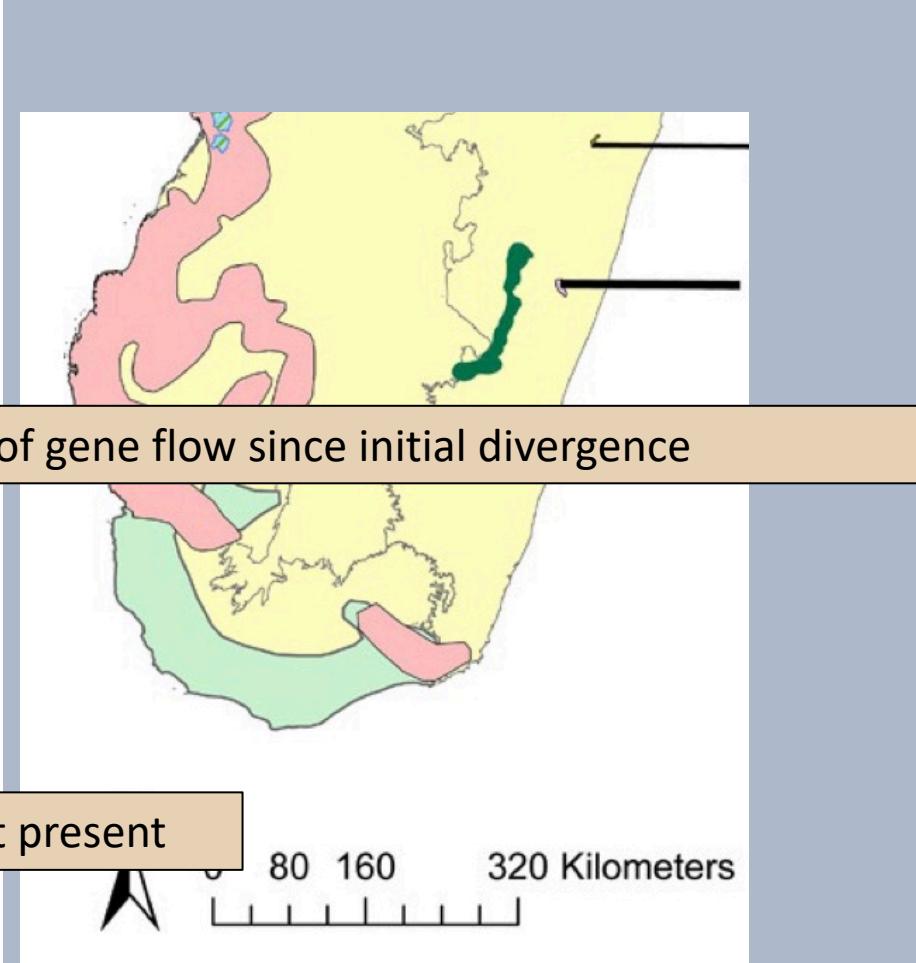
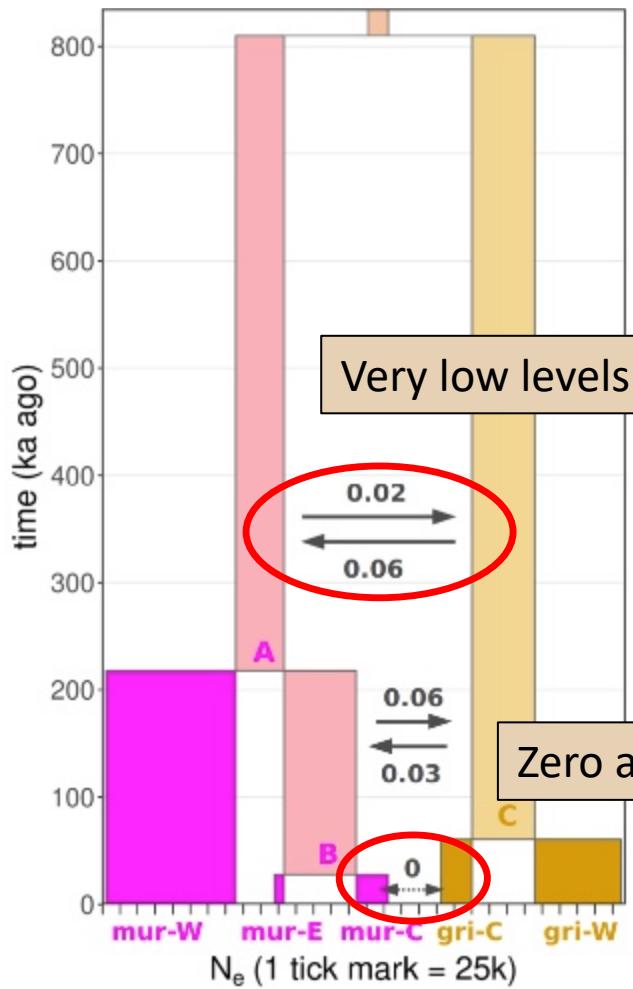
When K = 3



GPhoCS (with migration)



GPhoCS (with migration)





Slide courtesy of Jörg Ganzhorn

Conclusions (Case #4):

- RADseq data have revealed that previous hypotheses of hybridization between syntopic species occurrence is erroneous
- The two species show very strong ecological preferences which is likely to reinforce reproductive isolation
- It is unclear if F1 hybrids exist, and if they do, what is their fate?
- Future directions call for detailed field studies of individual interactions among and between *M. murinus* and *M. griseorufus*

Adaptive introgression in dwarf lemurs

The image shows a screenshot of a bioRxiv preprint page. At the top left is the CSHL logo (Cold Spring Harbor Laboratory) with the text "Cold Spring Harbor Laboratory". Next to it is the bioRxiv logo in red and black, followed by "THE PREPRINT SERVER FOR BIOLOGY". On the right side of the header are links for "HOME" and "ABOUT", and below them is a search bar. In the main content area, there are two buttons: "New Results" on the left and "Comment on this paper" on the right. Below these buttons is the title of the preprint: "Conservation genomic analysis reveals ancient introgression and declining levels of genetic diversity in Madagascar's hibernating dwarf lemurs". Underneath the title is a list of authors: "Rachel C. Williams, Marina B. Blanco, Jelmer W. Poelstra, Kelsie E. Hunnicutt, Aaron A. Comeault, Anne D. Yoder". Below the authors is the DOI: "doi: <https://doi.org/10.1101/620724>". At the bottom of the page, a note states: "This article is a preprint and has not been peer-reviewed [what does this mean?]."

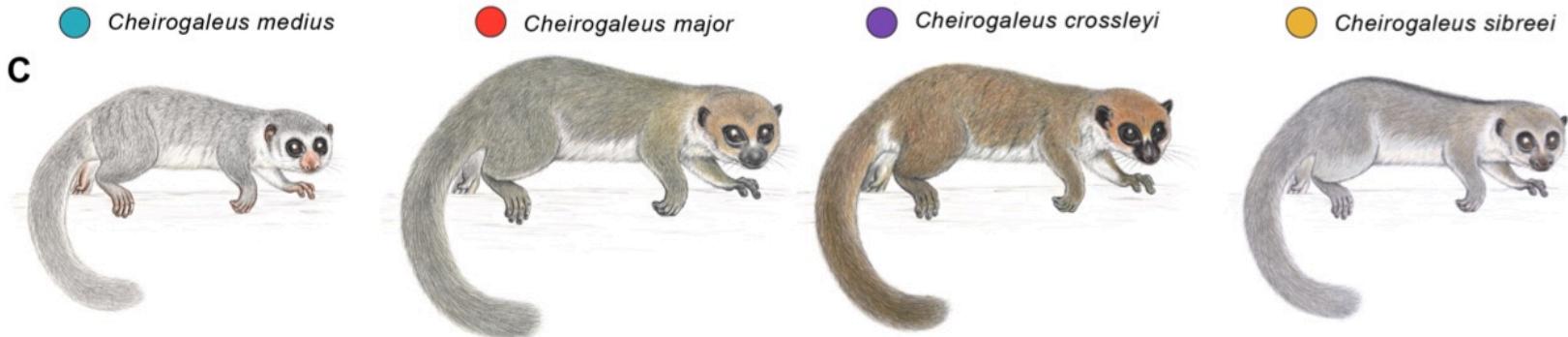


Rachel Williams

Heredity --- in press!

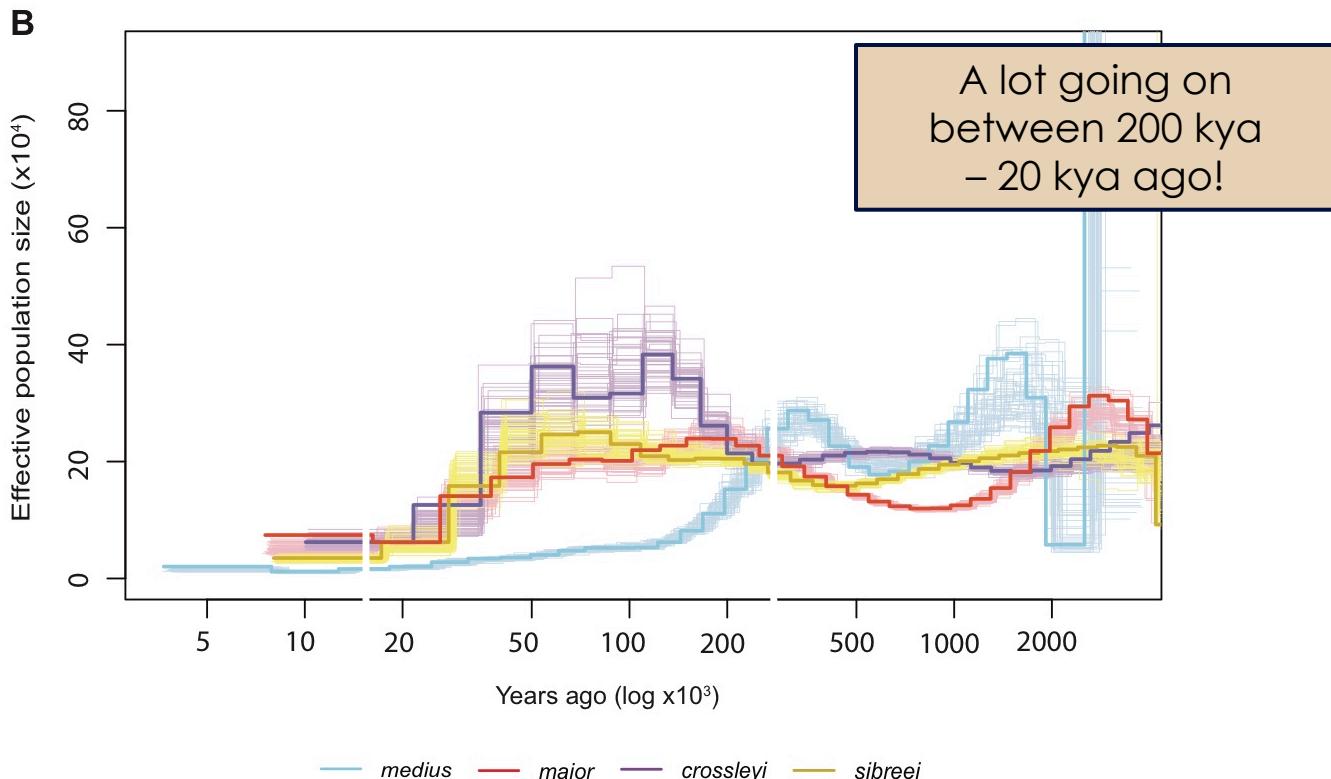
Conservation genomic analysis of Madagascar's hibernating dwarf lemurs reveals unexpected patterns of ancient introgression

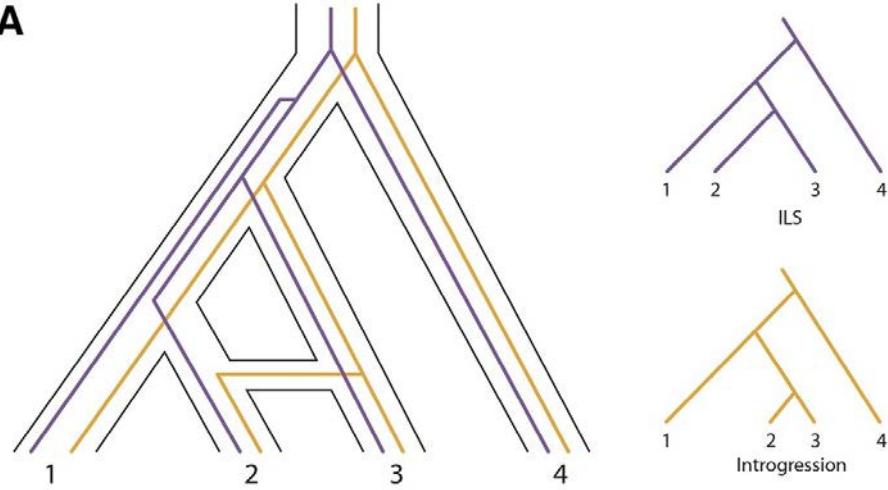
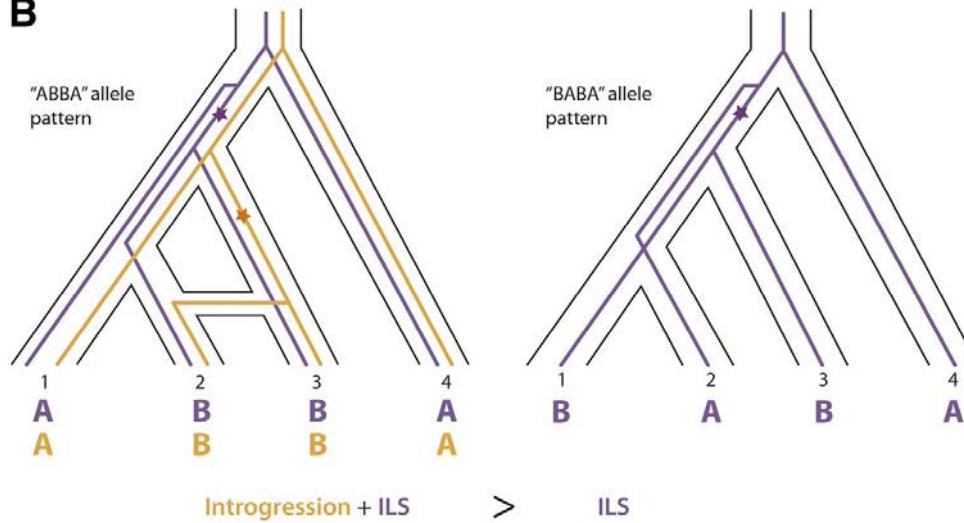
Rachel C. Williams^{1,2}, Marina B. Blanco¹, Jelmer W. Poelstra¹, Kelsie E. Hunnicutt¹, Aaron A. Comeault¹ & Anne D. Yoder^{1*}



Pairwise Sequentially Markovian Coalescent (PSMC)

Heterozygosity



A**B**

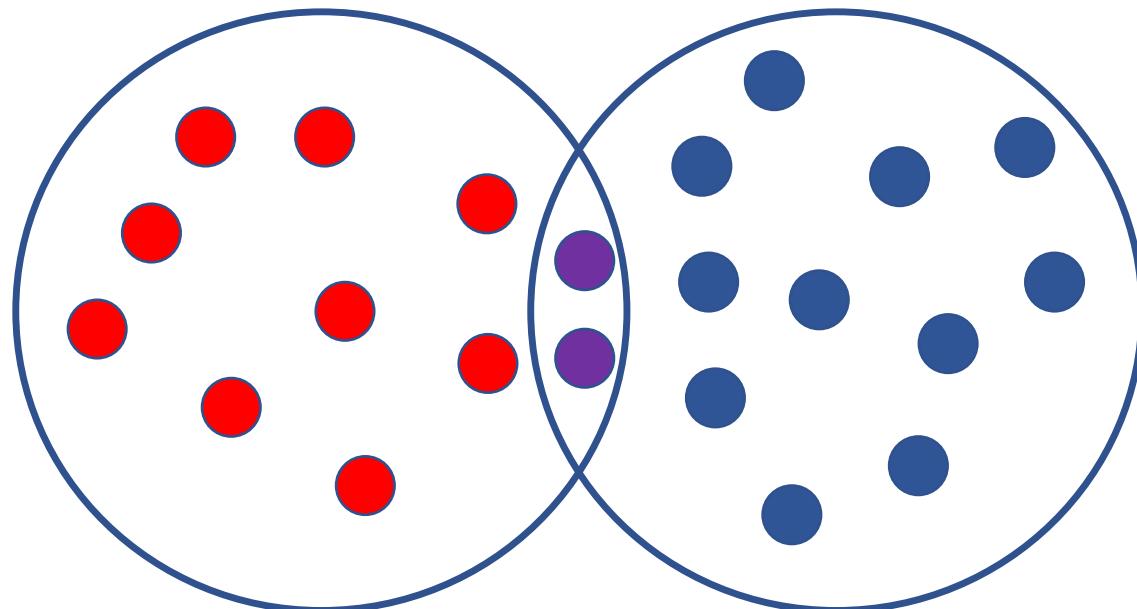
The “ABBA-BABA” test (i.e., Patterson’s D statistic)

- If incomplete lineage sorting (ILS) is causing most of the gene-tree/species-tree discordance, then ABBA and BABA sites will be roughly equal ($ABBA/BABA = 1.0$)
- If gene flow is acting to create discordance this relationship will break down

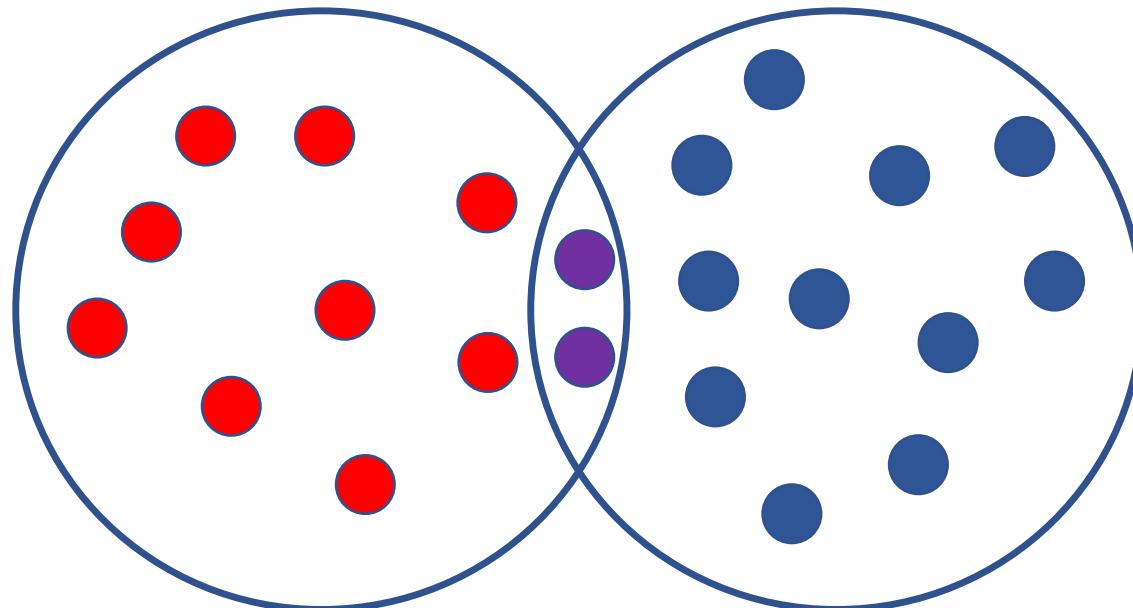
Figure from Goulet et al., 2017

Red Species

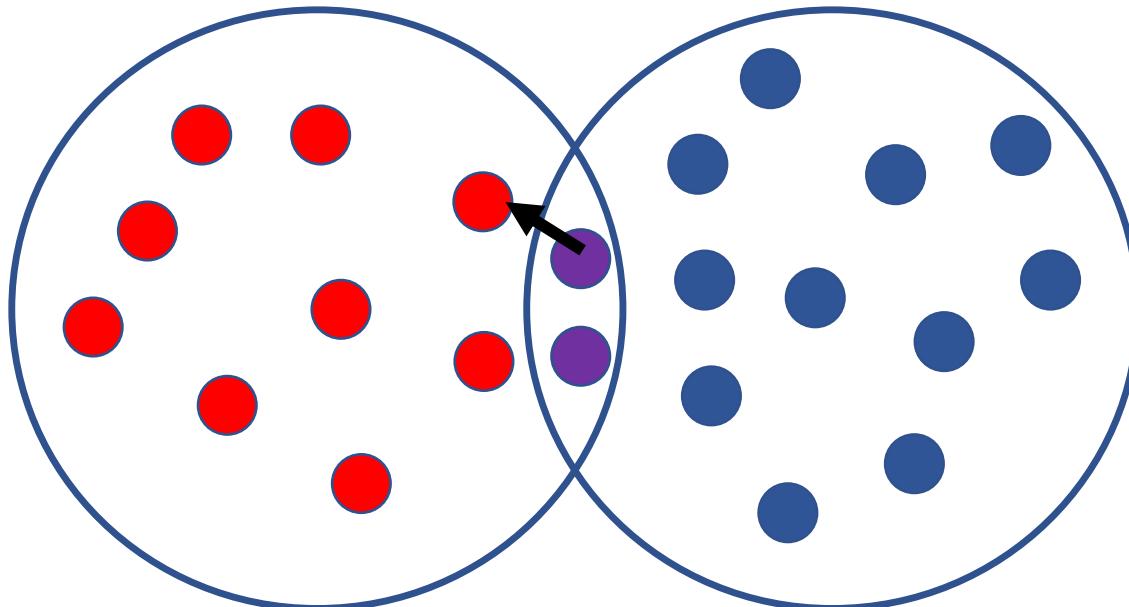
Blue Species



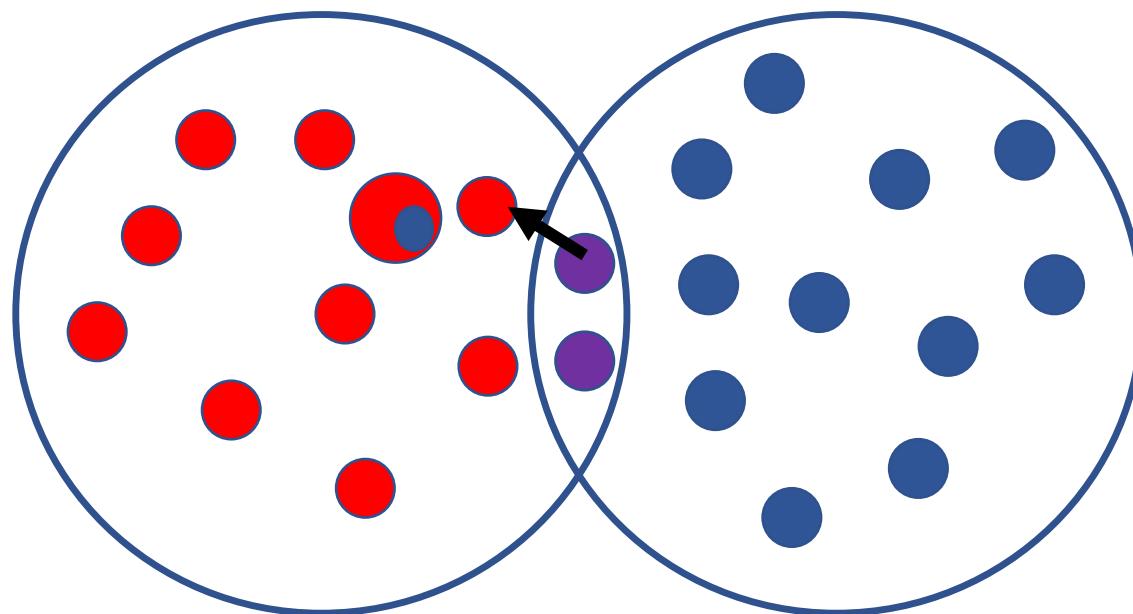
Hybrids (shown in purple) are less fit than parent species, the hybrid zone is said to be "reinforced"



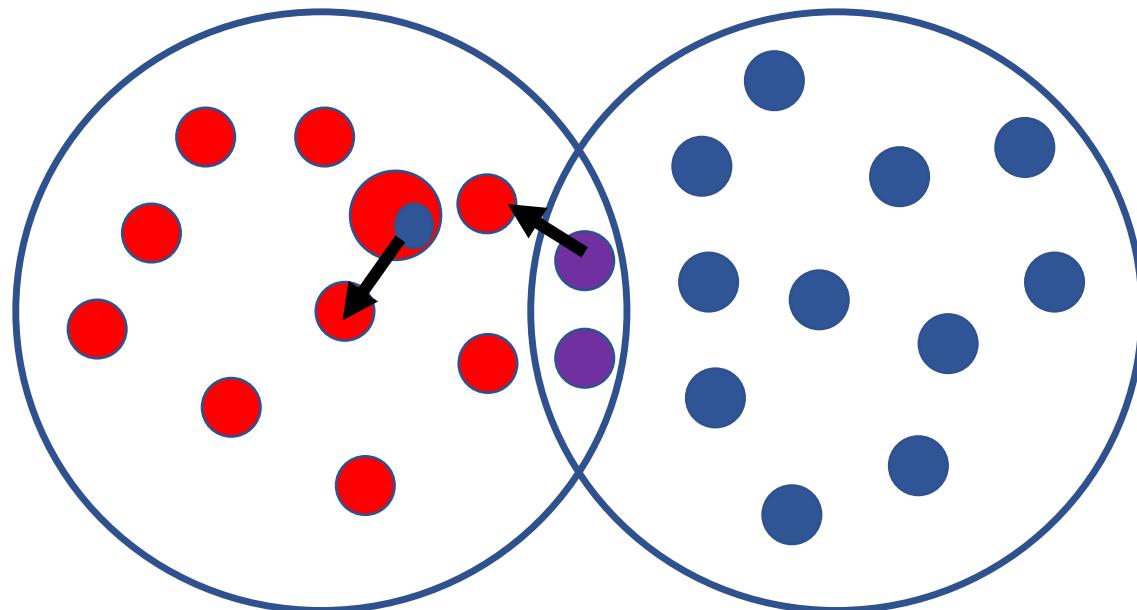
Even so, **back-crossing** can occur (when a hybrid mates with an individual from one of the parent species)



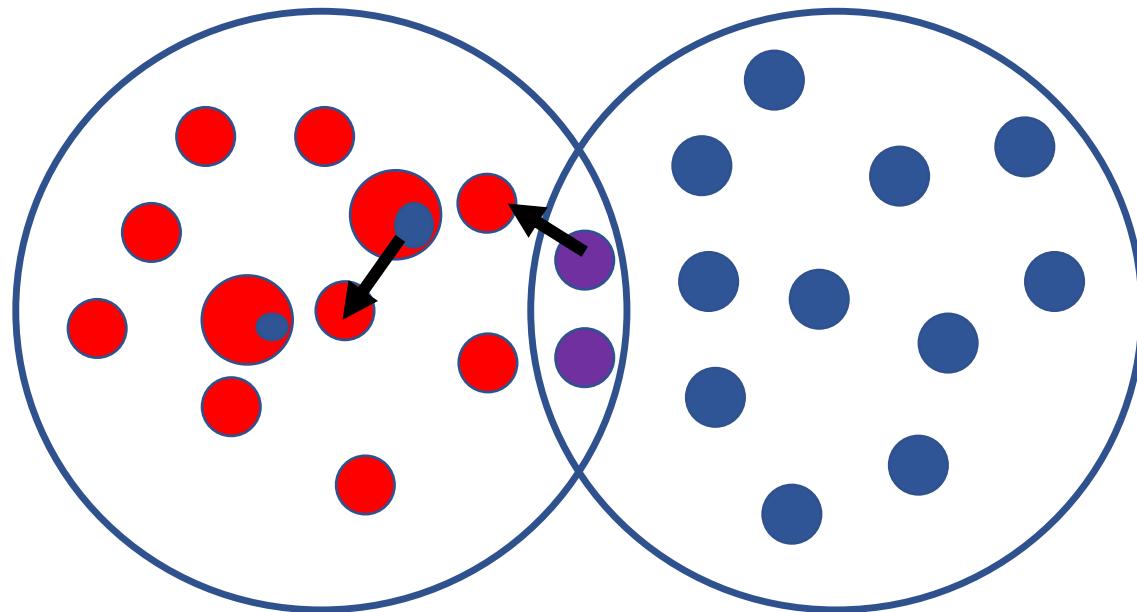
When that individual again crosses with a pure parent individual portions of the Blue species genome (blue circle) are transmitted even though the offspring is not a classic hybrid



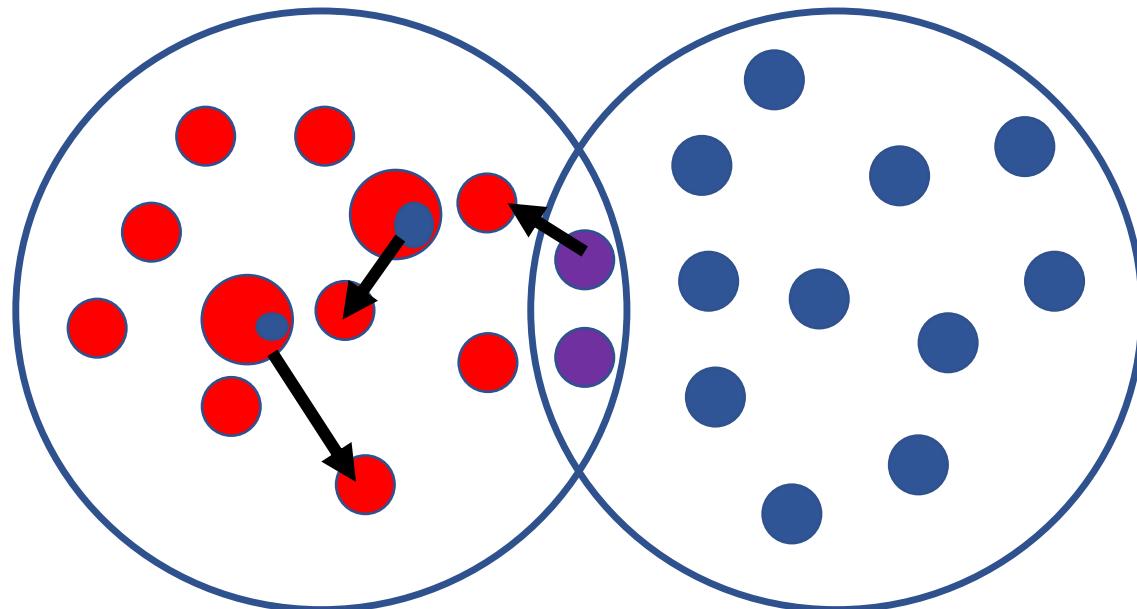
That individual can then mate with a pure parent individual, again transmitting portions of the Blue Species genome, but less will be represented in that offspring



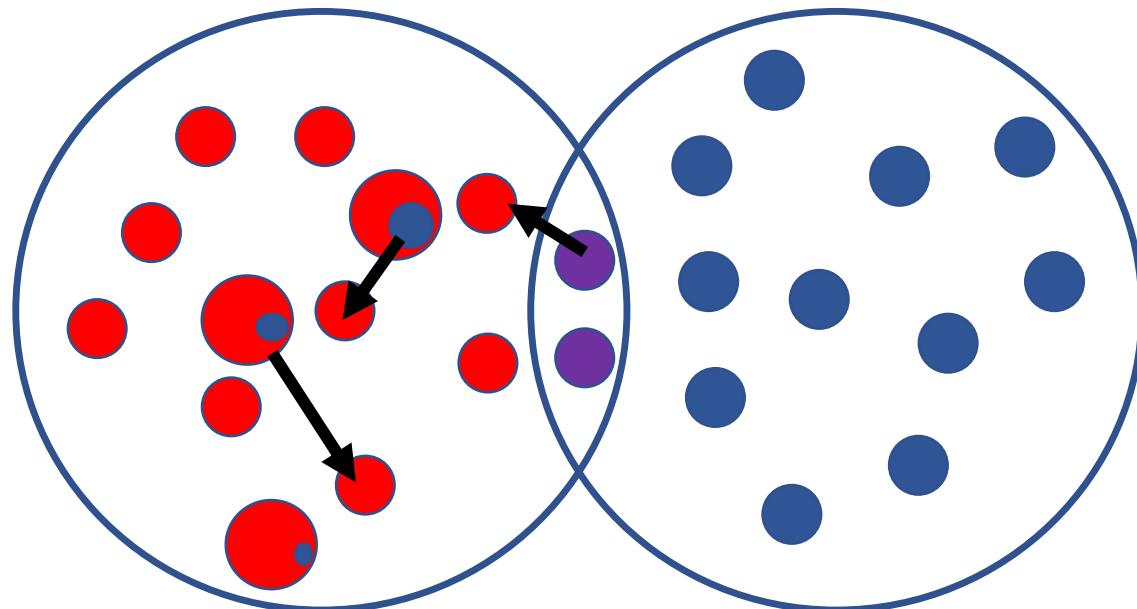
That individual can then mate with a pure parent individual, again transmitting portions of the Blue Species genome, but less will be represented in that offspring



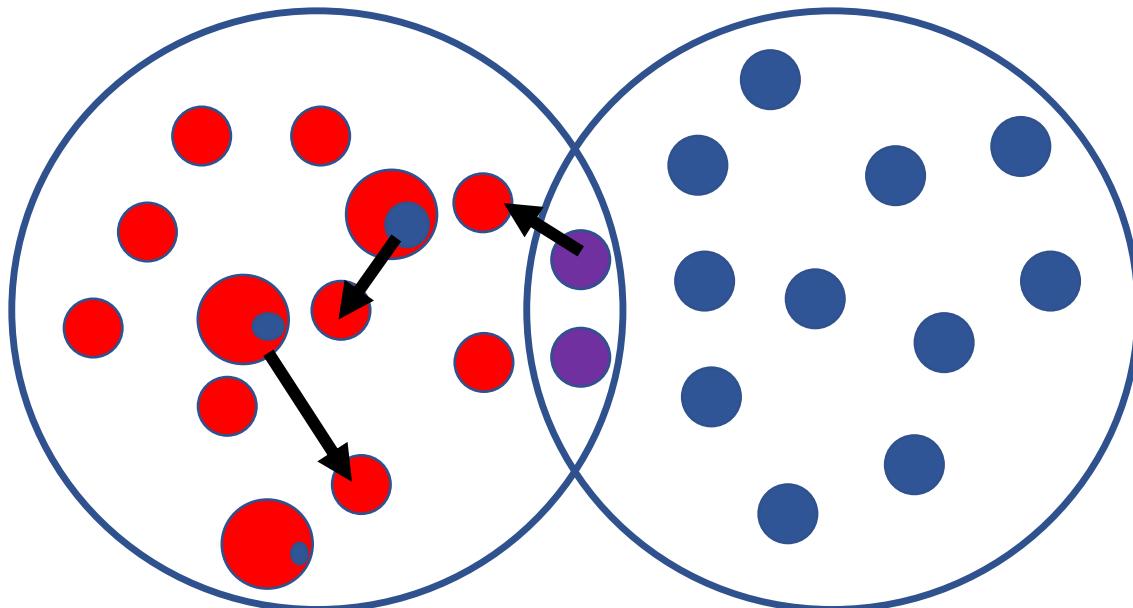
The process can continue, with less and less of the Blue Species genome transmitted, but those portions that persist probably do so because they are advantageous



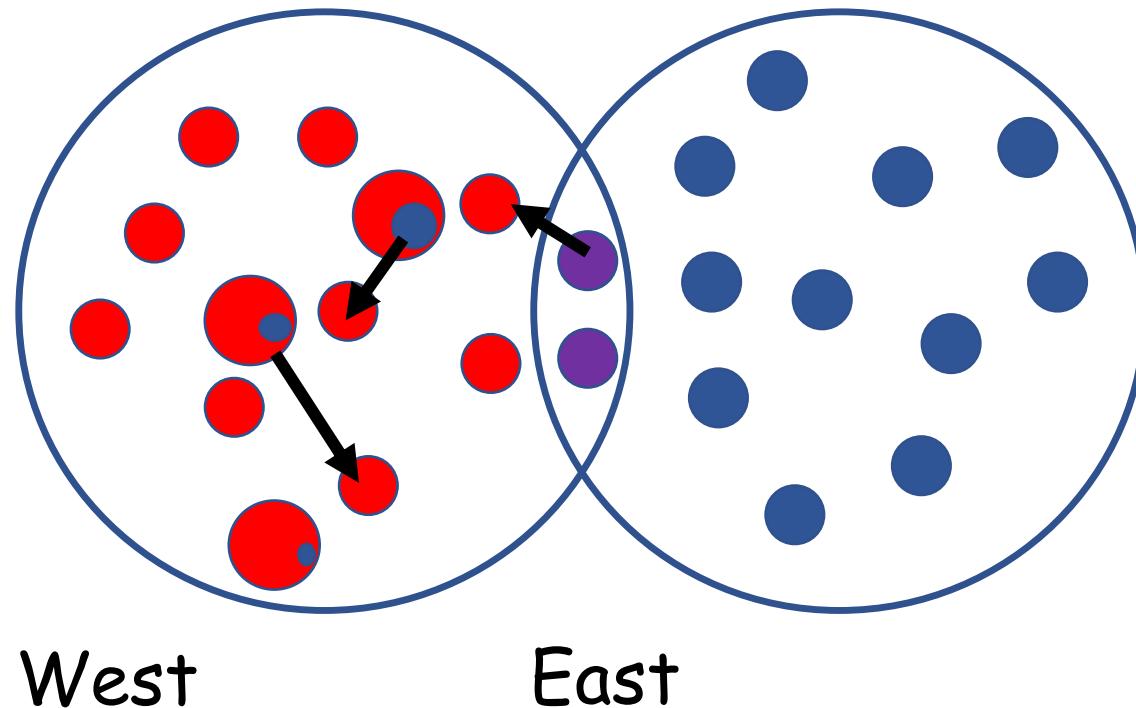
The process can continue, with less and less of the Blue Species genome transmitted, but those portions that persist probably do so because they are advantageous



A process called "Adaptive Introgression"

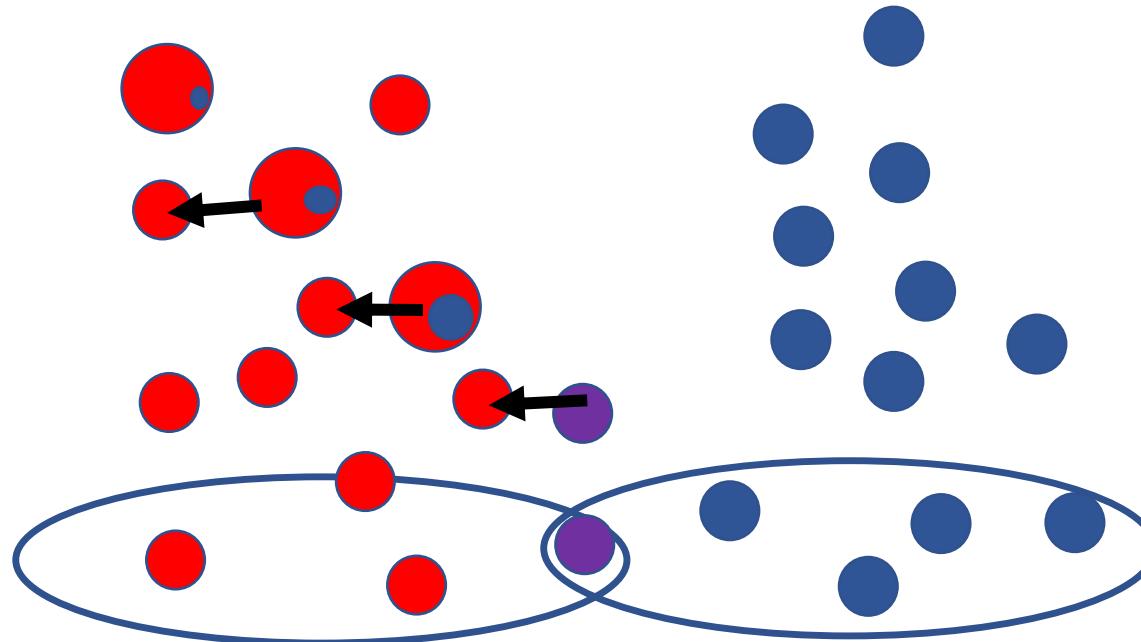


You can think about this occurring
over space



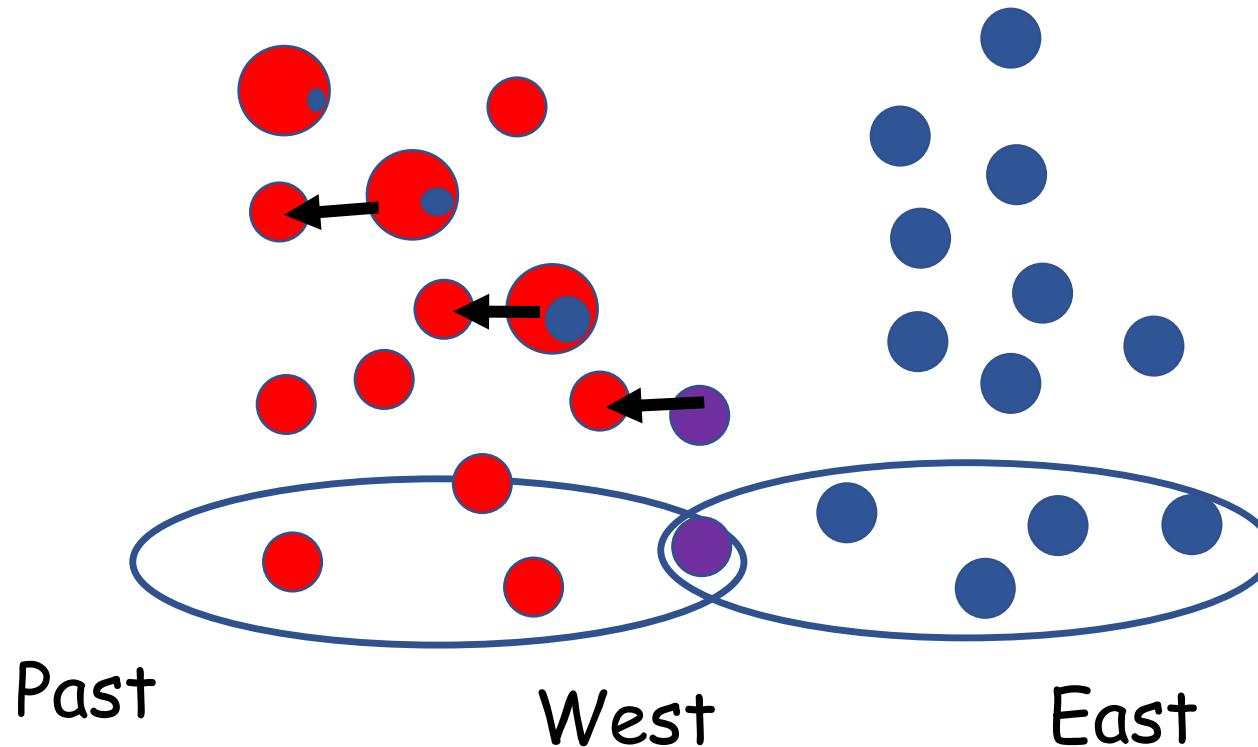
Or time

Present



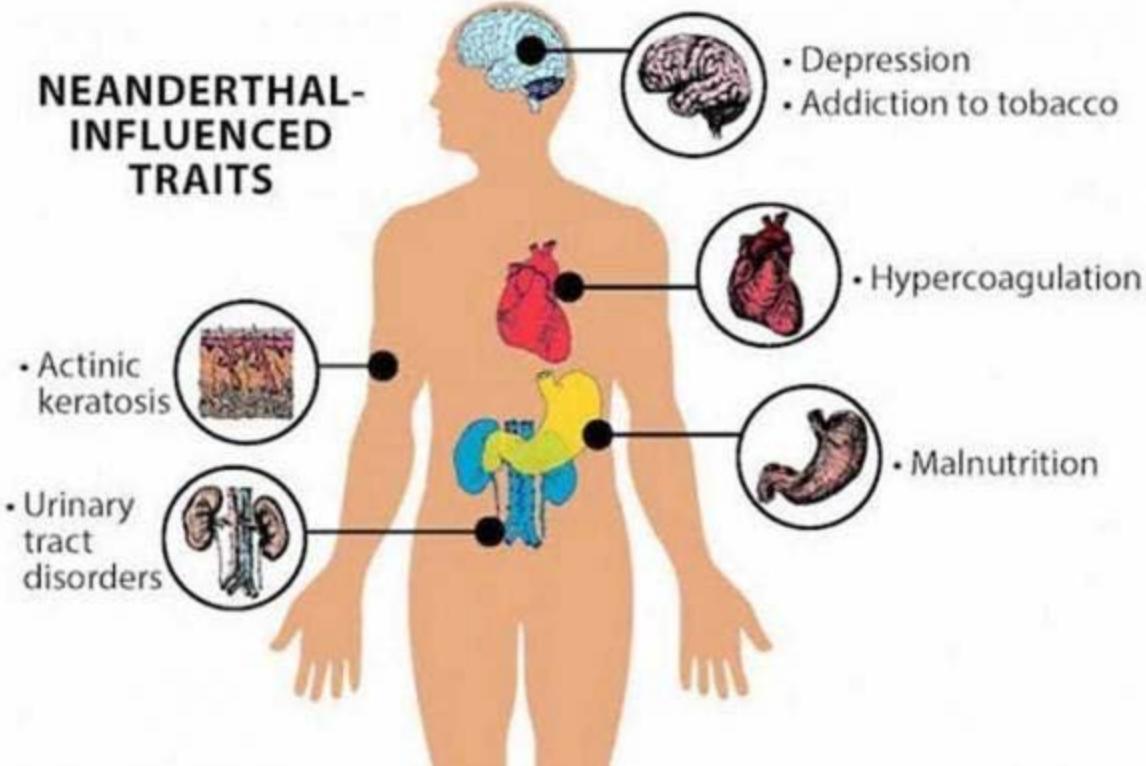
Past

And in fact, it is usually both
Present

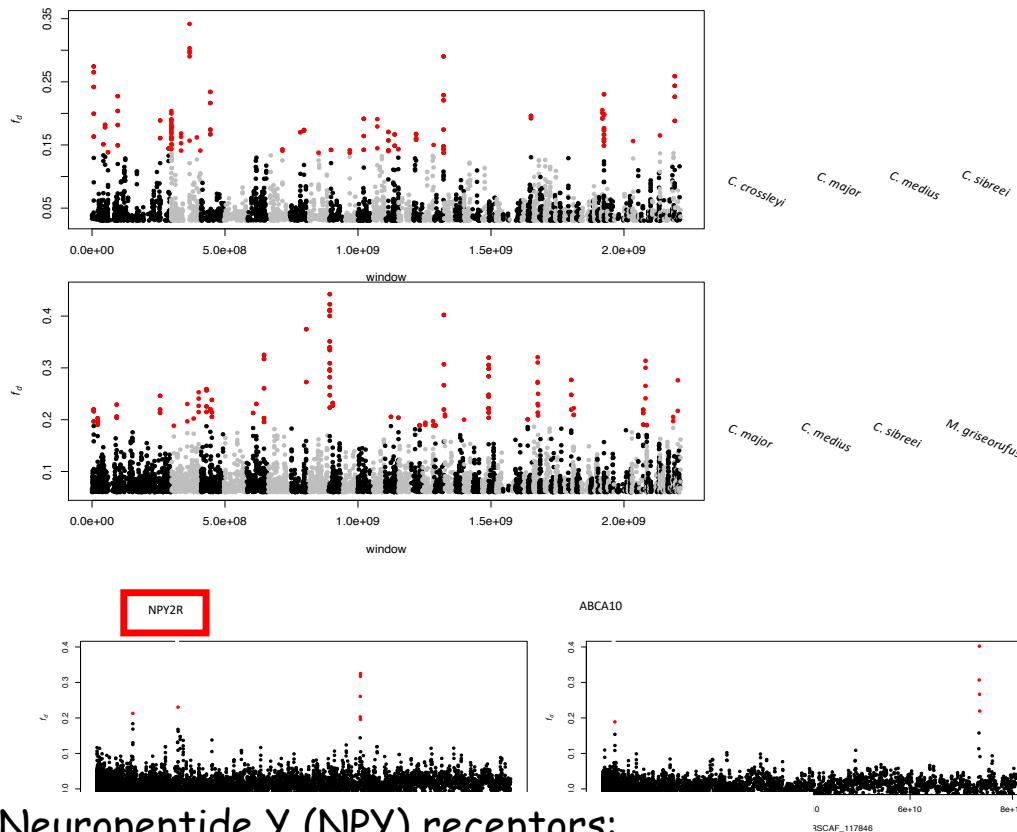


"Adaptive introgression"
can create classic cases
of gene tree/species
tree mismatches

NEANDERTHAL-INFLUENCED TRAITS



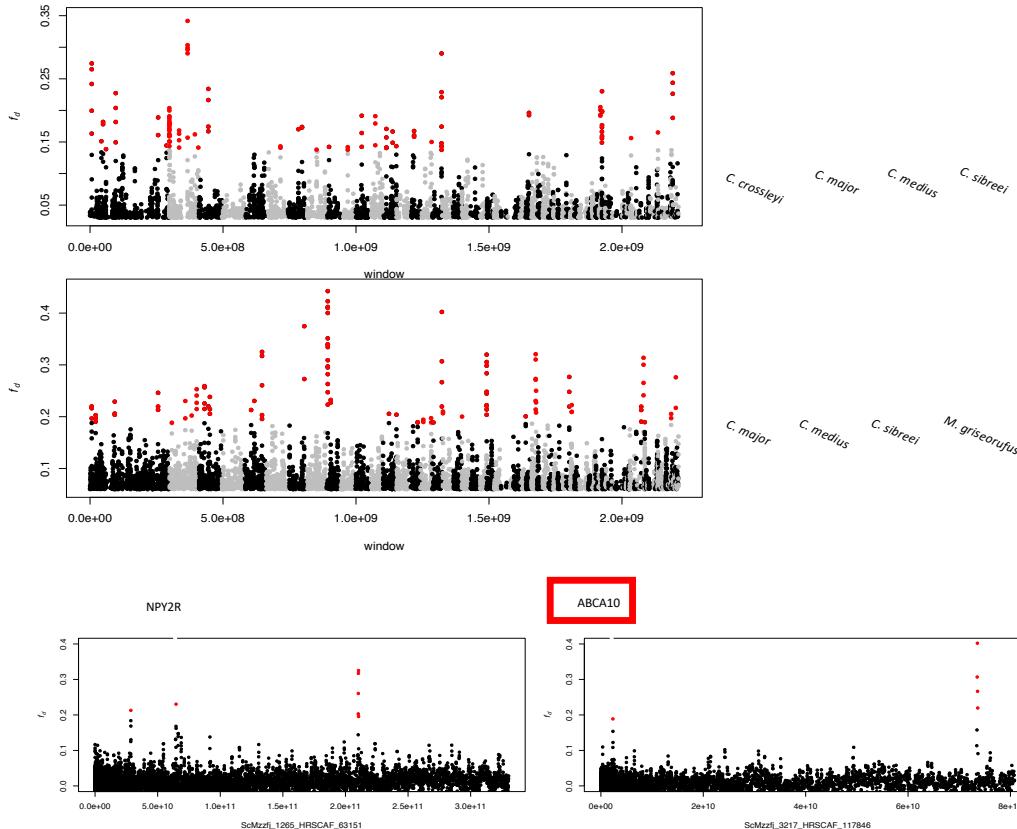
Looking at introgressed genes between dwarf lemur species



Neuropeptide Y (NPY) receptors:
stimulation of food intake and modulation of
circadian rhythm

Williams et al. (in press)

Looking at introgressed genes between dwarf lemur species



ABCA10: Probable transporter which may play a role in macrophage lipid homeostasis

Williams et al. (in press)

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

$p < 0.00006$, $p < 0.00009$

Wrapping it up ...

- There are many (exact count still unknown) species of mouse lemur
- It will take continuing and intensive field study, and international collaboration, to get to the bottom of it
- Speciation is a **process** so it should not be surprising that some lineages will be less diverged than others
- The point of it all is **both** to learn about the processes that drive speciation in these small mammals, **and** to identify those populations/species that are under immediate threat from human impacts

Taking genomics to the field

The image shows a screenshot of a bioRxiv preprint server page. At the top left is the CSHL logo with the text "Cold Spring Harbor Laboratory". Next to it is the bioRxiv logo with the tagline "THE PREPRINT SERVER FOR BIOLOGY". On the right side of the header are links for "HOME" and "ABOUT", and a search bar. Below the header, there are two buttons: "New Results" on the left and "Comment on this paper" on the right. The main title of the preprint is "Next-generation *in situ* conservation and educational outreach in Madagascar using a mobile genetics lab". Below the title, the authors are listed as Marina B. Blanco, Lydia K. Greene, Rachel C. Williams, Lanto Andrianandrasana, Anne D. Yoder, and Peter A. Larsen. The DOI is provided as [doi: https://doi.org/10.1101/650614](https://doi.org/10.1101/650614). A note at the bottom states, "This article is a preprint and has not been peer-reviewed [what does this mean?].". At the very bottom, there are buttons for "Abstract", "Full Text", "Info/History", "Metrics", and "Preview PDF".



Marina Blanco &
Lydia Greene





Biodiversity = (θ)

