

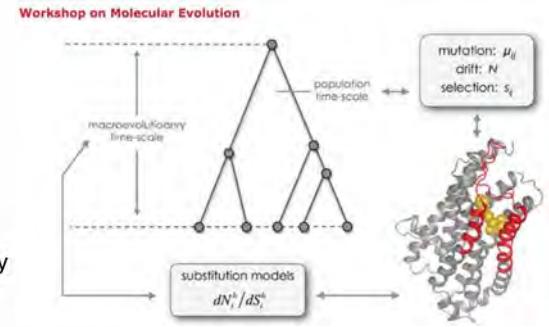


## Workshop on Molecular Evolution



## 2023 Course Faculty

Peter Beerli, Florida State University  
Joseph Bielawski, Dalhousie University  
Jeremy Brown, Louisiana State University  
Minh Quang Bui, Australian National University  
Belinda Chang, University of Toronto



Scott Edwards, Harvard University  
Laura Eme, University Paris-Saclay  
Tracy Heath, Iowa State University  
John Huelsenbeck, University of California-Berkeley  
Lacey Knowles, University of Michigan  
Laura Kubatko, Ohio State University  
Paul Lewis, University of Connecticut  
Emily Jane McTavish, University of California-Merced

Claudia Solís-Lemus, University of Wisconsin-Madison  
Megan Smith, Indiana State University  
Edward Susko, Dalhousie University  
David Swofford, Duke University  
Anne Yoder, Duke University

## 2023 Course TAs

Blake Fauskee, Duke University  
Kevin (Sungsik) Kong, Ohio State University  
Analisa Milkey, University of Connecticut  
Jordan Satler (lead TA), Ohio State University  
Kate Taylor, University of Maryland

# Capstone seminar

---

# Capstone: Evolutionary applications of genomic data

L. Lacey Knowles

Dept. of Ecology and Evolutionary Biology  
University of Michigan



Illustration credit: John Megahan

# Genomic data



## Model-based analyses

Model Formulation



Competing models



Marine Biological Laboratory

THE UNIVERSITY OF  
CHICAGO

## Evolutionary applications of genomic data:

- Codon substitution and analysis of natural selection
- Adaptive molecular evolution
- Divergence time estimation and biogeographic analysis
- Phylogenetic inference
- Inferring species boundaries (aka species delimitation)
- Demographic inference

- All models are flawed..., but ...  
models are **how we communicate our knowledge**  
**to a statistical apparatus**

# Evolutionary applications of genomic data

what I'll emphasize:

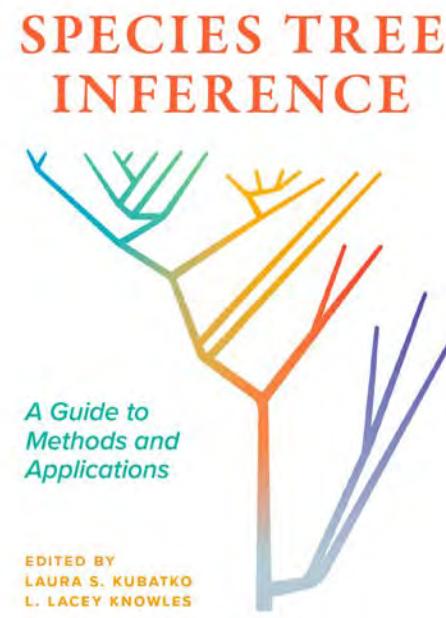
- Decisions/choices we make about model formulation
- Recognizing the subjectivity of model formulation itself when making inferences
- Decisions when applying to empirical data  
(e.g., all the data, subset of data, what subset of data)

# Evolutionary applications of model-based analyses:

- 
- (i) Inferring species boundaries (aka species delimitation)
- (ii) Phylogenetic inference (and beyond the species tree)
- (iii) Biogeographic study
- (iv) Phylogeography
- (v) Adaptive evolution

# Evolutionary applications of model-based analyses:

- (i) Inferring species boundaries (aka species delimitation)
- (ii) Phylogenetic inference (and beyond the species tree)
- (iii) Biogeographic study
- (iv) Phylogeography
- (v) Adaptive evolution



Laura S. Kubatko and  
L. Lacey Knowles  
*Species Tree Inference*

30% off  
with code P321 at  
[press.princeton.edu](http://press.princeton.edu)

## Model-based approaches for phylogeographic inference

Discussion points:

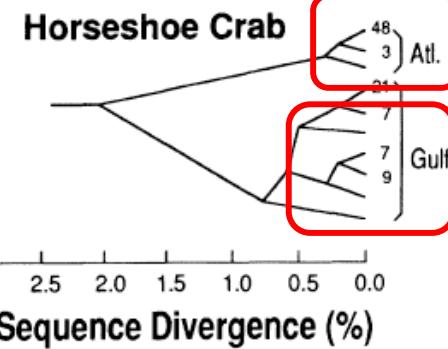
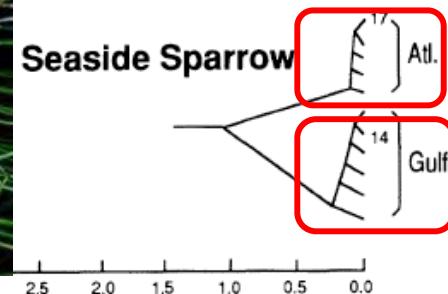
- Why models are important
- Generic versus informed models
- Species-specific expectations of genetic variation  
(e.g.. trait-based hypotheses, spatially explicit coalescent models, etc.)
- Concordance versus discord among species in communities  
(i.e.. lessons from comparative phyogeography)

# Why the transition from describing patterns of genetic variation to understanding process requires model-based approach

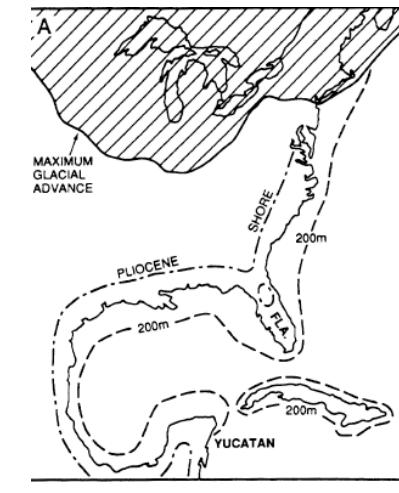
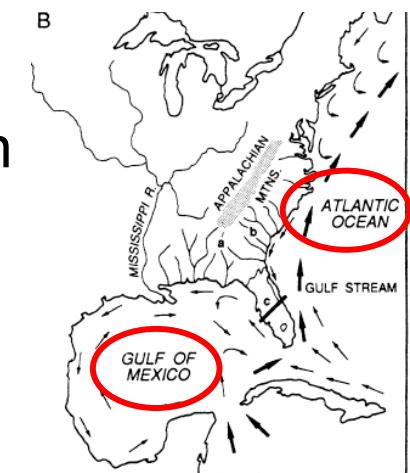
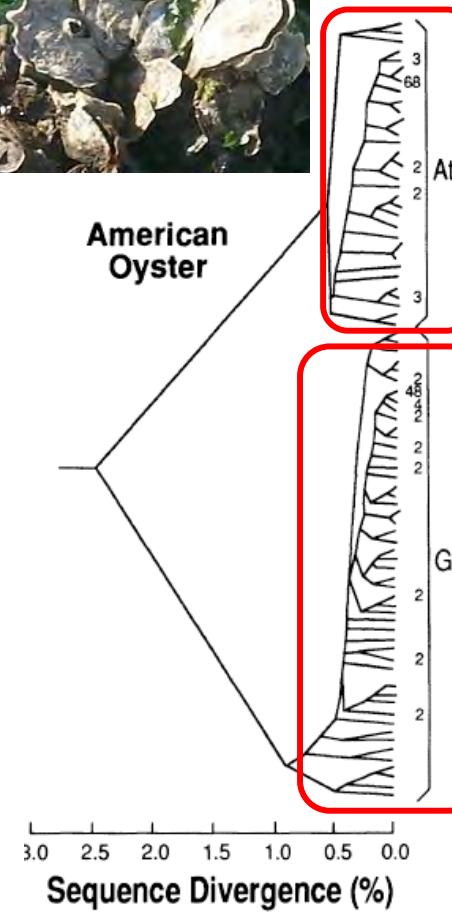
## Classics in phylogeography



Concordance reflects a common vicariant history of population separation



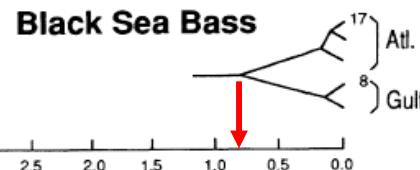
American Oyster



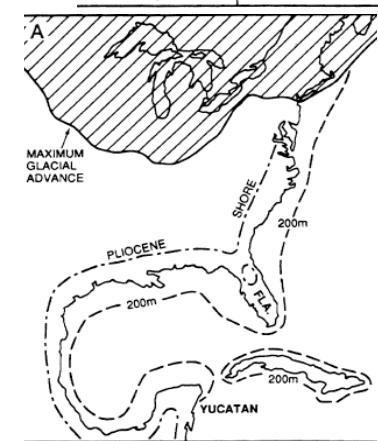
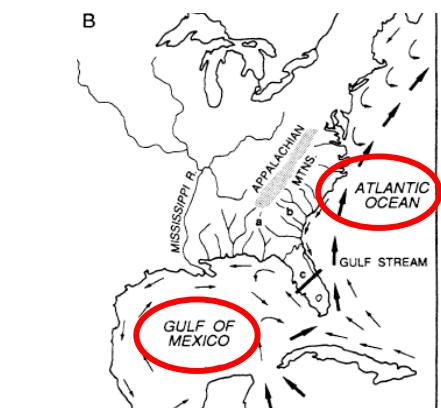
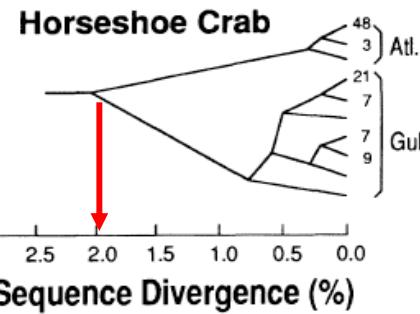
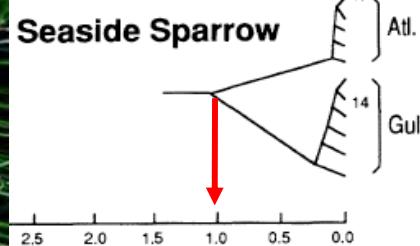
Avise 1992

The data may be consistent with a shared response to a specific geologic event, despite differing gene tree depths among taxa? Or maybe not?

By looking only at the gene trees,  
it isn't clear how the differences in gene tree depths  
should be interpreted!

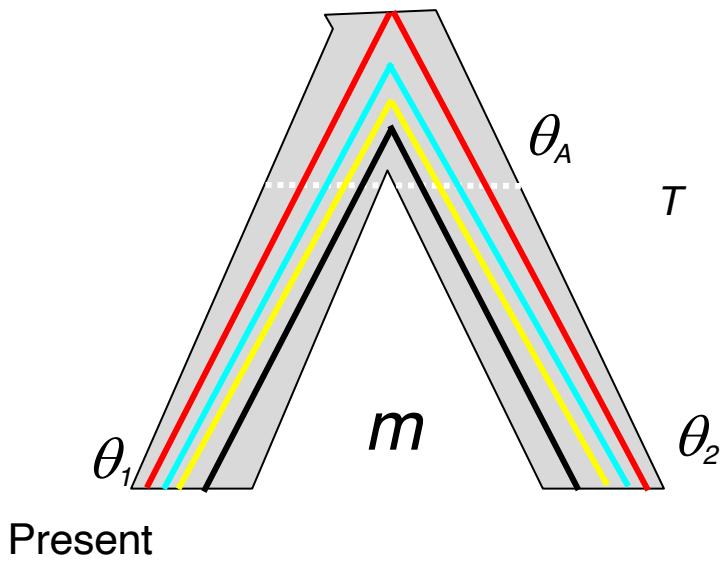


American Oyster

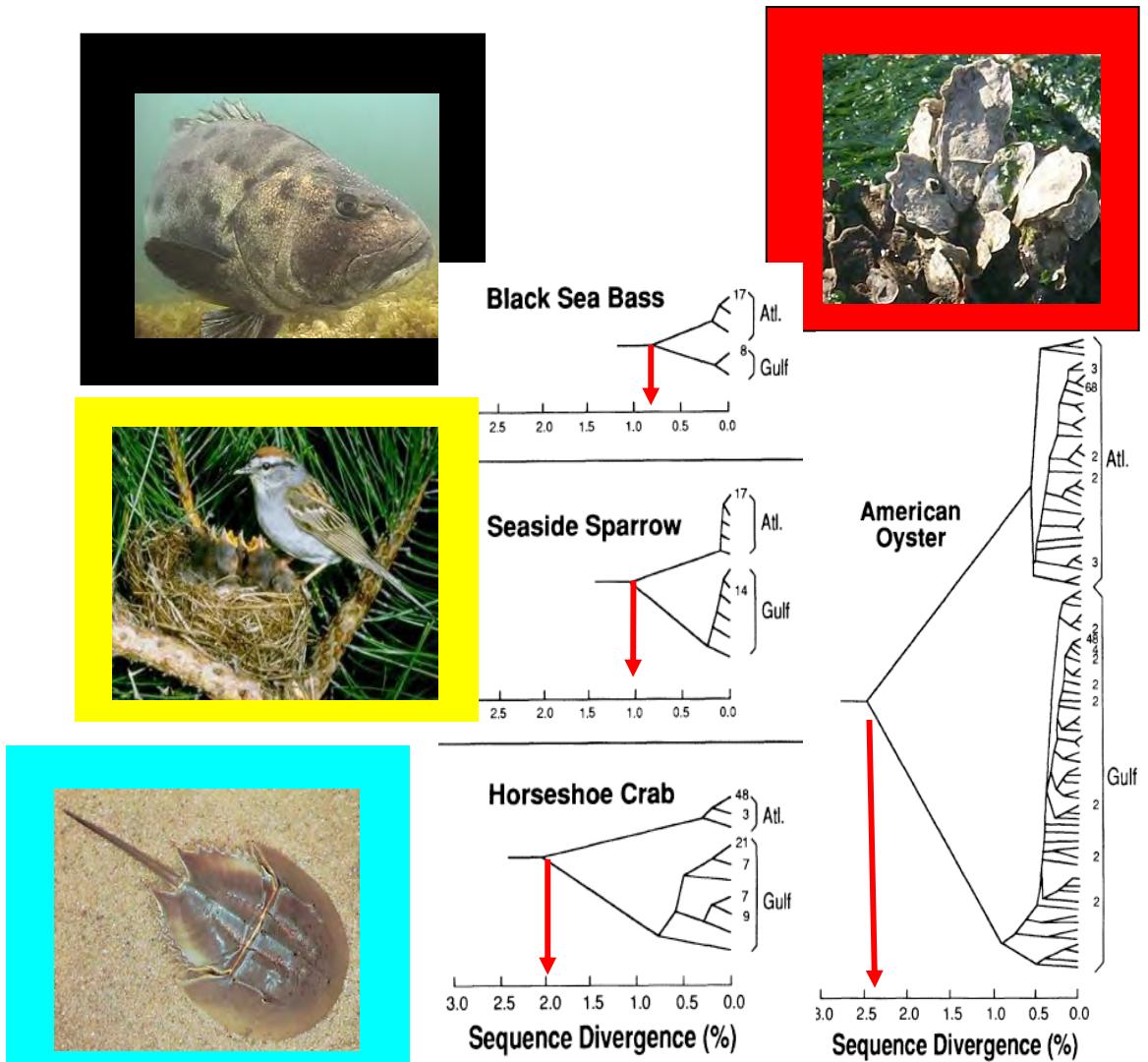


To test for shared vicariant history of the coastal community:

Assess statistically how much of a difference in the depths of the gene trees would still be consistent with the same geologic event based on the timing of divergence



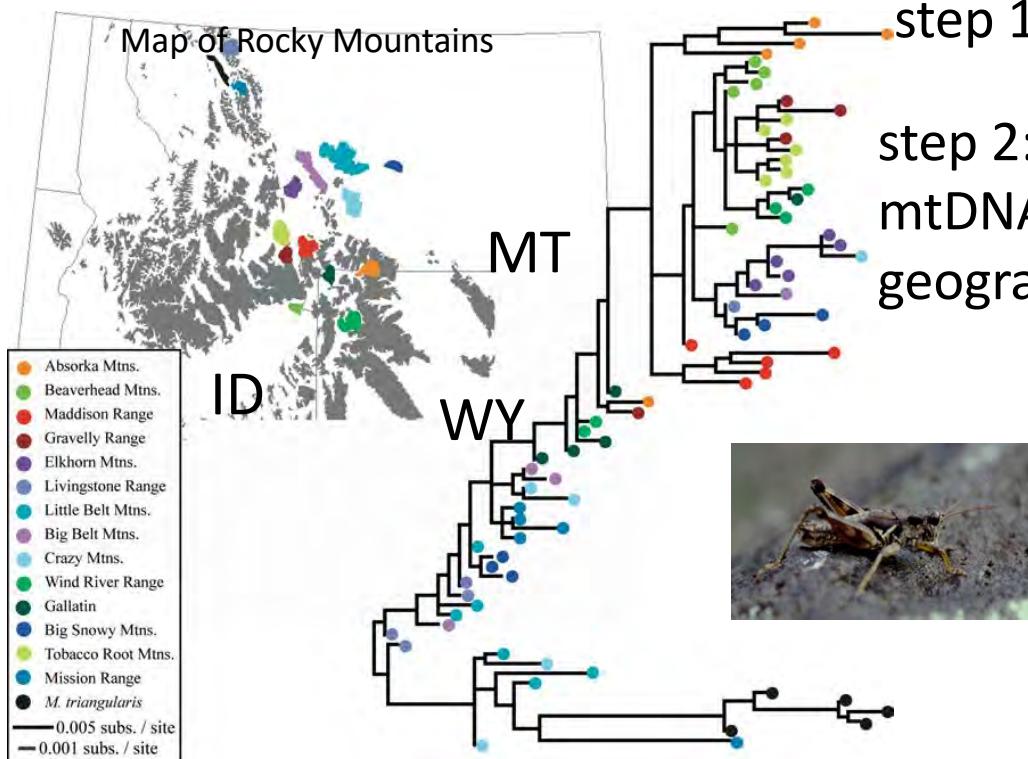
Expectation of  $T$  is based on the geologic event (i.e., sea level change) – that is, prediction based on information that is independent of the genetic data



In the past, the central focus was on the ‘phylo’ component

## PHYLOgeography

Use of gene trees predominated and genetic variation across populations described by:



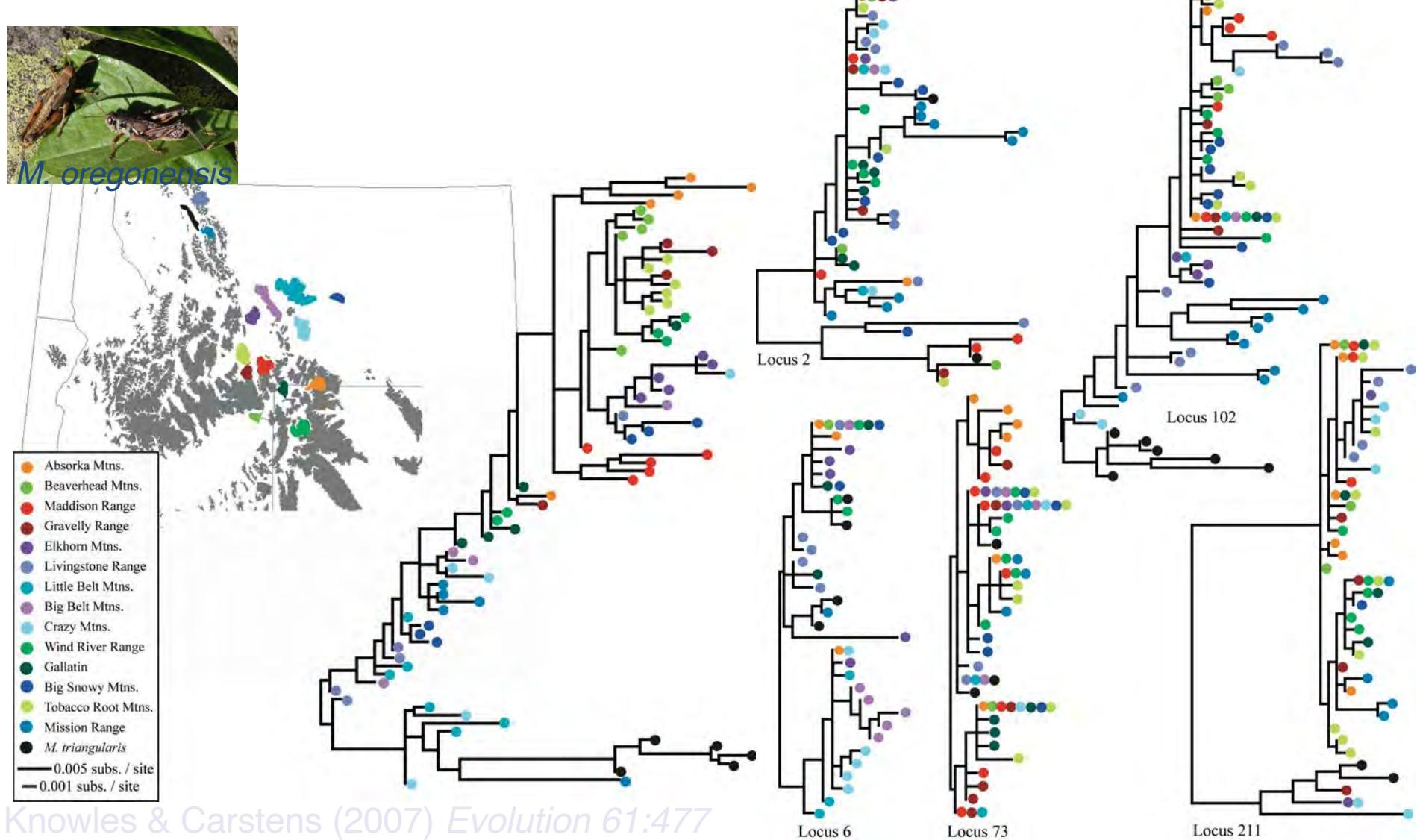
grasshopper haplotypes across populations  
(color coded by the mountain top where  
individual was collected)

step 1: reconstruct a gene tree

step 2: compare the relationships among mtDNA sequences/haplotypes to the geographic distribution of haplotypes

# But different loci have different gene trees

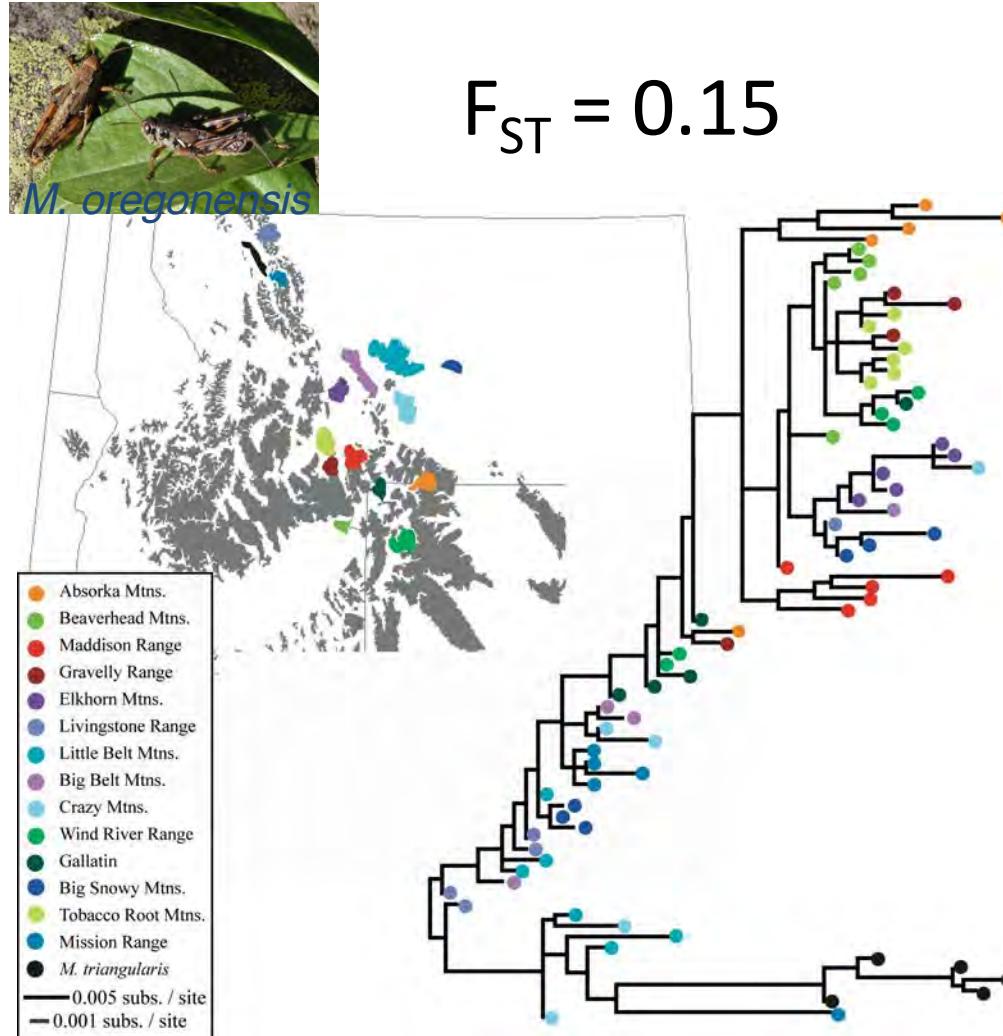
Phylogenetic relationships among populations (i.e., what's the underlying geographic history of divergence)?



Knowles & Carstens (2007) Evolution 61:477

# Different processes can produce similar genetic patterns

Recent isolation or migration?



Knowles & Carstens (2007) *Evolution* 61:477

# Interbreeding between Neanderthals and humans?



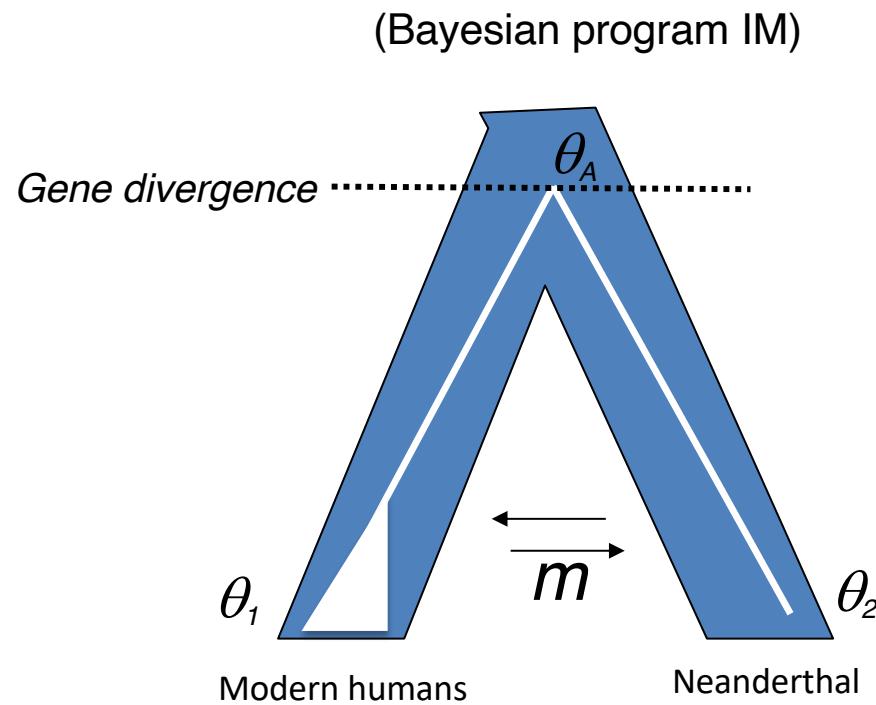
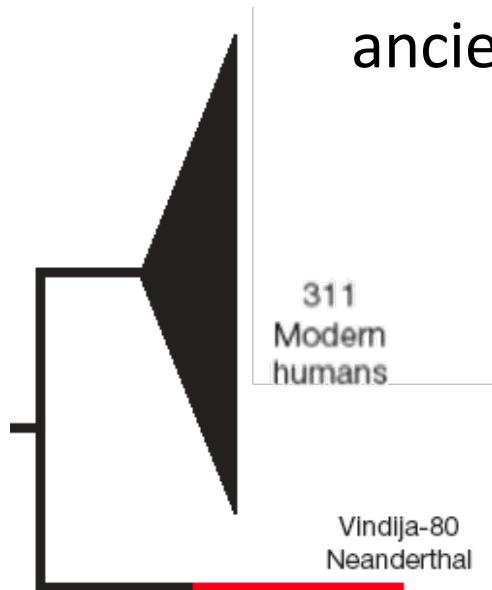
Hypothesis: humans replaced Neanderthals without gene flow between the separate species

- What are two bits of evidence for no gene flow based on this tree?

Problem with interpreting gene tree as evidence of  
“divergence with no gene flow”

# Interbreeding between Neanderthals and humans?

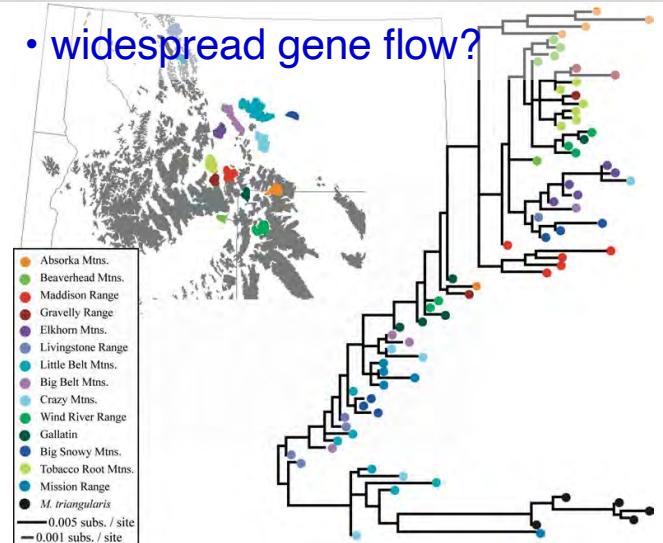
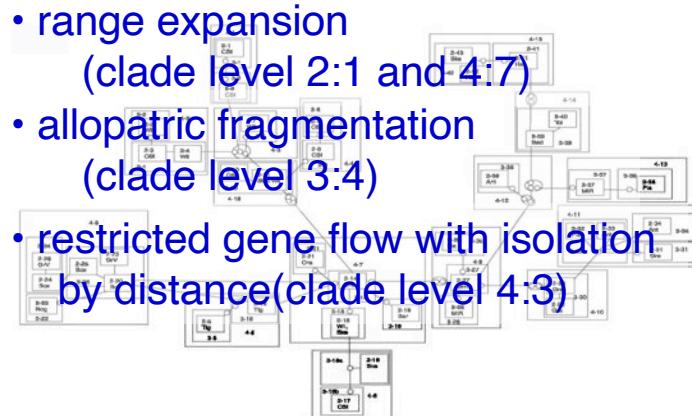
- Model based test of the hypothesis: what's the probability that this gene tree is compatible with ancient gene flow between humans and Neanderthal



Result: yes, tree is compatible; does this mean there was gene flow?

- Not necessarily because with single gene not a lot of power to evaluate the hypothesis

# Equating a gene tree (or network) with a species' history is not appropriate for making inferences about evolutionary processes



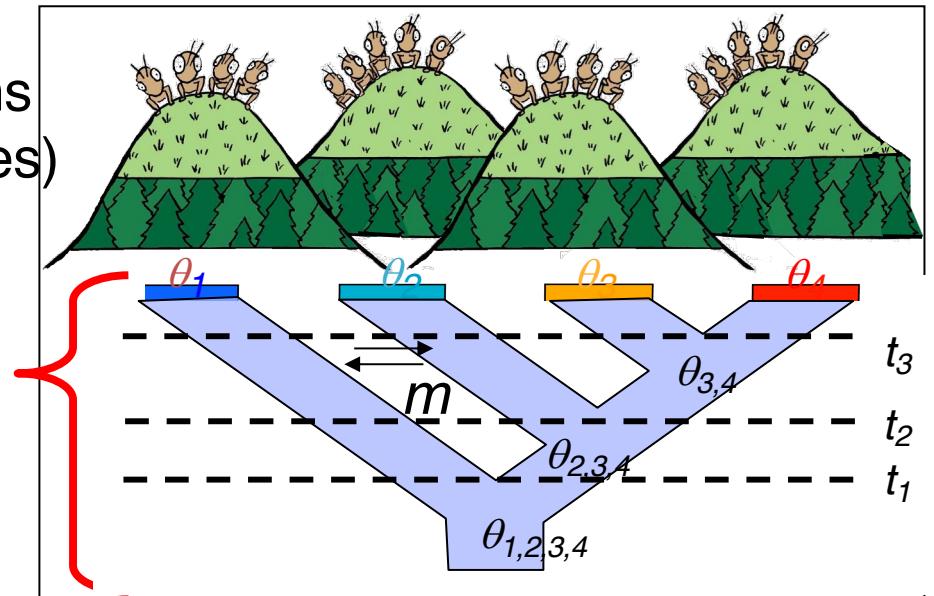
## Without a model:

- inferred processes may (*or may not*) be accurate because different processes can produce a similar pattern in genetic data and gene trees may differ across loci
- no measure of the uncertainty/support surrounding hypotheses or evaluating competing hypotheses
- no framework for incorporate additional data (e.g., geologic or ecological information)
- inherent lack of power when individual loci analyzed separately, and discordance among loci is uninterpretable

# Understanding historical process necessitates model-based approaches

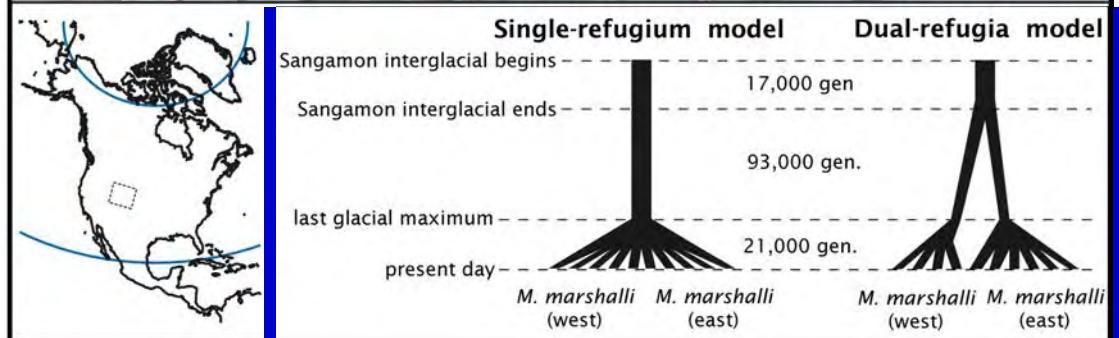
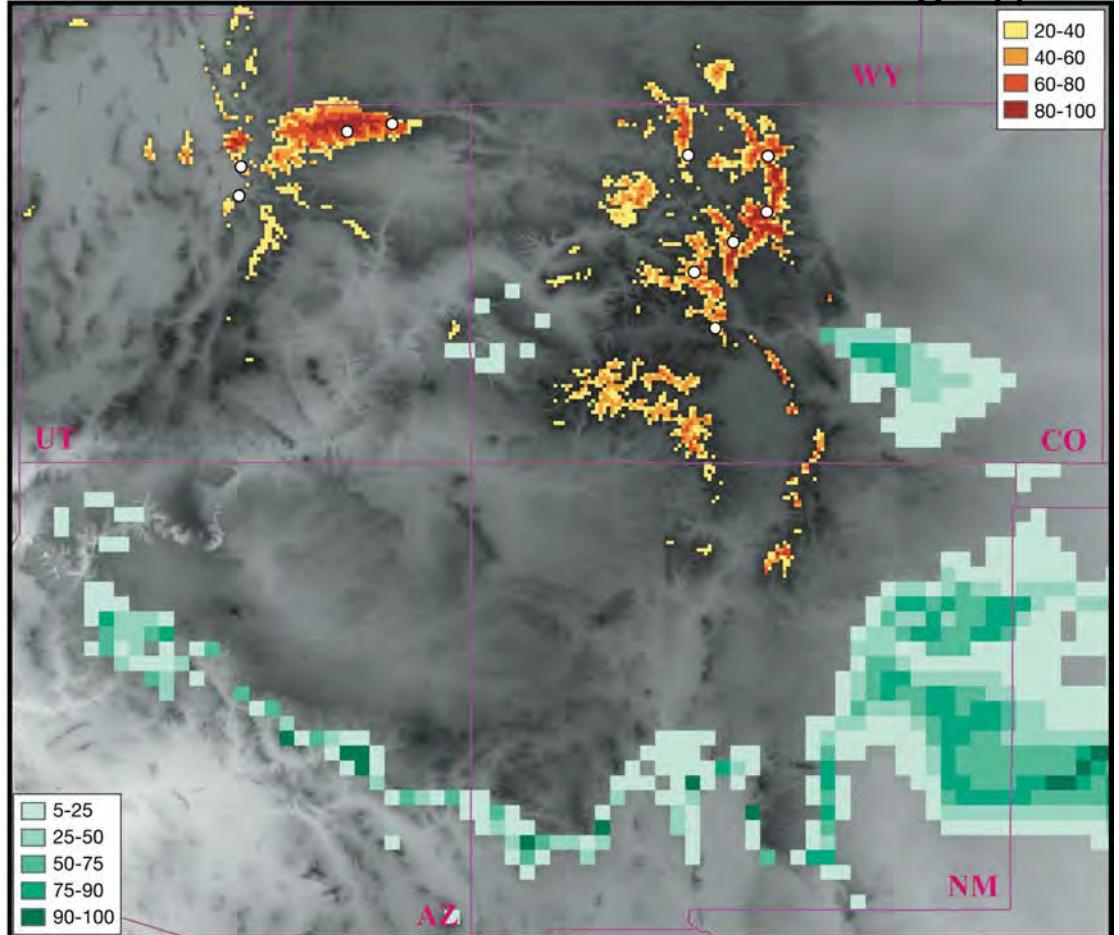
- accommodate and make full use of multilocus data (individual gene trees differ so trying to interpret their patterns would lead you to many different stories)

Explicit model of a species' history



- estimate evolutionary parameters (e.g., population size, migration rates, divergence times, or demographic changes like expansions or bottlenecks, the geographic coordinates of the ancestral population)
- test alternative hypotheses/models (e.g., distinguish between a hierarchical vicariant divergence model versus a stepping-stone colonization model, or isolation by distance)

- Incorporate additional non-genetic sources information to inform our choice of models for testing hypotheses



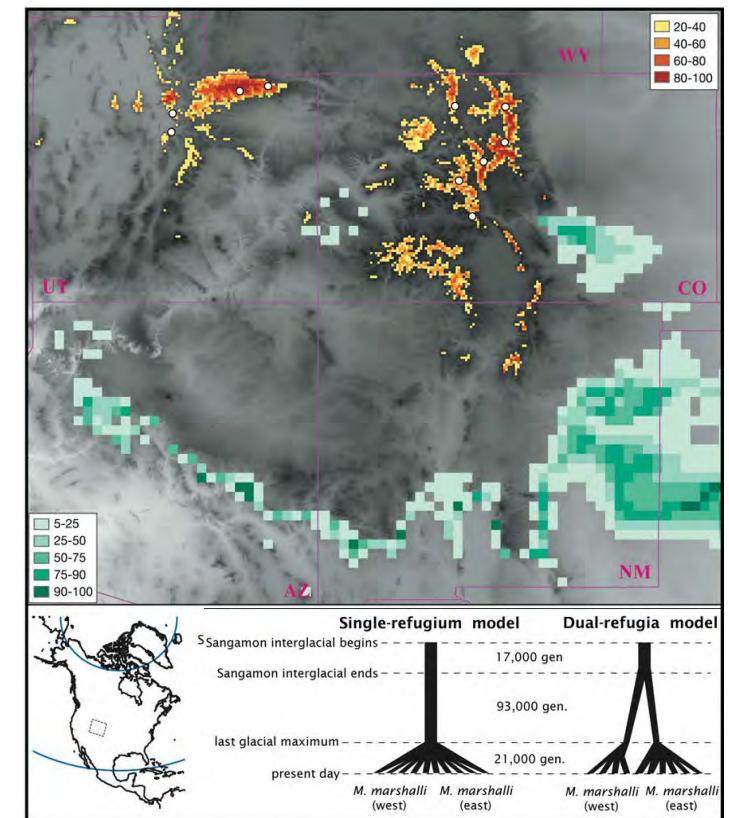
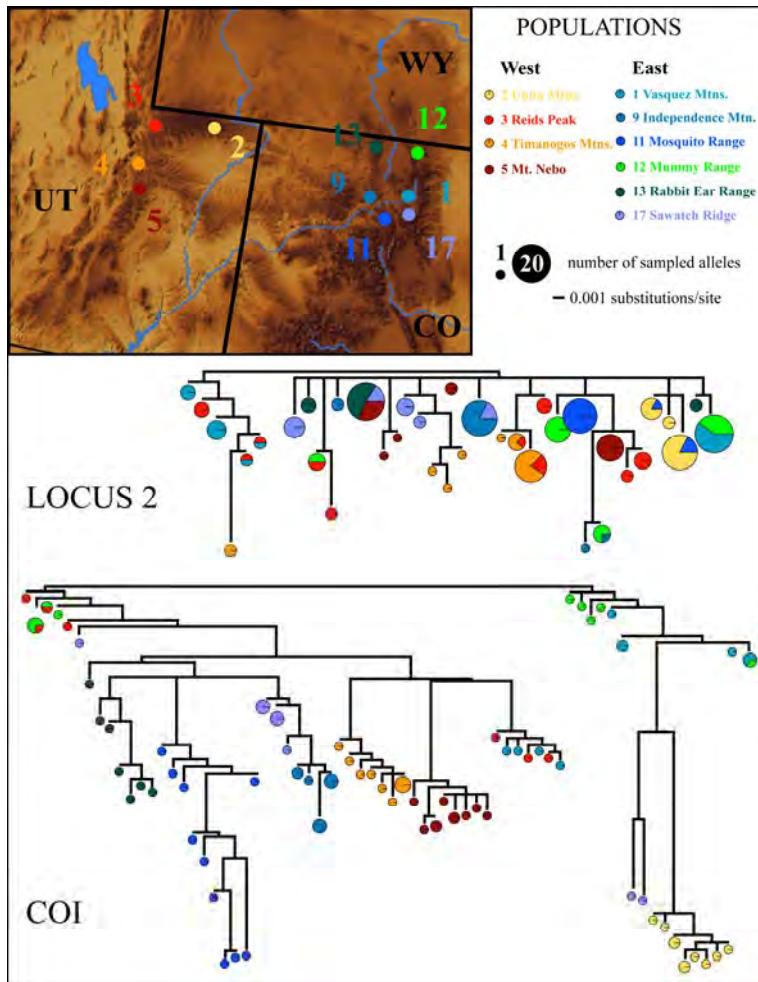
Coupled genetic and ecological-niche models to test hypotheses about ancestral refuges

- Projections of current distribution
- Projections of past distribution 21,000 years ago

(based on 19 bioclimatic variables; analyzed with MAXENT)

Knowles et al. 2007 Current Biology 17:1-7.

Coupled genetic and ecological-niche model:  
 With sequence data from multiple loci, we could reject the fragmentation  
 of a single refugial population, suggesting divergence among  
 multiple refugia promoted divergence.



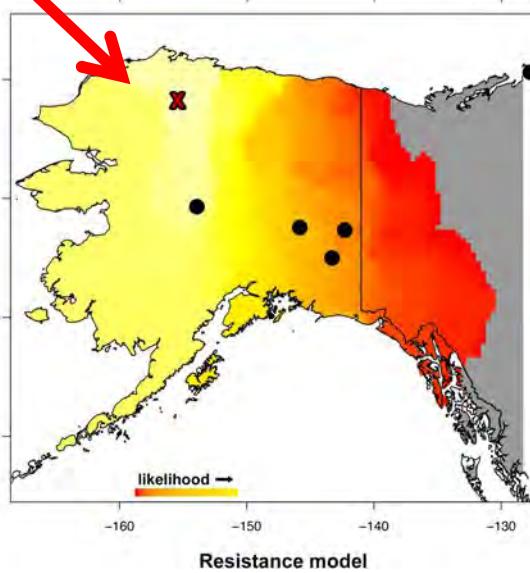
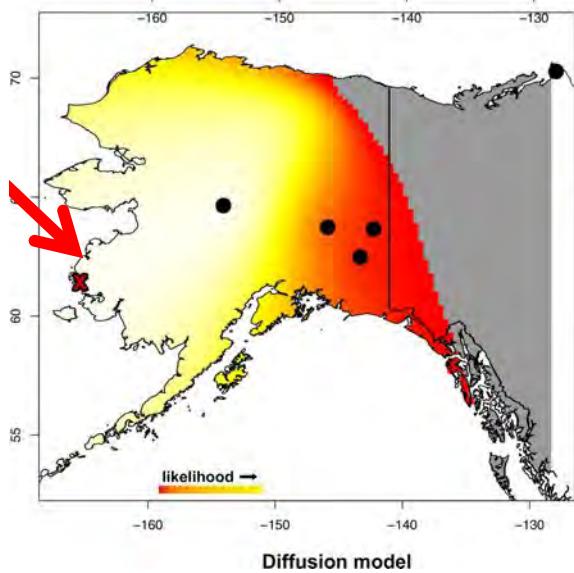
There are many different reasons why it is desirable to combine genetic data with other types of information (e.g., geographic or distributional data, ecological information, etc)

# Why is it desirable to combine genetic data with other types of information?



Capture biologically reality!

- Euclidean distance



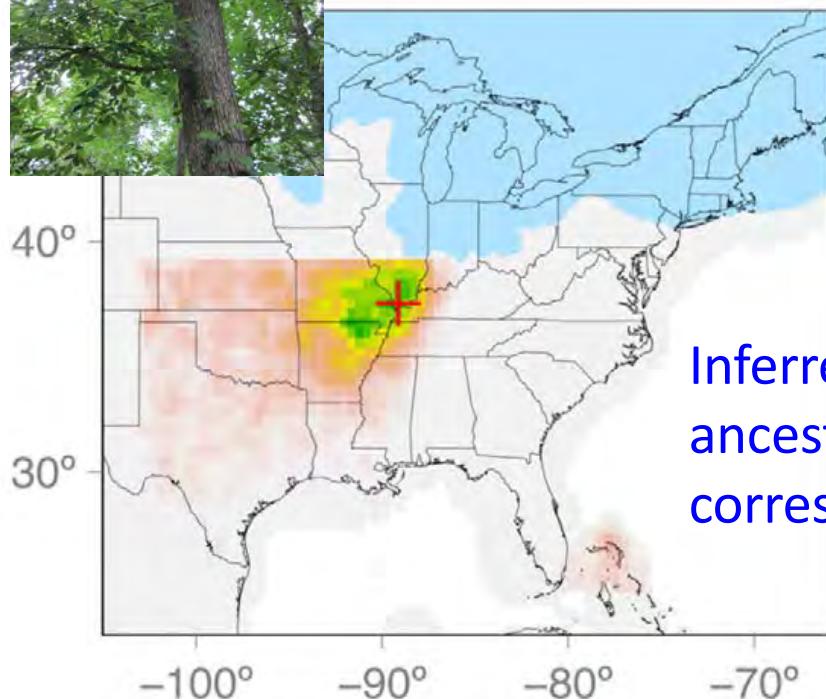
- resistance distance (based on underlying environmental setting)

- Direction and location of ancestral source of expanding population differs between Euclidean and resistance distance (He et al. 2017)

Likelihood surface of location of source population during expansion (He et al. 2017) based on allele frequency gradients, represented by  $\Psi$ -statistics (Peter & Slatkin 2013)

He et al. 2017. Inferring the geographic origin of a range expansion: latitudinal and longitudinal coordinates inferred from genomic data in an ABC framework with the program X-ORIGIN. *Mol. Ecol.* 26:6908-6920. DOI: 10.1111/mec.14380

# Use genetic data to corroborate inferences based on other data types



ENMs do not provide precise location of Pleistocene refuge for hickory trees

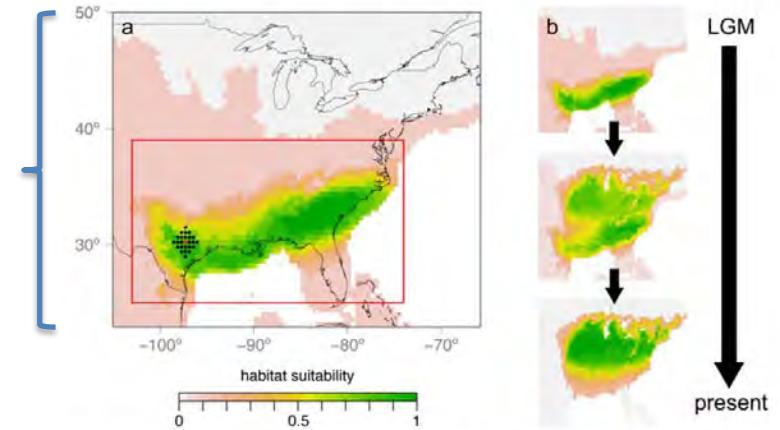


Fig. 1. Schematic overview of demographic simulations. (A) Simulations were initiated in the LGM landscape (shown here for *C. cordiformis*) from a central deme (see red dot as an example) plus an area extending three additional demes (black dots) in all directions. Different geographic sources of

Inferred likelihood of geographic coordinates of ancestral refugia population – this location corresponds to a macrofossil of the hickory species

Fig. 2. Estimated expansion origins ( $\Omega$ ; red cross) in *C. cordiformis* (A) and *C. ovata* (B). The shading of pixels depicts a probability surface (kernel density) showing the likelihood that each pixel served as the expansion origin relative to the pixel with the highest likelihood (i.e.,  $\Omega$ ). Glaciated regions are shown in blue. The results presented in A and B are based on retention of four and three PC axes of variation in genetic summary statistics, respectively. Results based on retaining additional PC axes are presented in *SI Appendix*, Figs. S2 and S3.

Bemmels JB, Knowles LL, Dick CW (2019) Genomic evidence of survival near ice sheet margins for some, but not all, North American trees. *PNAS* 116:8431-8436.

# Statistical inference in population genetics:

Need to define a model

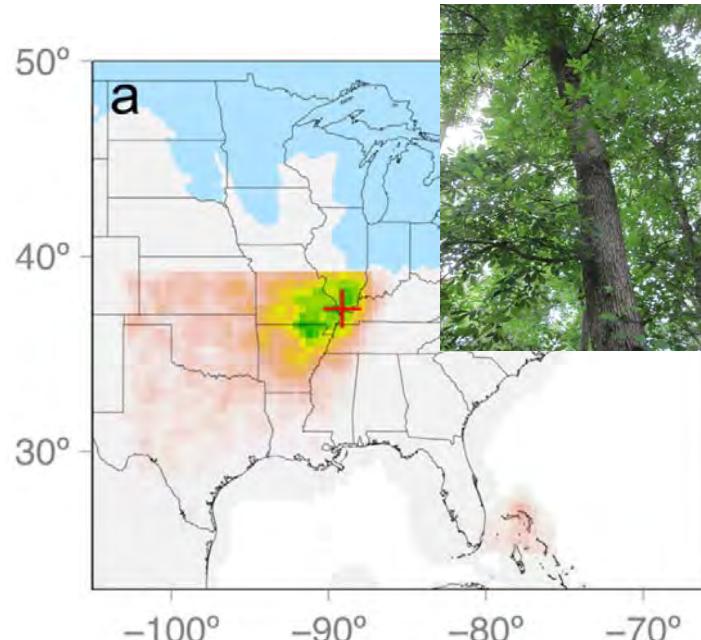
To see how variation in the parameters (e.g., mutation rates, migration rates, selection coefficients) leads to specific patterns of genetic variation (i.e., patterns of variation among DNA sequences, among SNPs, etc.)

## How do we decide upon a model\*:

- informed from information independent of the genetic data itself
  - that is, a specific biological narrative motivates the model
- models informed by genetic data
- generic models

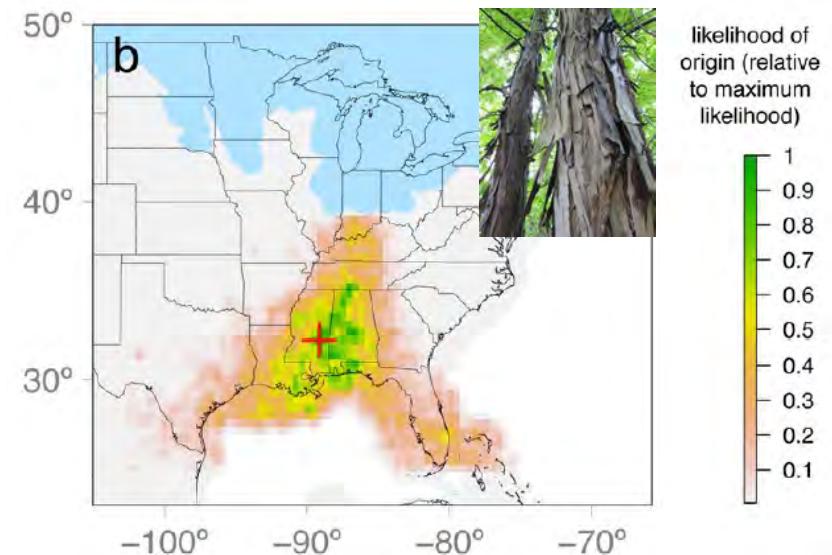
\* All models are simplifications, and vary in their relative degree of abstraction

Expansion model used because of known displacement of hickory trees from current distribution by glacial ice sheet.



Bemmels et al. 2019 PNAS 116:8431-8436

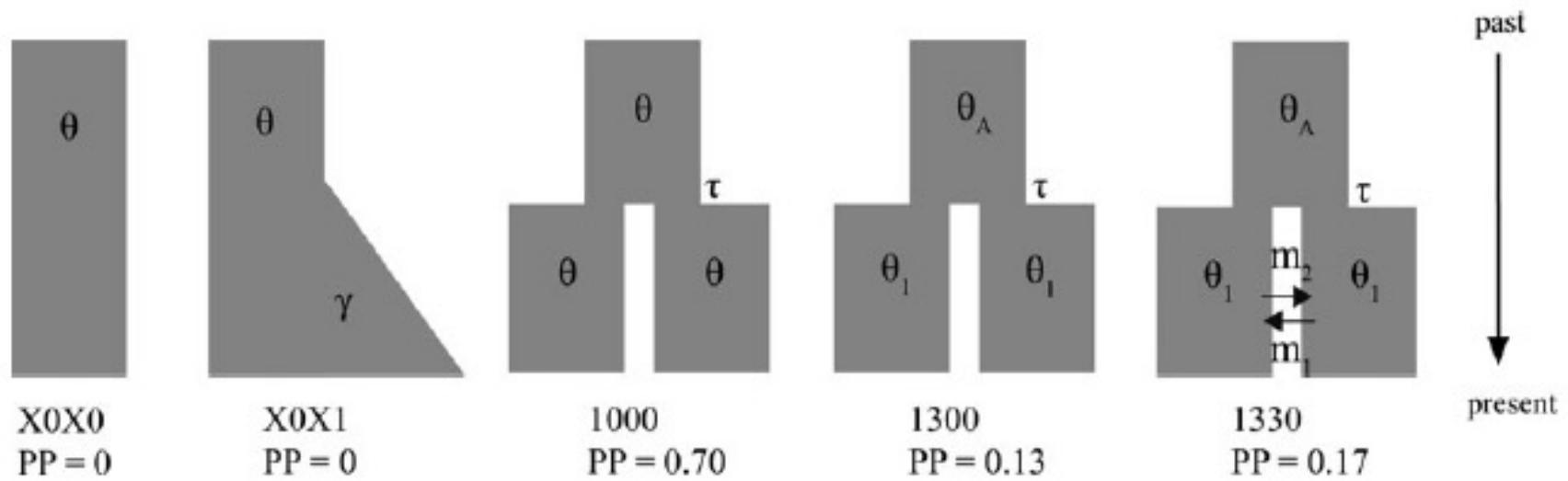
Inferred geographic coordinates of source of expansion, where the geographic coordinate is a parameter in the model (inferred using ABC; see [He et al. 2017. Inferring the geographic origin of a range expansion: latitudinal and longitudinal coordinates inferred from genomic data in an ABC framework with the program X-ORIGIN. Mol. Ecol. 26:6908-6920. DOI: 10.1111/mec.14380](#))



**Fig. 2.** Estimated expansion origins ( $\Omega$ ; red cross) in *C. cordiformis* (A) and *C. ovata* (B). The shading of pixels depicts a probability surface (kernel density) showing the likelihood that each pixel served as the expansion origin relative to the pixel with the highest likelihood (i.e.,  $\Omega$ ). Glaciated regions are shown in blue. The results presented in A and B are based on retention of four and three PC axes of variation in genetic summary statistics, respectively. Results based on retaining additional PC axes are presented in [SI Appendix, Figs. S2 and S3](#).

# Generic models in phylogeography

Tests of 142 objectively identified models (e.g., program like PHRAPL)



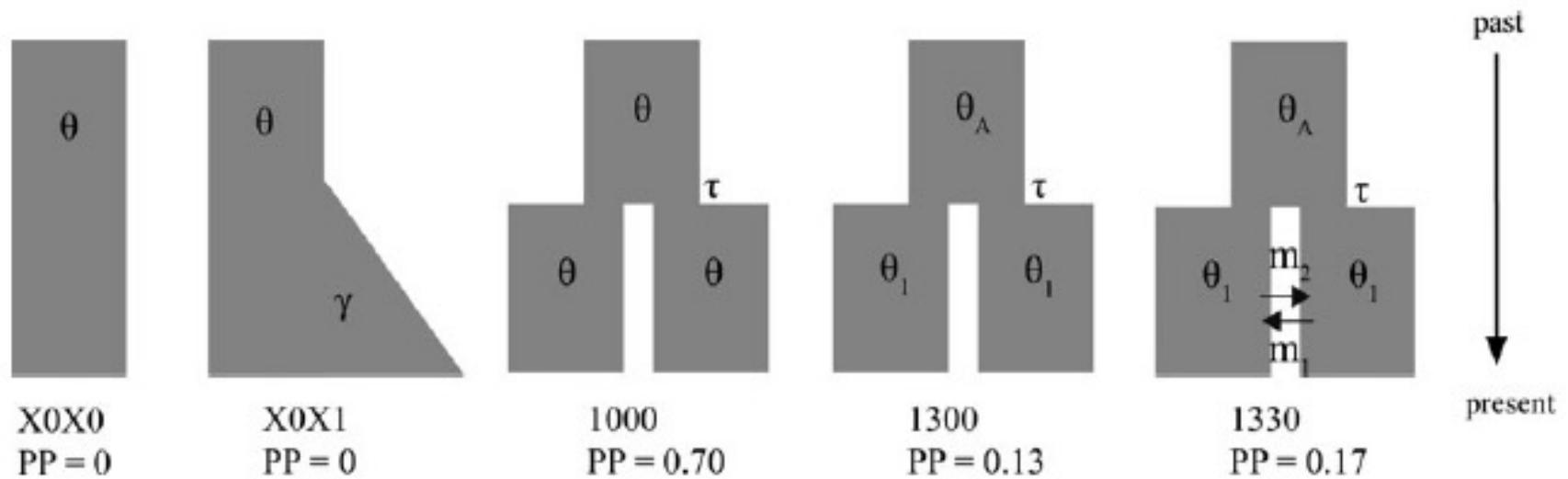
Pelletier & Carstens (2014 Mol. Ecol.)

- PHRAPL can create hundreds of possible histories that have a mixture of gene flow, population subdivision, and/or population size differences and compare these models using AIC (O'Meara)

# Model choice in phylogeography: generic versus informed

- generic models

Tests of 142 objectively identified models

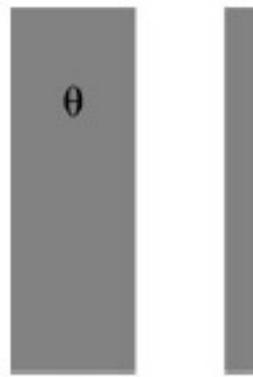


Pelletier & Carstens (2014 Mol. Ecol.)

Statistical procedures themselves may seem to provide a legitimacy to modeling approach – the advocacy of objective models in phylogeography

# Model choice

## Tests of 142 c



XOX0  
PP = 0

- generic models

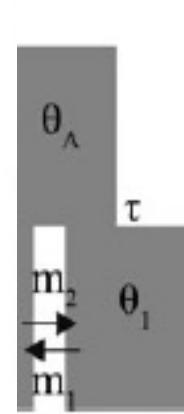
Table 3 List of all 143 models included in analyses. Model =  $\tau\theta\text{m}\gamma$

Model	Parameters	Mean	SD	Median	Posterior probability
1030	$\tau, \theta_A = \theta_1 = \theta_2, m_{12}, m_{21}$	0.792	1.124	0.000	0.024
1232	$\tau, \theta_A = \theta_2, \theta_1, m_{12}, m_{21}, \gamma_2$	0.822	0.856	0.772	0.007
1200	$\tau, \theta_A = \theta_2, \theta_1$	0.836	0.985	0.499	0.004
1222	$\tau, \theta_A = \theta_2, \theta_1, m_{21}, \gamma_2$	0.846	0.982	0.542	0.006
1220	$\tau, \theta_A = \theta_2, \theta_1, m_{21}$	0.849	0.957	0.647	0.006
1231	$\tau, \theta_A = \theta_2, \theta_1, m_{12}, m_{21}, \gamma_1$	0.863	0.877	0.859	0.006
1221	$\tau, \theta_A = \theta_2, \theta_1, m_{21}, \gamma_1$	0.870	0.878	0.862	0.011
1031	$\tau, \theta_A = \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_1$	0.886	1.133	0.000	0.020
1230	$\tau, \theta_A = \theta_2, \theta_1, m_{12}, m_{21}$	0.917	0.937	0.880	0.006
1033	$\tau, \theta_A = \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_1, \gamma_2$	0.923	1.170	0.000	0.018
0131	$\theta_A = \theta_1, \theta_2, m_{12}, m_{21}, \gamma_1$	0.930	1.024	0.779	0.007
0130	$\theta_A = \theta_1, \theta_2, m_{12}, m_{21}$	0.949	0.881	1.055	0.010
1023	$\tau, \theta_A = \theta_1 = \theta_2, m_{21}, \gamma_1, \gamma_2$	0.956	1.154	0.000	0.024
1201	$\tau, \theta_A = \theta_2, \theta_1, \gamma_1$	0.975	1.026	0.866	0.006
0030	$\theta_A = \theta_1 = \theta_2, m_{12}, m_{21}$	0.977	1.210	0.000	0.024
1211	$\tau, \theta_A = \theta_2, \theta_1, m_{12}, \gamma_1$	0.990	1.042	0.927	0.007
0020	$\theta_A = \theta_1 = \theta_2, m_{12}, m_{21}$	0.991	1.264	0.000	0.017
1132	$\tau, \theta_A = \theta_1, \theta_2, m_{12}, m_{21}, \gamma_2$	0.995	0.981	0.986	0.007
0031	$\theta_A = \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_1$	0.996	1.303	0.000	0.020
0022	$\theta_A = \theta_1 = \theta_2, m_{21}, \gamma_2$	1.003	1.241	0.000	0.025
1131	$\tau, \theta_A = \theta_1, \theta_2, m_{12}, m_{21}, \gamma_1$	1.011	0.967	1.013	0.004
1032	$\tau, \theta_A = \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_2$	1.013	1.212	0.000	0.031
1212	$\tau, \theta_A = \theta_2, \theta_1, m_{12}, \gamma_2$	1.015	0.986	1.083	0.003
1233	$\tau, \theta_A = \theta_2, \theta_1, m_{12}, m_{21}, \gamma_1, \gamma_2$	1.021	0.946	1.121	0.010
1203	$\tau, \theta_A = \theta_2, \theta_1, \gamma_1, \gamma_2$	1.024	1.058	1.002	0.010
0233	$\theta_A = \theta_2, \theta_1, m_{12}, m_{21}, \gamma_1, \gamma_2$	1.026	0.985	1.118	0.004
1110	$\tau, \theta_A = \theta_1, \theta_2, m_{21}, \gamma_1$	1.030	1.003	1.118	0.007
0222	$\theta_A = \theta_2, \theta_1, m_{21}, \gamma_2$	1.031	1.112	0.921	0.008
1130	$\tau, \theta_A = \theta_1, \theta_2, m_{12}, m_{21}$	1.031	0.976	1.084	0.006
0112	$\theta_A = \theta_1, \theta_2, m_{12}, \gamma_2$	1.032	0.991	1.121	0.007
0032	$\theta_A = \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_2$	1.033	1.212	0.000	0.020
0110	$\theta_A = \theta_1, \theta_2, m_{12}, \gamma_1$	1.034	1.031	1.070	0.004
1020	$\tau, \theta_A = \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_1, \gamma_2$	1.035	1.196	0.000	0.015
0012	$\theta_A = \theta_1 = \theta_2, m_{12}, \gamma_2$	1.038	1.272	0.000	0.018
1213	$\tau, \theta_A = \theta_2 = \theta_1, m_{12}, \gamma_1, \gamma_2$	1.041	1.053	1.121	0.003
0220	$\theta_A = \theta_2, \theta_1, m_{21}$	1.041	0.965	1.121	0.010
1013	$\tau, \theta_A = \theta_1 = \theta_2, m_{12}, \gamma_1, \gamma_2$	1.042	1.227	0.543	0.024
0231	$\theta_A = \theta_2, \theta_1, m_{12}, m_{21}, \gamma_1$	1.048	1.104	0.997	0.007
1111	$\tau, \theta_A = \theta_1, \theta_2, m_{12}, \gamma_1$	1.050	1.027	1.098	0.013
0013	$\theta_A = \theta_1 = \theta_2, m_{12}, \gamma_1, \gamma_2$	1.056	1.254	0.000	0.021
0133	$\theta_A = \theta_1, \theta_2, m_{12}, m_{21}, \gamma_1, \gamma_2$	1.057	1.107	1.028	0.001
0033	$\theta_A = \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_1, \gamma_2$	1.059	1.289	0.000	0.031
1002	$\tau, \theta_A = \theta_1 = \theta_2, \gamma_2$	1.084	1.261	0.000	0.008
1331	$\tau, \theta_A, \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_1$	1.098	1.093	1.081	0.000
0132	$\theta_A = \theta_1, \theta_2, m_{12}, m_{21}, \gamma_2$	1.101	0.991	1.129	0.007
0210	$\theta_A = \theta_2, \theta_1, m_{12}$	1.102	1.111	1.040	0.001
1321	$\tau, \theta_A, \theta_1 = \theta_2, m_{21}, \gamma_1$	1.108	1.012	1.124	0.000
1123	$\tau, \theta_A = \theta_1, \theta_2, m_{21}, \gamma_1, \gamma_2$	1.118	1.094	1.121	0.003
1021	$\tau, \theta_A = \theta_1 = \theta_2, m_{21}, \gamma_1$	1.119	1.323	0.000	0.036
1113	$\tau, \theta_A = \theta_1, \theta_2, m_{12}, \gamma_1, \gamma_2$	1.132	1.042	1.129	0.003
1010	$\tau, \theta_A = \theta_1 = \theta_2, m_{12}$	1.135	1.284	0.558	0.013
1112	$\tau, \theta_A = \theta_1, \theta_2, m_{12}, \gamma_1$	1.135	0.943	1.137	0.006
1101	$\tau, \theta_A = \theta_1, \theta_2, \gamma_1$	1.136	1.048	1.129	0.006
1011	$\tau, \theta_A = \theta_1 = \theta_2, m_{12}, \gamma_1$	1.148	1.274	0.739	0.021
0023	$\theta_A = \theta_1 = \theta_2, m_{21}, \gamma_1, \gamma_2$	1.154	1.311	0.500	0.020

d

330  
P = 0.17

& Carstens (2014 Mol. Ecol.)



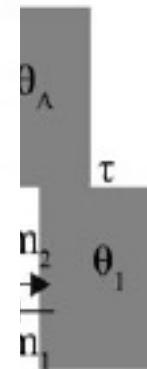
past  
present

# Model choice in

Table 3 *Continued*

Model	Parameters	Mean	SD	Median	Posterior probability
0230	$\theta_A = \theta_2, \theta_1, m_{12}, m_{21}$	1.172	1.022	1.135	0.003
0321	$\theta_A, \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_1$	1.173	1.106	1.129	0.003
1000*	$\tau, \theta_A = \theta_1 = \theta_2$	1.178	1.261	0.971	0.015
1202	$\tau, \theta_A = \theta_1 = \theta_2, \gamma_2$	1.180	1.163	1.124	0.004
0223	$\theta_A = \theta_2, \theta_1, m_{21}, \gamma_1, \gamma_2$	1.181	1.173	1.124	0.007
1001	$\tau, \theta_A = \theta_1 = \theta_2, \gamma_1$	1.187	1.328	0.752	0.021
0011	$\theta_A = \theta_1 = \theta_2, m_{12}, \gamma_1$	1.198	1.298	0.931	0.022
0213	$\theta_A = \theta_2, \theta_1, m_{12}, \gamma_1, \gamma_2$	1.199	1.117	1.135	0.004
1102	$\tau, \theta_A = \theta_1, \theta_2, \gamma_2$	1.205	1.217	1.129	0.004
1121	$\tau, \theta_A = \theta_1, \theta_2, m_{21}, \gamma_1$	1.211	1.141	1.137	0.010
1022	$\tau, \theta_A = \theta_1 = \theta_2, m_{21}, \gamma_2$	1.214	1.308	1.011	0.021
1012	$\tau, \theta_A = \theta_1 = \theta_2, m_{12}, \gamma_2$	1.270	1.324	1.129	0.021
1332	$\tau, \theta_A, \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_2$	1.271	1.159	1.179	0.003
1322	$\tau, \theta_A, \theta_1 = \theta_2, m_{21}, \gamma_2$	1.280	1.087	1.233	0.000
0212	$\theta_A = \theta_2, \theta_1, m_{12}, \gamma_2$	1.281	1.181	1.140	0.001
1312	$\tau, \theta_A, \theta_1 = \theta_2, m_{12}, \gamma_2$	1.286	1.105	1.221	0.001
1323	$\tau, \theta_A, \theta_1 = \theta_2, m_{21}, \gamma_1, \gamma_2$	1.312	1.075	1.239	0.001
0123	$\theta_A = \theta_1, \theta_2, m_{21}, \gamma_1, \gamma_2$	1.312	1.189	1.192	0.007
1003	$\tau, \theta_A = \theta_1 = \theta_2, \gamma_1, \gamma_2$	1.321	1.443	1.122	0.007
0313	$\theta_A, \theta_1 = \theta_2, m_{12}, \gamma_1, \gamma_2$	1.327	1.207	1.182	0.001
1433	$\tau, \theta_A, \theta_1, \theta_2, m_{12}, m_{21}, \gamma_1, \gamma_2$	1.327	0.998	1.269	0.000
0312	$\theta_A, \theta_1 = \theta_2, m_{12}, \gamma_2$	1.328	1.201	1.209	0.004
0211	$\theta_A = \theta_2, \theta_1, m_{12}, \gamma_1$	1.333	1.195	1.256	0.006
1320	$\tau, \theta_A, \theta_1 = \theta_2, m_{21}$	1.336	1.235	1.180	0.001
1403	$\tau, \theta_A, \theta_1, \theta_2, \gamma_1, \gamma_2$	1.350	1.011	1.298	0.000
1330*	$\tau, \theta_A, \theta_1 = \theta_2, m_{12}, m_{21}$	1.351	1.274	1.225	0.006
0323	$\theta_A, \theta_1 = \theta_2, m_{21}, \gamma_1, \gamma_2$	1.353	1.170	1.259	0.003
1333	$\tau, \theta_A, \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_1, \gamma_2$	1.357	1.127	1.277	0.003
1103	$\tau, \theta_A = \theta_1, \theta_2, \gamma_1, \gamma_2$	1.400	1.186	1.408	0.003
1423	$\tau, \theta_A, \theta_1, \theta_2, m_{21}, \gamma_1, \gamma_2$	1.408	1.502	1.182	0.001
0331	$\theta_A, \theta_1 = \theta_2, m_{21}, m_{12}, \gamma_1$	1.424	1.314	1.368	0.000
0311	$\theta_A, \theta_1 = \theta_2, m_{12}, \gamma_1$	1.475	1.353	1.353	0.003
1432	$\tau, \theta_A, \theta_1, \theta_2, m_{12}, m_{21}, \gamma_2$	1.500	1.297	1.360	0.000
1402	$\tau, \theta_A, \theta_1, \theta_2, \gamma_2$	1.543	1.101	1.545	0.003
0413	$\theta_A, \theta_1, \theta_2, m_{12}, \gamma_1, \gamma_2$	1.570	1.139	1.545	0.006
0412	$\theta_A, \theta_1, \theta_2, m_{12}, \gamma_2$	1.575	1.172	1.516	0.001
0322	$\theta_A, \theta_1 = \theta_2, m_{21}, \gamma_2$	1.591	1.493	1.481	0.001
1303	$\tau, \theta_A, \theta_1 = \theta_2, \gamma_1, \gamma_2$	1.591	1.303	1.610	0.003
1301	$\tau, \theta_A, \theta_1 = \theta_2, \gamma_1$	1.621	1.428	1.554	0.001
1300*	$\tau, \theta_A, \theta_1 = \theta_2$	1.630	1.342	1.562	0.004
1313	$\tau, \theta_A, \theta_1 = \theta_2, m_{12}, \gamma_1, \gamma_2$	1.676	3.419	1.164	0.007
0423	$\theta_A, \theta_1, \theta_2, m_{21}, \gamma_1, \gamma_2$	1.710	1.358	1.593	0.000
0430	$\theta_A, \theta_1, \theta_2, m_{12}, m_{21}$	1.715	1.294	1.620	0.000
0113	$\theta_A, \theta_1 = \theta_2, m_{12}, \gamma_1, \gamma_2$	1.715	5.727	1.068	0.004
0411	$\theta_A, \theta_1, \theta_2, m_{12}, \gamma_1$	1.717	1.259	1.665	0.003
0422	$\theta_A, \theta_1, \theta_2, m_{21}, \gamma_2$	1.759	1.417	1.614	0.000
1401	$\tau, \theta_A, \theta_1, \theta_2, \gamma_1$	1.781	1.835	1.505	0.001
0433	$\theta_A, \theta_1, \theta_2, m_{12}, m_{21}, \gamma_1, \gamma_2$	1.843	1.773	1.597	0.000
0021	$\theta_A = \theta_1 = \theta_2, m_{21}, \gamma_1$	1.867	4.813	0.673	0.014
0221	$\theta_A = \theta_2, \theta_1, m_{21}, \gamma_1$	1.934	6.915	0.937	0.006
1400	$\tau, \theta_A, \theta_1, \theta_2$	2.098	1.697	1.899	0.000
0232	$\theta_A = \theta_2, \theta_1, m_{12}, m_{21}, \gamma_2$	2.186	7.859	1.121	0.007
0122	$\theta_A = \theta_1, \theta_2, m_{21}, \gamma_2$	2.356	7.532	1.254	0.006
1122	$\tau, \theta_A = \theta_1, \theta_2, m_{21}, \gamma_2$	2.551	8.798	1.283	0.003
1133	$\tau, \theta_A = \theta_1, \theta_2, m_{12}, m_{21}, \gamma_1, \gamma_2$	2.748	12.927	0.814	0.008
1410	$\tau, \theta_A, \theta_1, \theta_2, m_{12}$	2.790	7.890	1.673	0.003

past



present

$$= 0.17$$

Carstens (2014 Mol. Ecol.)

- generic models

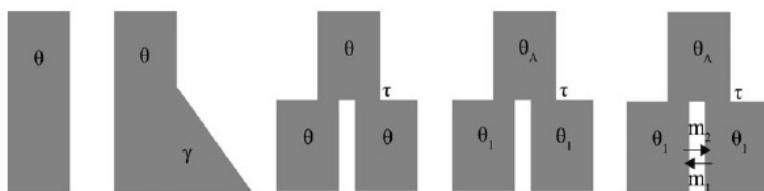
# Model choice in phylogeography: generic versus informed

Table 3 *Continued*

Model	Parameters	Mean	SD	Median	Posterior probability
1420	$\tau, \theta_A, \theta_1, \theta_2, m_{21}$	2.819	9.142	1.557	0.001
0330	$\theta_A, \theta_1 = \theta_2, m_{12}, m_{21}$	3.156	11.980	1.608	0.000
0431	$\theta_A, \theta_1, \theta_2, m_{12}, m_{21}, \gamma_1$	3.388	12.338	1.687	0.001
0432	$\theta_A, \theta_1, \theta_2, m_{12}, m_{21}, \gamma_2$	3.769	15.818	1.606	0.003
1210	$\tau, \theta_A = \theta_2, \theta_1, m_{12}$	4.007	21.699	0.880	0.010
0310	$\theta_A, \theta_1 = \theta_2, m_{12}$	4.405	20.648	1.670	0.001
0421	$\theta_A, \theta_1, \theta_2, m_{21}, \gamma_1$	4.761	18.586	1.563	0.000
1223	$\tau, \theta_A = \theta_2, \theta_1, m_{21}, \gamma_1, \gamma_2$	4.813	27.942	0.880	0.007
0410	$\theta_A, \theta_1, \theta_2, m_{12}$	4.840	19.483	1.684	0.000
0333	$\theta_A, \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_1, \gamma_2$	4.841	24.764	1.304	0.004
1411	$\tau, \theta_A, \theta_1, \theta_2, m_{12}, \gamma_1$	4.949	22.725	1.182	0.000
0320	$\theta_A, \theta_1 = \theta_2, m_{21}$	5.184	25.275	1.771	0.000
1431	$\tau, \theta_A, \theta_1, \theta_2, m_{12}, m_{21}, \gamma_1$	5.539	28.987	1.440	0.000
1421	$\tau, \theta_A, \theta_1, \theta_2, m_{21}, \gamma_1$	5.618	22.805	1.418	0.001
1311	$\tau, \theta_A, \theta_1 = \theta_2, m_{12}, \gamma_1$	5.721	32.177	1.137	0.001
0111	$\theta_A = \theta_1, \theta_2, m_{12}, \gamma_1$	5.804	32.950	1.143	0.008
0420	$\theta_A, \theta_1, \theta_2, m_{21}$	6.037	28.946	1.629	0.001
1412	$\tau, \theta_A, \theta_1, \theta_2, m_{12}, \gamma_2$	6.186	23.177	1.611	0.003
0010	$\theta_A = \theta_1 = \theta_2, m_{12}$	6.223	36.293	0.000	0.017
1413	$\tau, \theta_A, \theta_1, \theta_2, m_{12}, \gamma_1, \gamma_2$	8.209	48.083	1.344	0.000
1430	$\tau, \theta_A, \theta_1, \theta_2, m_{12}, m_{21}$	8.661	50.499	1.516	0.001
1422	$\tau, \theta_A, \theta_1, \theta_2, m_{21}, \gamma_2$	9.269	45.089	1.344	0.006
0121	$\theta_A = \theta_1, \theta_2, m_{21}, \gamma_1$	9.369	56.607	1.327	0.004
1302	$\tau, \theta_A, \theta_1 = \theta_2, \gamma_2$	9.386	44.243	1.233	0.004
0120	$\theta_A = \theta_1, \theta_2, m_{21}$	9.466	57.924	1.189	0.004
1310	$\tau, \theta_A, \theta_1 = \theta_2, m_{12}$	9.812	60.333	1.206	0.000
1100	$\tau, \theta_A = \theta_1, \theta_2$	10.795	68.438	1.121	0.007
0332	$\theta_A, \theta_1 = \theta_2, m_{12}, m_{21}, \gamma_2$	13.053	82.999	1.415	0.004
1120	$\tau, \theta_A = \theta_1, \theta_2, m_{21}$	14.667	54.818	1.365	0.007
X0X1*	$\theta_A, \gamma_1$	16.013	5.576	15.576	0.000
X0X0*	$\theta_A$	17.048	7.013	16.115	0.000
0000	$\theta_A = \theta_1 = \theta_2$				

For each model:  $\tau\theta m\gamma$

The answer is model 1023!



Divergence time ( $\tau$ )	Theta ( $\theta$ )	Migration (m)	Population expansion ( $\gamma$ )
0: island model	0: $\theta_A = \theta_1 = \theta_2$	0: no migration	0: no expansion
1: divergence at time ( $\tau$ )	1: $\theta_A = \theta_1, \theta_2$	1: $m_{12}$	1: $\gamma_1$
X: panmixia	2: $\theta_A = \theta_2, \theta_1$ 3: $\theta_A, \theta_1 = \theta_2$ 4: $\theta_A, \theta_1, \theta_2$	2: $m_{21}$ 3: $m_{12}, m_{21}$	2: $\gamma_2$ 3: $\gamma_1, \gamma_2$
Prior: 0.001–5 (4N generations)	Prior: 0.01–10 per locus	X: na/panmixia	Prior: 0.1–9 (exponential)
		Prior: 0–5 migrants per generation	

Biological insights depend on the questions we (the scientist) ask!

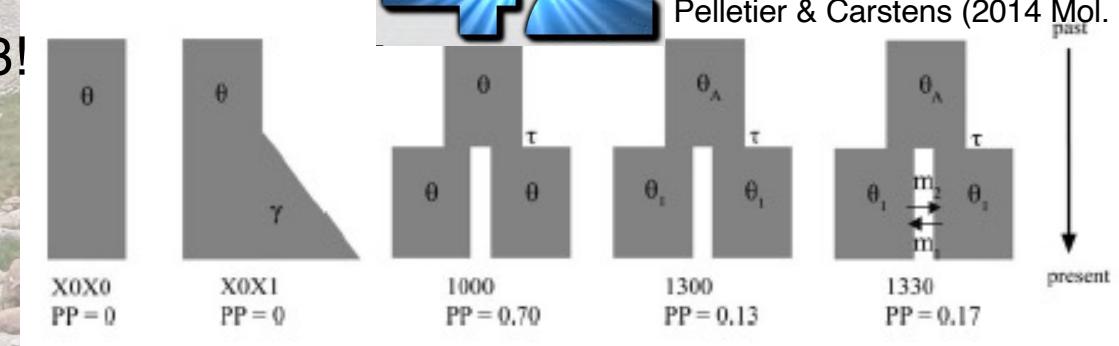
- Should we expect (or want) or computer programs to define the questions we ask!?!

The answer is:

42

Pelletier & Carstens (2014 Mol. Ecol.)

The answer is model 1023!



- Model formulation is a way of communicating our expert knowledge to statistical apparatus to test hypotheses



## Model-based approach: Forecasting spatial patterns of diversity in poorly explored, highly threatened ecosystems

Model-based approach:  
Directly model historical processes through a combination of ecological-niche models under paleoclimates and genetic analyses, discovered a central region in the Brazilian Atlantic forest that served as a biodiversity refuge during climatic extremes.

*H. semilineatus*

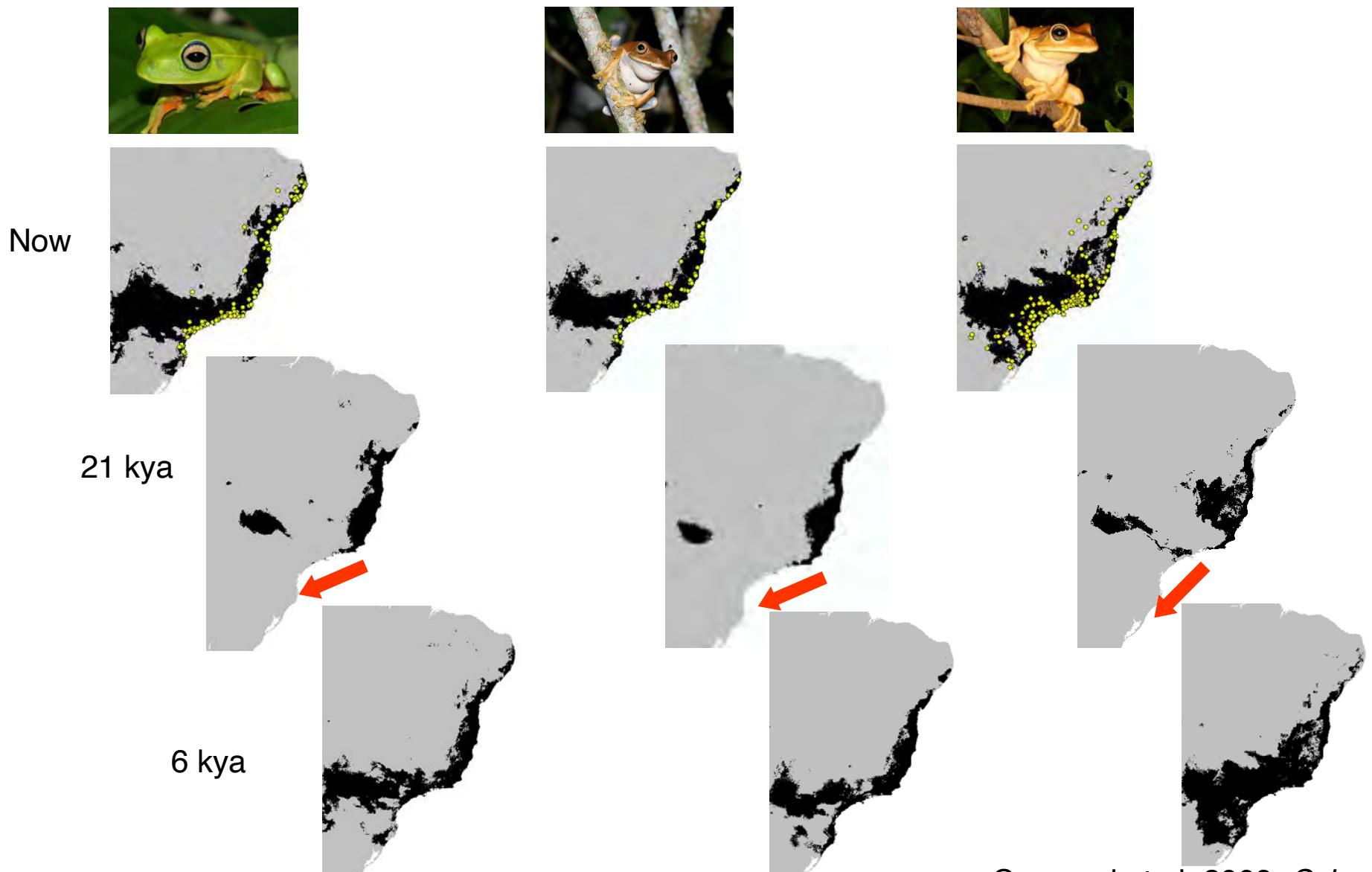


*H. faber*



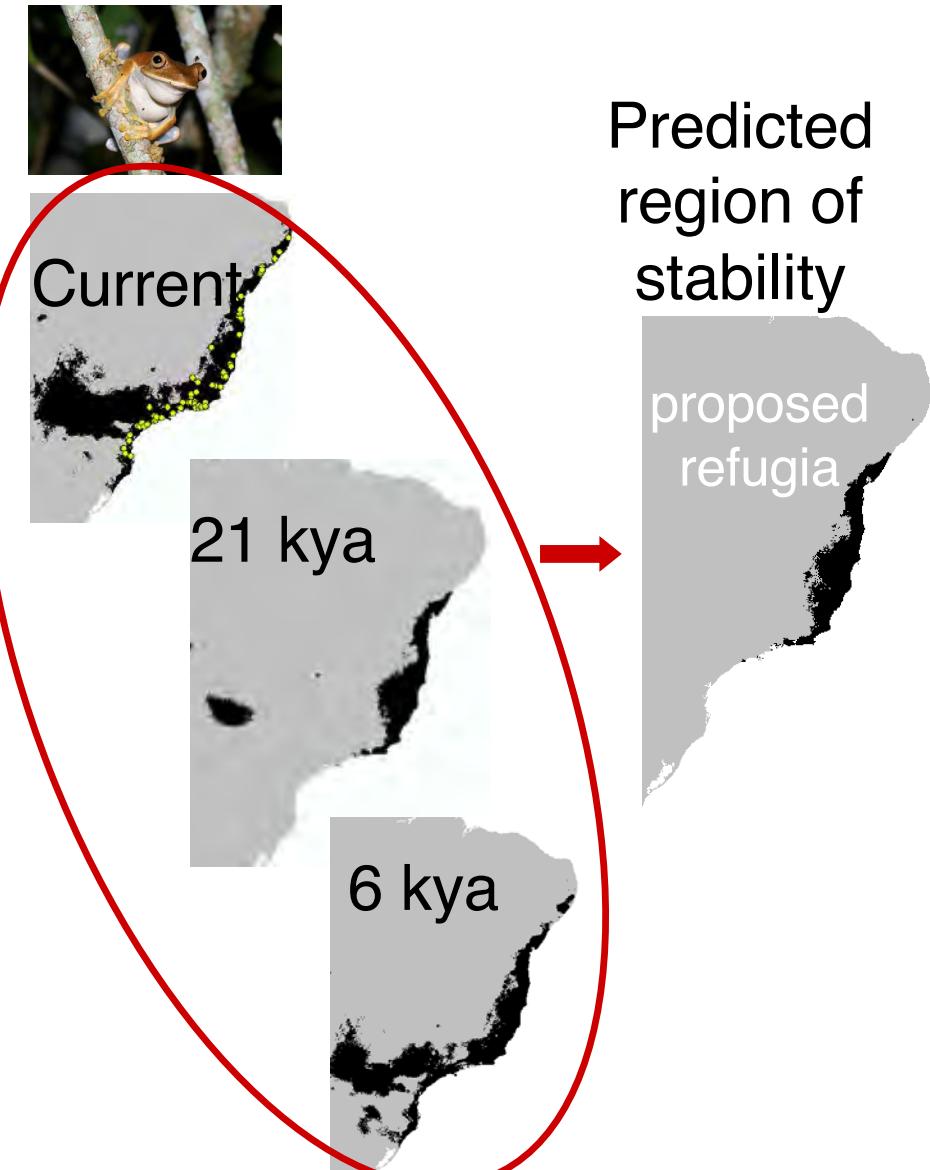
Carnaval et al. 2009. *Science*

## Model species distributions under current conditions and climatic extremes (based on climatic niches with MAXENT)



Carnaval et al. 2009. *Science*

## Model species distributions under current conditions and climatic extremes (based on climatic niches with MAXENT)



Maps of stable and unstable areas raise specific hypotheses about regional differences in persistence and hence diversity, which lead to phylogeographic predictions that can be tested with molecular data

## Different demographic scenarios motivated by stable/unstable areas:

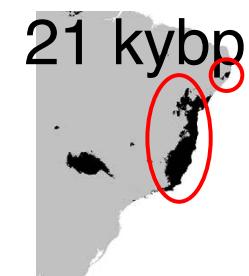


Results support community responses for both models using hierarchical Approximate Bayesian Computation:

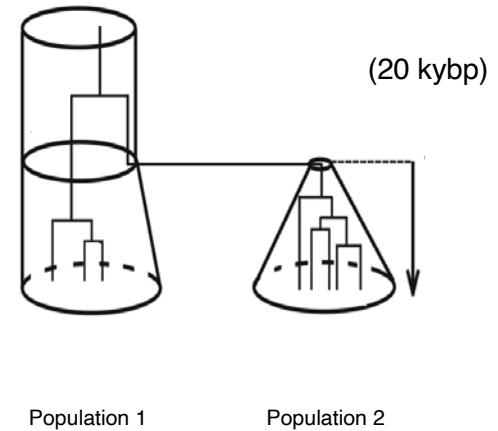
(i) simultaneous, multi-species colonization of unstable areas from adjacent refugial populations since the LGM



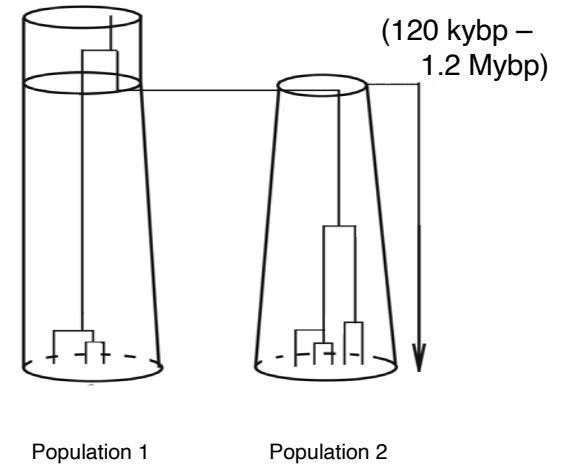
(ii) assemblage-scale, long-term persistence of populations in isolated refugial areas (i.e., temporally stable regions)



recent colonization



long-term persistence

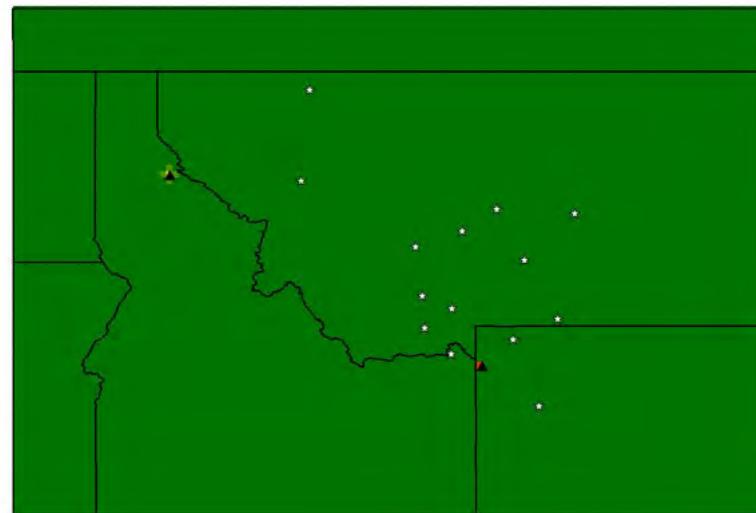
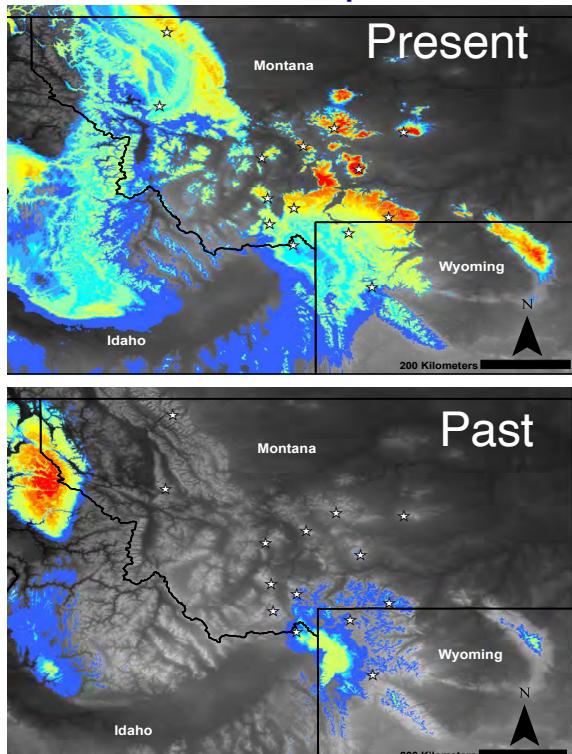


\* All models are simplifications, but they vary in their relative degree of abstraction

Different ways to model population expansion:

- (i) Model as population size change with no spatial aspect of expansion  
(e.g., Brazilian Atlantic forest areas of instability associated with recent expansion)
- (ii) Model expansion process across landscape explicitly

ENM based on paleoclimatic data 6kya



# iDDC: Generate species-specific expectations for patterns of genetic variation

He, Edwards & Knowles, Evolution 2013

## integrative Distributional Demographic Coalescent modeling

Distributional model  
(i.e., ecological niche model) with  
predictions on probability of occurrence  
across the landscape



Demographic model  
informed by habitat  
suitabilities



Spatially-explicit coalescent  
simulations based on  
demographic model



Tests of hypotheses/models  
using ABC

Habitat suitability  
scores

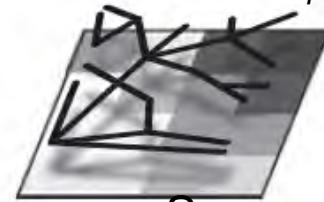
40	20	10	5
100	60	20	10
100	100	40	40
80	80	60	60

$K(m)$

400	200	100	50
(40)	(20)	(10)	(5)
1000	600	200	100
(100)	(60)	(20)	(10)
1000	1000	400	400
(100)	(100)	(40)	(40)
800	800	600	600
(80)	(80)	(60)	(60)

Carrying capacity:  $k_i$

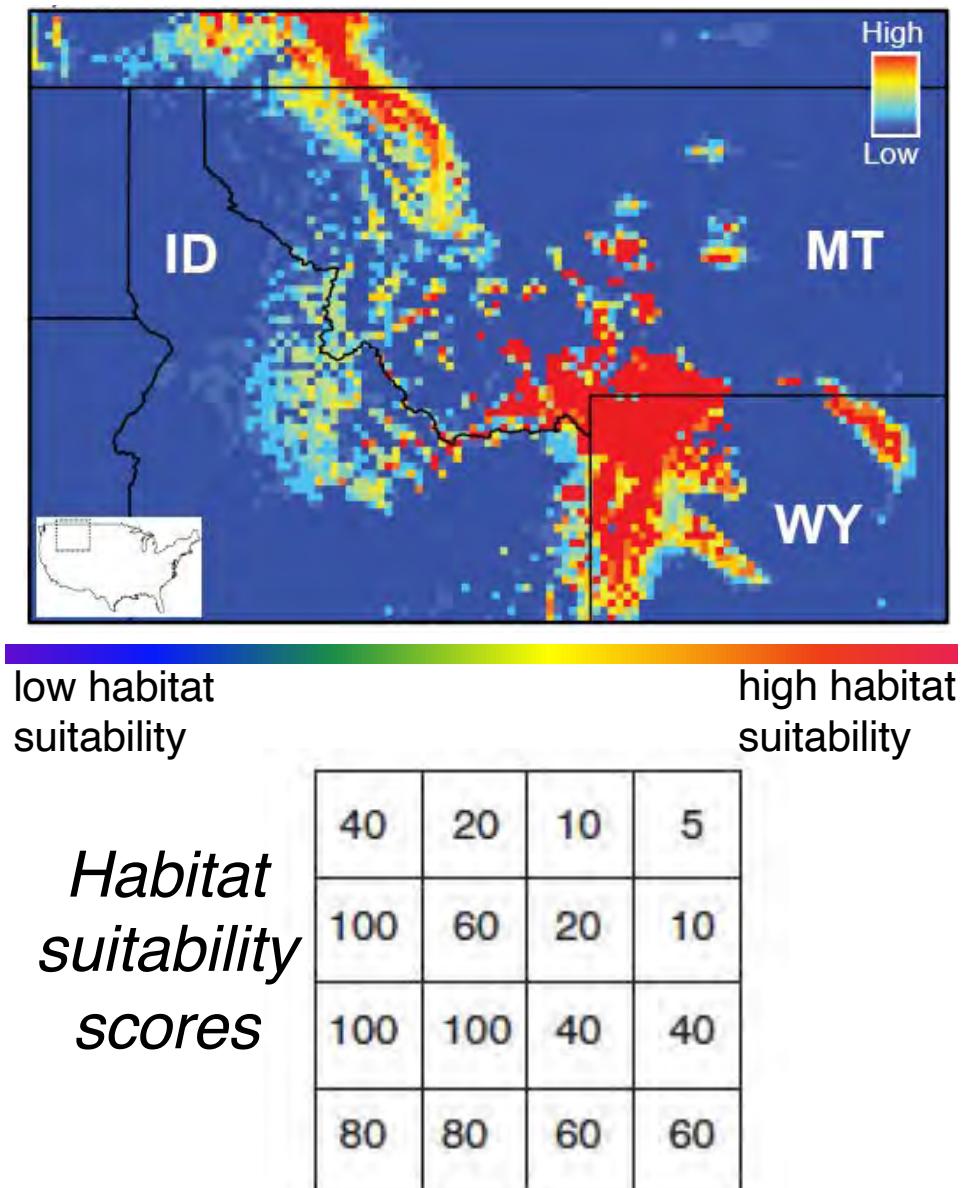
Gene coalescence  
across the landscape



SPLATCHE2

# iDDC: integrative Distributional, Demographic, Coalescent modeling

SPECIES-SPECIFIC  
Spatially explicit  
quantitative information  
about probabilities of  
occurrence based on  
habitat suitability



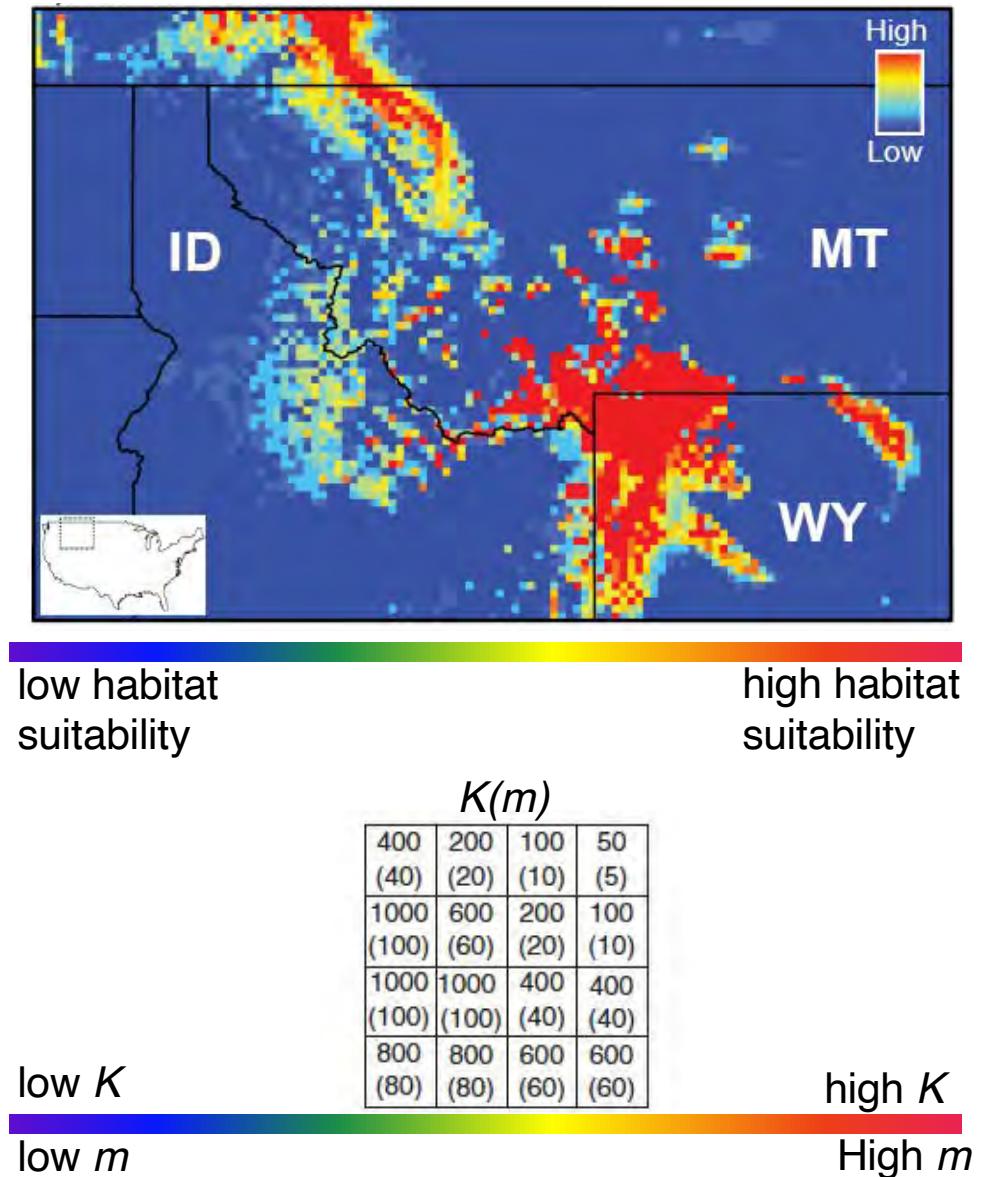
# iDDC: integrative Distributional, Demographic, Coalescent modeling

Spatially explicit  
probabilities of occurrence based on  
habitat suitability



## SPECIES-SPECIFIC Spatially explicit demographic model

- carrying capacity:  $k$
- migration rate:  $m$
- logistic growth rate:  $r$



## e.g.: SPECIES-SPECIFIC Demographic model:

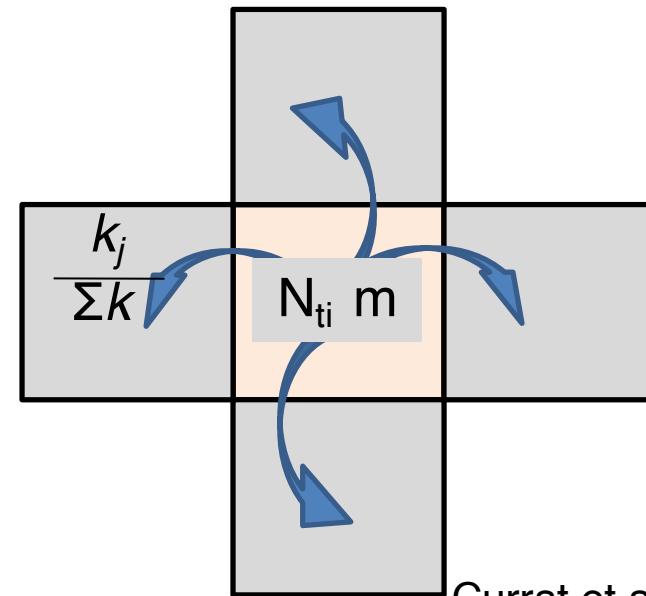
At each generation:

- the population density,  $N_{ti}$ , of each deme is logically regulated
- followed by a migration step
- the population densities and number of immigrants ( $N_{ti}$  and  $m$ ) are stored and used during the genetic simulations**

- carrying capacity:  $k_i$

- # of **emigrants** leaving deme i:  $N_{ti} m$

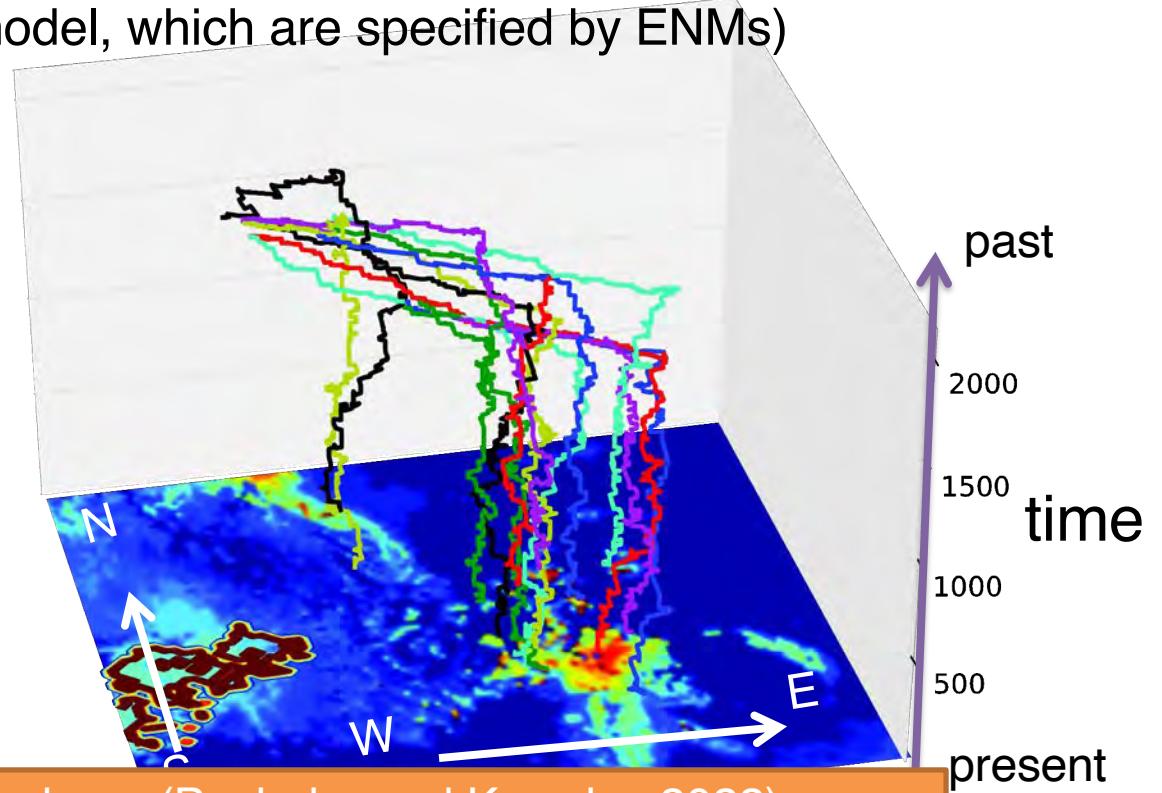
- # of **immigrants** entering deme j:  $\frac{k_j}{\sum k}$



Currat et al. 2004

# iDDC: integrative Distributional, Demographic, Coalescent modeling

- spatially-explicit genealogies to generate genetic patterns
  - at each generation (looking backwards in time), genes have probability of:
    - (i) staying in the same deme,
    - (ii) move to a different deme, or
    - (iii) coalesce with another gene lineage  
(depending upon the population densities and migration rates from the demographic model, which are specified by ENMs)

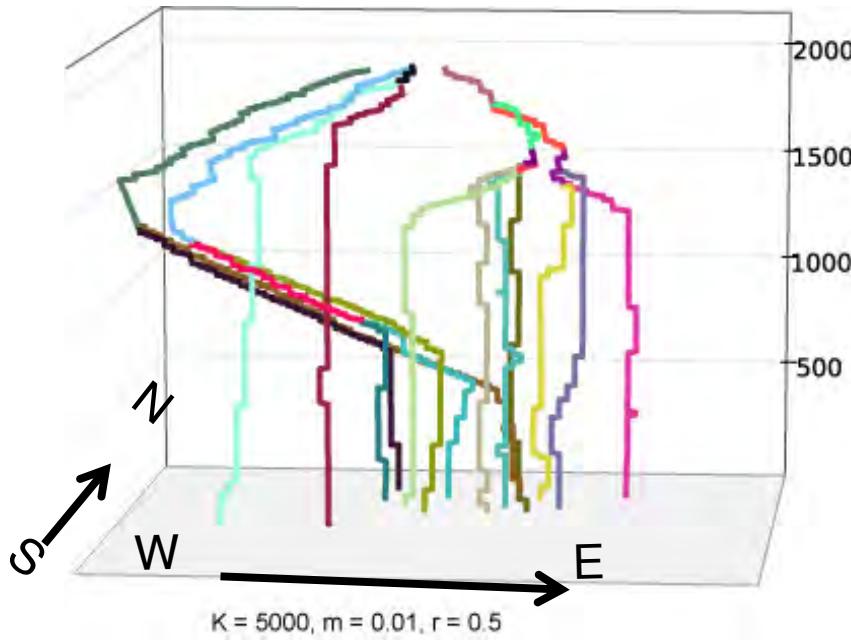


Suggested software: Quesztal package (Becheler and Knowles 2022)

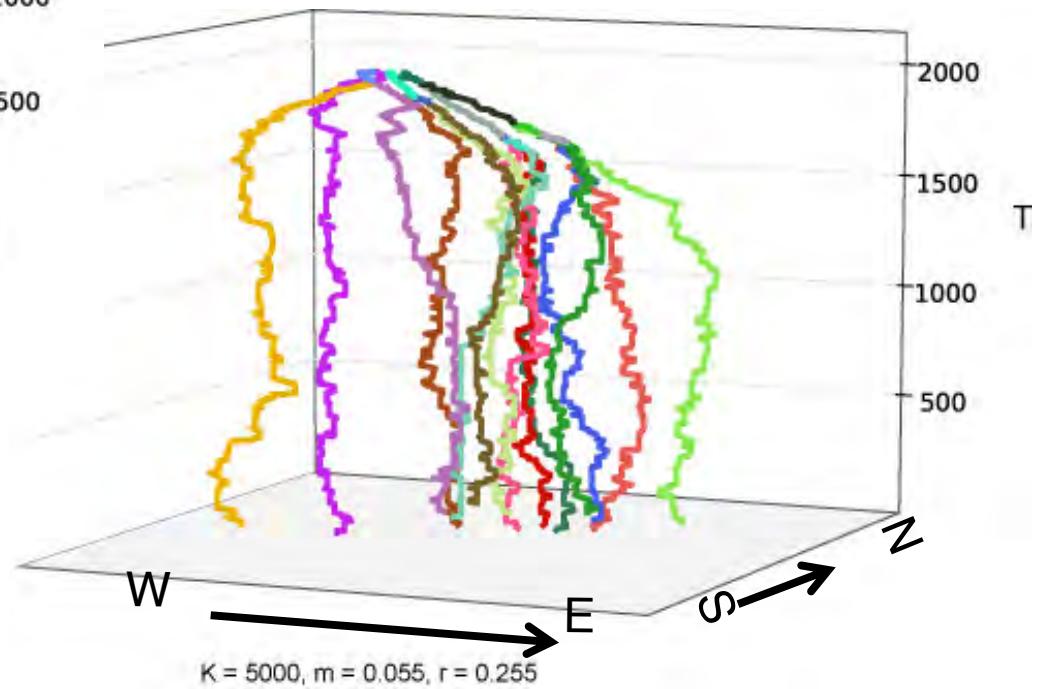
Under different demographic parameters (e.g., different  $k$  and  $m$ ), same set of sampled populations would have different coalescent histories

because of different probabilities to:

- (i) stay in the same deme,
- (ii) move to a different deme, or
- (iii) coalesce with another gene lineage



- (iv) Mutations accumulate along the branches of the genealogy according to a Poisson process with rate  $\mu t$

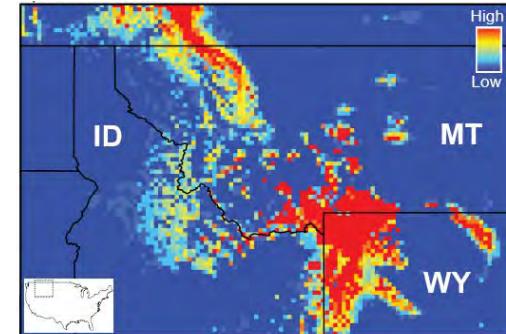


- Simulate predicted patterns of genetic variation for set of parameters under the model

Species-distribution model (SDM) generates predictions on probability of occurrence across the landscape



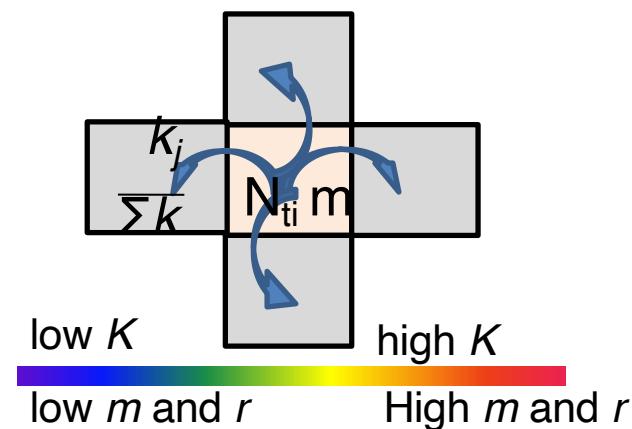
Spatially explicit demographic model (localized population densities, migration and growth rates)



Habitat suitability scores

40	20	10	5
100	60	20	10
100	100	40	40
80	80	60	60

low habitat suitability      high habitat suitability

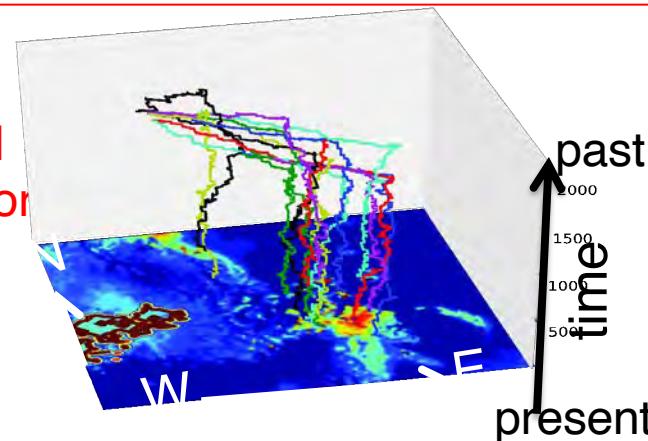


$K(m)$

400 (40)	200 (20)	100 (10)	50 (5)
1000 (100)	600 (60)	200 (20)	100 (10)
1000 (100)	1000 (100)	400 (40)	400 (40)
800 (80)	800 (80)	600 (60)	600 (60)

Carrying capacity:  $k_i$

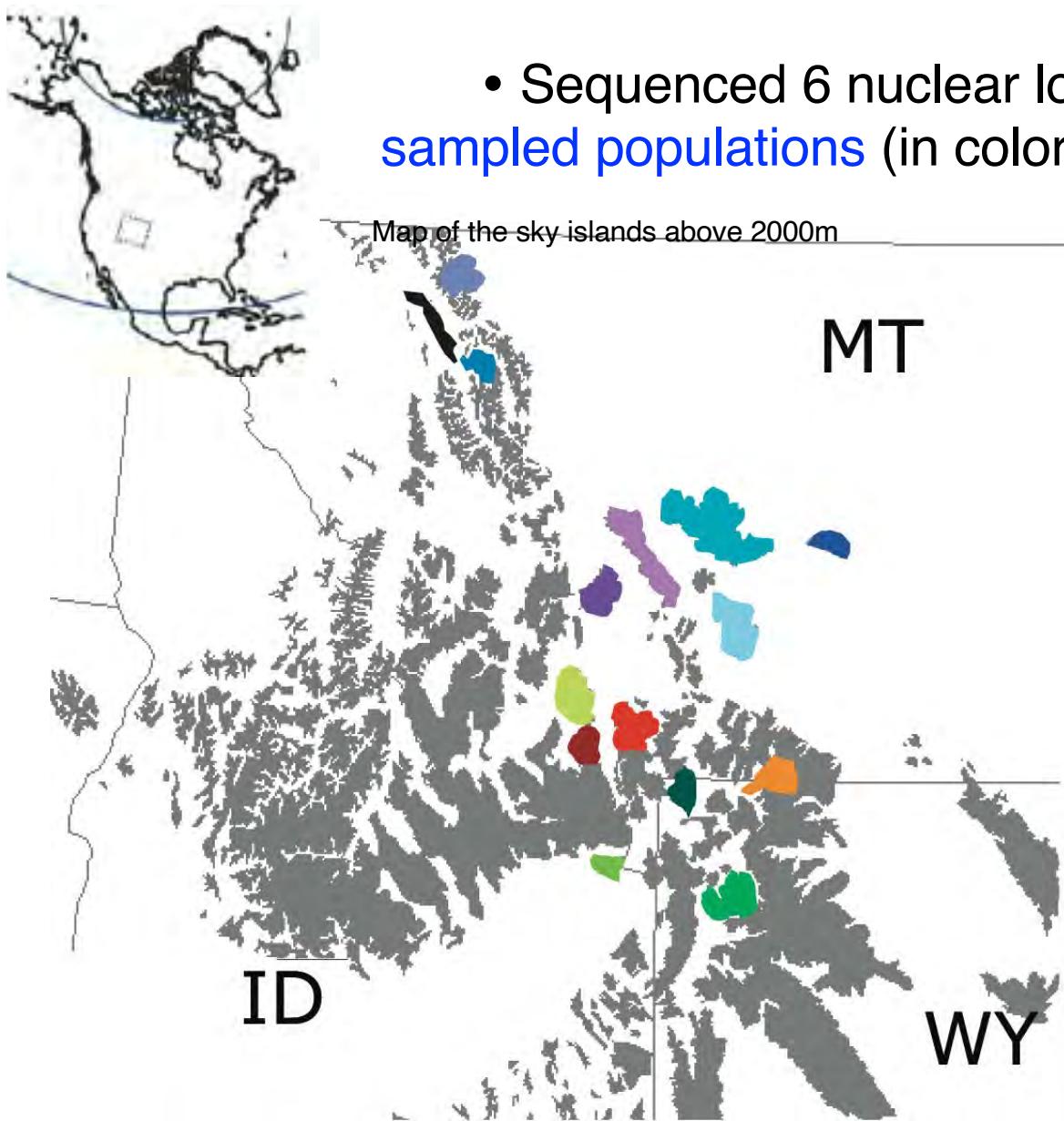
Spatially explicit coalescent model to generate predicted patterns of genetic variation for the empirically sampled population localities



Gene coalescence across the landscape

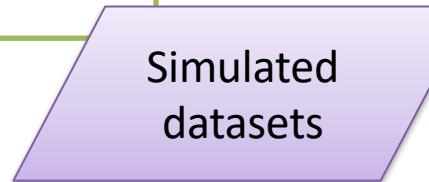


- Sequenced 6 nuclear loci in 114 individuals of **sampled populations** (in color) across the species range



## iDDC : Model Selection & Parameter Estimation using Approximate Bayesian Computation (ABC)

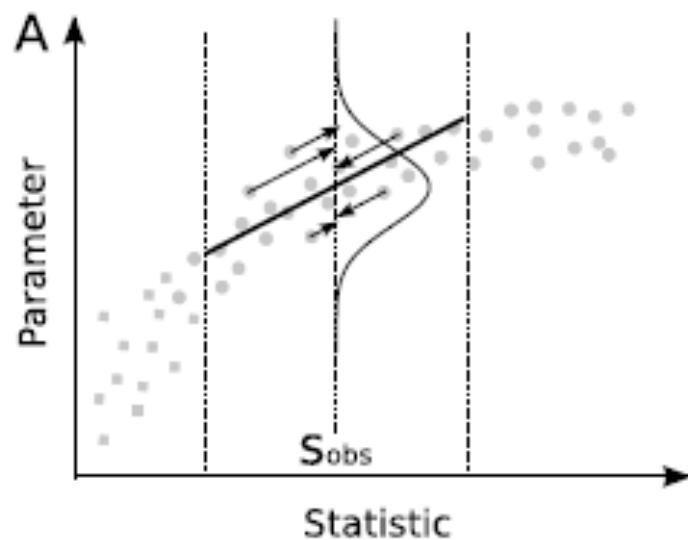
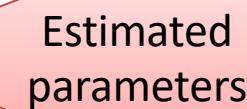
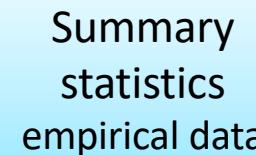
Model



See Beaumont et al. 2002

Advantages:

- computational efficiency compared to ML methods
- allow for complex models



We can identify sets of parameters for specific models that produce simulated data that matches the empirical data.

Suggested software: abctoolbox (Wegmann et al. 2010)

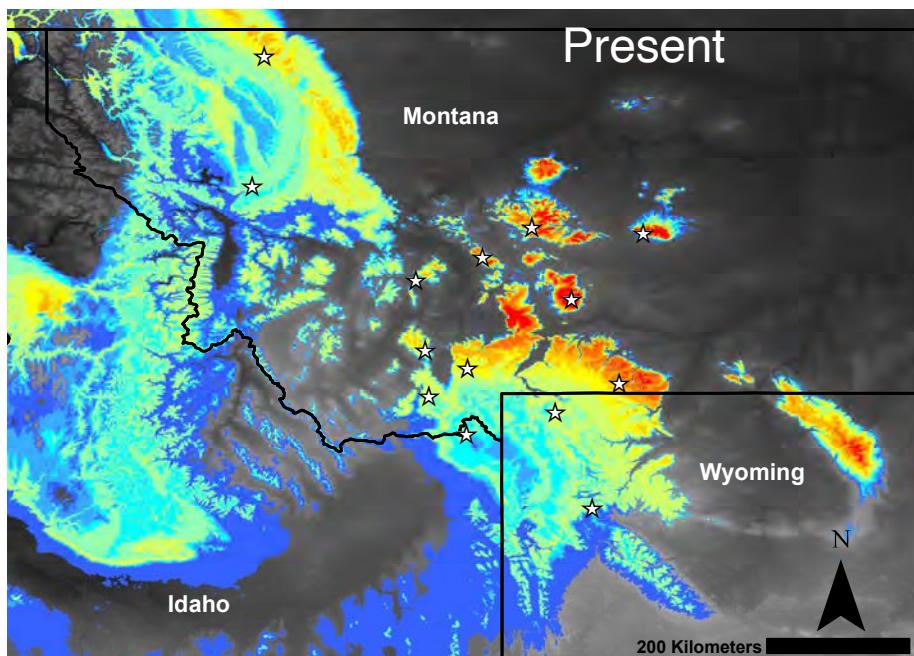
## What geographic configuration of sky island populations promotes species divergence?

Population connectivity determined by contemporary sky island distribution

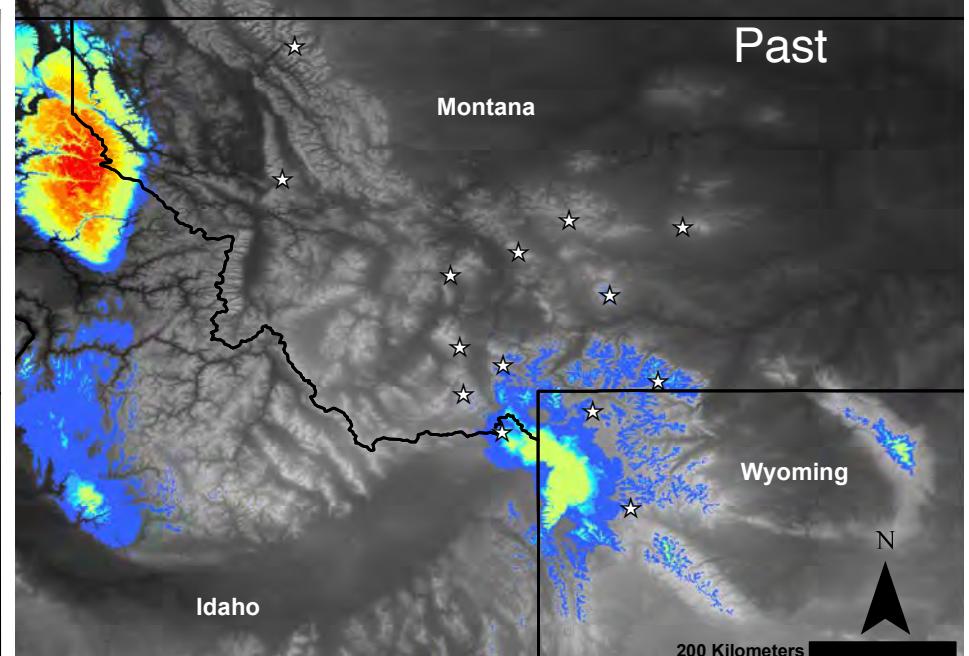
versus

Colonization of present sky island distribution from glacial refugia

19 bioclimatic variables used in modeling distributions



ENM based on current environmental data

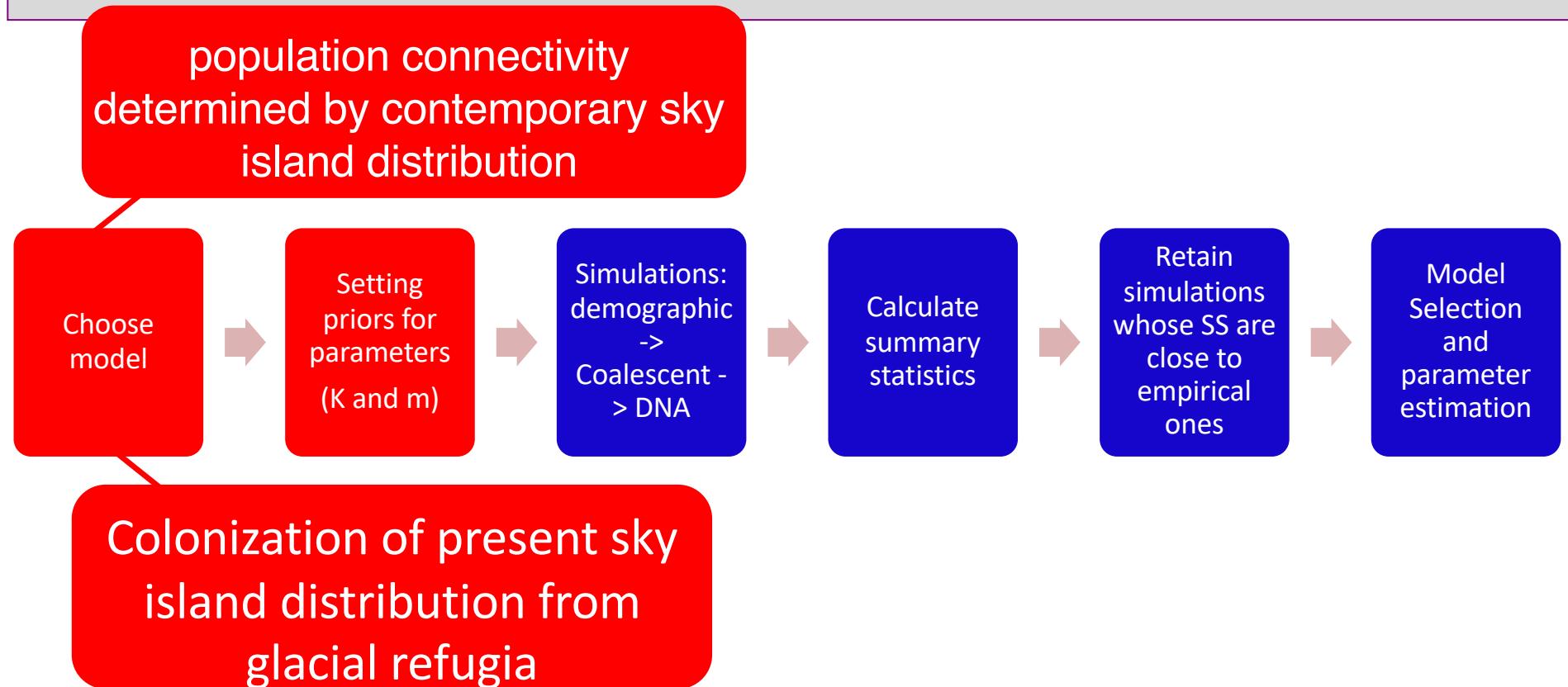


ENM based on paleoclimatic data 6kya



- grasshoppers are flightless habitat specialists restricted to montane meadows

# iDDC tests of drivers of divergence



6 anonymous nuclear loci from 114 individuals sampled across the range of *M. oregonensis*



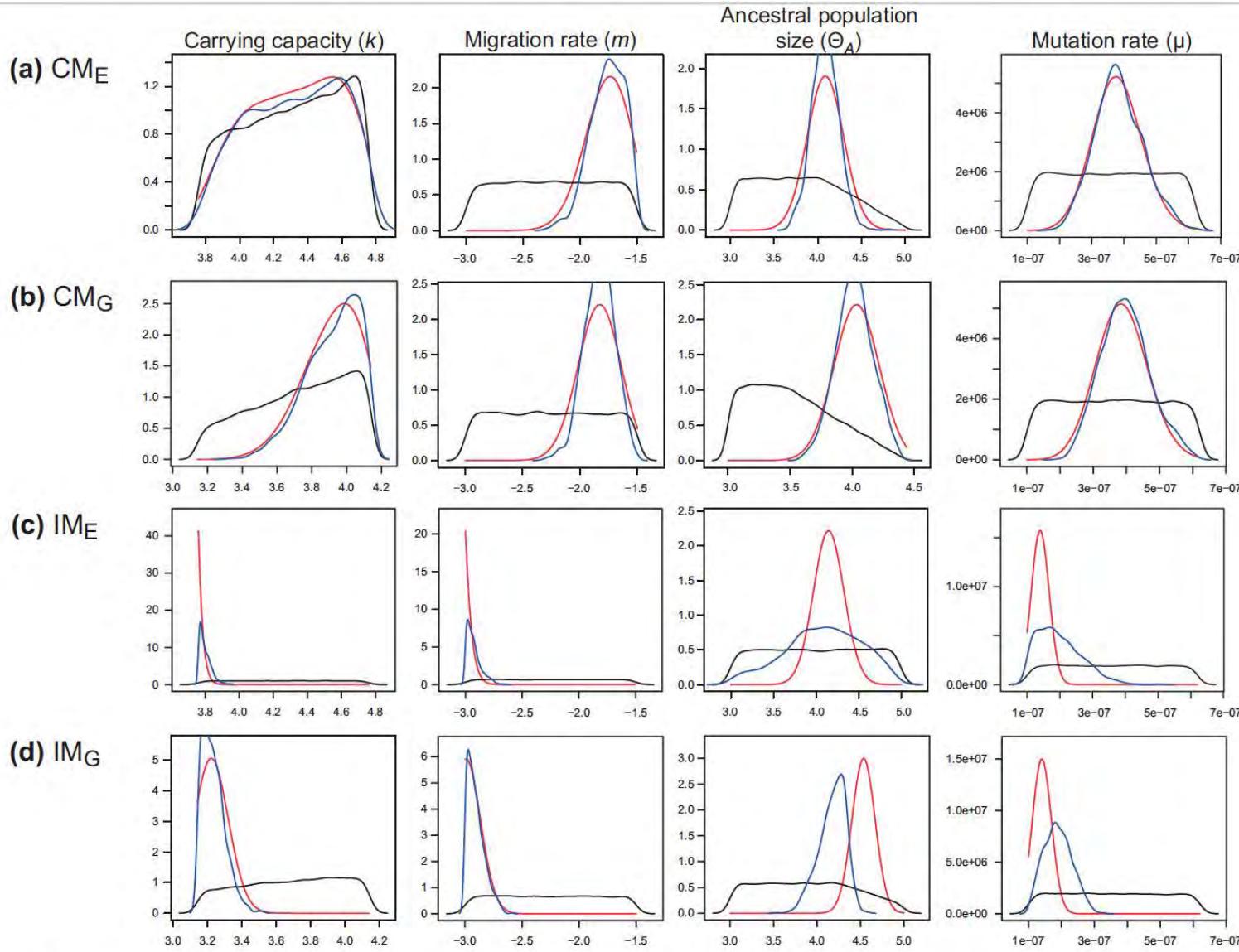
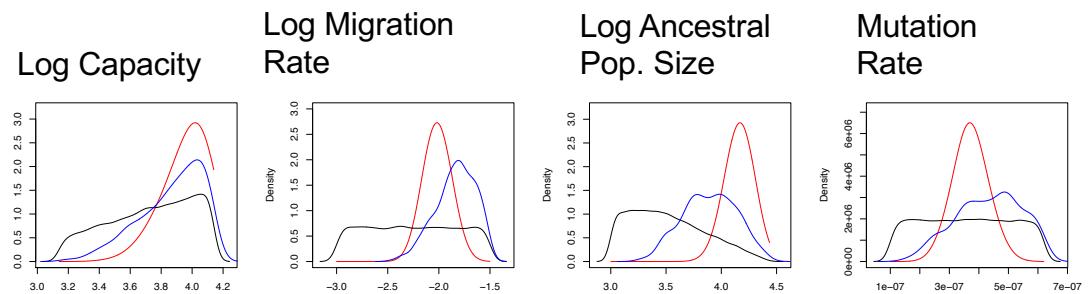
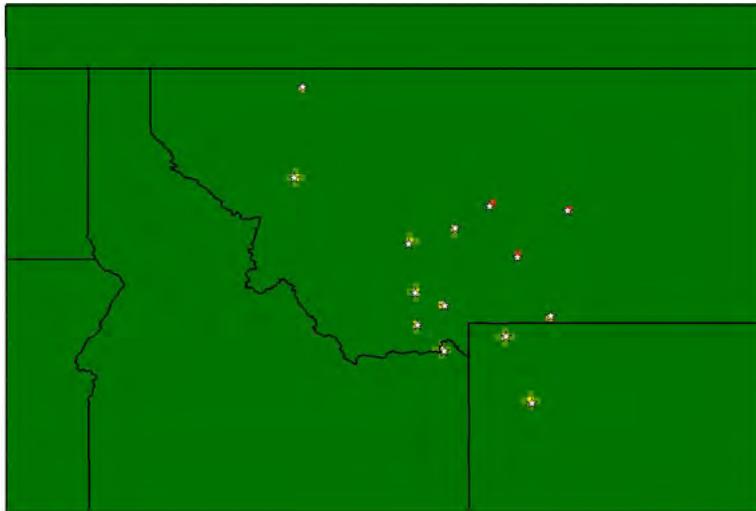
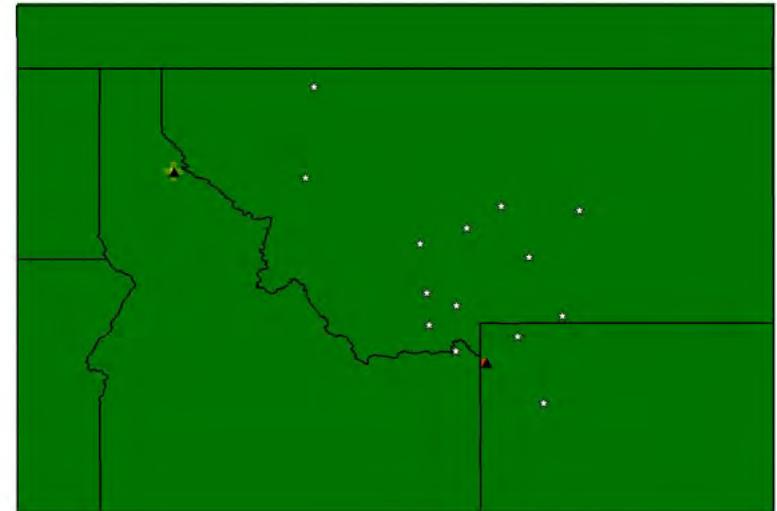


Figure 4. Posterior distribution (red line) of parameter estimates (i.e. carrying capacity,  $k$ , migration rate,  $m$ , ancestral population size  $\Theta_A$ , and mutation rate,  $\mu$ ) for each of the two colonization models, (a)  $\text{CM}_E$  and (b)  $\text{CM}_G$ , and the two sky island isolation models, (c)  $\text{IM}_E$  and (d)  $\text{IM}_G$ , where the subscripts E and G refer to connectivity patterns determined by either environmental heterogeneity or geographic distance, respectively. Results are based on a GLM regression adjustment of the 5000 closet simulations to each model. The distribution of the retained simulations (blue line) and the prior (black line) demonstrate the improvement that the GLM procedure had on parameter estimates and that the data contained information relevant to estimating the parameters.



Model tests based on comparing marginal likelihoods:

(i) population connectivity determined by contemporary sky island distribution



Patterns of genetic variation reflect:

(ii) genetic data predicted by a colonization history from glacial refugia to present sky island distribution

Knowles LL, Massatti R (2017) Distributional shifts – not geographic isolation – as a probable driver of montane species divergence. *Ecography* 40:1475-1485.



# Explicit modeling of movement across landscape in phylogeography models



*D. ornatus*



*Luc. alboguttatum*



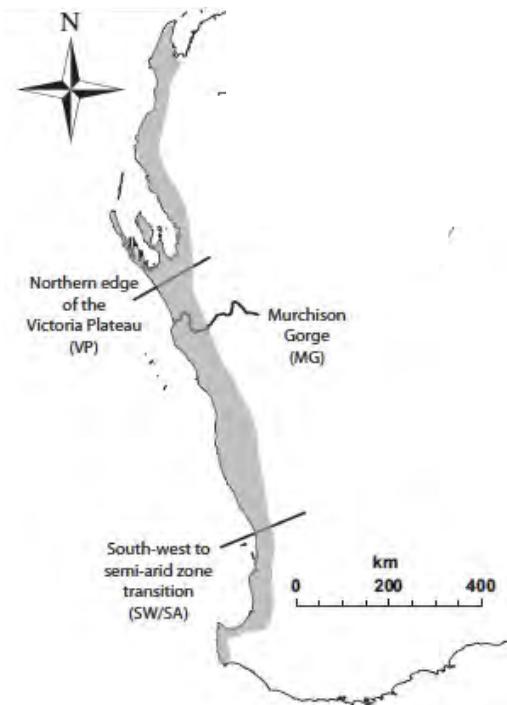
*L. lineopunctulata*



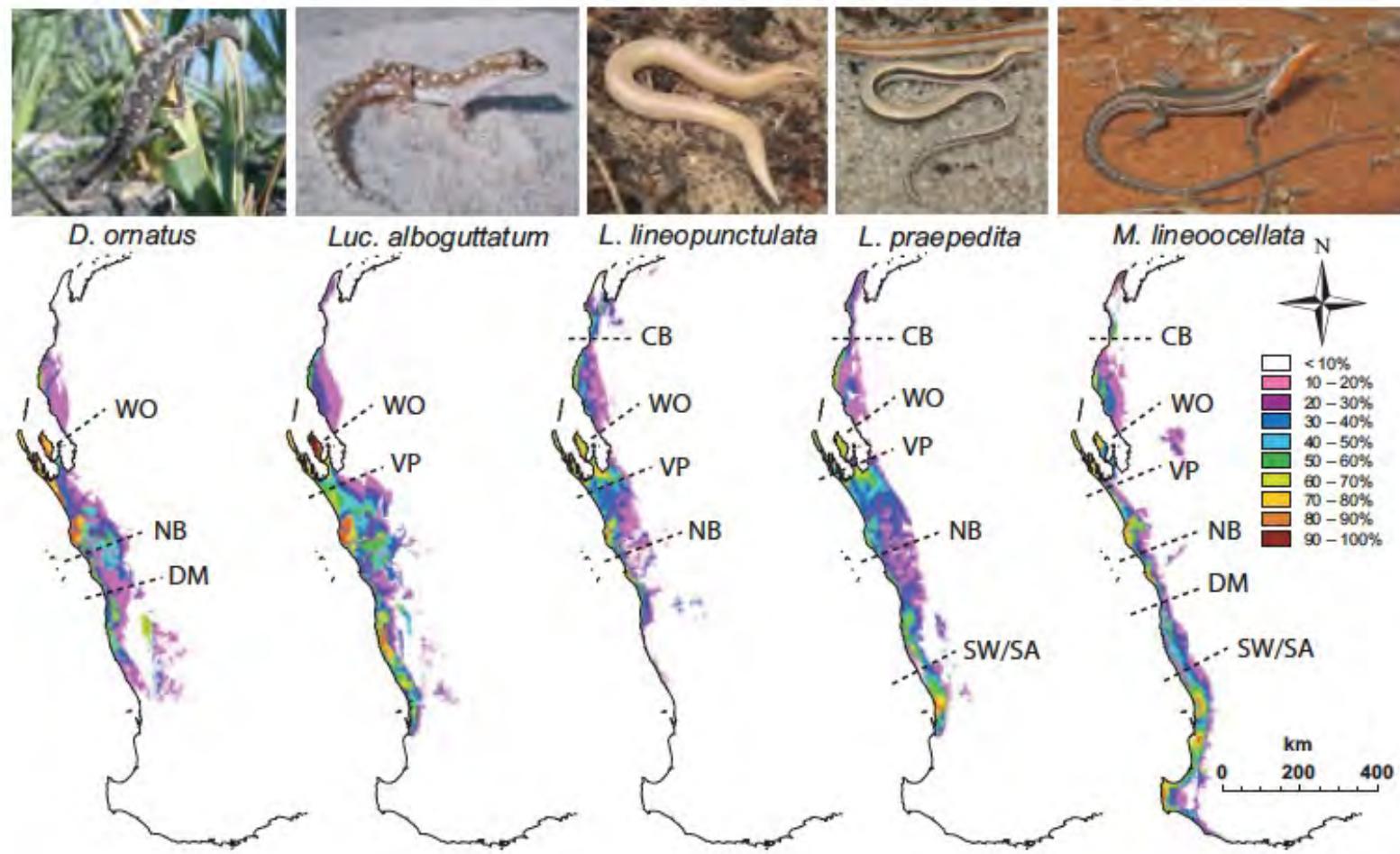
*L. praepedita*



*M. lineoocellata* N



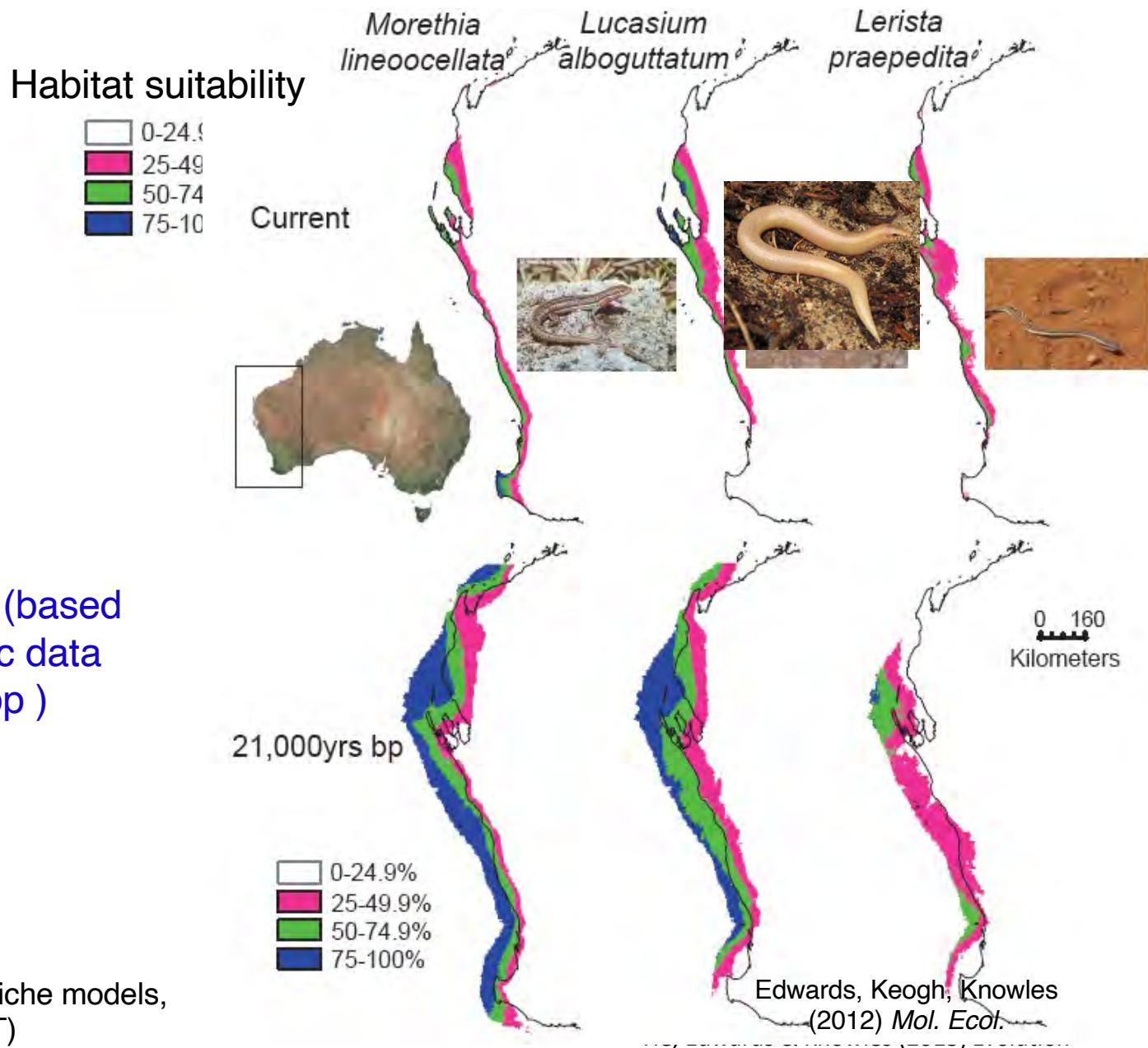
Linear distribution of populations along SW coast suggests isolation-by-distance may be important in structuring patterns of genetic variation



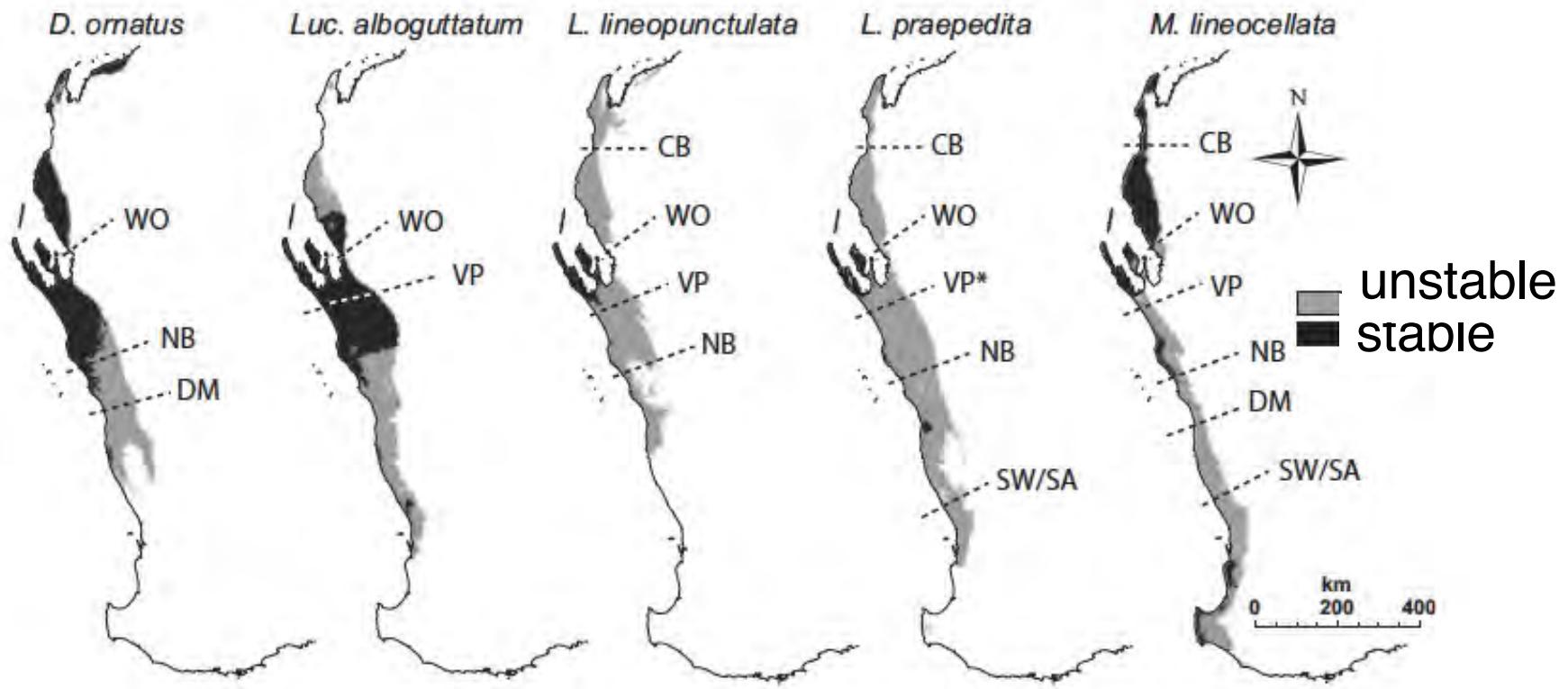
But species vary in their specialization to sand-dunes,  
suggesting habitat differences across space may be important  
in structuring patterns of genetic variation

# Climatic conditions have changed over time

Current distribution  
(contemporary climatic data)



(based on ecological-niche models,  
ENMs, with MAXENT)

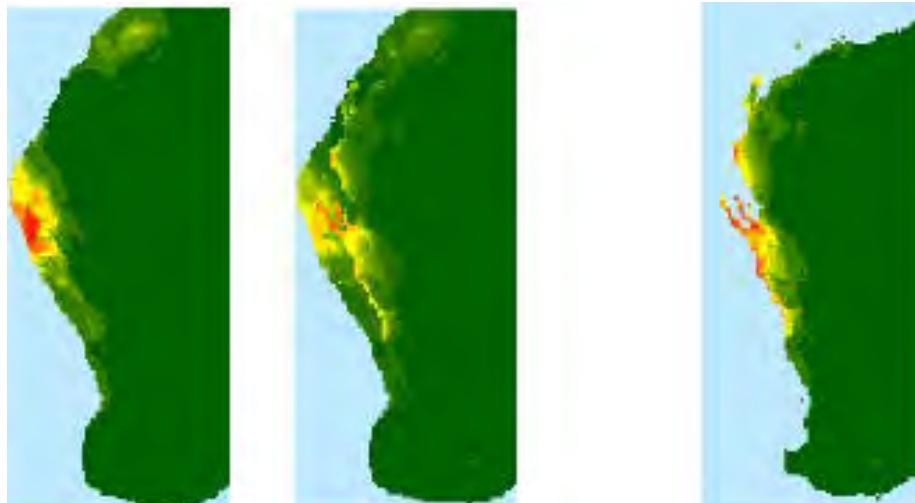


Climate-induced distributional shifts may structure genetic variation, given differences in stability of habitat over time

## Transforming hypotheses into testable phylogeographic models:

Incorporate history of shifts in species distribution in explicit spatial framework

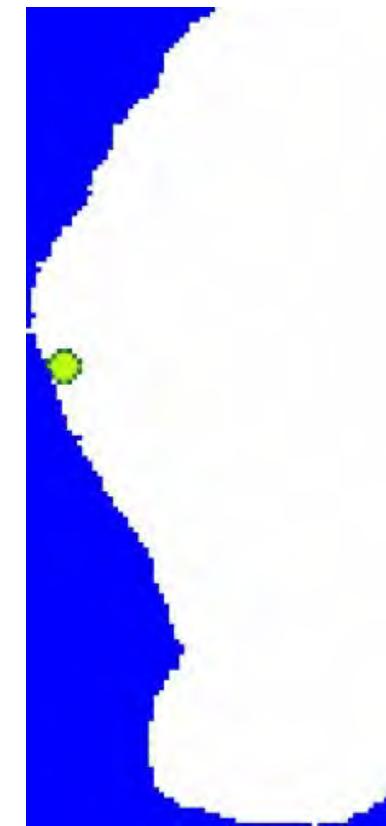
Colonization by dynamic niche



Past

Present

Dynamic  
ENM



- Start from LGM refugia
- Colonize with changing layers of ENM

Spatially explicit coalescent model to capture movement across space

integrative  
Distributional  
Demographic  
Coalescent  
modeling

iDDC:  
Distributional model  
(i.e., ecological niche model)

Hypotheses

- geographic isolation alone (IBD)
- population connectivity determined by current landscape, as measured from ENM
- population connectivity determined by distributional shifts associated with climate change, as modeled by current and paleoclimatic data

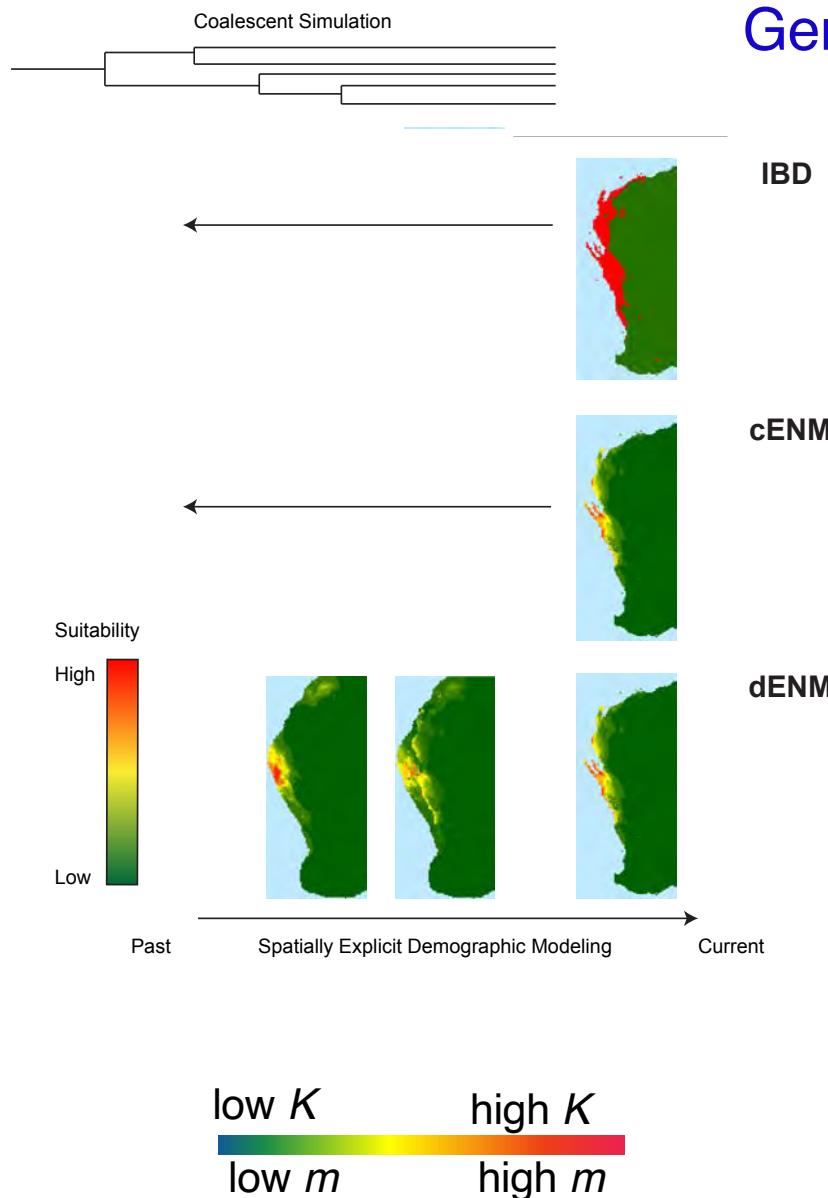


24 anonymous nuclear loci from 89 individuals sampled across the range of *Lerista* (shown by dots)

↓  
Demographic model

↓  
Coalescent model

# iDDC modeling:

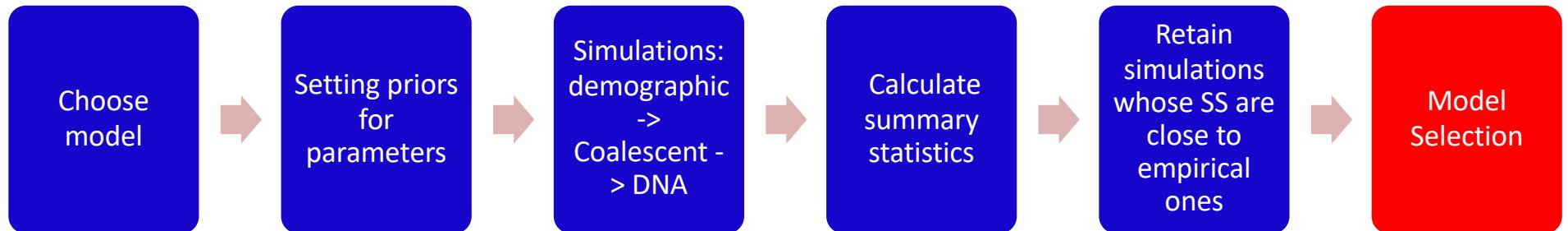


Generate lots of simulated data sets under each model (IBD, cENM, dENM).

We can identify sets of parameters for specific models that produce simulated data that matches the empirical data.

**Model Selection** using Approximate Bayesian Computation (ABC)

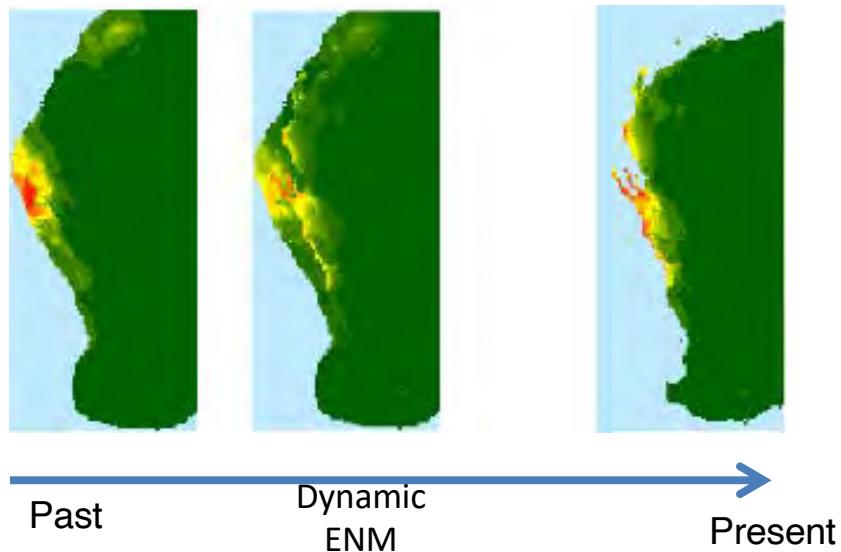
# Tests of hypotheses/models using ABC



Comparison of Bayes factor showed that

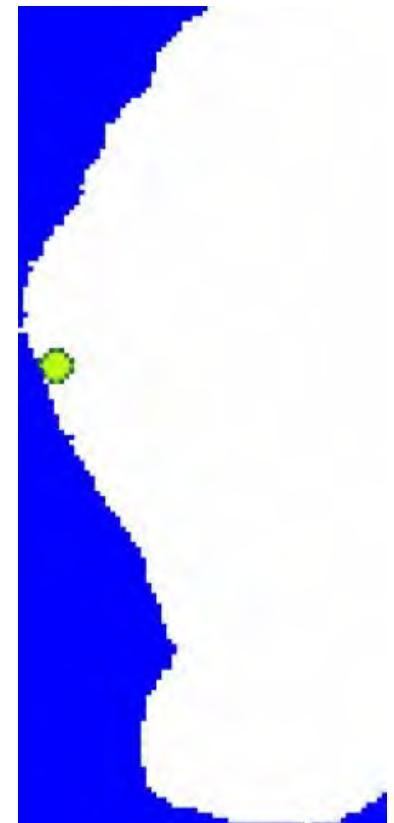
Colonization by dynamic ENM

- >> Isolation by contemporary ENM
- > Isolation by distance



- Start from LGM refugia
- Colonize with changing layers of ENM

He, Edwards & Knowles (2013) *Evolution*



Advantages of iDDC:

- Flexible (expand to multiple species)
- Complex history
- Test of processes
- Model verifications for ABC, e.g.:

- Is the model capable of generating the observed data: the likelihood of the empirical data can be compared with the likelihoods of other retained simulations (a  $p$ -value of 0 means all the simulations had a better likelihood than the observed data)
- Compute the coefficient of variation of each parameter explained by each PLSs of the summary statistics as an indicator for the power of the estimation
- Accuracy of parameter estimation in the most supported model evaluate using 1000 PODs generated from prior distributions of the parameters

Challenges:

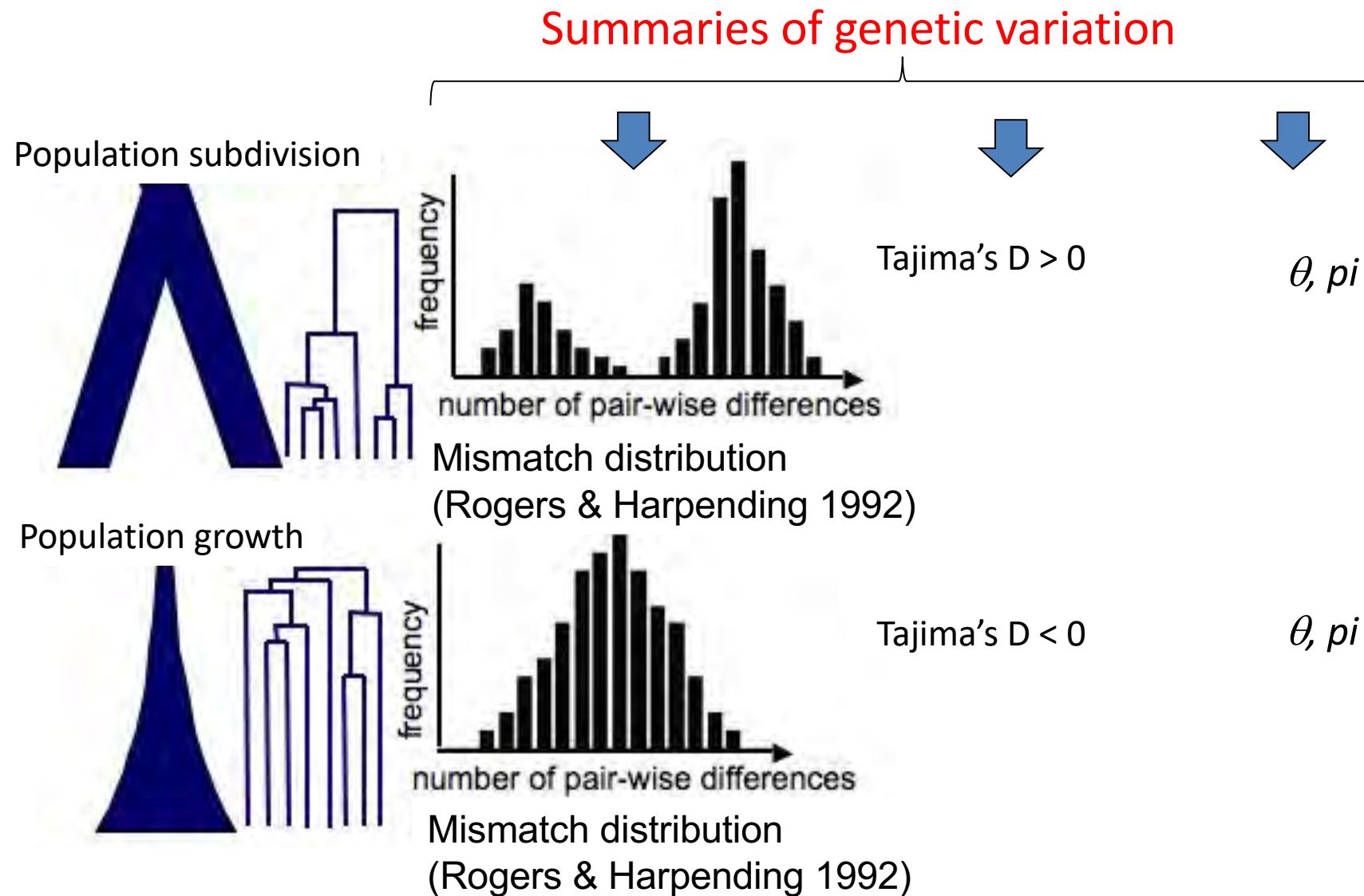
- Computationally intensive

# Evolutionary applications of genomic data

what I'll emphasize:

- Decisions/choices we make about model formulation
- Recognizing the subjectivity of model formulation itself when making inferences
- Decisions when applying to empirical data  
(e.g., all the data, subset of data, what subset of data)
  - Decide how to extract information from genetic data

Summary statistics of genetic variation will have different values depending upon the biogeographic and demographic processes generating the genetic data

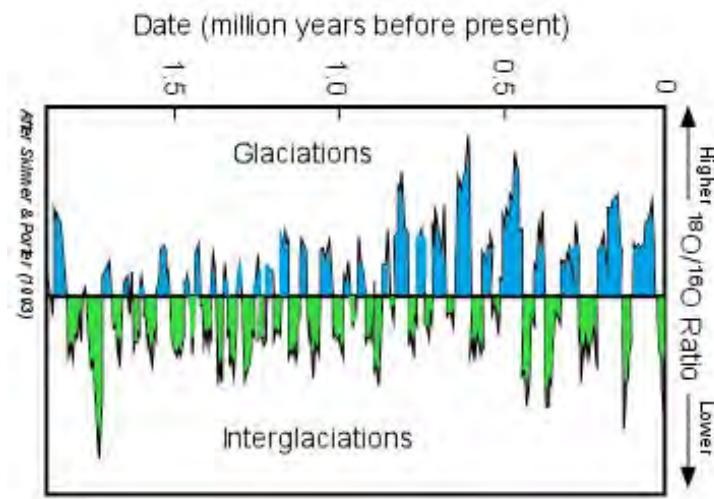


# Decisions about how to extract information from genetic data

- ⇒ use of summary statistic (sacrifices information content for simplification and ease)
  - observed quantities are compared to expectations
- 
- ⇒ calculate full likelihood of the sequence data  
(computationally demanding, and may not work for complex models, but makes full use of the data)

# Understanding the effects of rapid climate change on species diversity:

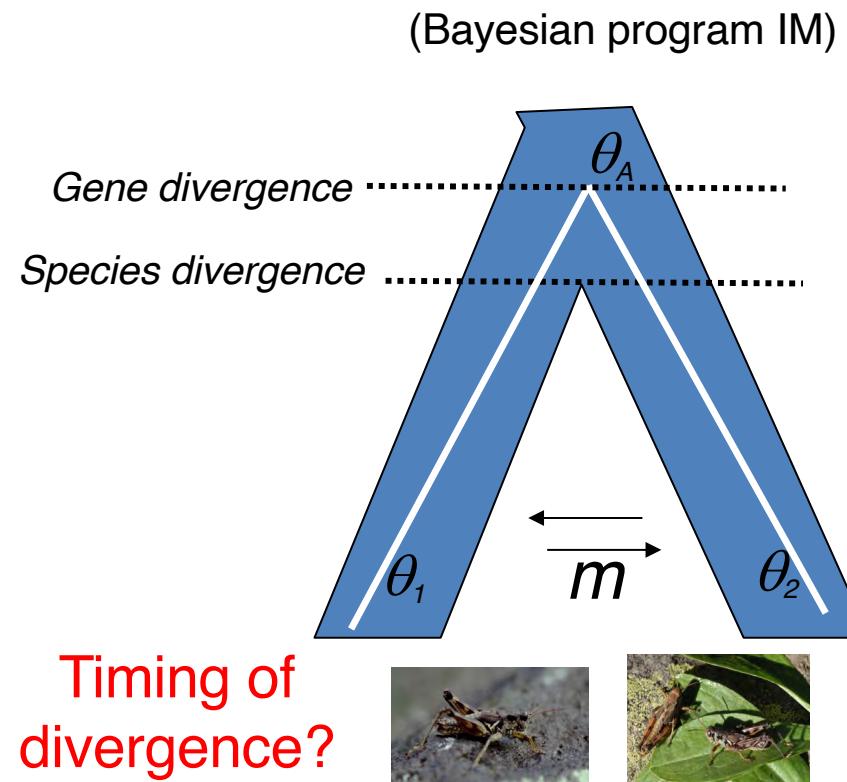
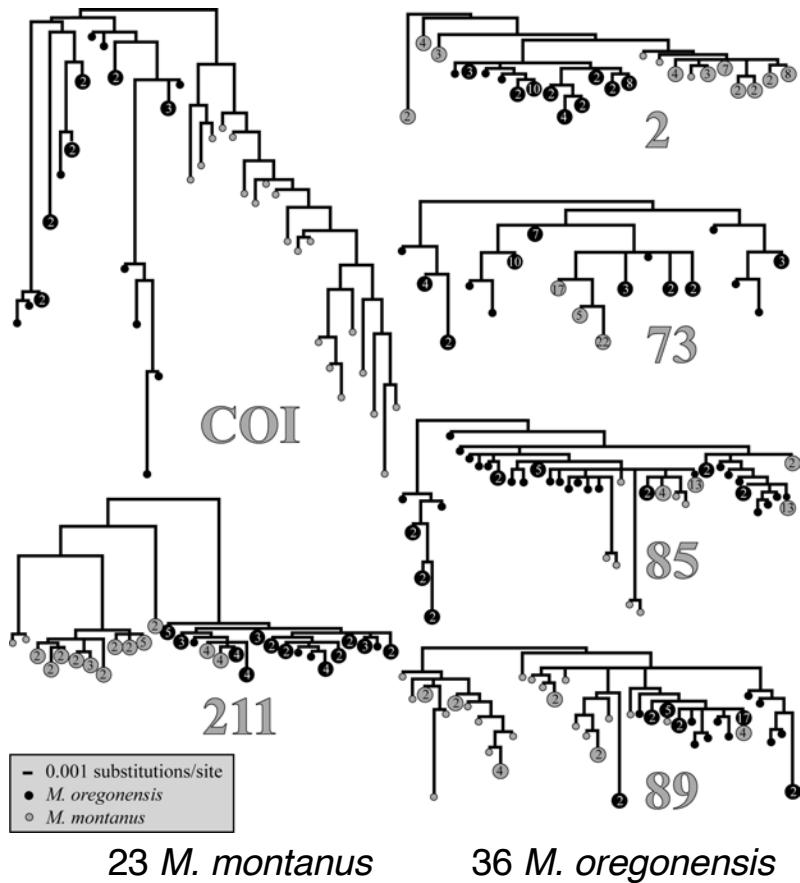
Did the frequent and repeated shifts in species distribution in response to the Pleistocene glacial cycles promote or inhibit divergence?



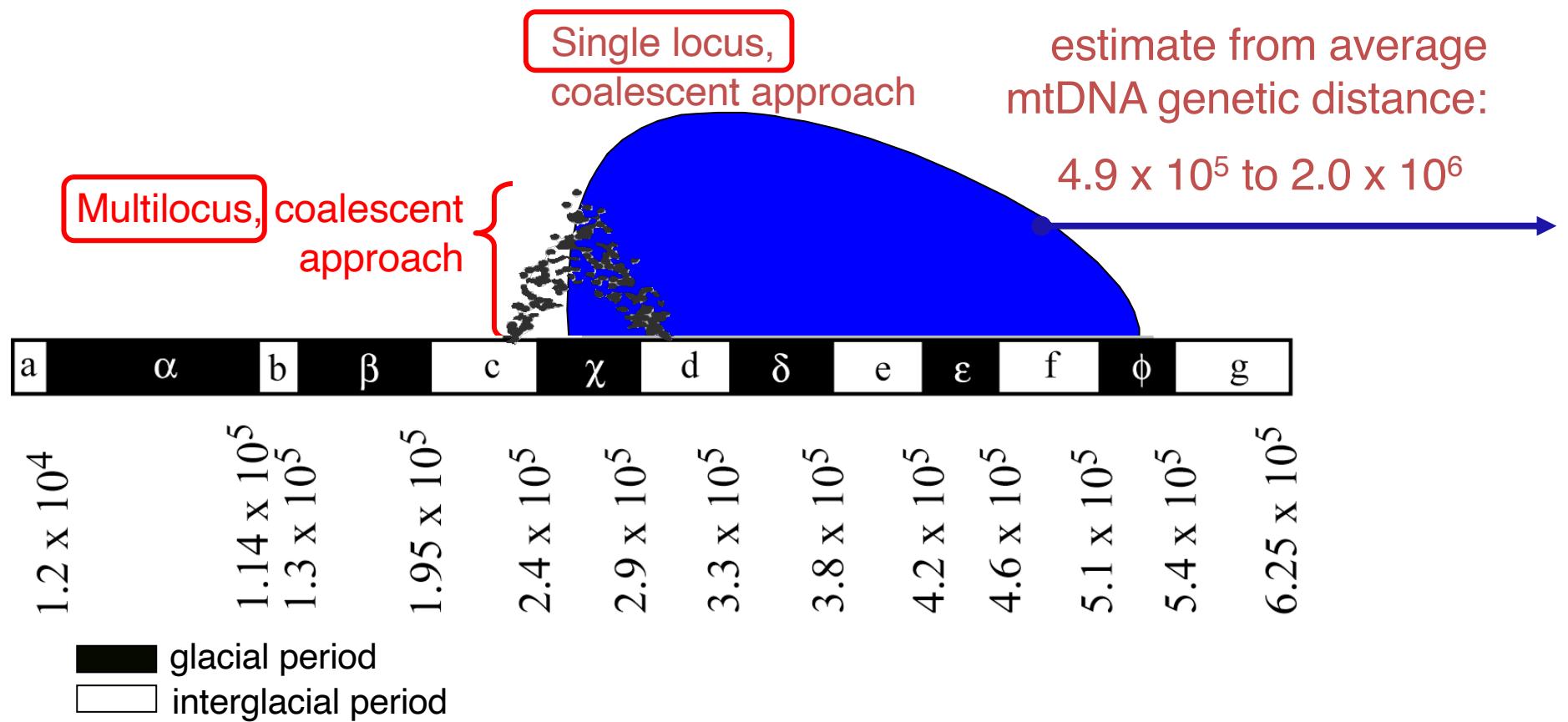
What is the timing of divergence?

- Pleistocene versus pre-Pleistocene?
- Glacial versus inter-glacial?

- Use multilocus data and a coalescent framework to estimate the timing of divergence



Precise estimate of T suggests species diverged during a glacial period

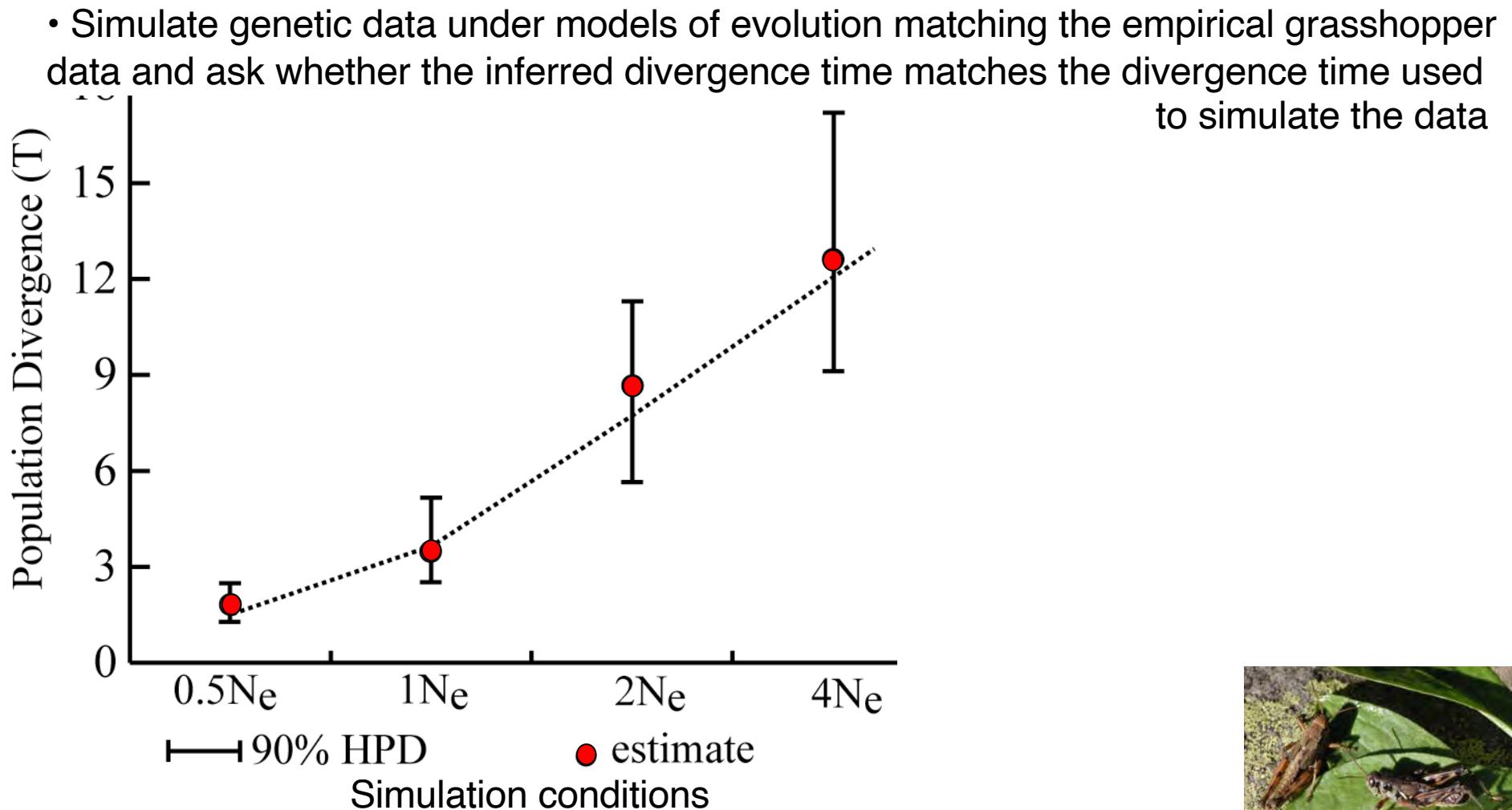
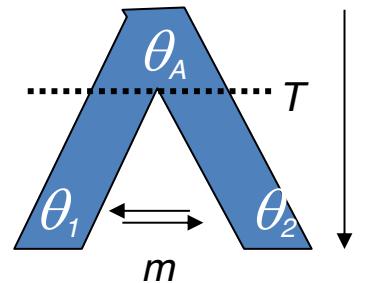


\*same mutation rate used in the different approaches

Carstens & Knowles 2007, Mol. Ecol. 16:619-27.

Verified the accuracy of the speciation model given the data (only 6 loci)

(estimates may be compromised when the complexity of the model exceeds the information content of the genetic data)



Carstens & Knowles 2007, Mol. Ecol. 16:619-27.

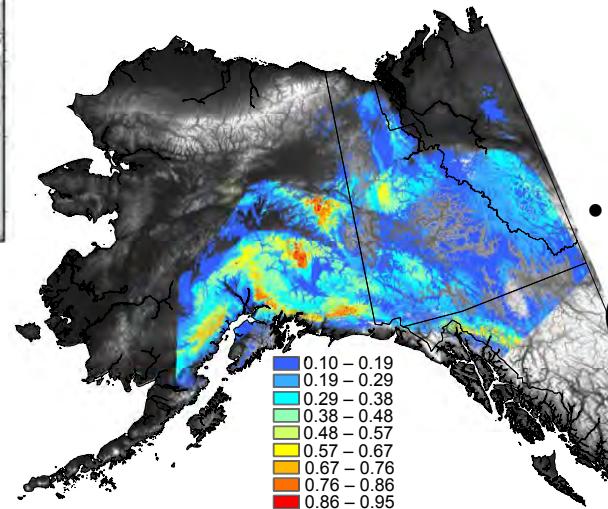


# How do we decide upon a model\*:

- informed from information independent of the genetic data itself
  - that is, a specific biological narrative motivates the model
- models informed by the genetic data (...but be careful not to use same data twice)
- arbitrary/generic models

\* All models are simplifications, and vary in the degree of their relative degree of abstraction

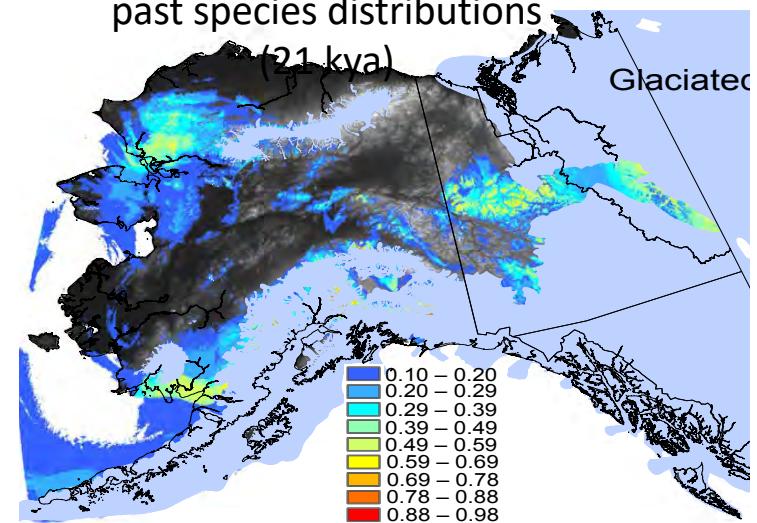
# Informing model based on preliminary tests based on genetic data

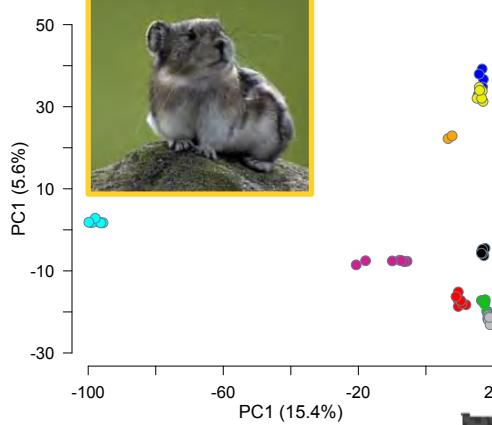


Sometimes ENMs not sufficient to define model

- Projected distribution from MAXENT based on contemporary bioclimatic variables (e.g., max and minimum temperatures and precipitation, etc)

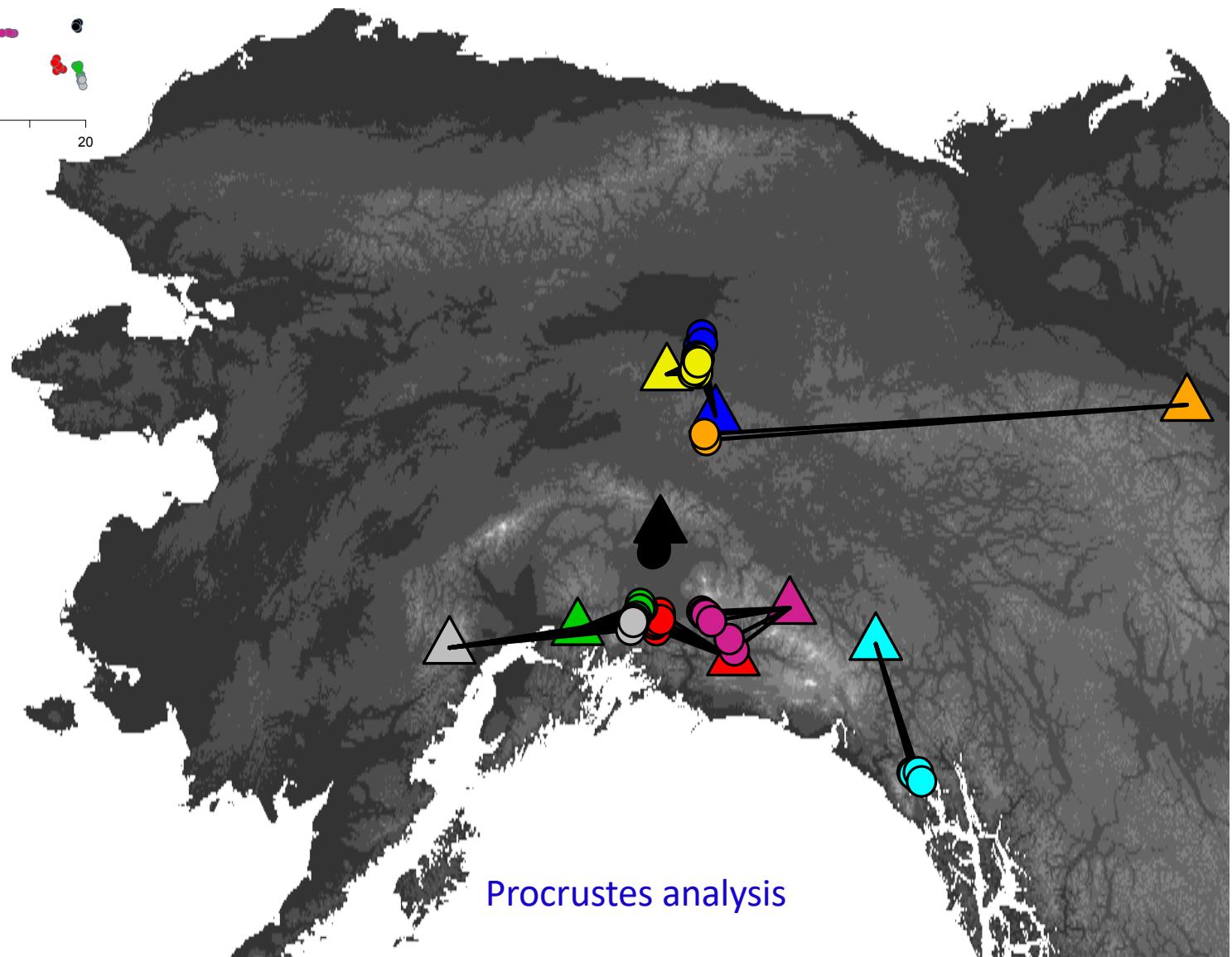
- Paleoclimatic data used to model past species distributions





- Informing model based on preliminary tests of genetic data

- Allie's Valley
- Anchorage
- Crescent Creek
- Denali Hwy
- Eagle Summit
- Jawbone Lake
- Lake Kenibuna
- Pika Camp
- Rock Lake





To better understand the historical demographic trends for pika populations, we estimated divergence time, gene flow and population size changes among different populations using the site-frequency spectrum (SFS) using FastSimCoal.

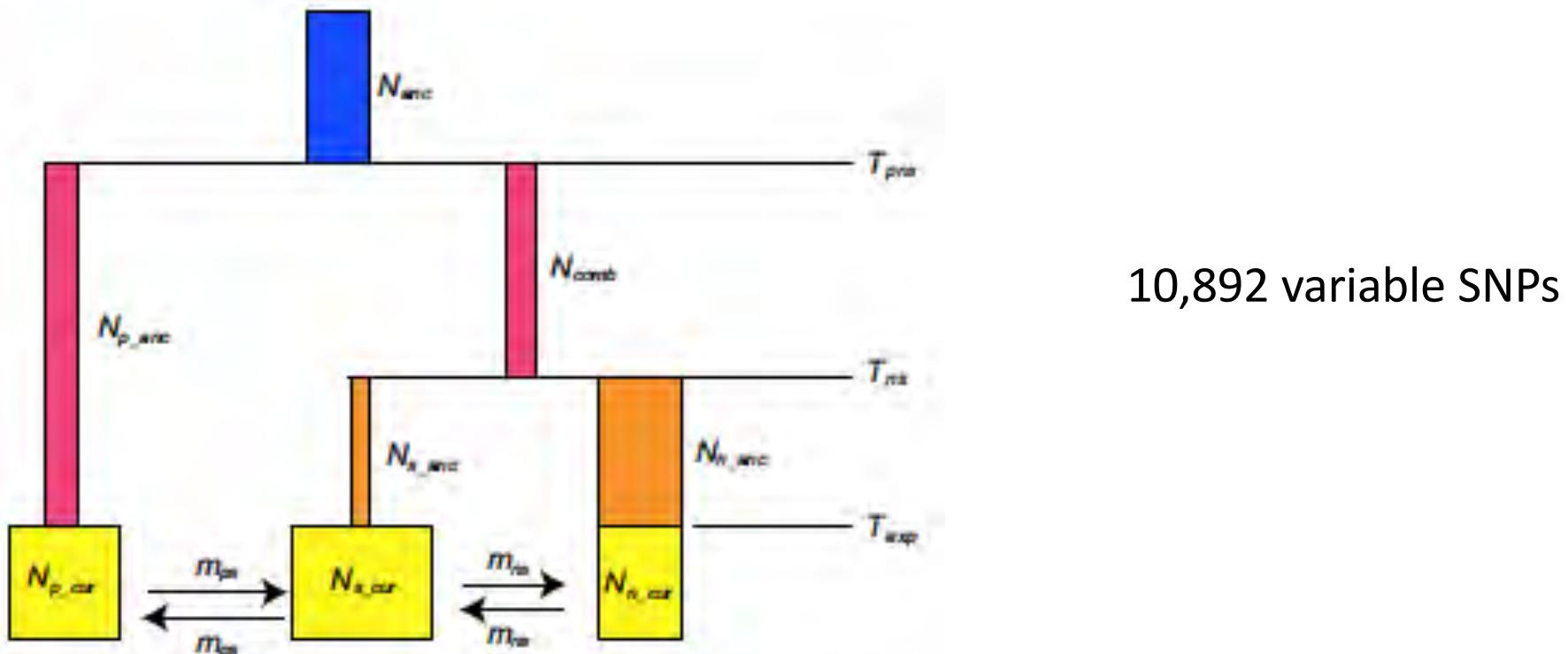


Fig. 2 Hypothesized demographic history of pika populations used in FASTSIMCOAL2 analyses. Pika ancestors diverged ( $T_{pns}$  generations ago) into ancestral populations of Pika Camp ( $N_{p,anc}$ ) and the other populations ( $N_{ns}$ ). Later, the divergence into southern ( $N_{s,anc}$ ) and northern refugia ( $N_{n,anc}$ ) occurred, and populations experienced recent expansions and exchanged migrants. The estimates of these parameters are listed in Table 4.

Lanier et al. (2015) *Mol. Ecol.* 24:3688-3705



- Our results indicate that contemporary factors alone (i.e., current habitat continuity and glacial corridors) are not sufficient to explain connectivity among populations of Collared Pikas across their range
- Instead, the results provide strong support for the predominance of three divergent lineages, likely separated in different Pleistocene refugia, with population expansion among lineages predating the Last Glacial Maximum

Lanier HC, Massatti R, He Q, Olson LE, Knowles LL (2015) Colonization from divergent ancestors: glaciation signatures on contemporary patterns of genetic variation in Collared Pikas (*Ochotona collaris*). Mol. Ecol. 24:3688-3705.

# How do we know if we used the “right” model?

In practice we can never completely model the evolutionary process, all we can hope for is that we have captured the important features.

(i.e., YOUR knowledge about a biological system is key!)

"The purpose of models is not to fit the data  
but to sharpen the questions."

- *Samuel Karlin*

## Evolutionary applications of genomic data

- Accounting for species-specific traits
- Spatially explicit coalescent models
- Comparative analyses of genetic variation across species



## Evolutionary applications of genomic data

- Accounting for species-specific traits
- Spatially explicit coalescent models
- Comparative analyses of genetic variation across species

# Evolutionary applications of genomic data

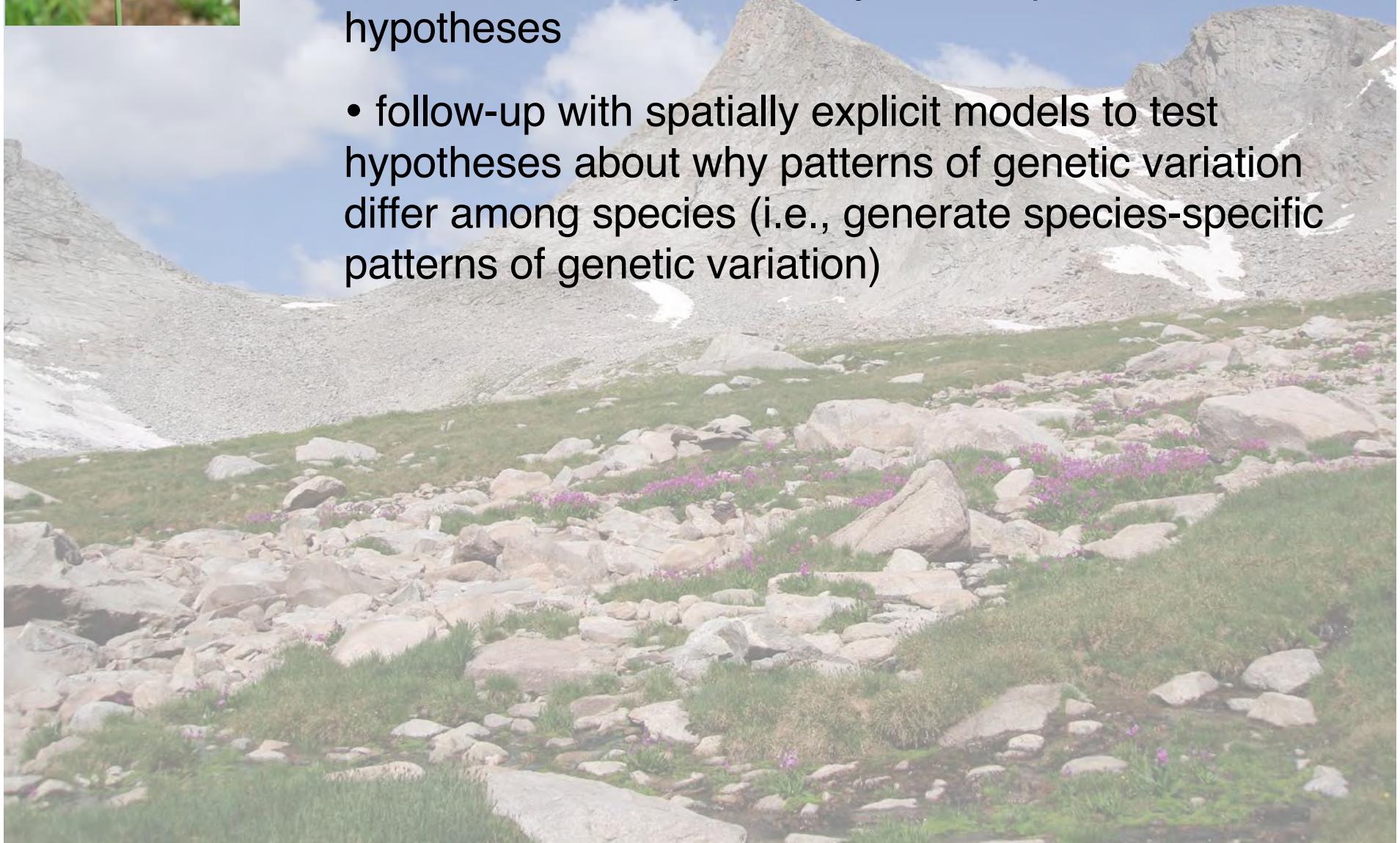
What I'll emphasize:

- Decisions/choices we make about model formulation
- Recognizing the subjectivity of model formulation itself when making inferences
- Decisions when applying to empirical data  
(e.g., all the data, subset of data, what subset of data)



## Does microhabitat differences affect species responses to climate change?

- start with descriptive analysis to explore hypotheses
- follow-up with spatially explicit models to test hypotheses about why patterns of genetic variation differ among species (i.e., generate species-specific patterns of genetic variation)



# Sky island community responses to climate change similarly (based on patterns of genetic differentiation)

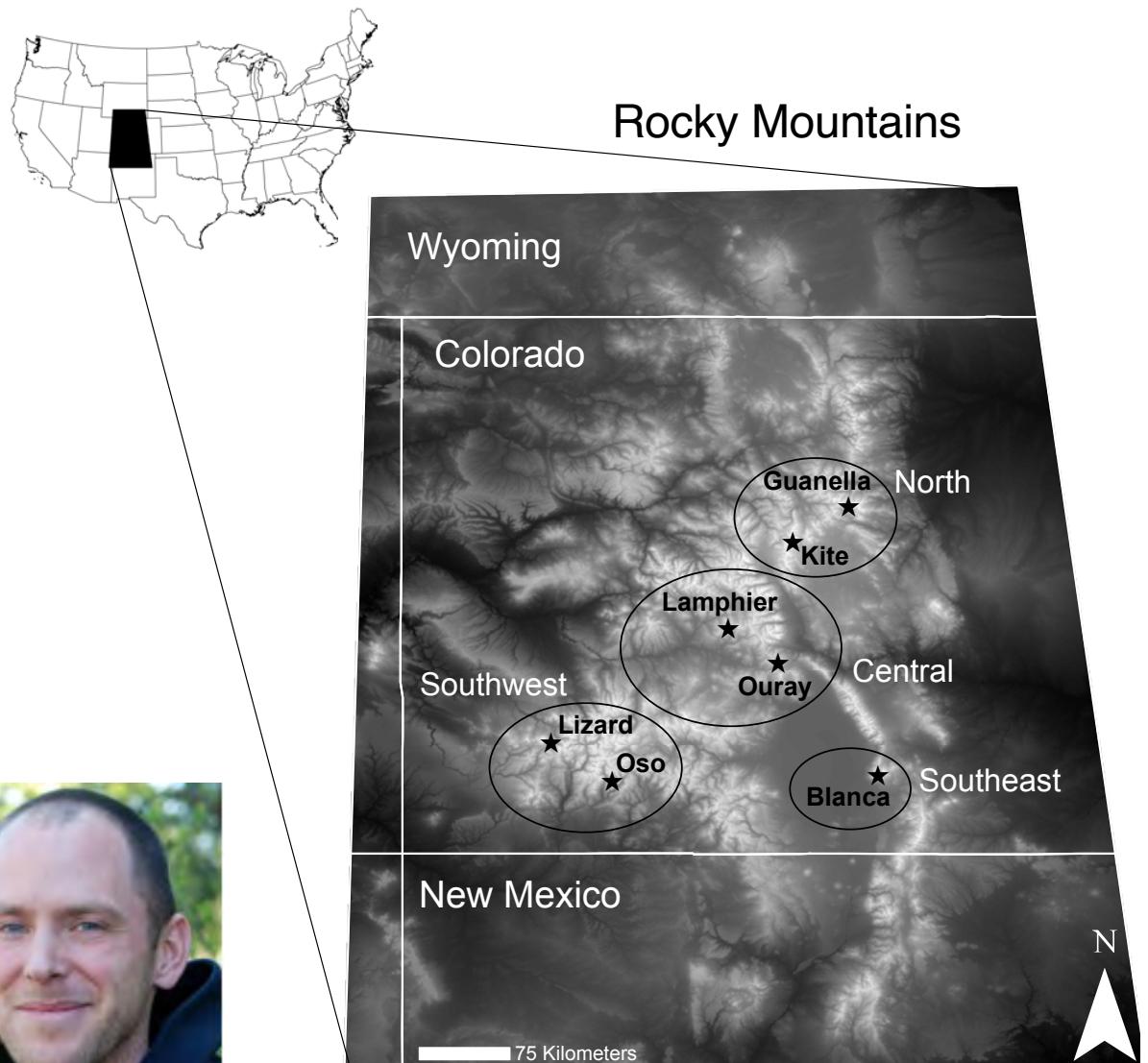
*Carex chalciolepis*



*Carex nova*



Massatti & Knowles  
(2014 Evolution)



# Sky island communities: responses to climate change

- co-distributed, abundant taxa with similar natural histories and dispersal abilities

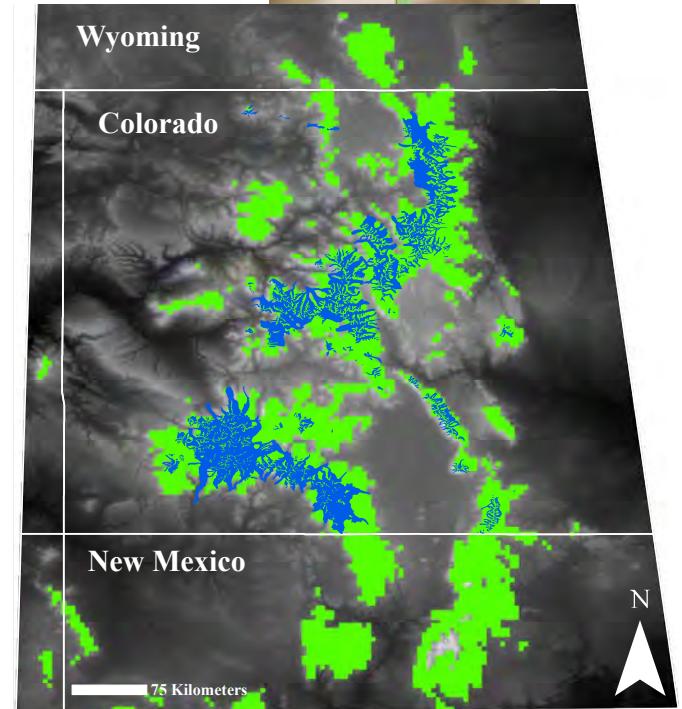
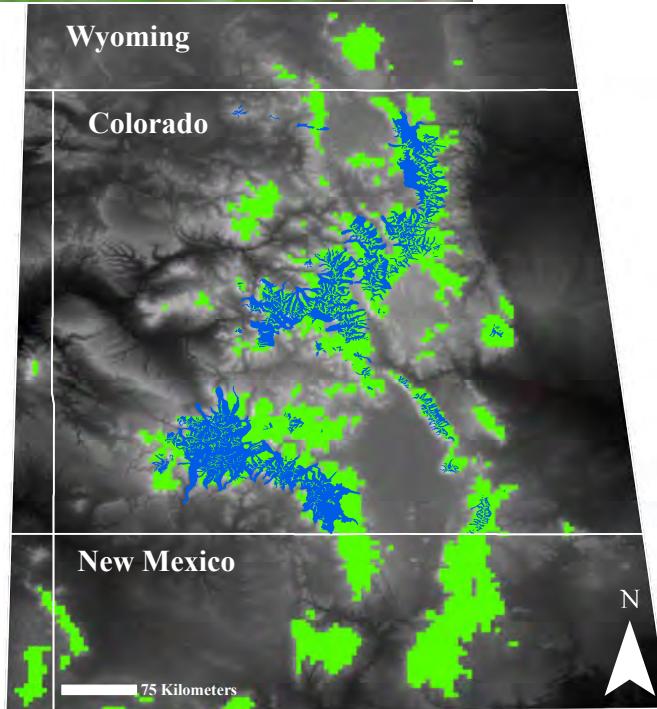
*Carex chalciolepis*



*C. nova*



- so similar that ENMs project very similar past distributions



- taxa differ in microhabitats

inhabits slopes and ridges

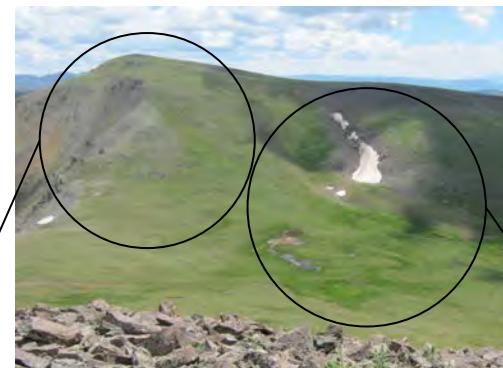


*Carex chalciolepis*

restricted to wetlands  
a

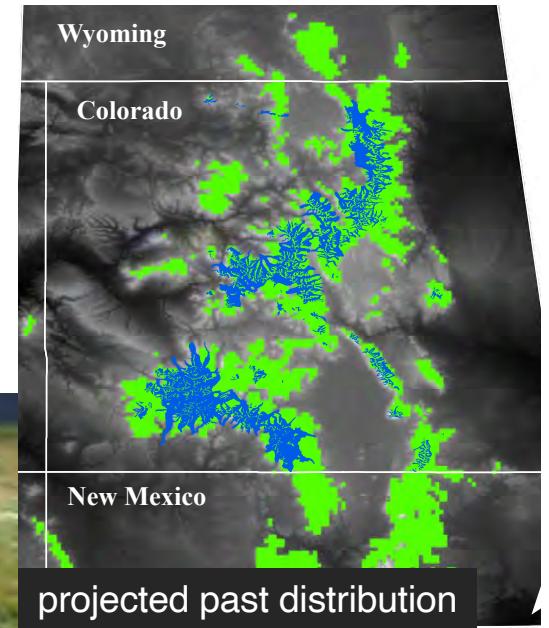
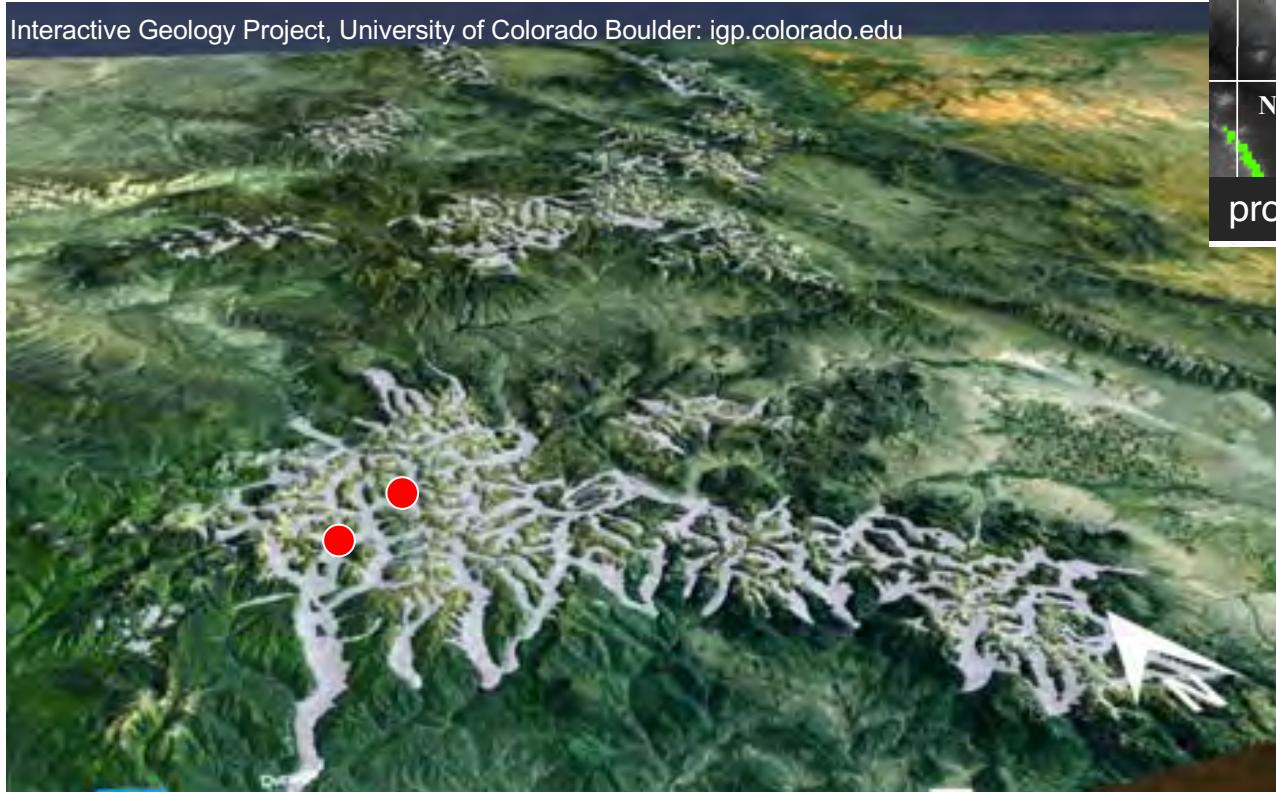


*Carex nova*



Given that ecological niche models (ENMs) are similar between species (both present and during LGM)...

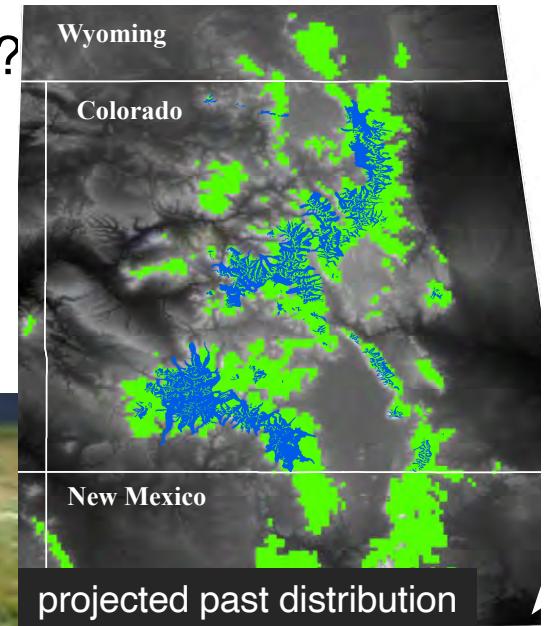
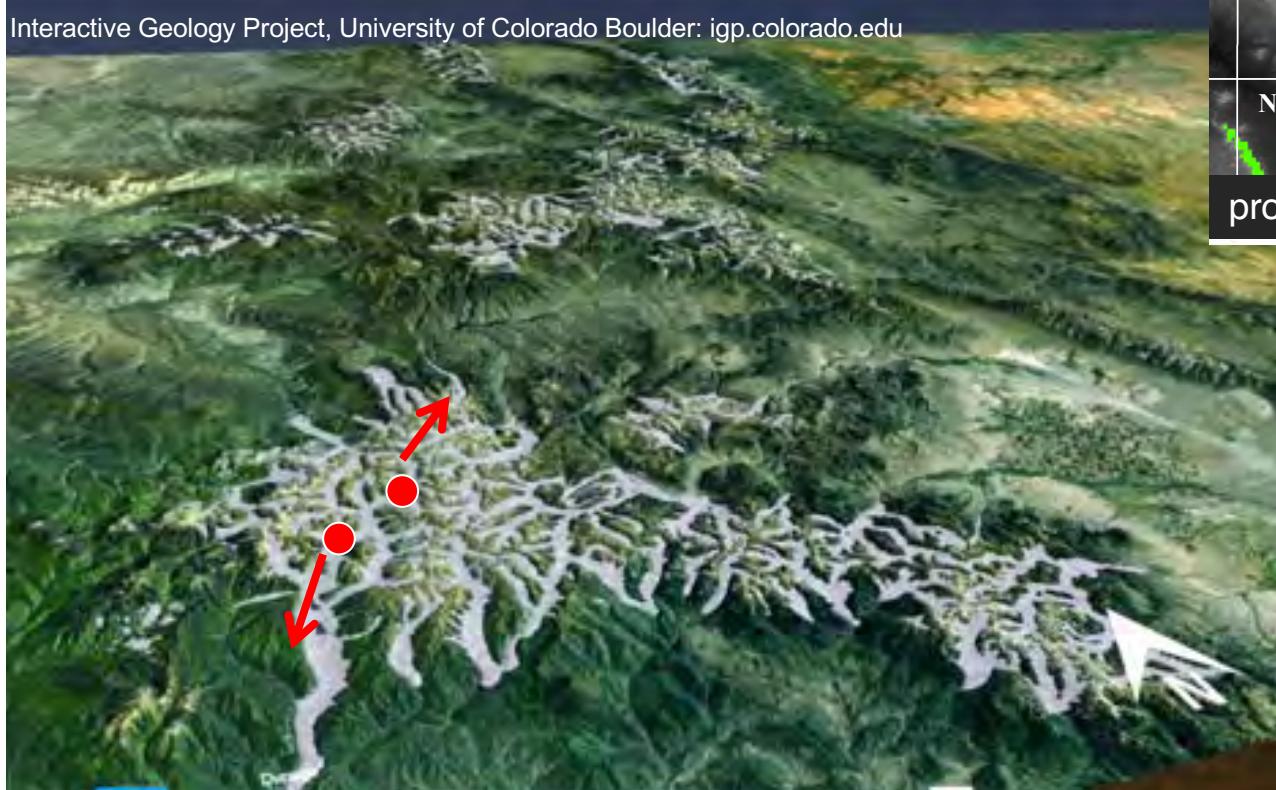
why would we predict discord in patterns of genetic variation between the plant species?



If microhabitat matters...

- glaciers in drainages would have displaced populations of wetland specialist

## Why should microhabitat matter for sky island inhabitants?

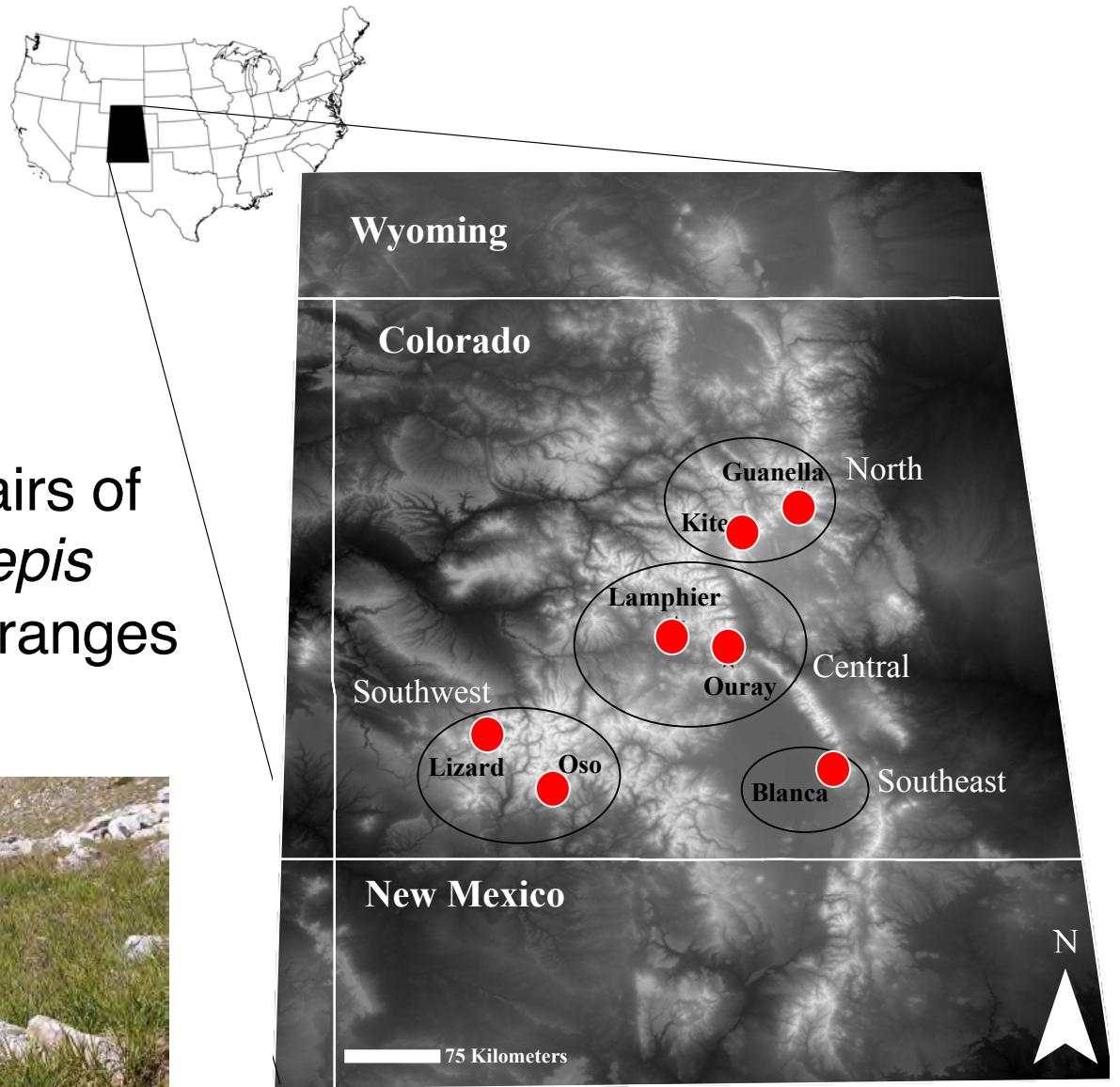


If microhabitat matters...

- distances separating populations may have been considerable greater in the past – *but only in the wetland specialist*

# 1. Sky island communities: microhabitat differences and responses to climate change

- SNPs from over 22,000 loci (RADseq)
- sampled population pairs of *C. nova* and *C. chalciolepis* from different mountain ranges

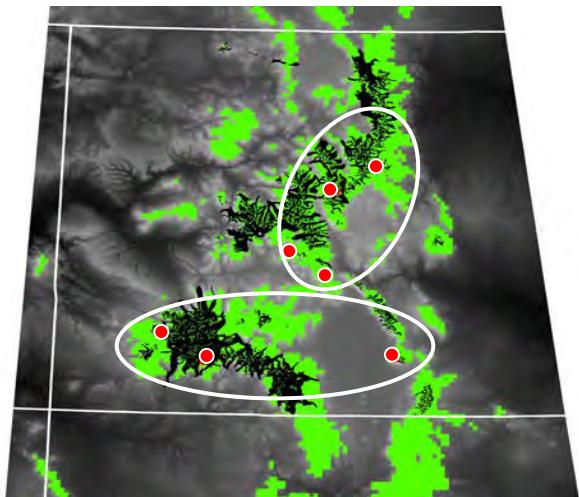


Massatti and Knowles, Evolution (in press)

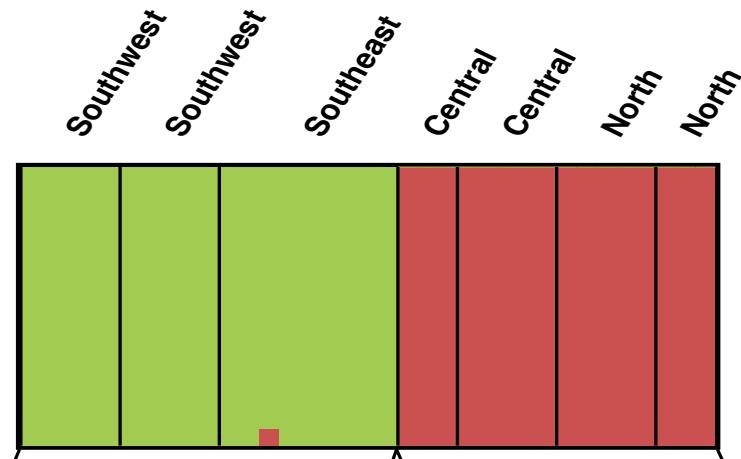


*C. nova*

restricted to wetlands



projected past distribution

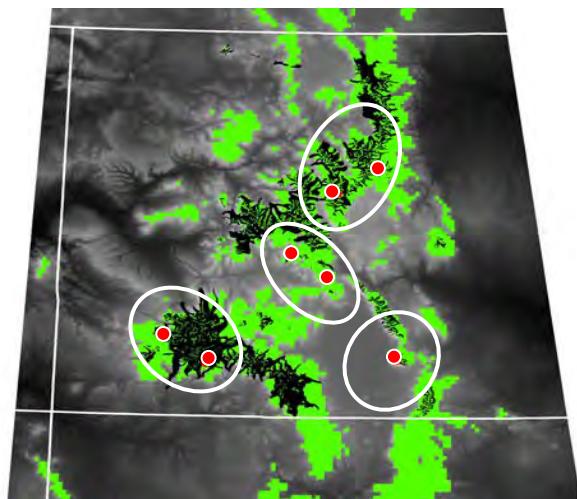


- Structure analysis of SNPs from over 22,000 loci

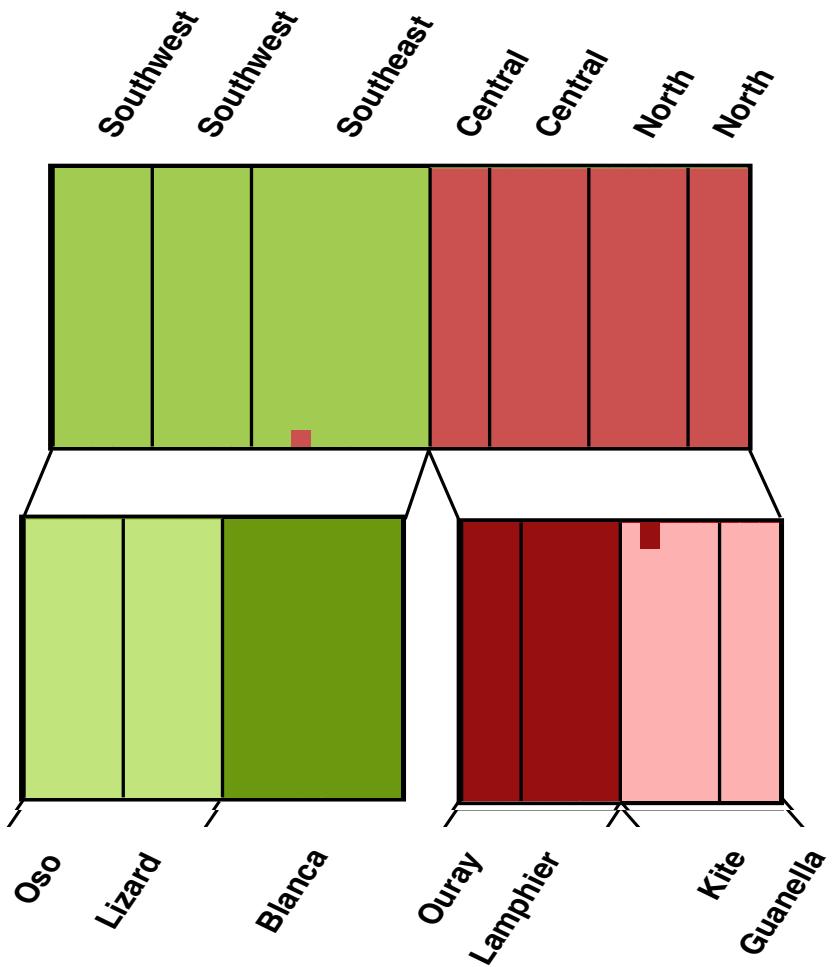


*C. nova*

restricted to wetlands



projected past distribution

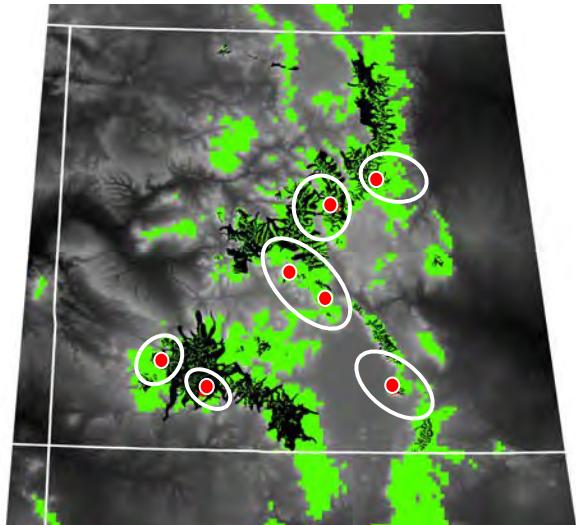


- Structure analysis of SNPs from over 22,000 loci



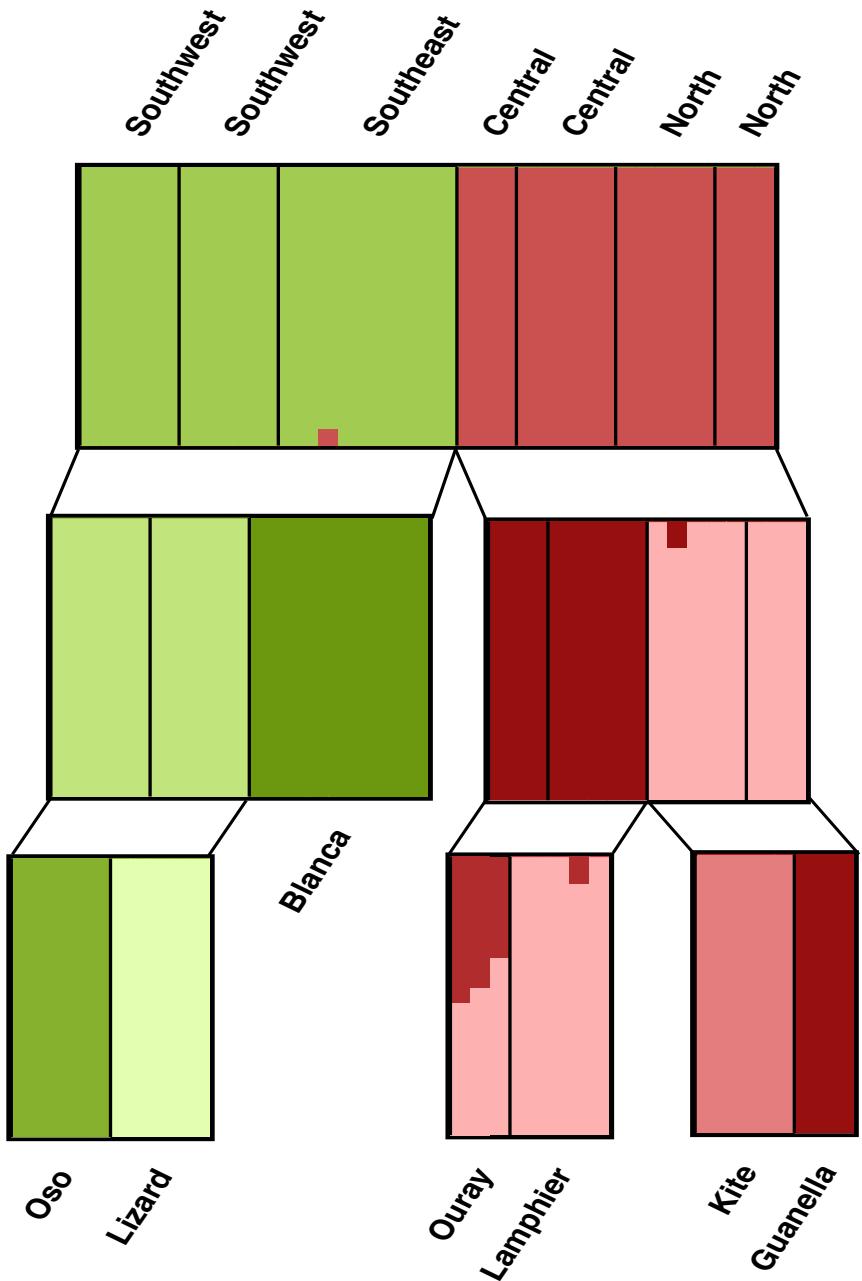
*C. nova*

restricted to wetlands



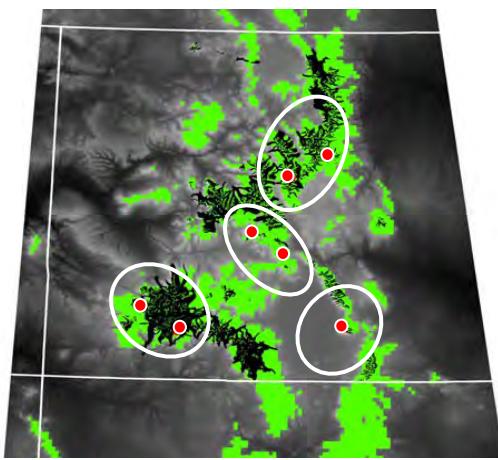
projected past distribution

Massatti and Knowles, Evolution (in press)



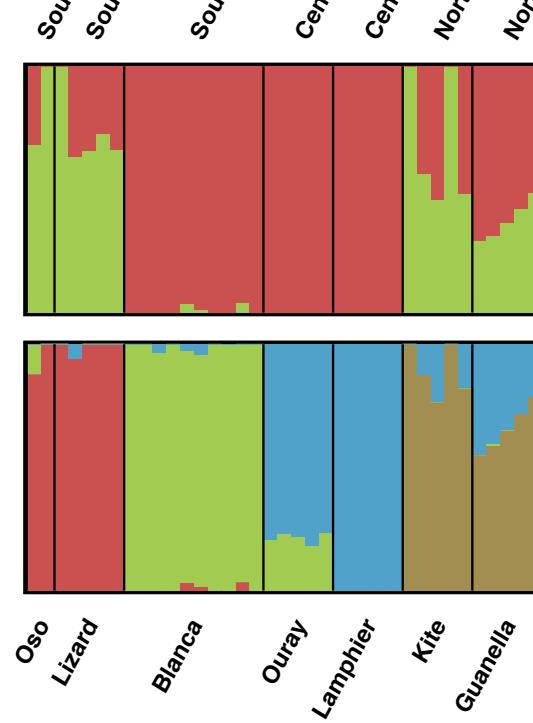
- STRUCTURE analysis of SNPs from over 22,000 loci

inhabits slopes and ridges



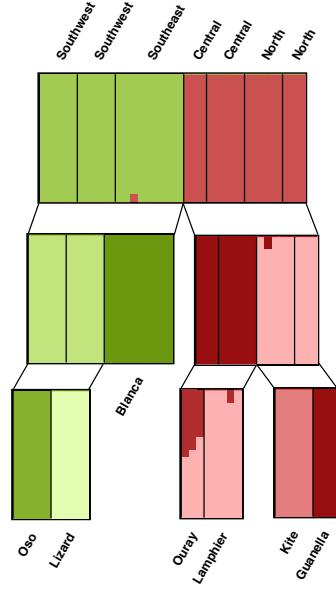
projected past distribution

$K = 2$



### *C. chalciolepis*

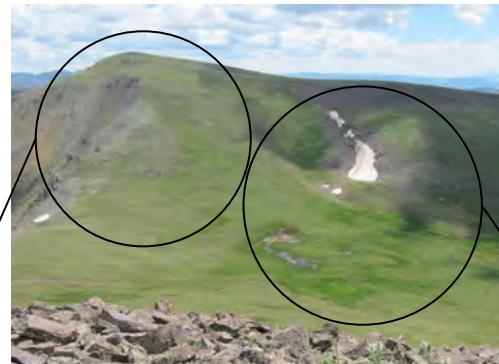
### *C. nova*



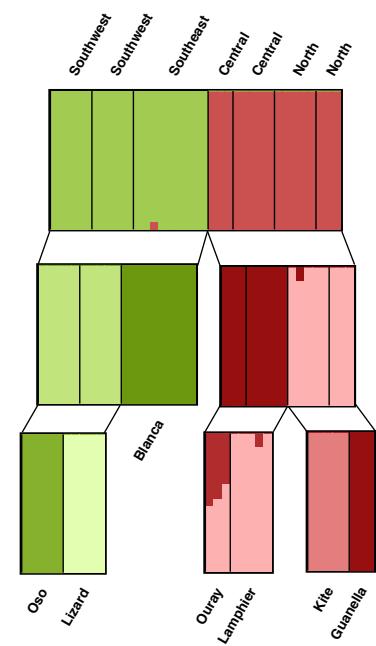
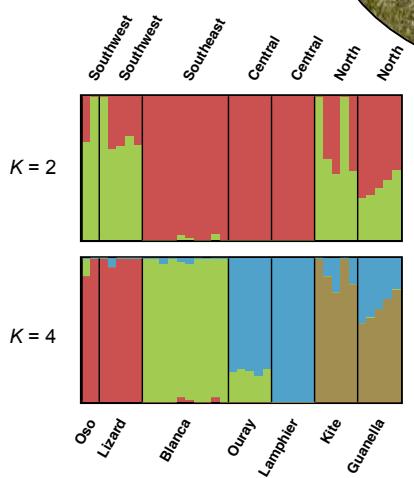
# Genomic patterns support predictions of an interaction between microhabitat affinity and climate change (glaciers are barrier for movement of wetland specialists only)



*Carex chalciolepis*  
dry ridges



*Carex nova*  
wetland specialists



# Genomic patterns support prediction of an interaction between microhabitat affinity and climate change

Massatti & Knowles (2014) *Evolution*



*Carex chalciolepis*  
dry ridges



*Carex nova*  
wetland specialists



Test if observed discordant phylogeographic structure could be caused by differences in microhabitat affinity ....

- generate species-specific expectations for patterns of genetic variation (i.e., glaciers are barrier for movement of wetland specialists only)

# iDDC: Generate species-specific expectations for patterns of genetic variation

He, Edwards & Knowles, Evolution 2013

## integrative Distributional Demographic Coalescent modeling

Distributional model  
(i.e., ecological niche model) with  
predictions on probability of occurrence  
across the landscape



Demographic model  
informed by habitat  
suitabilities



Spatially-explicit coalescent  
simulations based on  
demographic model



Tests of hypotheses/models  
using ABC

Habitat suitability  
scores

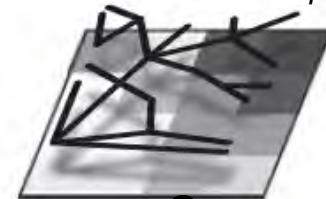
40	20	10	5
100	60	20	10
100	100	40	40
80	80	60	60

$K(m)$

400	200	100	50
(40)	(20)	(10)	(5)
1000	600	200	100
(100)	(60)	(20)	(10)
1000	1000	400	400
(100)	(100)	(40)	(40)
800	800	600	600
(80)	(80)	(60)	(60)

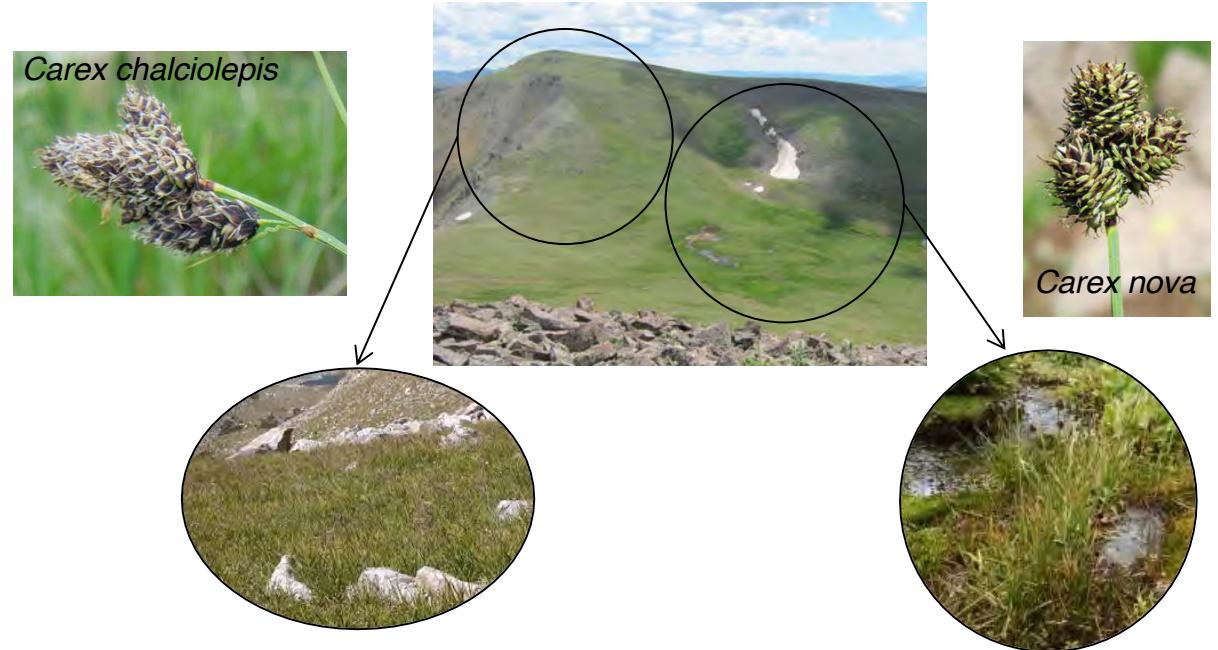
Carrying capacity:  $k_i$

Gene coalescence  
across the landscape



SPLATCHE2

## iDDC: Generate species-specific expectations for patterns of genetic variation

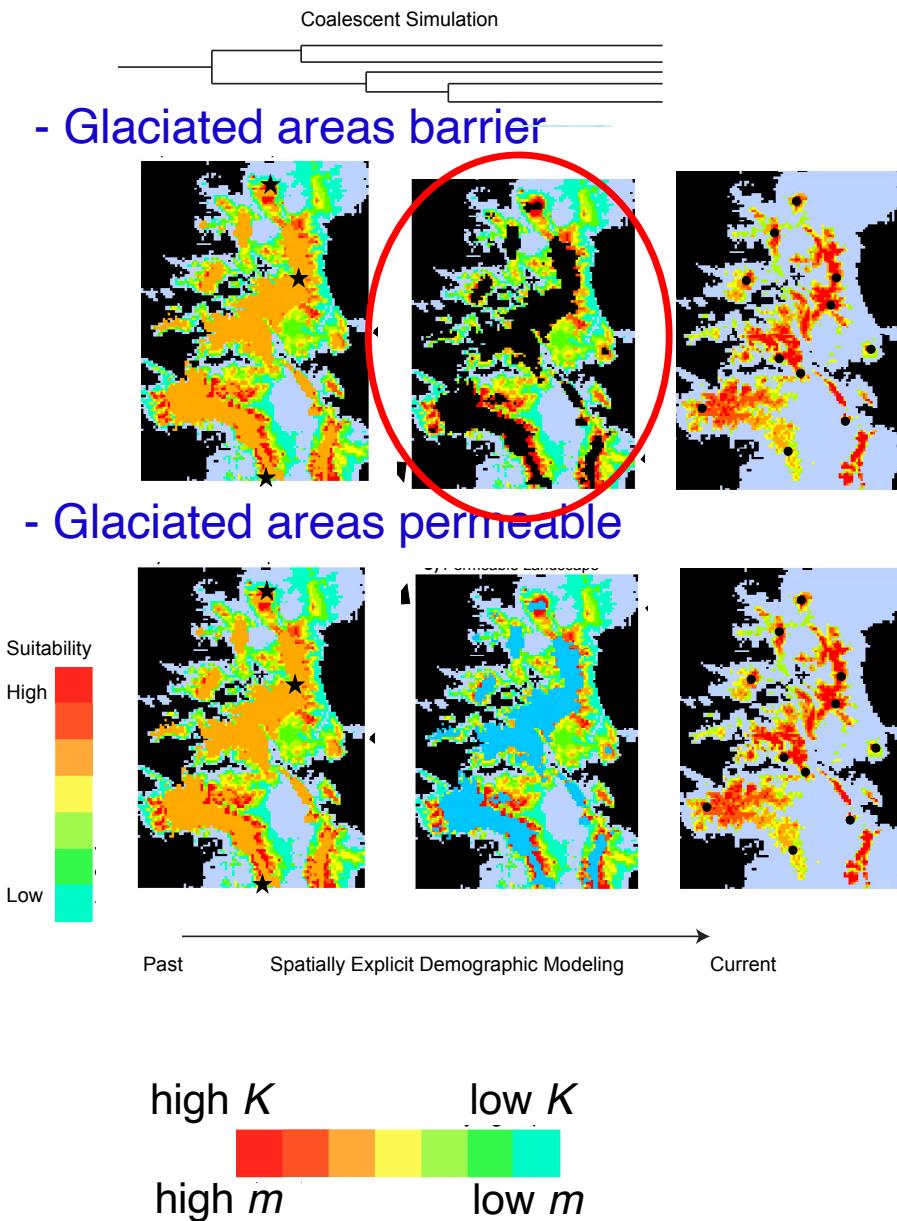


H: species-specific responses to climate change

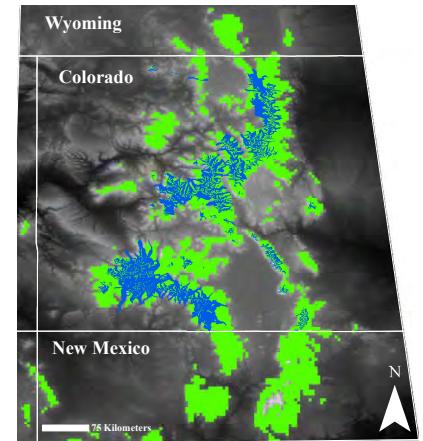
- Glaciated areas act as barriers,  
but only in wetland specialist



# iDDC modeling:



Glaciers shown  
in blue

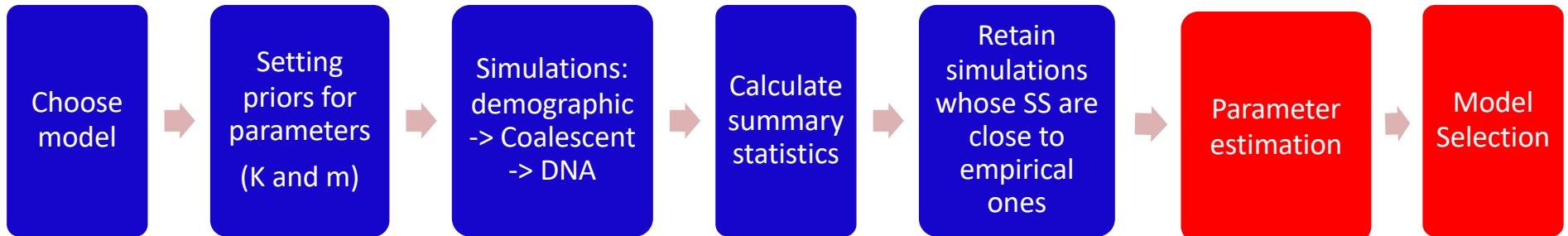


Generate lots of simulated data  
sets under each model

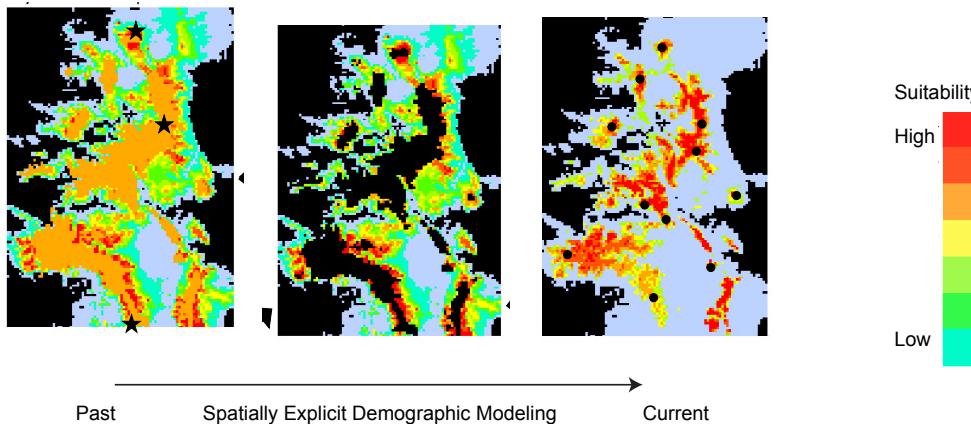
We identify sets of parameters for the  
models that produce simulated data  
that match the empirical data.

**Model Selection** using  
Approximate Bayesian  
Computation (ABC)

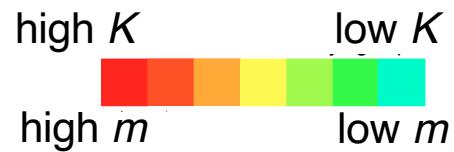
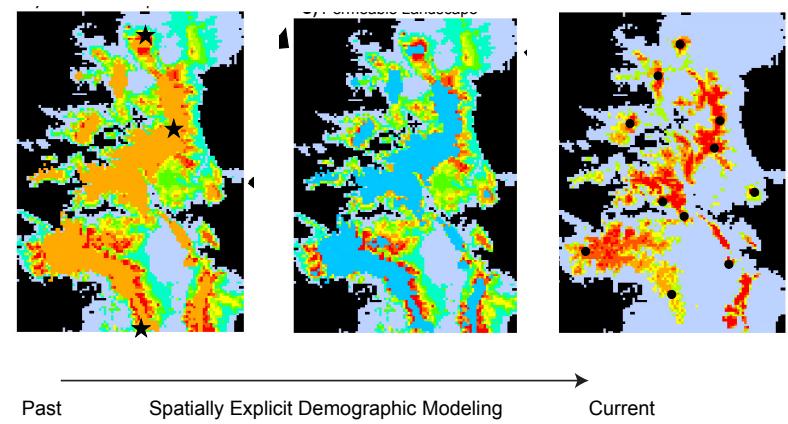
# Tests of hypotheses/models using ABC



Model: Glaciated areas barrier

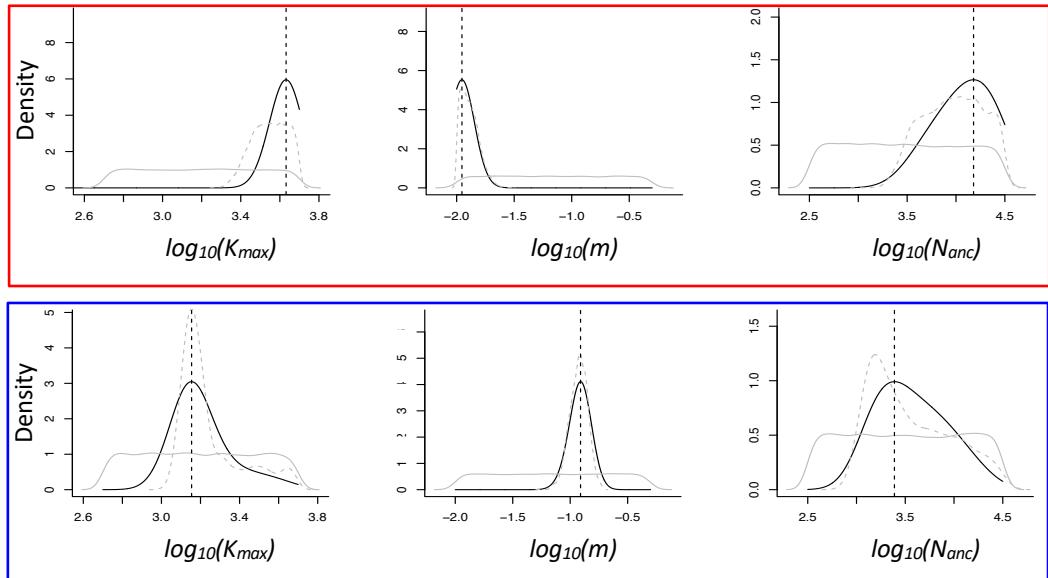


Model: Glaciated areas permeable





5000 simulations closest to empirical data retained for parameter estimation



Marginal densities:

*Carex chalciolepis*  
Bayes factor ~3

*Carex nova*  
Bayes factor ~23

### Barrier Model

$4.87 \times 10^{-5}$   
(0.65)

$1.29 \times 10^{-4}$   
(0.84)

### Permeable Model

$1.38 \times 10^{-4}$   
(0.97)

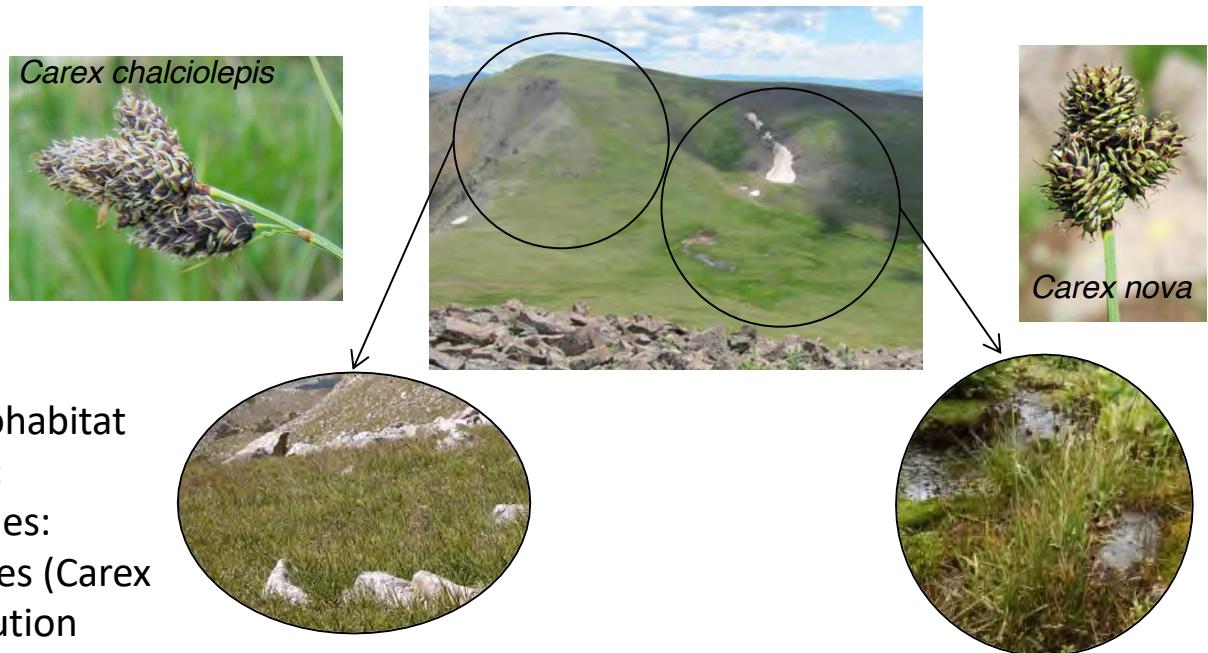
$5.68 \times 10^{-6}$   
(0.08)

Is the most probable model capable of generating the observed data ?  
(compare the L of retained simulated data sets to the L for the empirical data: “P-value”)

Massatti & Knowles (2016) Mol. Ecol.

# Refined hypotheses based on taxon-specific traits in comparative phylogeography

- statistical tests of discordant phylogeographic structure that is predicted from differences in taxon-specific traits



Massatti & Knowles LL (2014) Microhabitat differences impact phylogeographic concordance of co-distributed species: genomic evidence in montane sedges (Carex L.) from the Rocky Mountains. Evolution 68:2833-2846.

- Glaciated areas act as barriers, but only in wetland specialist



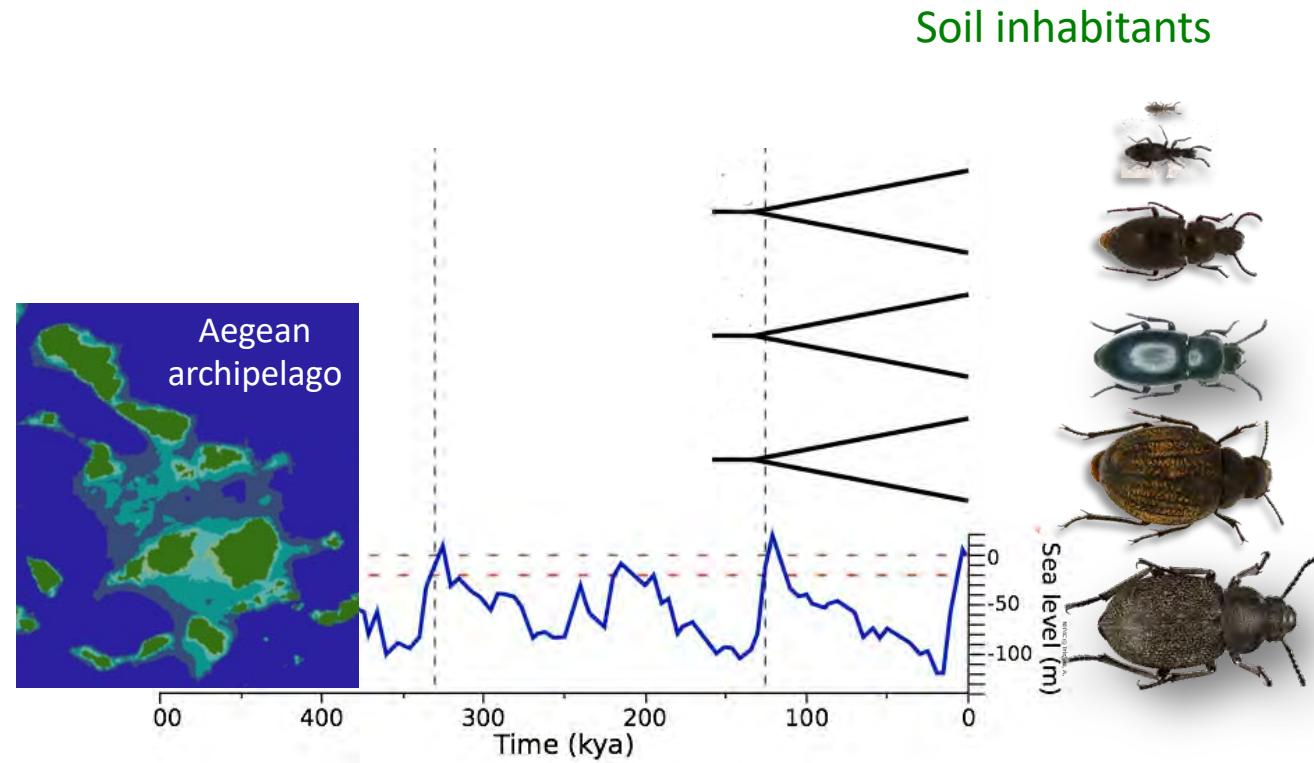
Communities may be characterized by species-specific responses to climate change



# Inference based on samples from communities

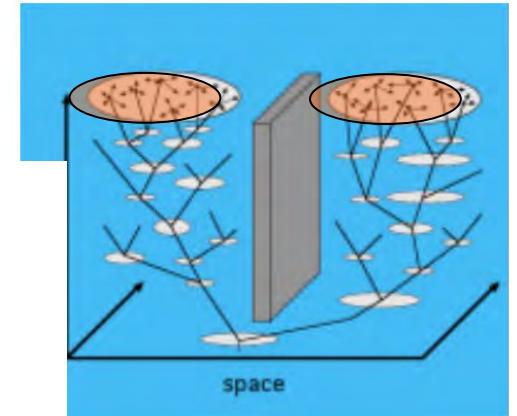
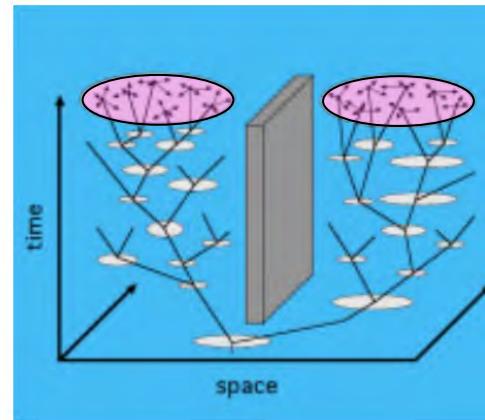
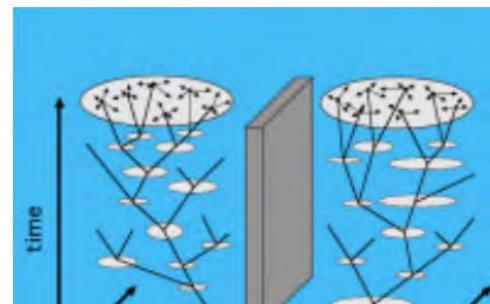
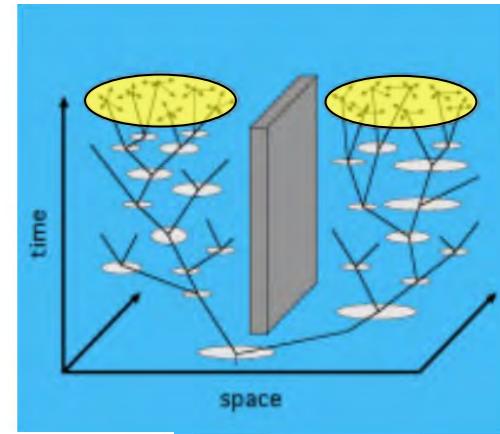
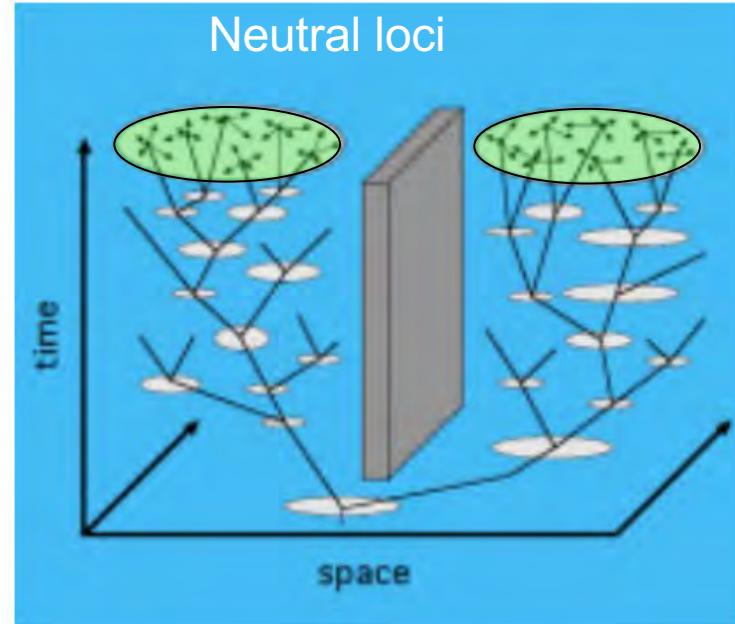
- How we use similarity of the association between genes and geography across species to test evolutionary hypotheses
- Importance of considering refined-hypotheses based on taxon-specific traits

# Refined hypotheses based on taxon-specific traits in comparative phylogeography



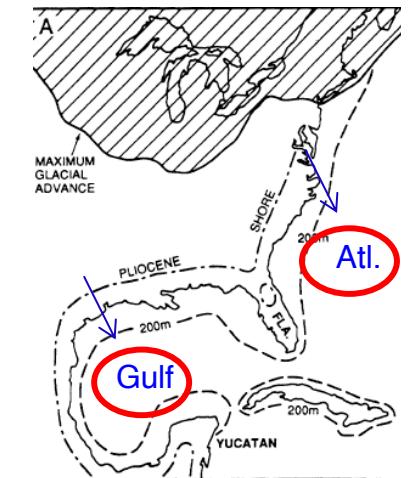
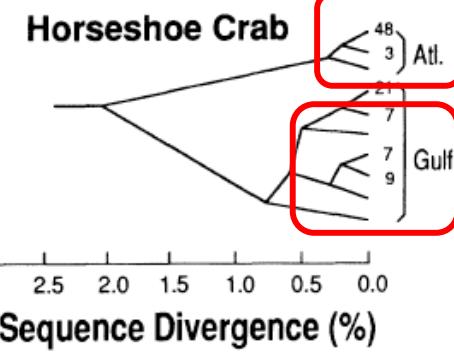
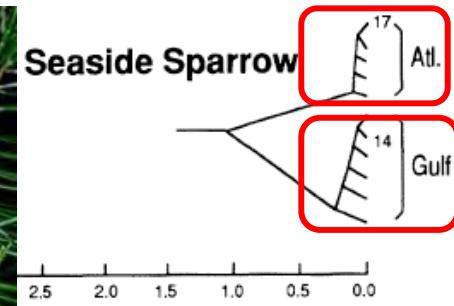
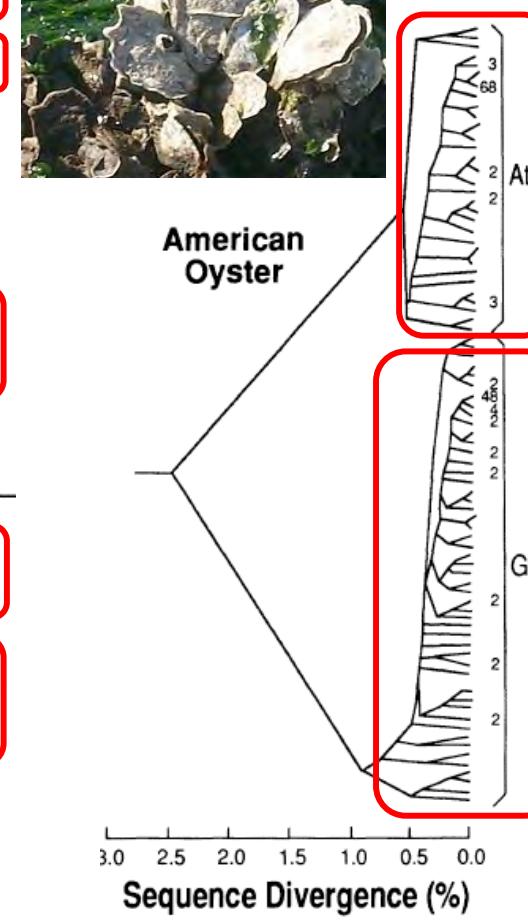
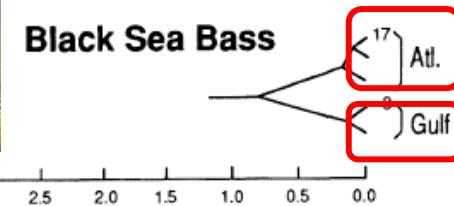
- key to avoid misleading inference
- bias toward tests of the effects of abiotic factors if rely on similarity in genetic structure across taxa for hypothesis testing

# Genes and Geography Across Species



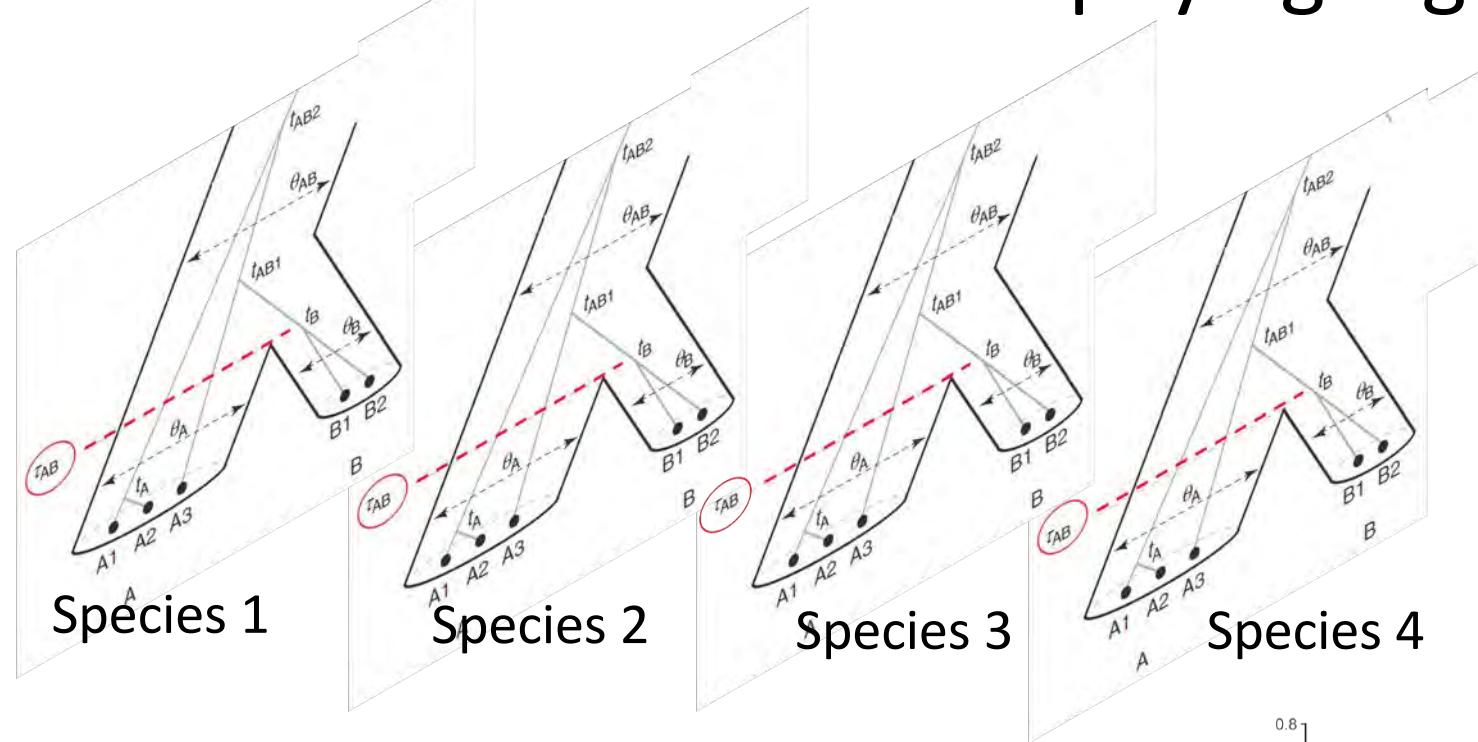
similarity of the association between genes and geography across species – **CONCORDANCE** – is typically used to test evolutionary hypotheses

# Concordance used in descriptive studies

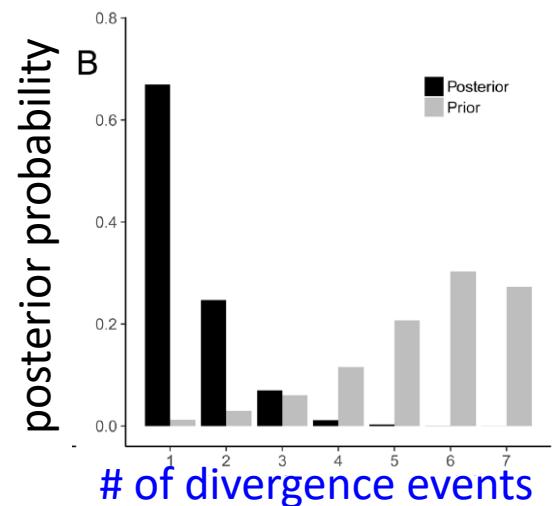


Avise 1992

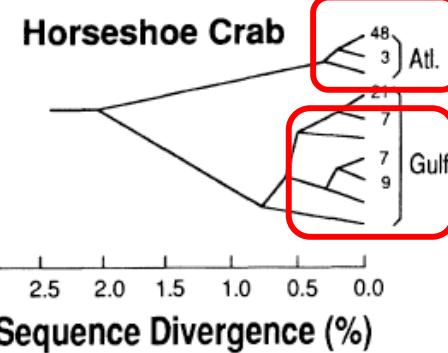
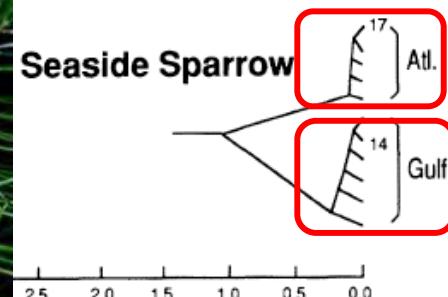
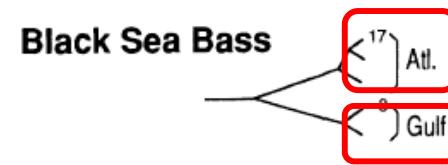
# Concordance used in statistical phylogeography



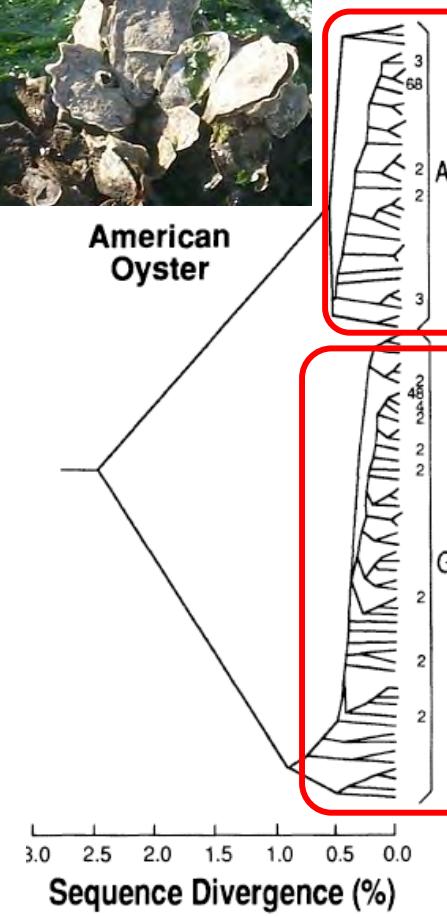
Statistically evaluate a parameterized model  
of co-divergence among species using  
hierarchical Approximate Bayesian  
Computation (hABC)



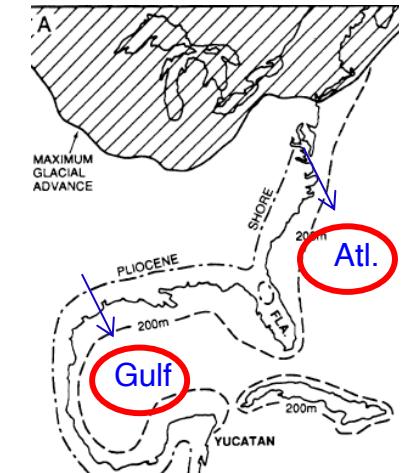
# Concordance to test hypotheses



American Oyster



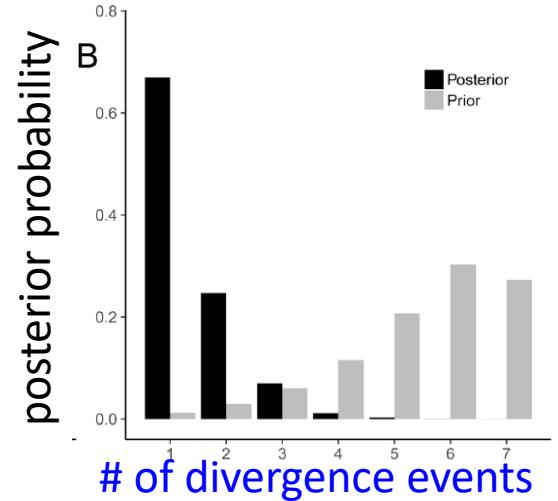
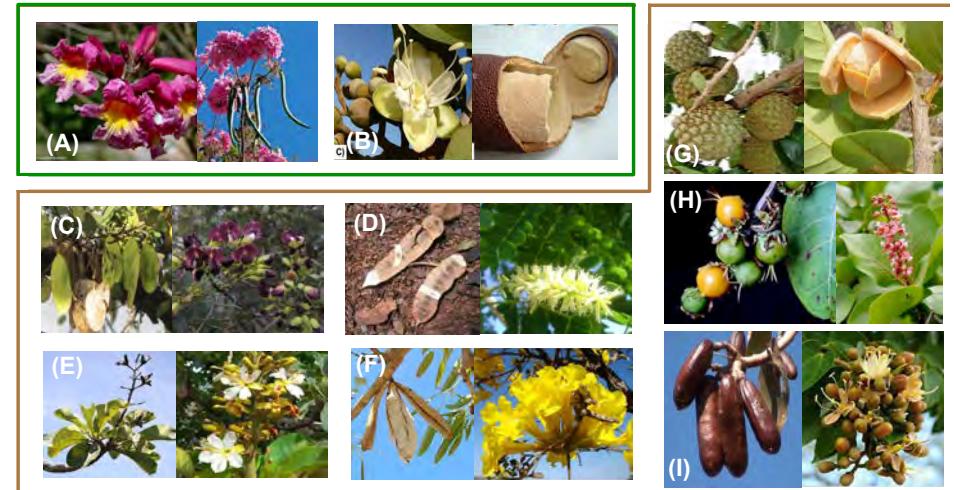
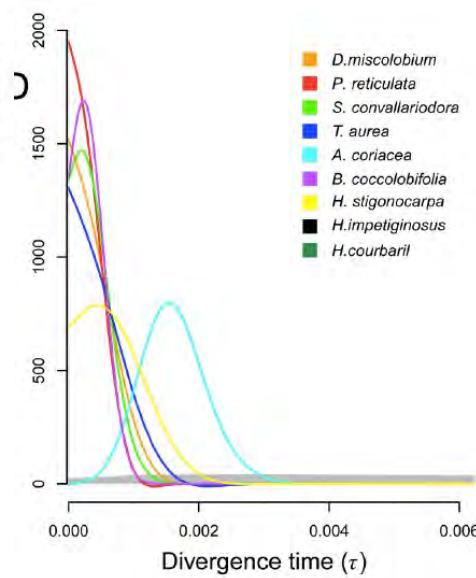
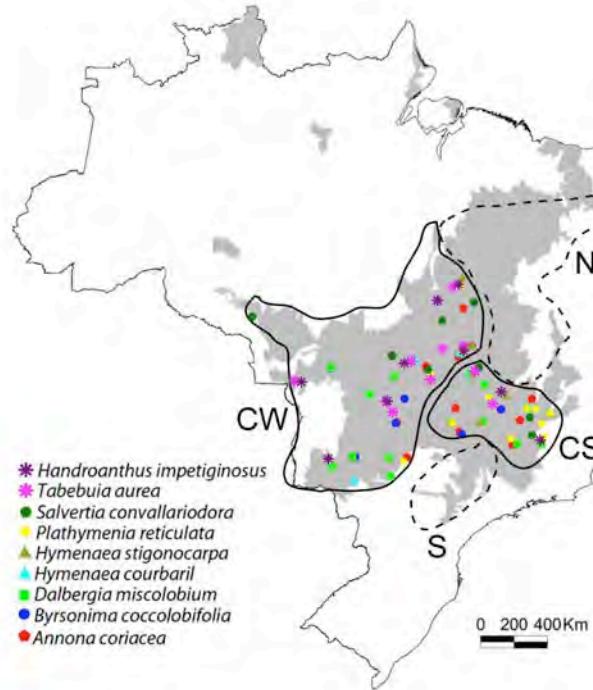
Biogeographic barrier



Avise 1992

# Concordance to test hypotheses using hABC

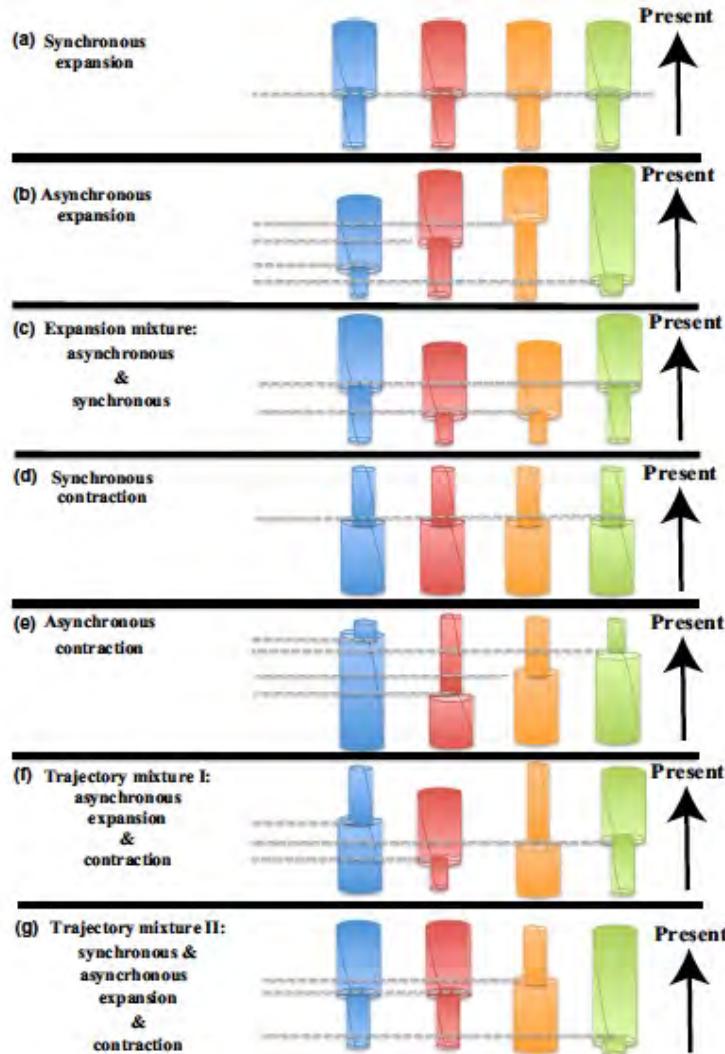
Estimate degree of co-divergence among species to evaluate hypothesized barrier associated with floristic provinces in Cerrado



concordance is a hyper-parameter in model that is estimated from genetic data across multiple species

Resende et al. (in prep)

# Concordance to test hypotheses of co-expansion



ECOLOGY LETTERS

*Ecology Letters*, (2016) 19: 1457–1467

doi: 10.1111/ele.12695

Asynchronous demographic responses to Pleistocene climate change in Eastern Nearctic vertebrates

Burbrink et al. 2016

# Genes and Geography across species



## CONCORDANCE

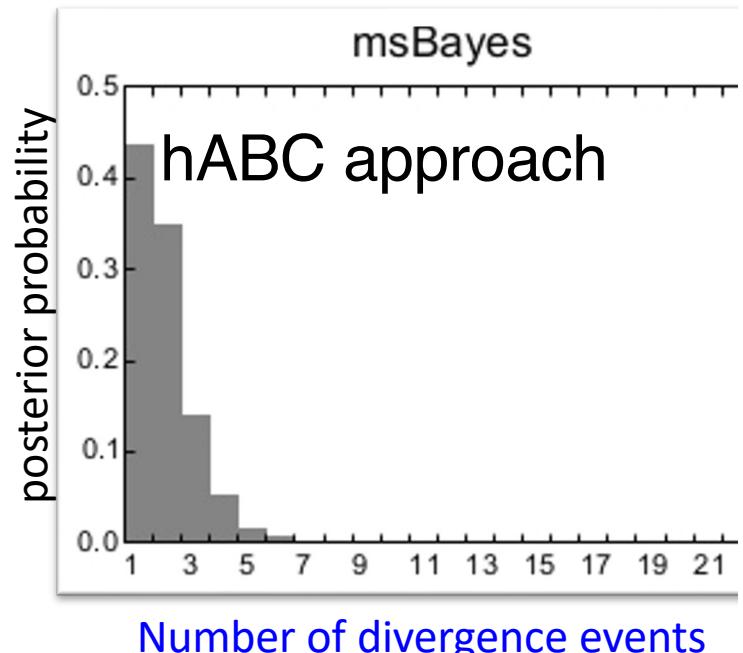
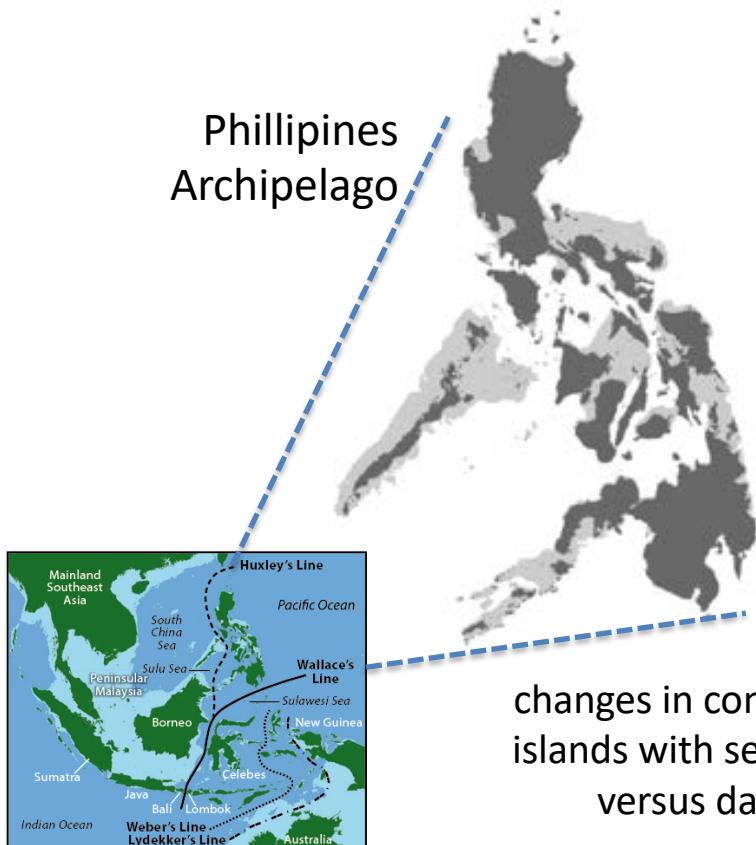
for testing hypotheses  
about evolutionary history

- potential for misleading inference by not considering both biotic and abiotic components

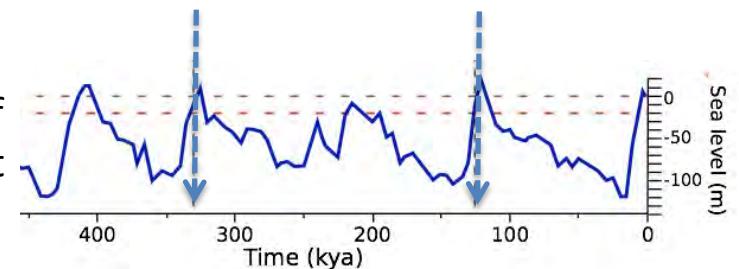
# Concordance criteria for hypothesis testing

Hypothesis of simultaneous divergence to test whether sea-level oscillations during the Pleistocene caused diversification

Oaks et al. (2012) *Evolution*

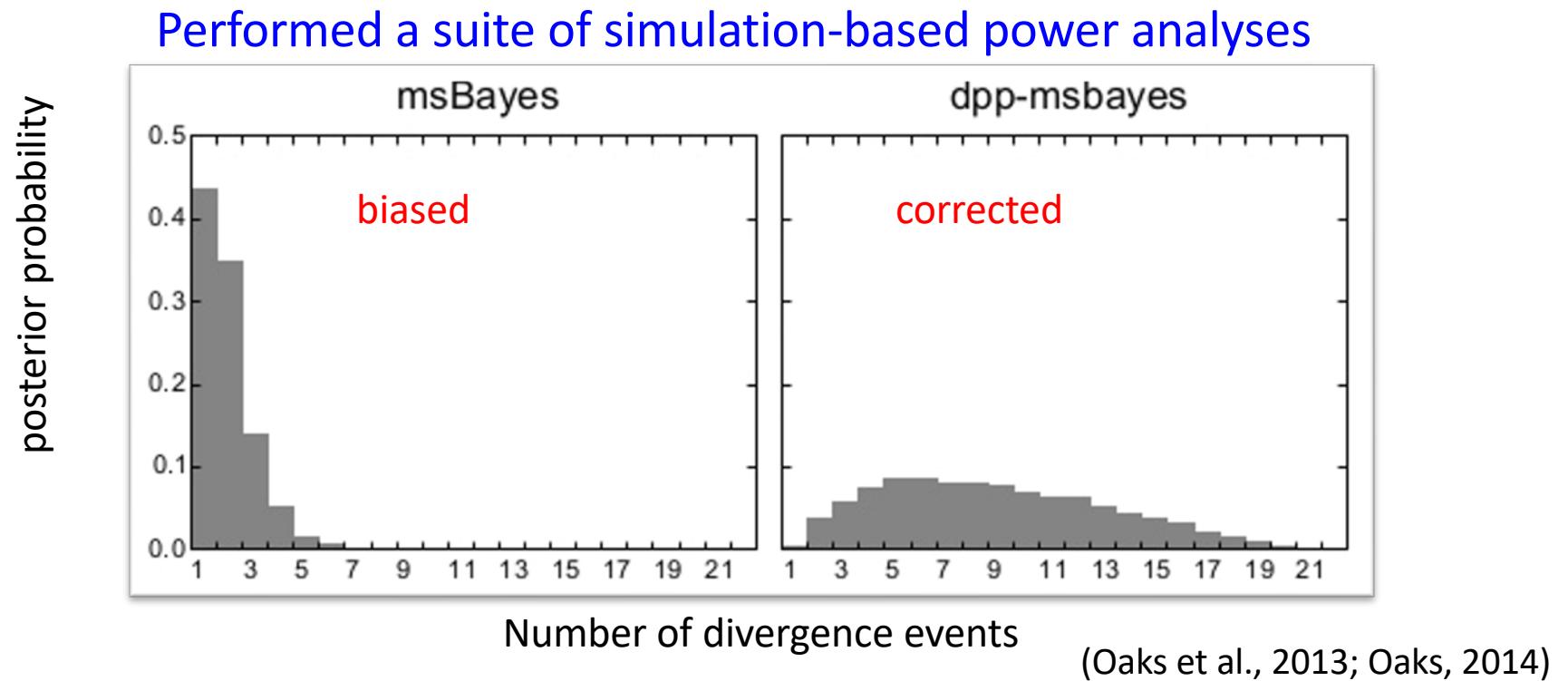


- Inferred the distribution of divergence times among 22 pairs of co-distributed vertebrate taxa



## Concordance criteria for hypothesis testing

Hypothesis of simultaneous divergence to test whether sea-level oscillations during the Pleistocene caused diversification



Should this be interpreted as a rejection of the “species pump” model of diversification in which sea-level changes drive divergence?

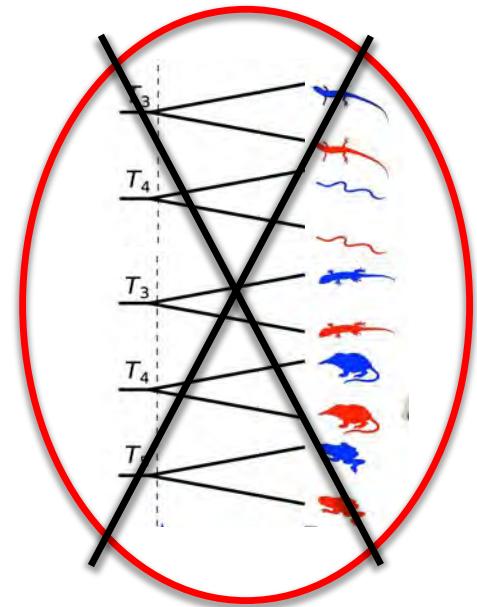
# Hypothesis of phylogeographic concordance Is TOO generic

Hypothesis of simultaneous divergence to test whether sea-level oscillations during the Pleistocene caused diversification



(Oaks et al., 2013; Oaks, 2014)

Should this be interpreted as a rejection of the “species pump” model of diversification in which sea-level changes drive divergence?



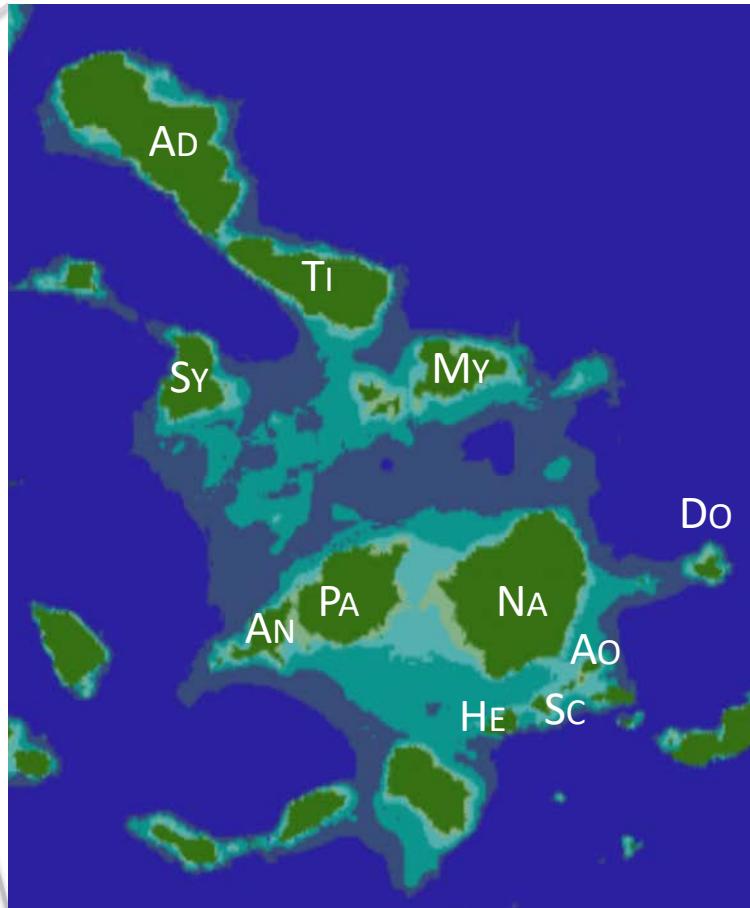
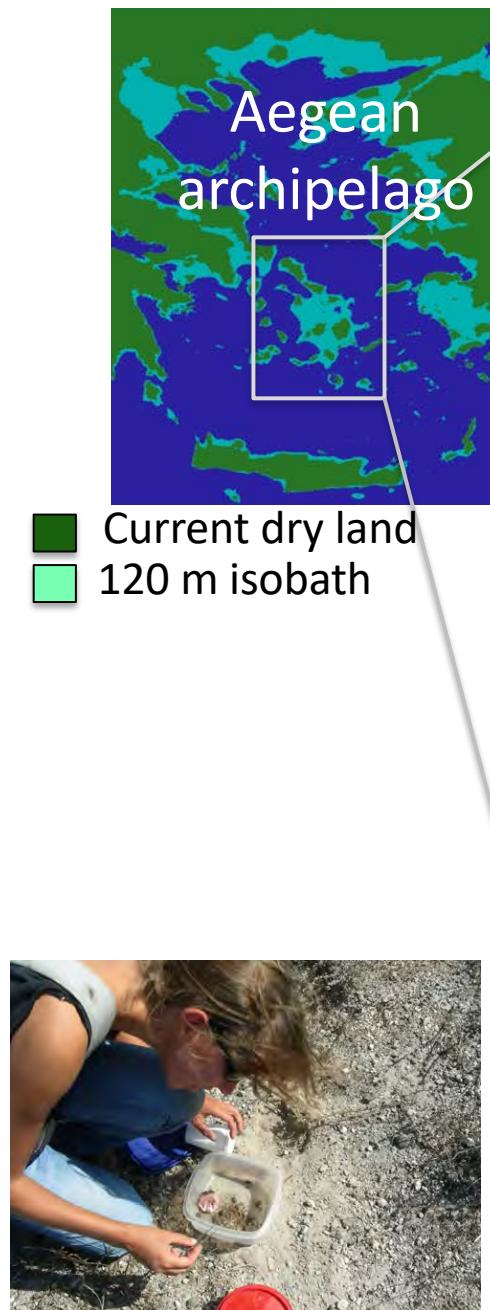
# ~~Generic~~ Refined hypothesis of phylogeographic concordance

- a study design that considers **taxon attributes**

Hypothesis of simultaneous divergence to test whether sea-level oscillations during the Pleistocene caused diversification

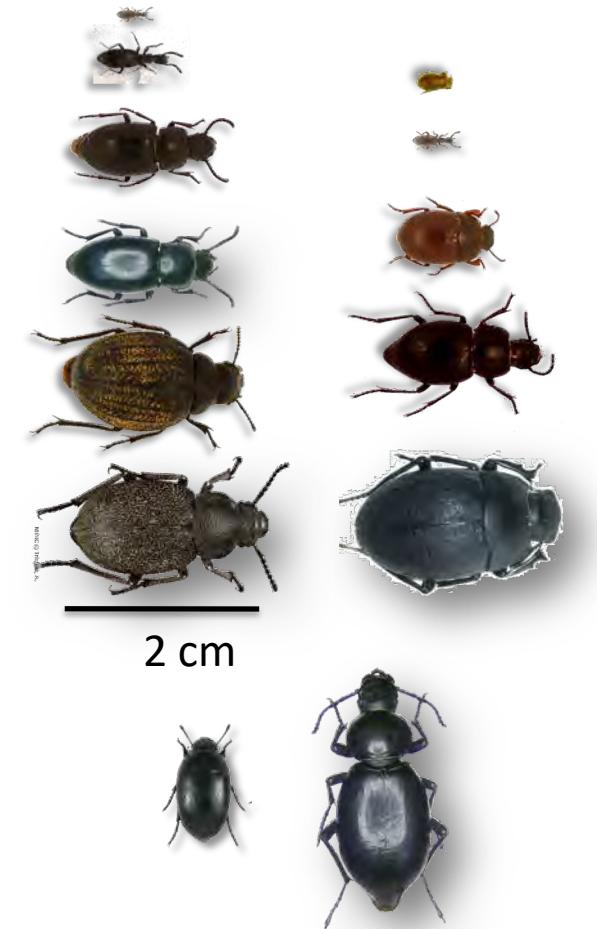


## Refined models of phylogeographic concordance to test the “species pump” model



current dry land  
0 to -30 m  
-30 to -60 m

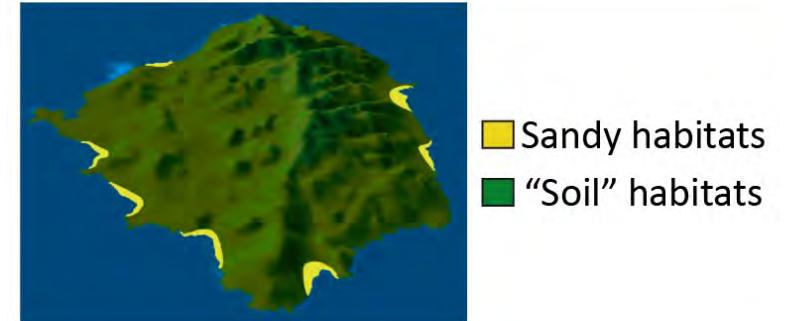
-60 to -90 m  
-90 to -120 m  
below -120 m



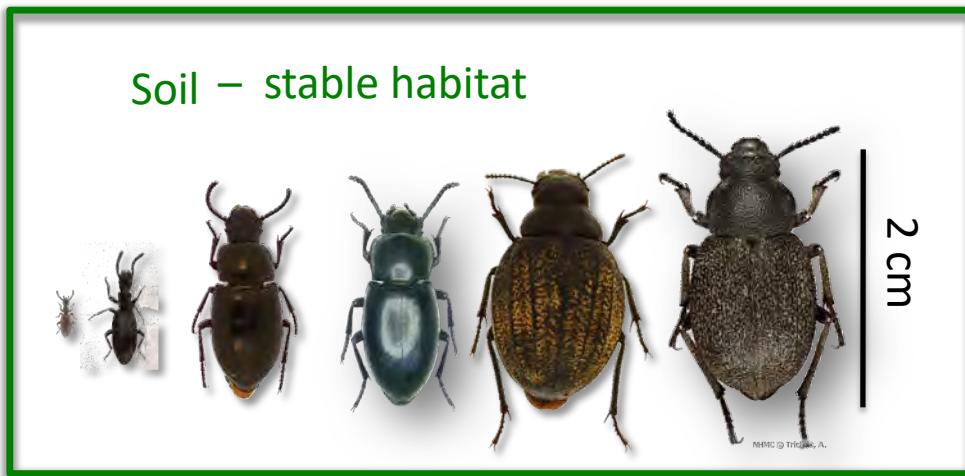
13 species of darkling beetles  
(Coleoptera: Tenebrionidae)

Papadopoulou & Knowles(2015) *Mol. Ecol.*

- taxa differ in their soil associations



Ephemerality of sand habitats may supersede effects of sea-level connections!

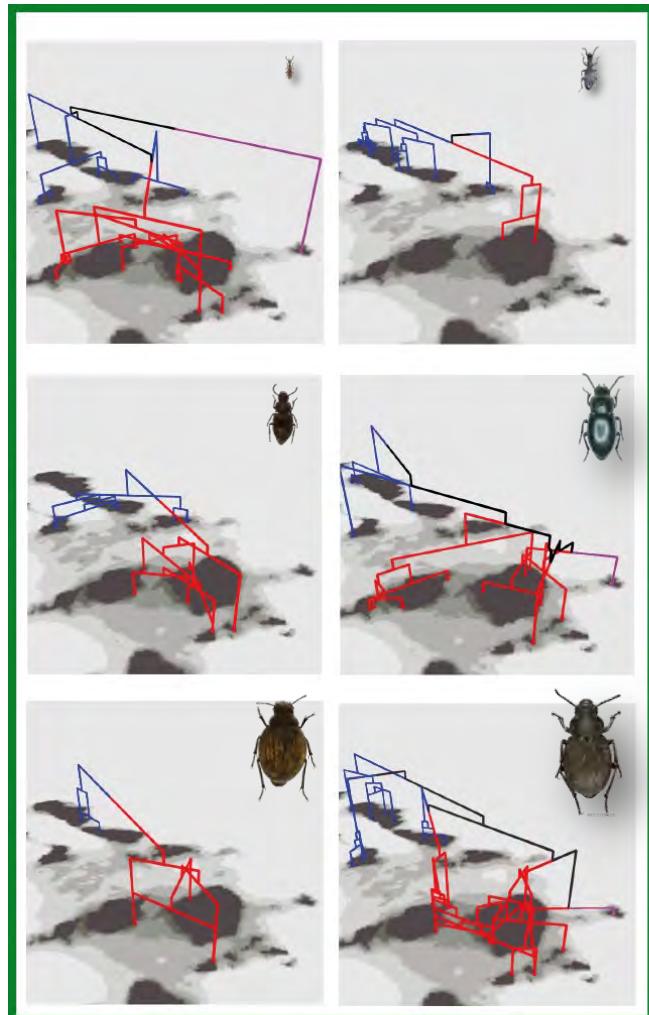


- uniform trophic ecology & inherent dispersal abilities

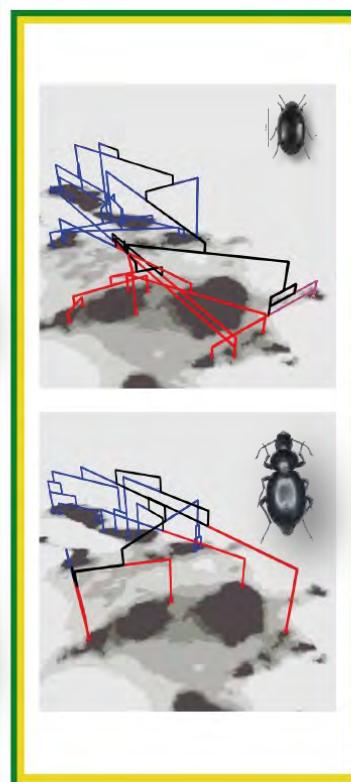
Papadopoulou & Knowles(2015) *Mol. Ecol.*

## Different degrees of structure of mtDNA gene trees suggestive of differences in habitat stability

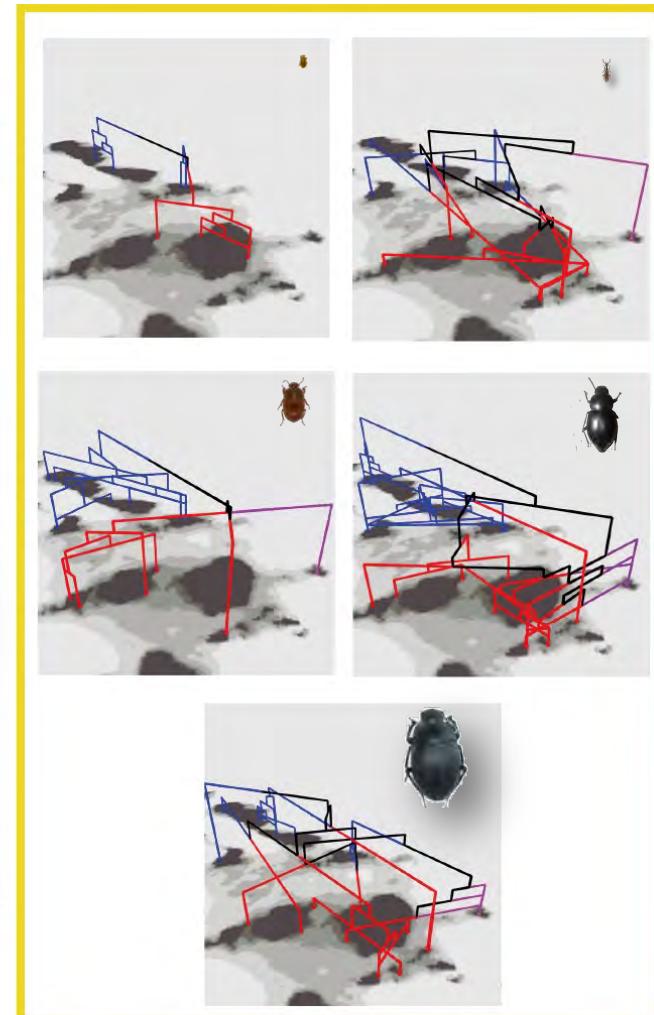
Soil – stable habitat



generalists  
both stable  
and disturbed  
habitats

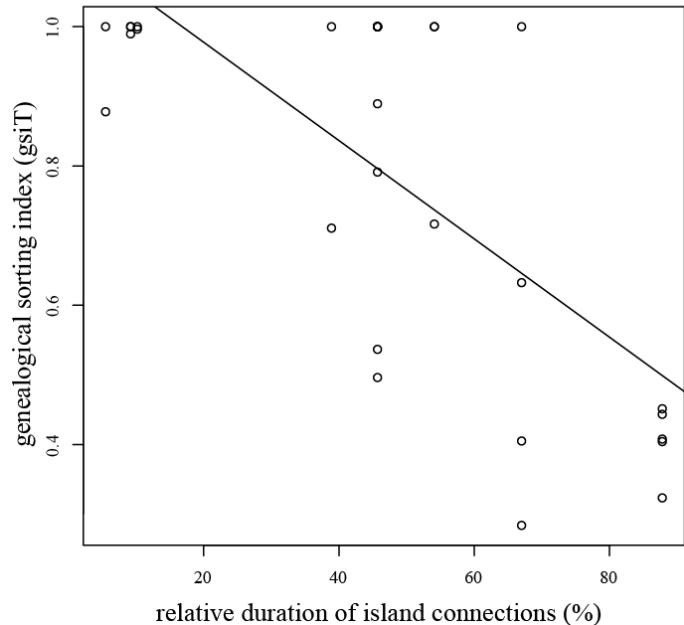


Sand – disturbed habitat

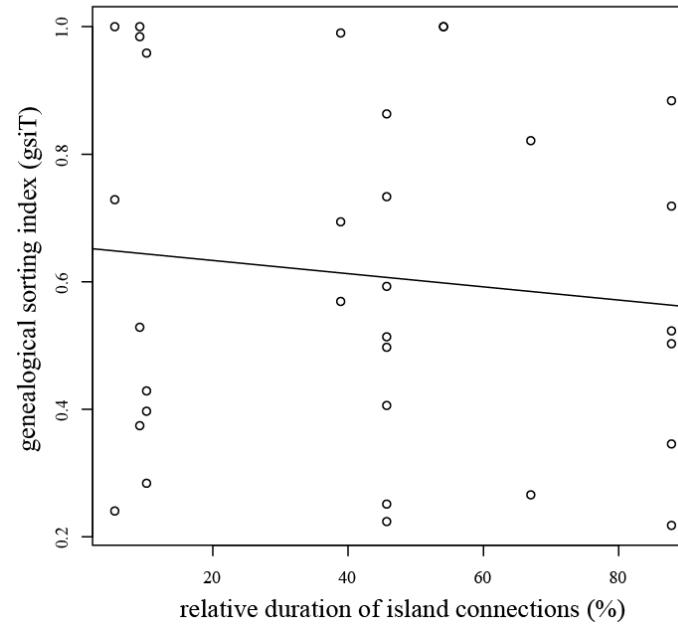


■ Northern Islands bathymetrically separated by 95m trench from ■ Southern islands

## Degree of lineage sorting correlated with duration of island connections?



Soil – stable habitat:  $R^2_{adj}=0.48$ ,  $p<<0.001$

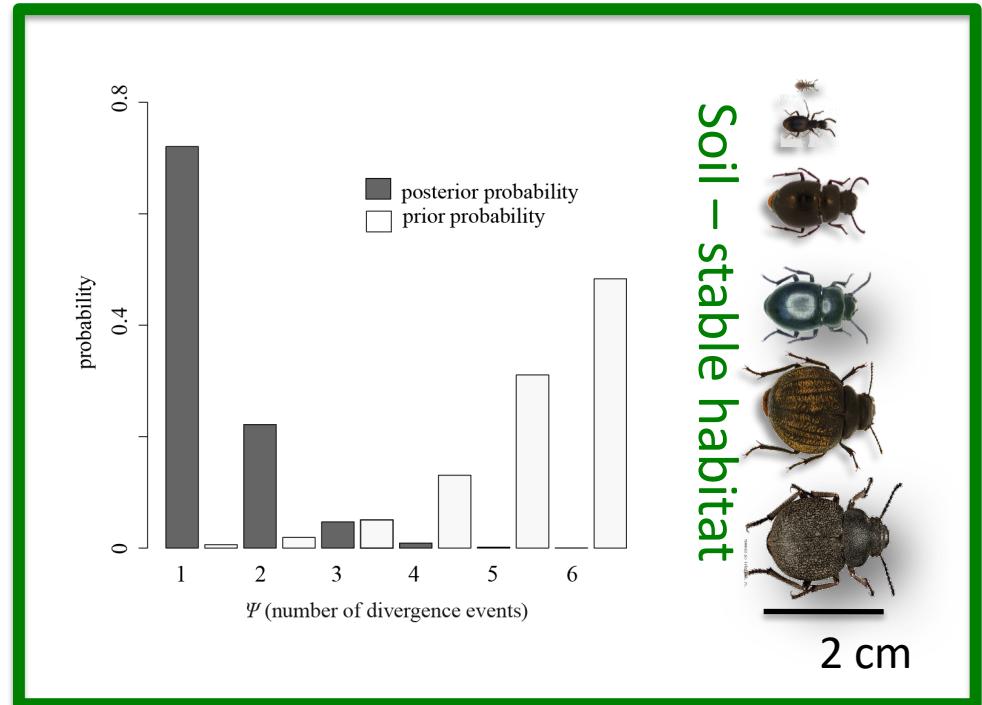


Sand – disturbed habitat:  $R^2_{adj}=0.01$ ,  $p=0.54$

Model comparisons in subsequent analyses also identified the relative duration of island connection in combination with habitat type as the best predictors of genealogical sorting (in contrast to other explanatory variables such as body size or island size) based on AICs

Refined hypothesis for tests of concordance that focus on stable-habitat taxa

### Test of simultaneous divergence

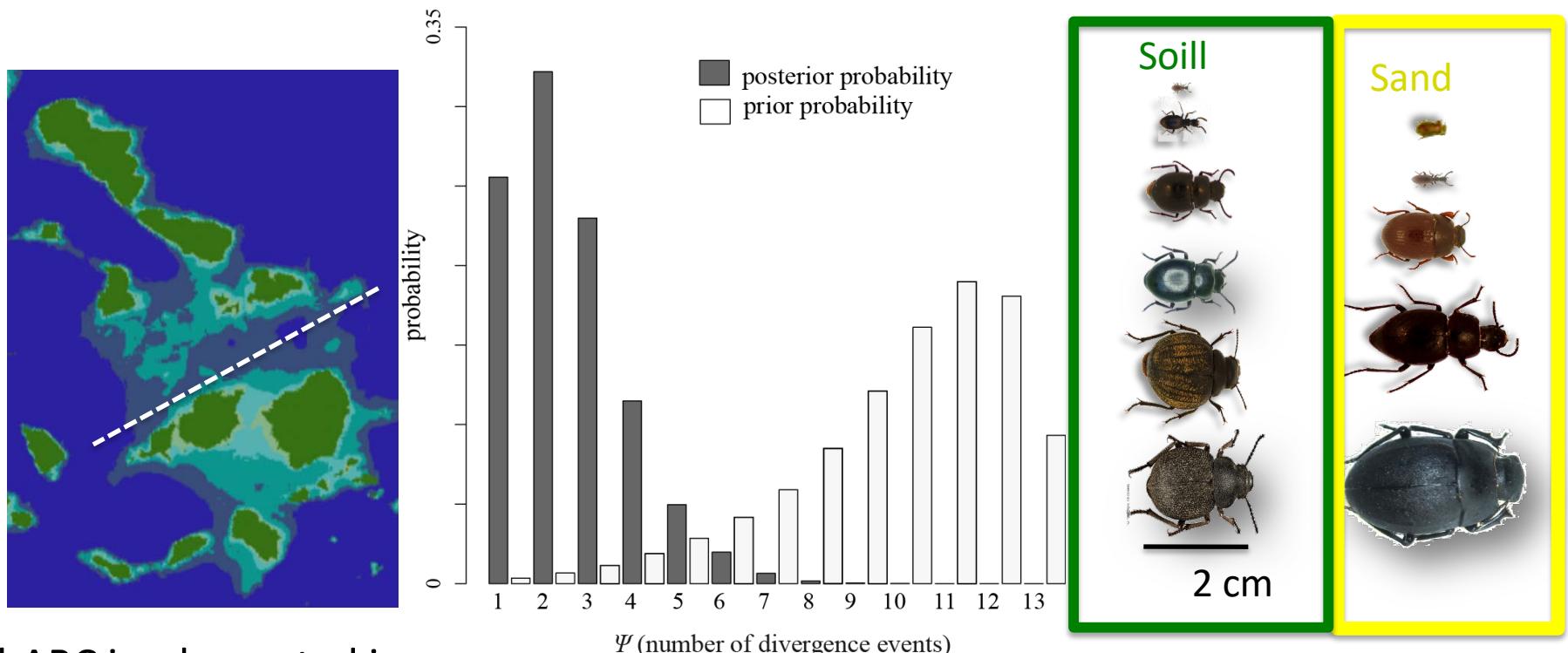


hABC: hierarchical Approximate Bayesian Computation;  
Implemented in dpp-msbayes (Oaks, 2014)

By focusing on ecologically equivalent taxa, test of concordance supported the species pump model of divergence

# Generic hypotheses of global phylogeographic concordance

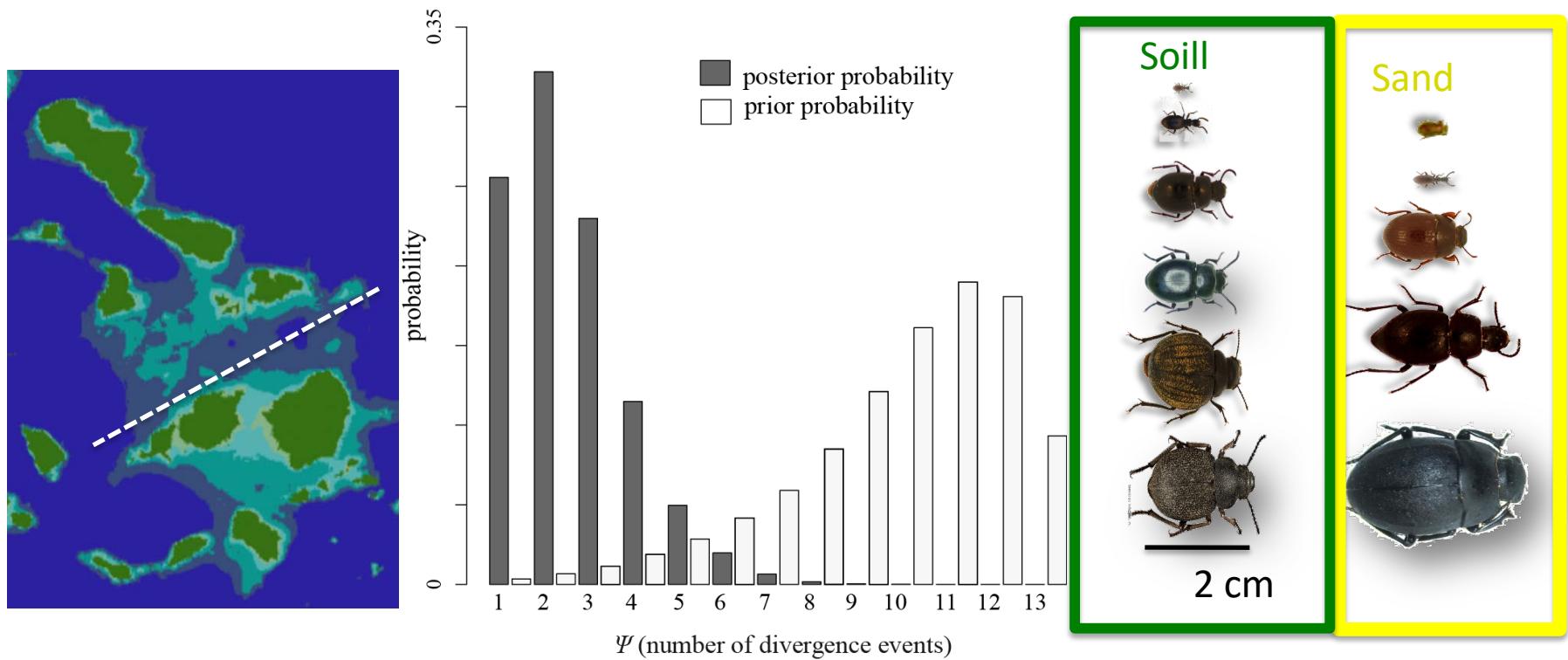
No evidence for simultaneous divergence



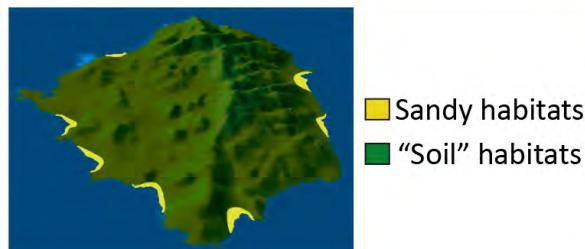
hABC implemented in  
[dpp-msbayes](#) (Oaks, 2014)

# Generic hypotheses of global phylogeographic concordance

No evidence for simultaneous divergence



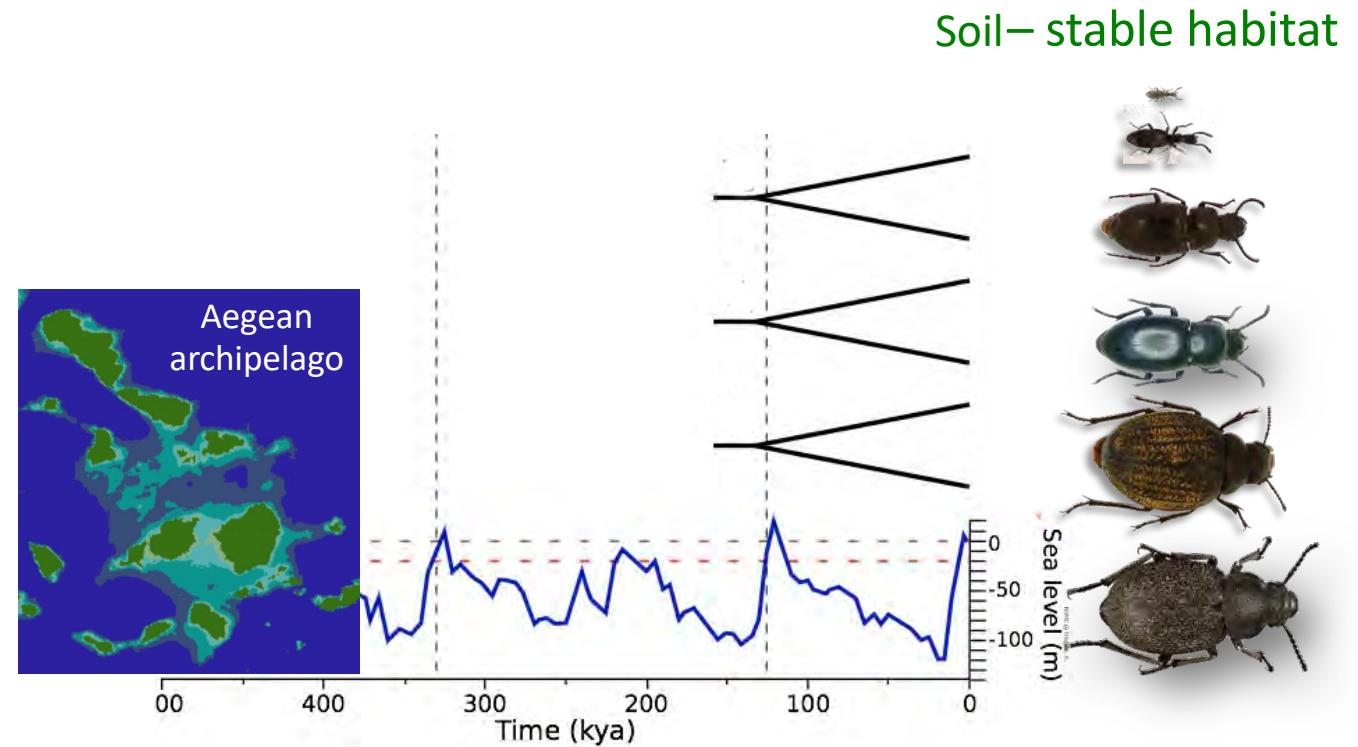
Ephemerality of  
sand habitats!



Lack of global concordance → rejection of species pump model of divergence ???

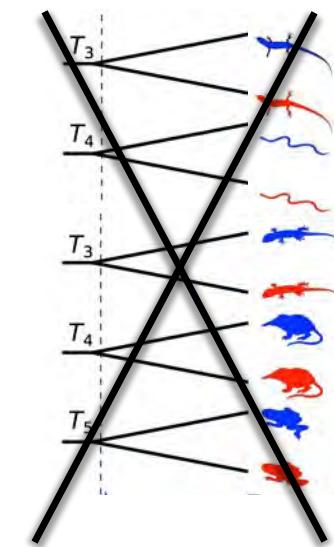
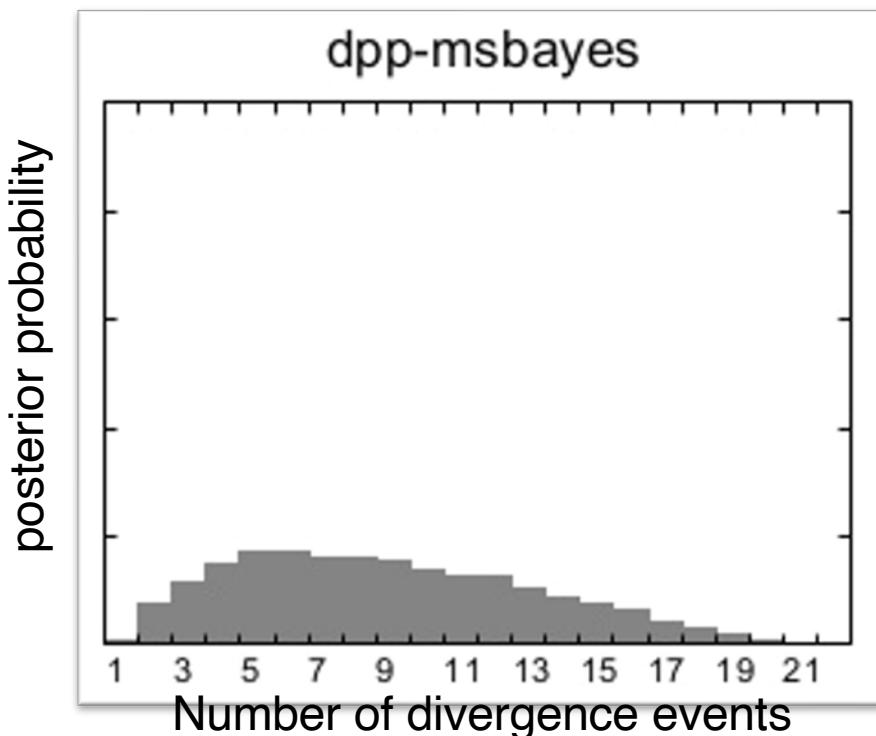
Papadopoulou & Knowles (2015) *Mol. Ecol.* 24: 4252-4268

# Refined hypotheses based on taxon-specific traits in comparative phylogeography



- refinement of the expectation for concordance is needed for concordance itself to be a meaningful metric
- reduced predictive power of **generic** hypotheses – their rejection leads to inconclusive statements that do not offer particularly meaningful insights

- comparative phylogeographic methods are designed to quantify congruence, rather than gain insights from discordant patterns
  - indirectly encourages users to emphasize idiosyncratic aspects of history!



- ad hoc interpretations of discordance
- NEED development/application of methods for statistical evaluation of phylogeographic discord as an expectation

- Model formulation is a way of communicating our expert knowledge to statistical apparatus to test hypotheses

## Biological insights:

- (i) hypotheses that capture processes structuring genetic variation, and
- (ii) model-based approaches to evaluate statistical support for alternative hypotheses

Does microhabitat affect responses to climate change

Massatti & Knowles (2014, 2016)  
*Evolution, Mol. Ecol.*



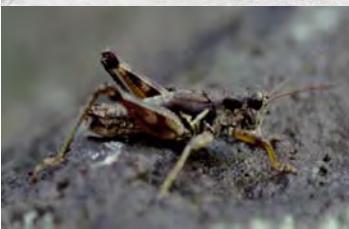
Role of habitat stability in structuring genetic variation

He et al (2013) *Evolution*



Present versus past distributions as drivers of divergence

Knowles & Massatti ( 2017) *Ecography*



Extent of distributional shifts or rate of climatic change as determinants of concordant patterns of genetic structure

Knowles et al. (2016) *J. Biogeogr.*  
He et al. (2017) *Mol Ecol.*

## Biological insights:

- (i) hypotheses that capture processes structuring genetic variation, and
- (ii) model-based approaches to evaluate statistical support for alternative hypotheses

Does microhabitat affect responses to climate change



Massatti & Knowles (2014, 2016)  
*Evolution, Mol. Ecol.*

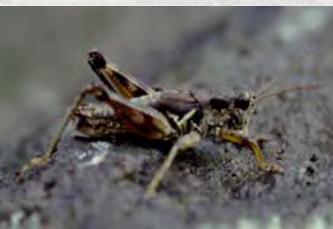
Role of habitat stability in structuring genetic variation

He et al (2013) *Evolution*



Present versus past distributions as drivers of divergence

Knowles & Massatti ( 2017) *Ecography*



Extent of distributional shifts or rate of climatic change as determinants of concordant patterns of genetic structure

Knowles et al. (2016) *J. Biogeogr.*  
He et al. (2017) *Mol Ecol.*

"The purpose of models is not to fit the data  
but to sharpen the questions."

- *Samuel Karlin*

# Evolutionary applications of model-based analyses:

- (i) Inferring species boundaries (aka species delimitation)
- (ii) Phylogenetic inference (and beyond the species tree)
- (iii) Biogeographic study
- (iv) Phylogeography
- (v) Adaptive evolution





*Myotis lucifugus*

Little brown bats are widespread in North America and were the most abundant species in the eastern US prior to white nose syndrome (WNS), which is caused by introduced fungal pathogen



## Little brown bats decimated by white nose syndrome (WNS)



Population declines > 90% since introduction of fungal pathogen that causes WNS

Dead bats in underground hibernation sites (shown here on the floor of a mine)



Others leave hibernating sites prematurely, like these dead bats on the outer screen of a house < 1 km from a hibernation site (note the snowy landscape).



*Myotis lucifugus*

Survival of the species may ultimately depend upon its capacity for adaptive change

- Compare the genetic makeup of wild survivors and non-survivors of WNS to tests for adaptive change

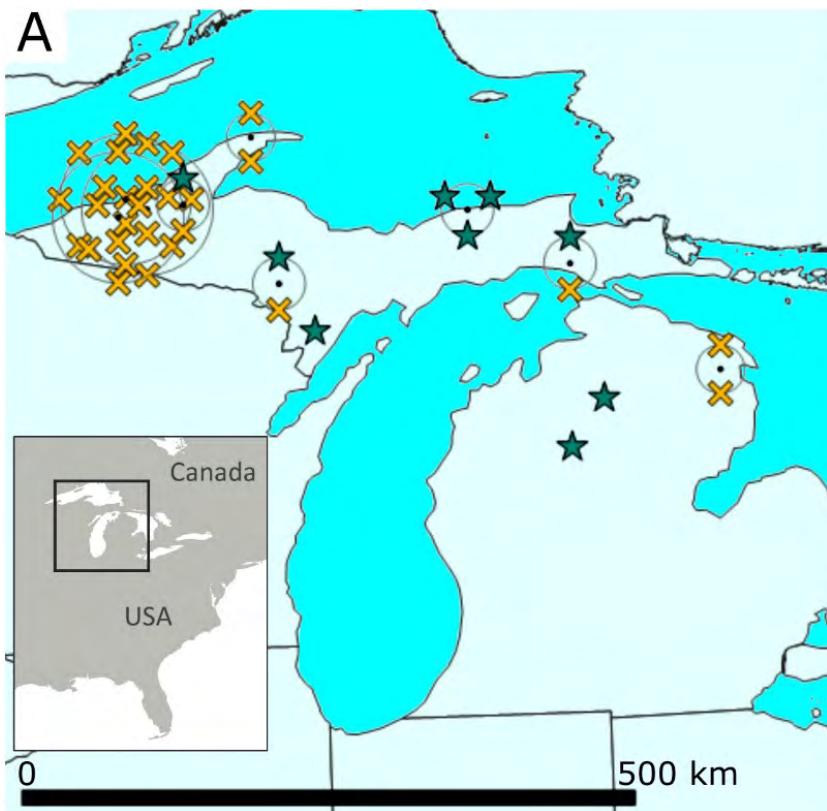
Auteri GG, Knowles LL (2020) Decimated little brown bat population show potential for adaptive change. *Scientific Reports*. 10:3023. [doi.org/10.1038/s41598-020-59797-4](https://doi.org/10.1038/s41598-020-59797-4)

Giorgia G. Auteri



Studied geographically isolated population of little brown bats

✖ non-survivor  
★ survivor

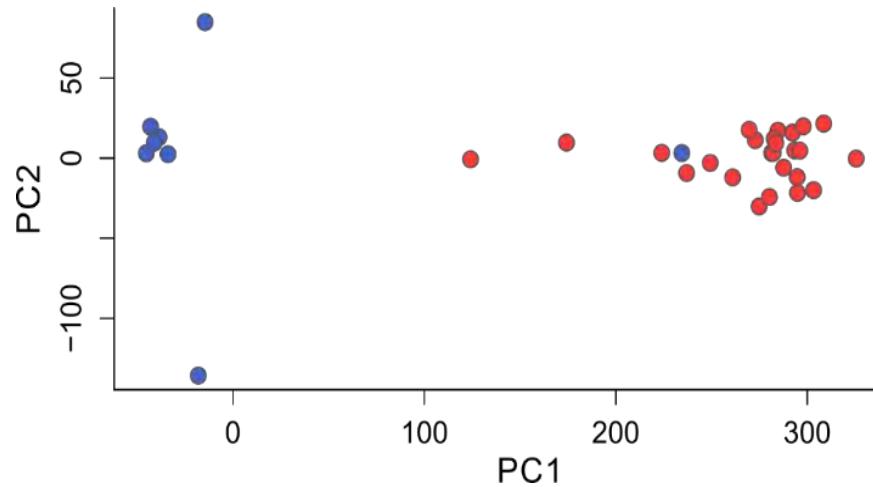


WNS arrived in 2014



- RADseq: 14,345 loci , 19,797 SNPs

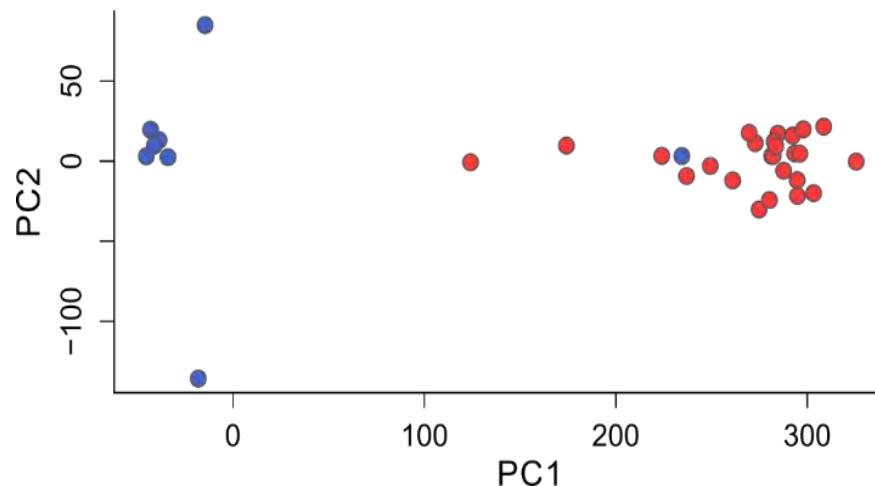
Evidence of strong genetic drift caused by the massive population losses in little brown bats.



PCA of survivors of WNS (in blue)  
with non-survivors (in red)  
projected onto the PC axes

- 14,345 SNPs and 33 individuals

Evidence of strong genetic drift caused by the massive population losses in little brown bats.



Quantified rate of evolutionary change  
from inferred ancestor  
(using F-model in STRUCTURE)

Survivor



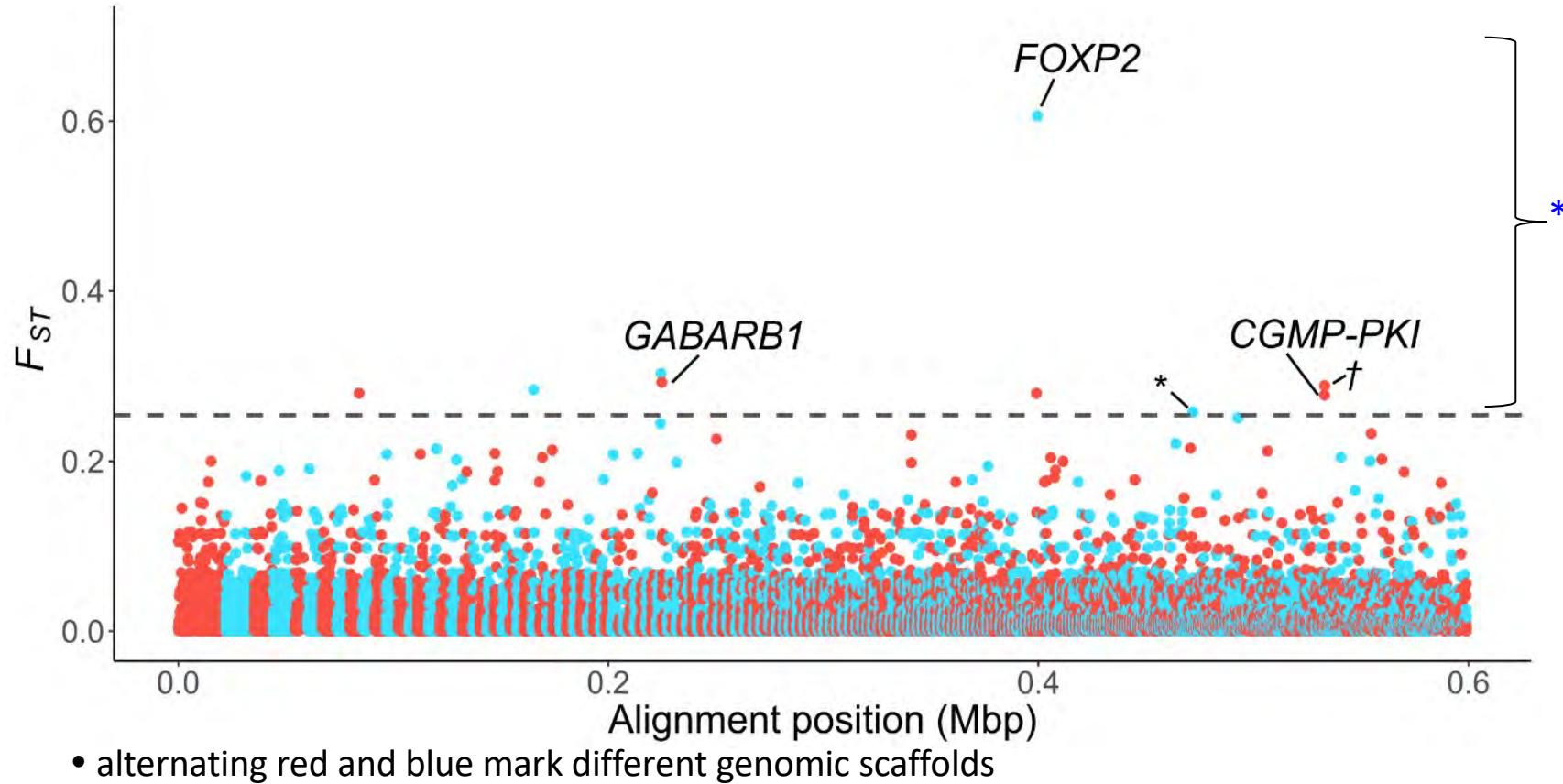
$F = 0.04$   
 $SE \pm 0.0001$

Non-survivors



$F = 0.0006$   
 $SE \pm 0.0003$

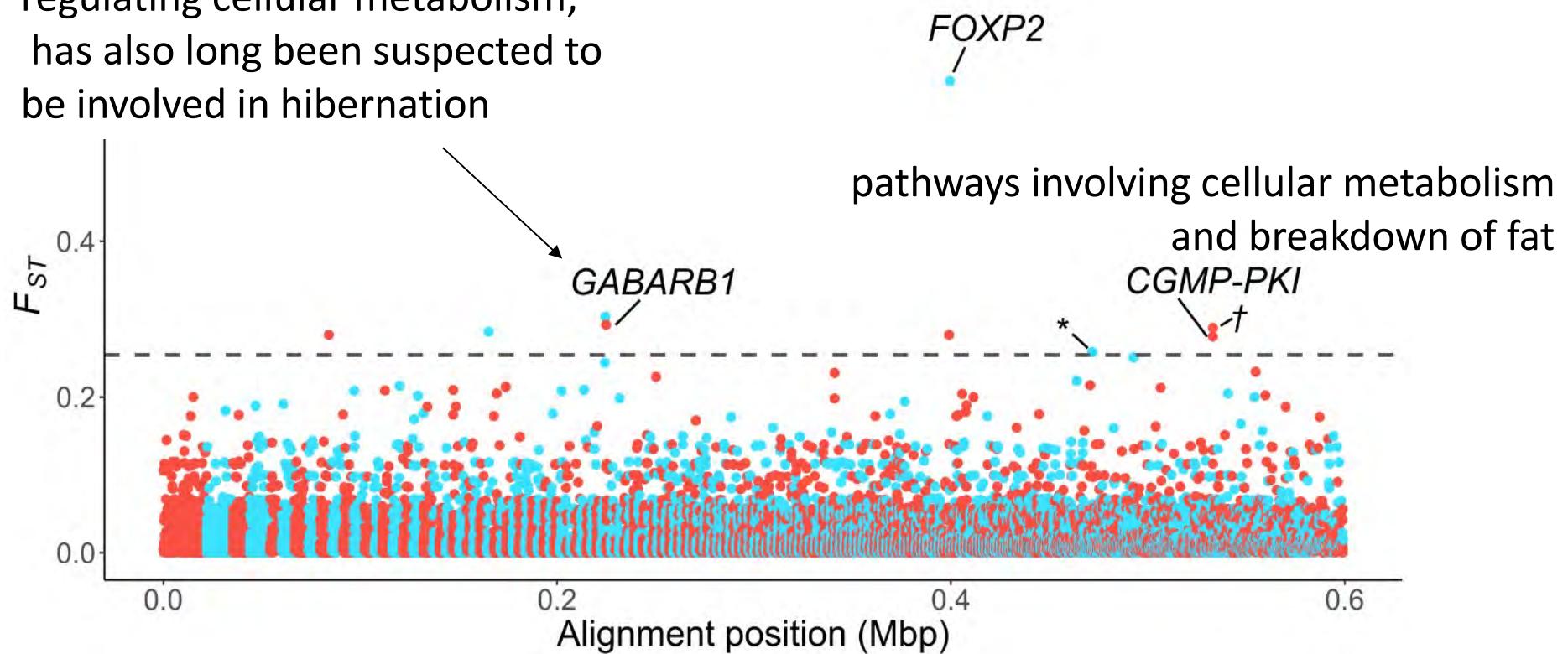
To identify genetic changes among individuals that might have contributed to their survival of WNS, as opposed to changes due to strong genetic drift, used an  $F_{ST}$ -outlier



\*signature of selection can be detected by levels of genetic differentiation at a gene that exceeds background levels across the genome

## Links between metabolic demands and survival

regulating cellular metabolism;  
has also long been suspected to  
be involved in hibernation

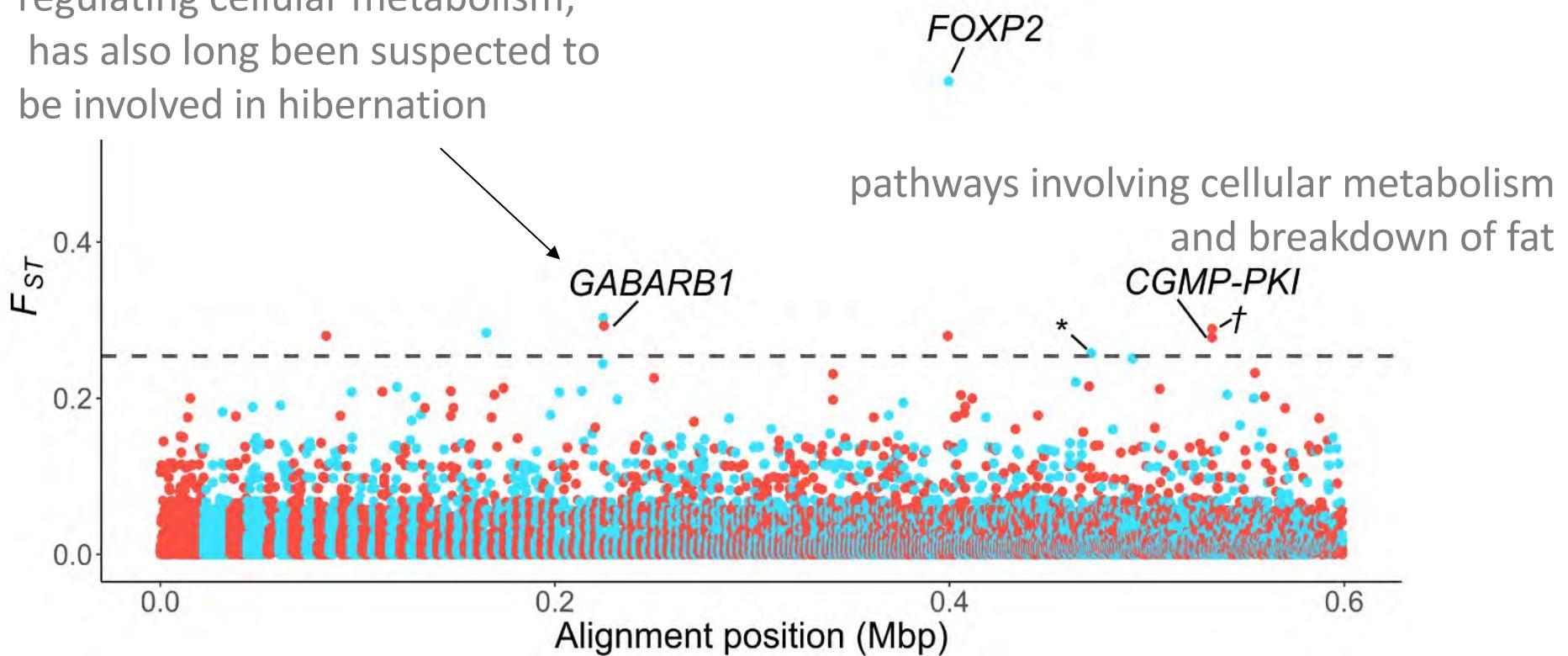


Physiological functions that make immediate sense in an adaptive context—deaths from the WNS fungus occur as a result of too frequent arousals from hibernation that causes starvation.

## Links between metabolic demands and survival

regulating cellular metabolism;  
has also long been suspected to  
be involved in hibernation

associated with vocalizations, and  
echolocation in bats



Variation in calls is closely associated with type of prey and the habitat  
bats must navigate, potentially adaptive shifts might result from  
selective pressures related to proficient hunting or prey preferences



Too soon to claim that the species will be “saved” via an evolutionary rescue effect.

Evidence of potentially adaptive evolution in the survivors of little brown bats is particularly notable on several fronts:





Too soon to claim that the species will be “saved” via an evolutionary rescue effect.

Evidence of potentially adaptive evolution in the survivors of little brown bats is particularly notable on several fronts:



- We detected selectively driven divergence, despite strong genetic drift caused by the massive population losses in little brown bats.



Too soon to claim that the species will be “saved” via an evolutionary rescue effect.

Evidence of potentially adaptive evolution in the survivors of little brown bats is particularly notable on several fronts:



- We detected selectively driven divergence, despite strong genetic drift caused by the massive population losses in little brown bats.
- These evolutionary changes were detected in less than three generations since exposure to WNS



Too soon to claim that the species will be “saved” via an evolutionary rescue effect.

Evidence of potentially adaptive evolution in the survivors of little brown bats is particularly notable on several fronts:



© Steve Byland

- We detected selectively driven divergence, despite strong genetic drift caused by the massive population losses in little brown bats.
- These evolutionary changes were detected in less than three generations since exposure to WNS
- Putatively selected loci and their potential adaptive functions point to multifaceted nature of selection (i.e., genes linked to physiological and behavioral traits, whose roles vary across habitats of highly seasonal environments)

# Evolutionary applications of model-based analyses:

- (i) Inferring species boundaries (aka species delimitation)
- (ii) Phylogenetic inference (and beyond the species tree)
- (iii) Biogeographic study
- (iv) Phylogeography
- (v) Adaptive evolution

# Species delimitation (discovery)

Learning goals:

- Describe applications of the multispecies coalescent (MSC) to species delimitation
- Explain the merit/limitations of the multispecies coalescent (MSC) to delimitation
- Describe (i) how over-estimation of species numbers might occur with applications based on the MSC (ii) what determines the degree of overestimation
- Explain the relevance of the speciation process to delimitation approaches



# Hypotheses about species boundaries

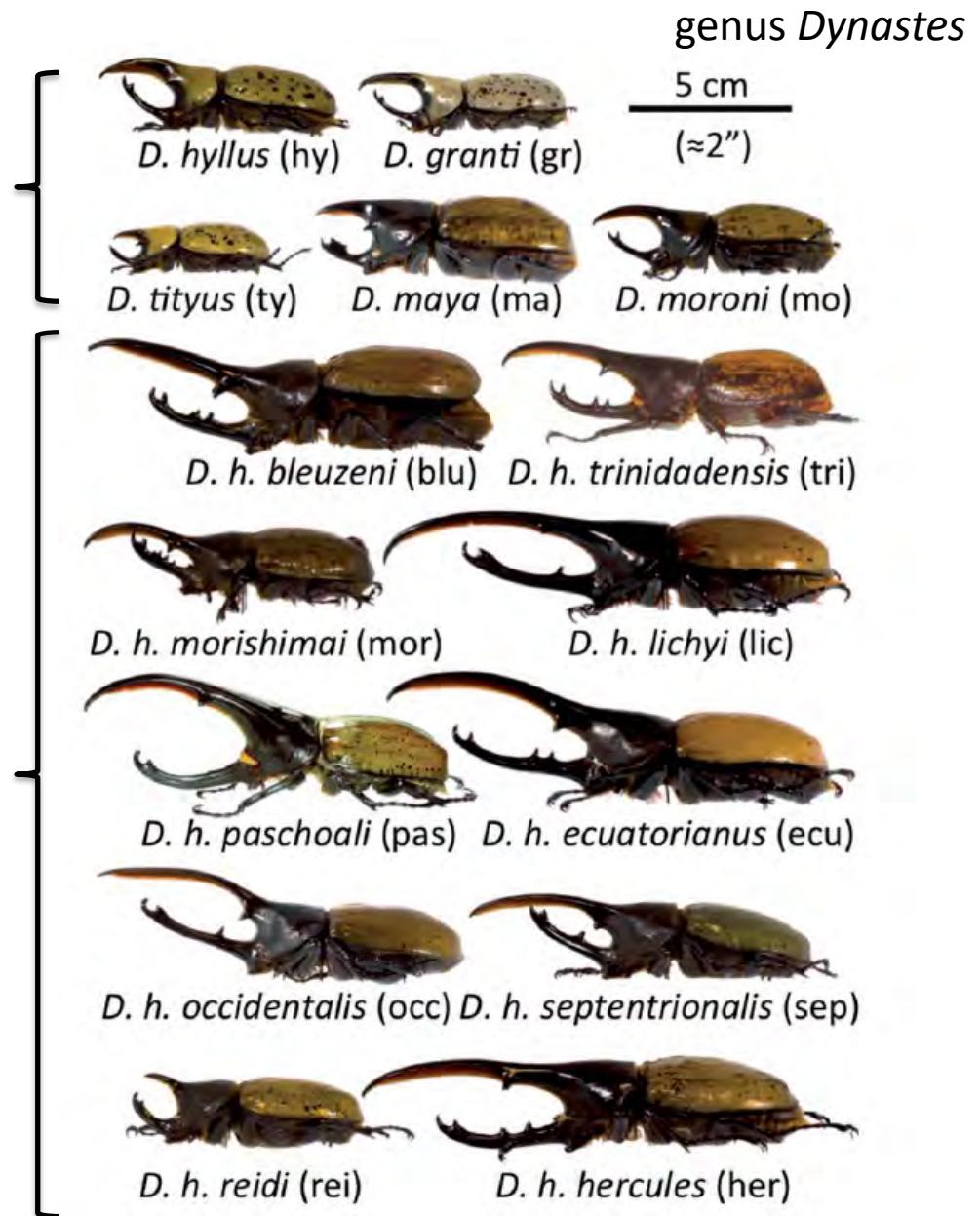


# Model-based inference of species boundaries

Statistical evaluation of a hypothesized species delimitation model

1 species

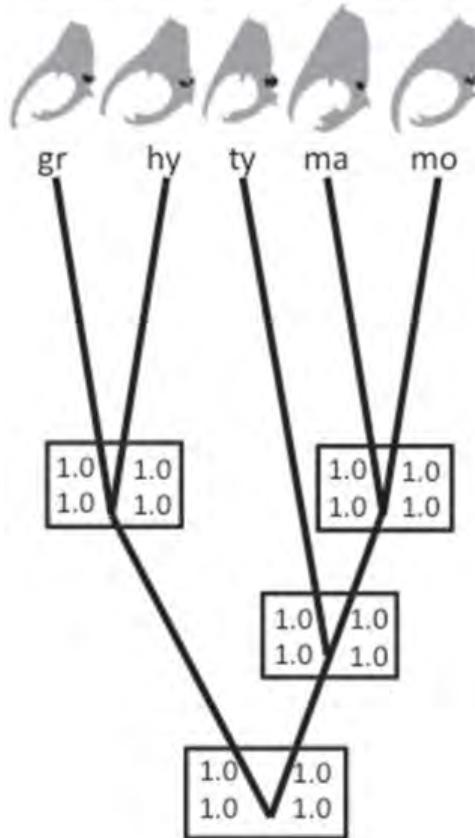
5 species



# Model-based inference of species boundaries

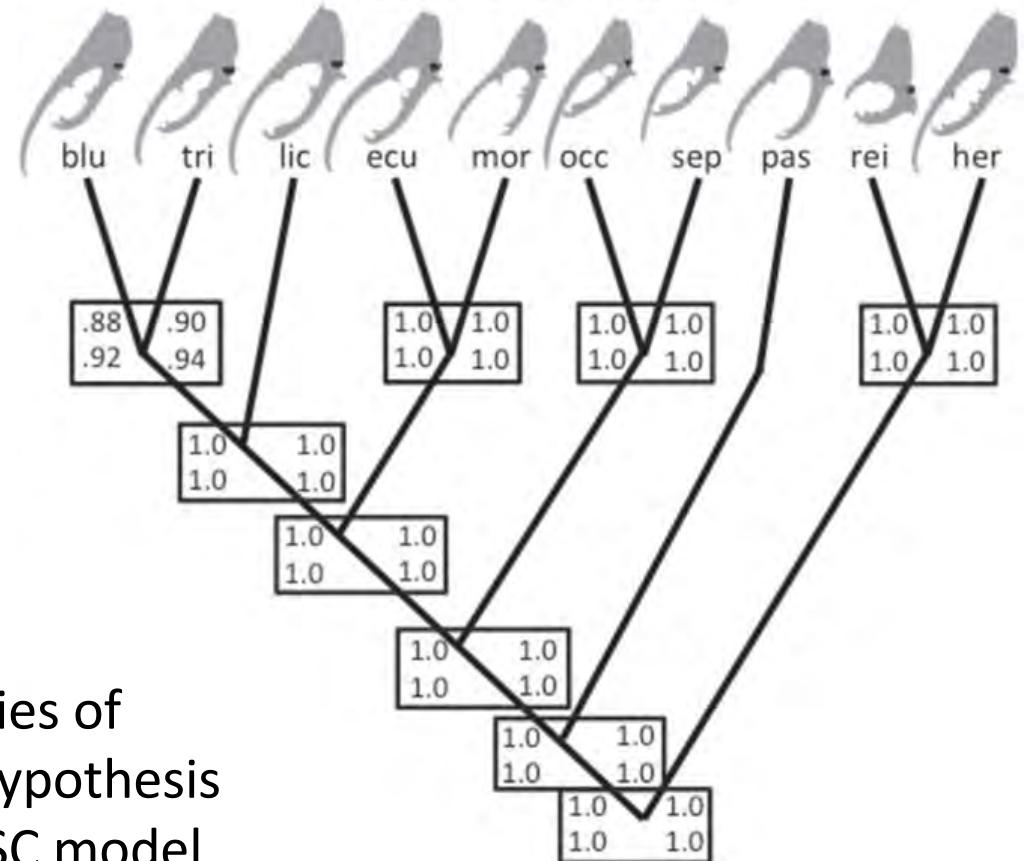
5 recognized species  
In North America

## White Hercules



1 recognized species  
In South America

## Subspecies of Giant Hercules



Probabilities of  
delimitation hypothesis  
under the MSC model

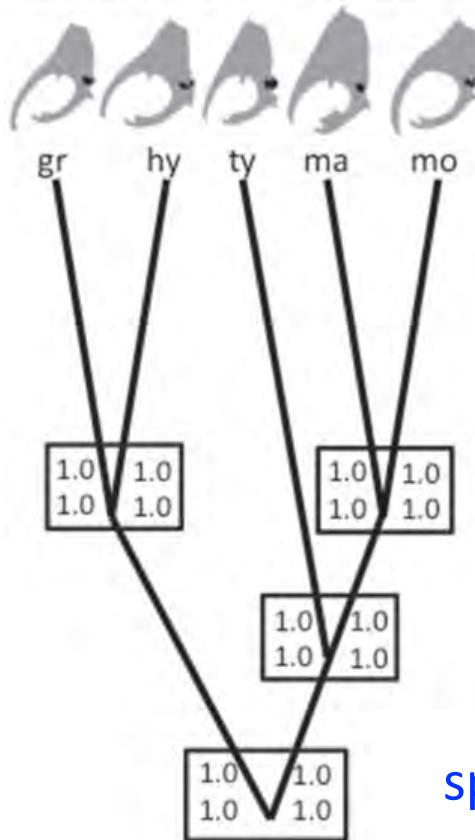
genus *Dynastes*

Huang & Knowles (2016) *Syst. Biol.*

# Model-based inference of species boundaries

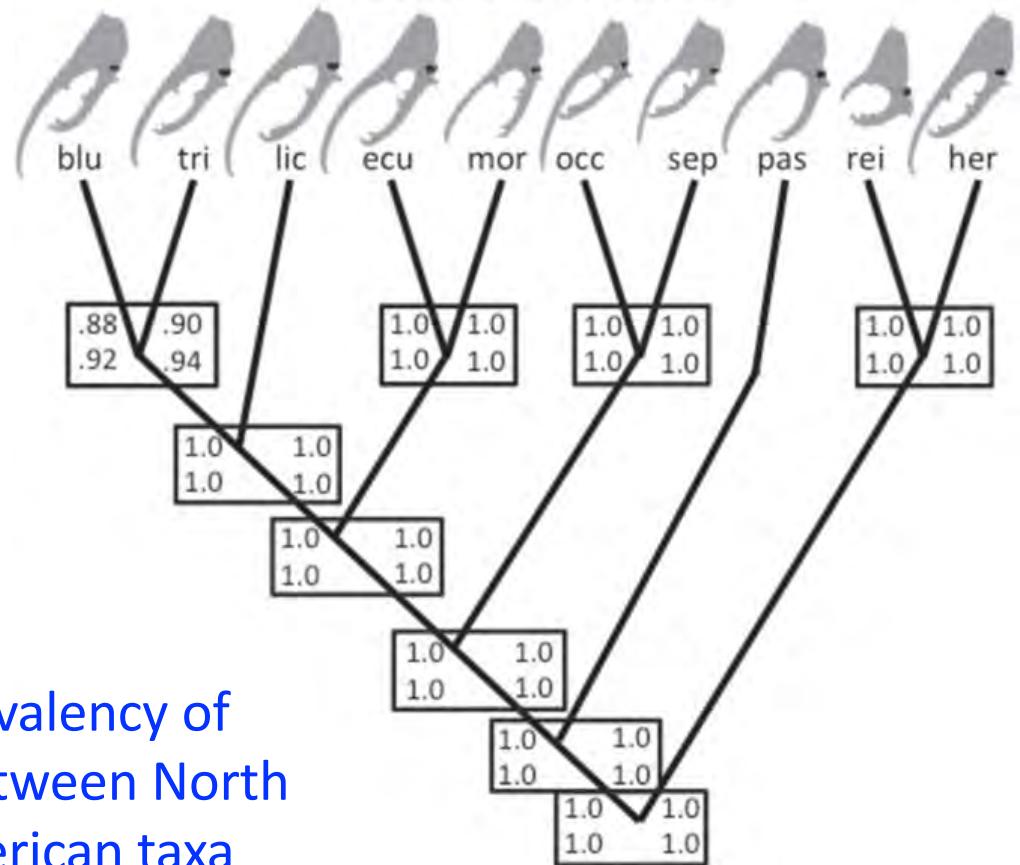
5 recognized species  
In North America

## White Hercules



1 recognized species  
In South America

## 10 inferred species of Giant Hercules



Statistical equivalency of  
species status between North  
and South American taxa

genus *Dynastes*

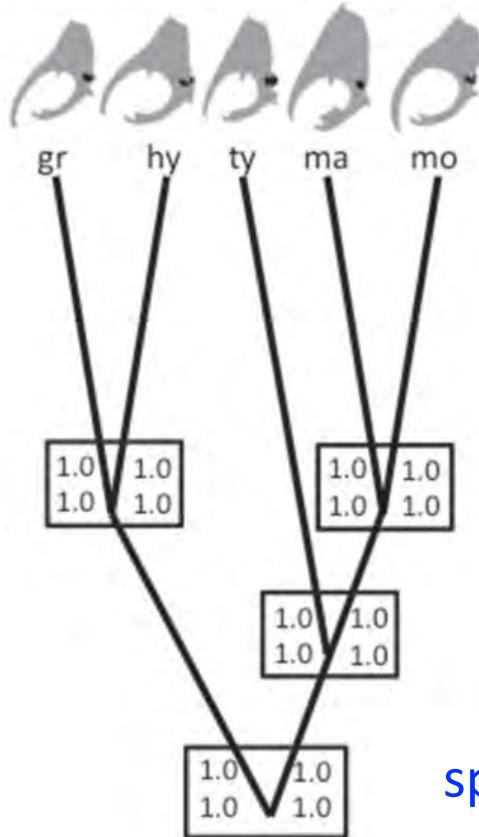
Huang & Knowles (2016) *Syst. Biol.*

# Model-based inference of species boundaries

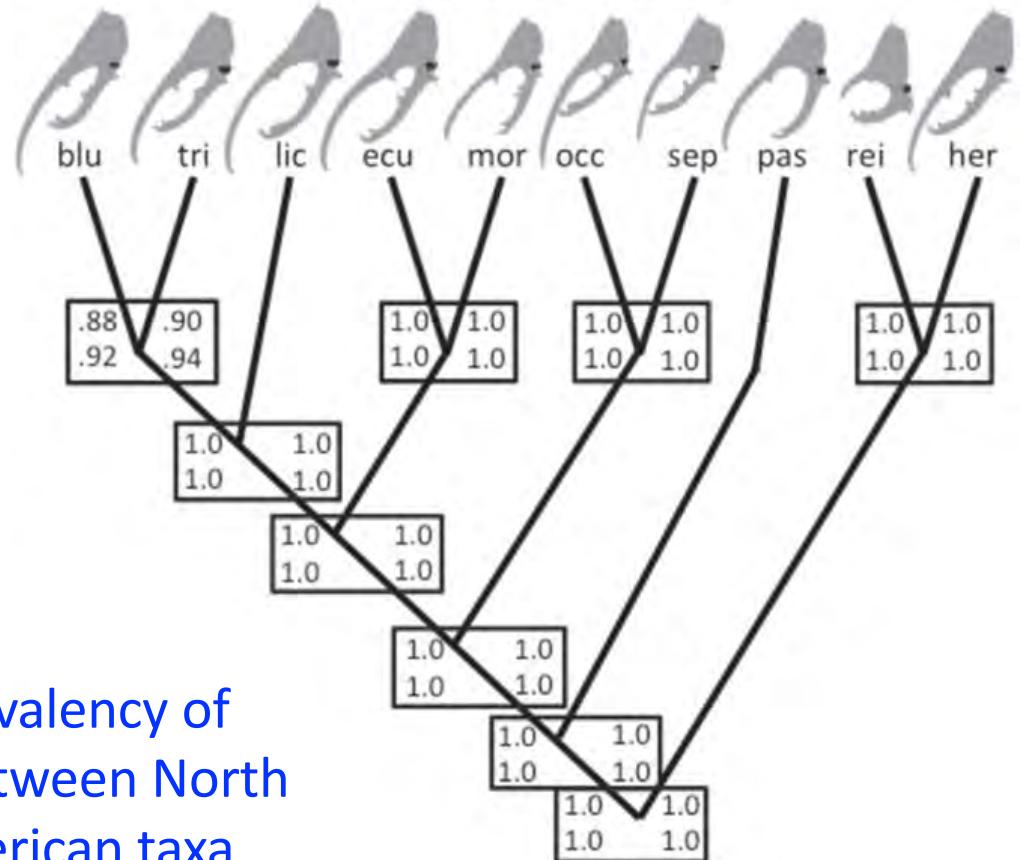
5 recognized species  
In North America

1 recognized species  
In South America

## White Hercules



## 10 inferred species of Giant Hercules



Statistical equivalency of  
species status between North  
and South American taxa

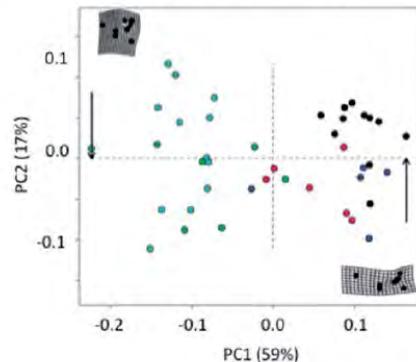
genus *Dynastes*

Huang & Knowles (2016) *Syst. Biol.*

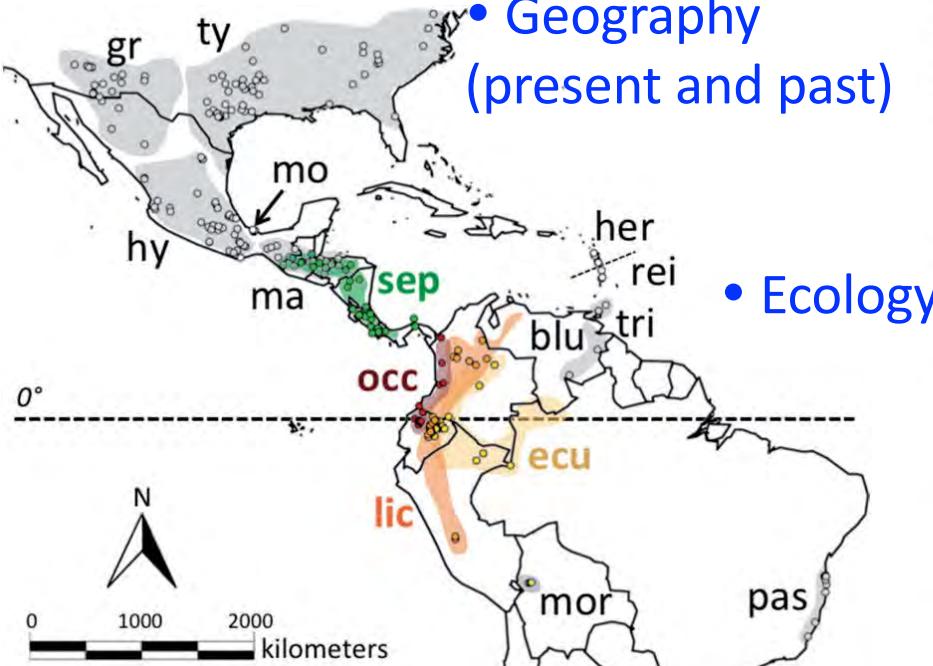
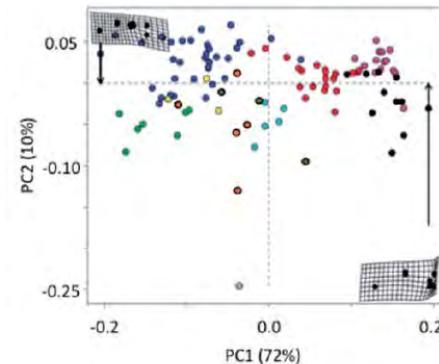
# Integration across data types to corroborate delimited taxa

- Quantification of phenotype

Thoracic horn (White Hercules)

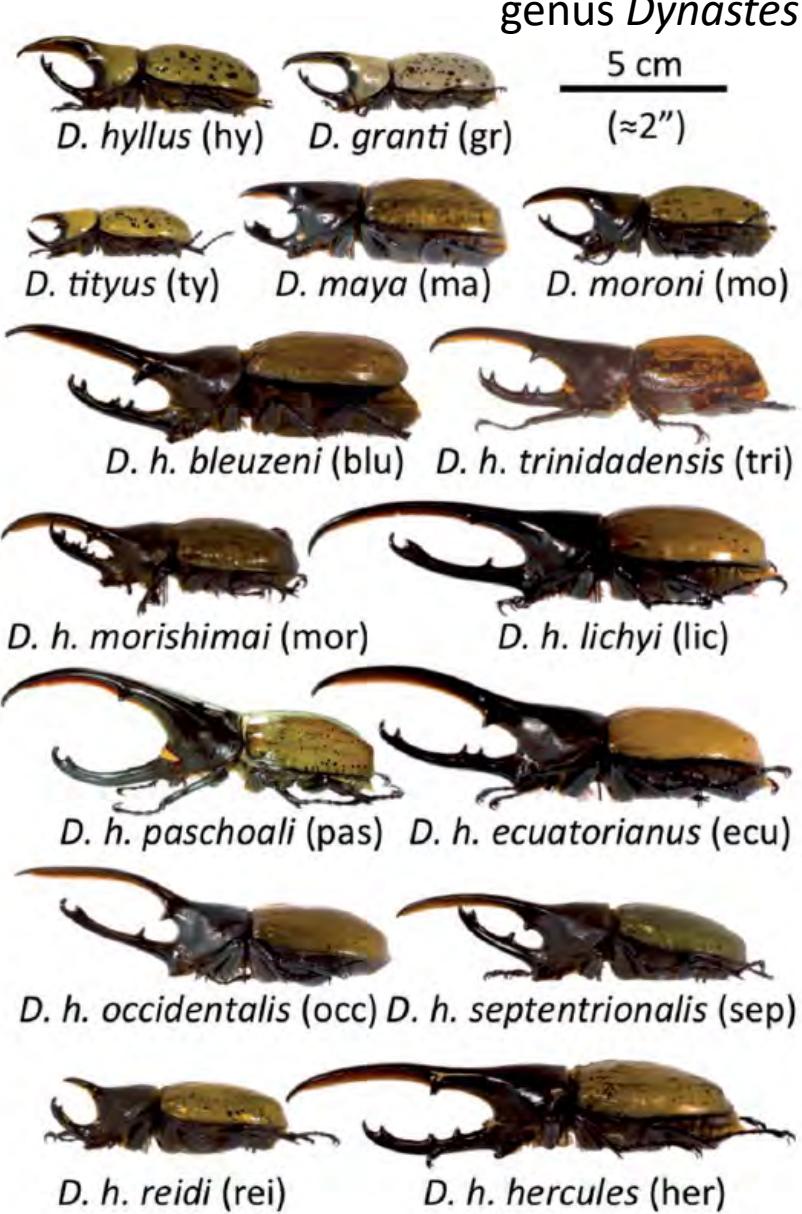


Thoracic horn (Giant Hercules)



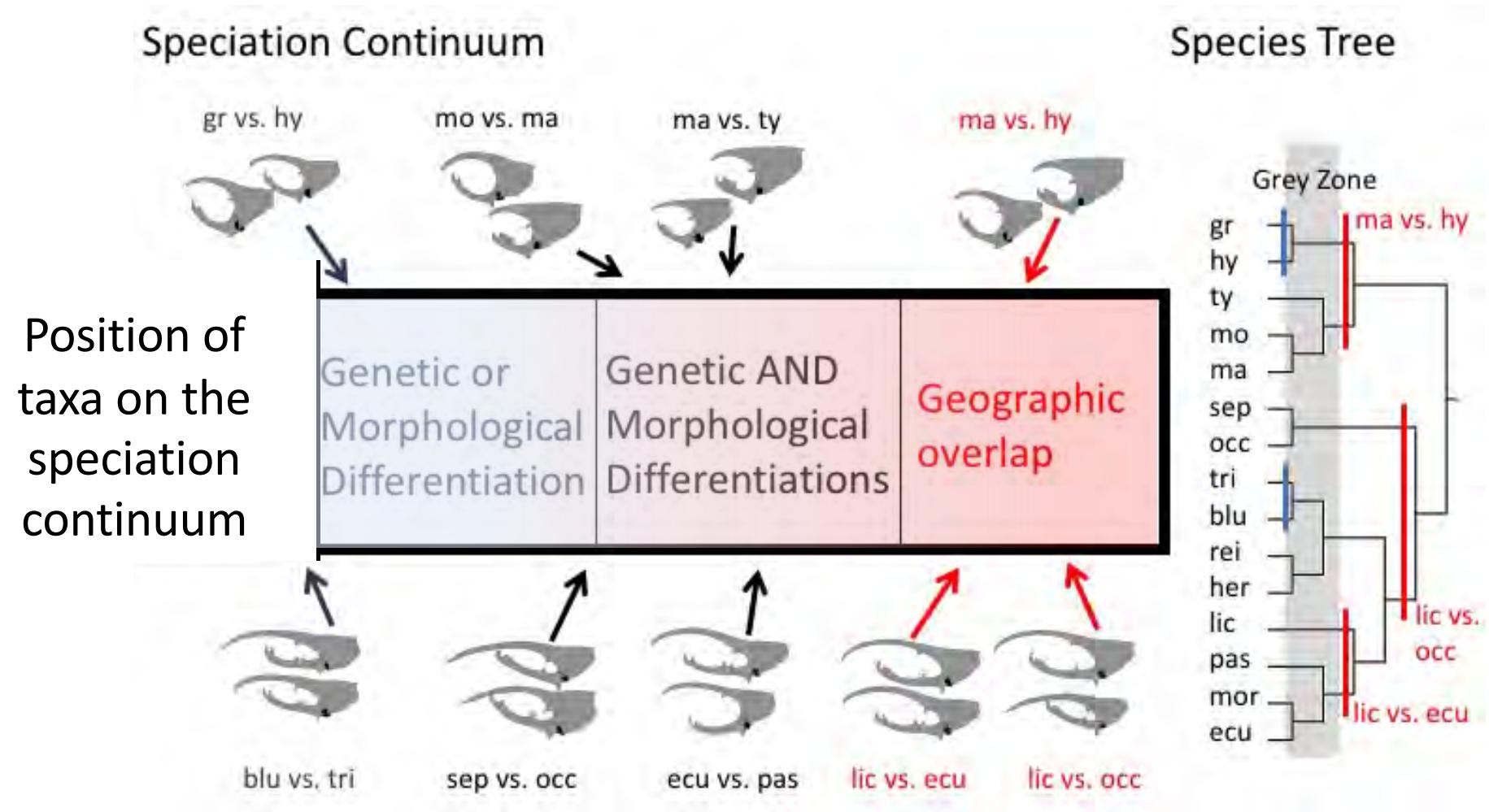
- Geography (present and past)

- Ecology



Huang & Knowles (2016) *Syst. Biol.*

Integrative data also provides insights into the divergence process



Huang & Knowles (2016) *Syst. Biol.*

# Transformative potential of model-based analyses:

- Codon substitution and analysis of natural selection
- Adaptive molecular evolution
- Divergence time estimation and biogeographic analysis
- Phylogenetic inference
- Species delimitation
- Demographic inference

# Transformative potential of model-based analyses:

- Codon substitution and analysis of natural selection
- Adaptive molecular evolution
- Divergence time estimation and biogeographic analysis
- Phylogenetic inference
- Species delimitation
- Demographic inference

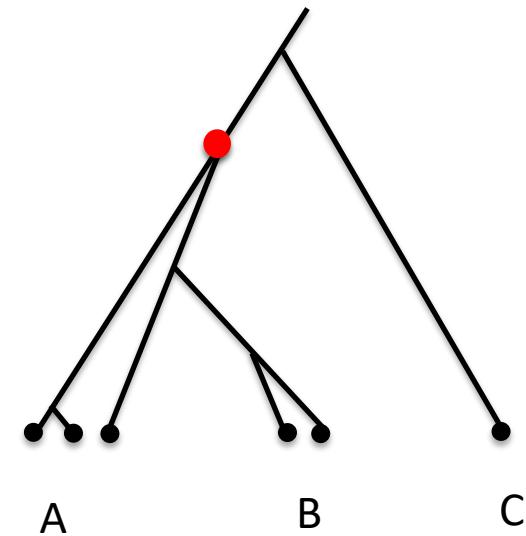
....models are how we communicate  
our knowledge to a statistical apparatus

# Transformative potential of model-based analyses:

- Codon substitution and analysis of natural selection
  - Adaptive molecular evolution
  - Divergence time estimation and biogeographic analysis
  - Phylogenetic inference
  - Species delimitation
  - Demographic inference
- 
- All models are flawed..., some are more or less useful  
....models are how we communicate  
our knowledge to a statistical apparatus

# Transformative potential of model-based analyses:

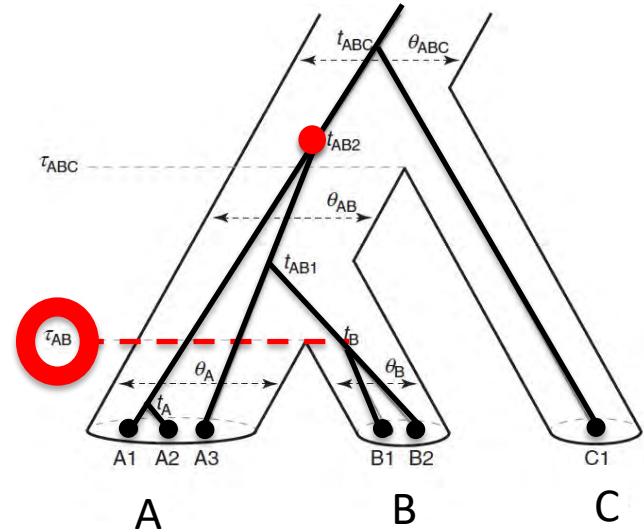
- Codon substitution and analysis of natural selection
- Adaptive molecular evolution
- Divergence time estimation and biogeographic analysis
- Phylogenetic inference
- Species delimitation
- Demographic inference  
(e.g., estimate divergence  
between population A and B)



Model of gene lineage divergence under  
an assumption of a molecular clock

# Transformative potential of model-based analyses:

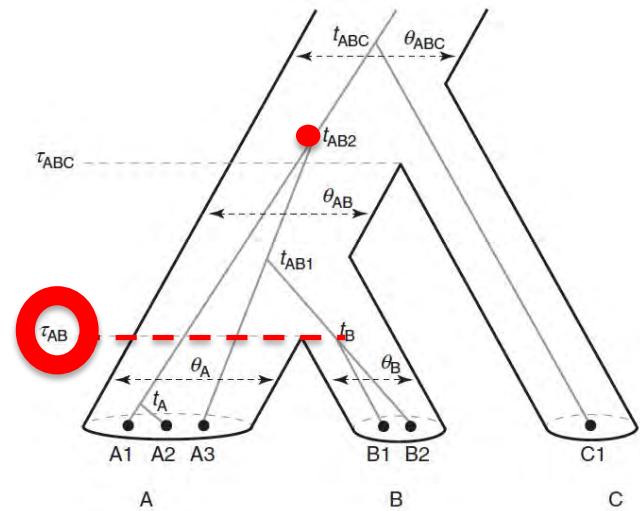
- Codon substitution and analysis of natural selection
- Adaptive molecular evolution
- Divergence time estimation and biogeographic analysis
- Phylogenetic inference
- Species delimitation
- Demographic inference  
(e.g., estimate divergence between population A and B)



Coalescent model of gene lineage sorting process

# Transformative potential of model-based analyses:

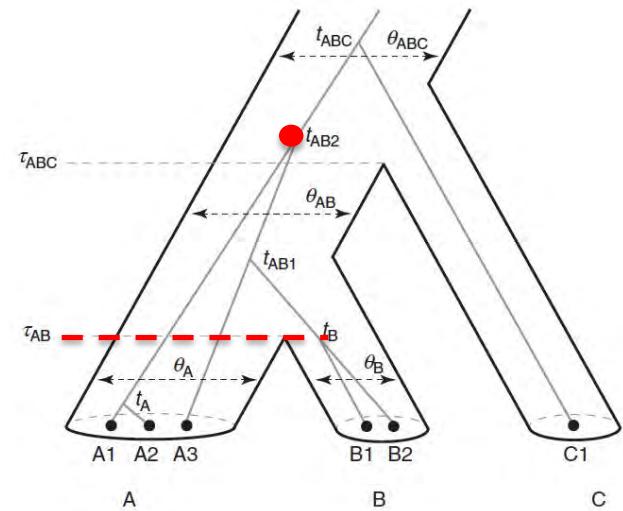
- Codon substitution and analysis of natural selection
- Adaptive molecular evolution
- Divergence time estimation and biogeographic analysis
- Phylogenetic inference
- Species delimitation
- Demographic inference  
(e.g., time of divergence)



- All models are flawed..., some are more or less useful  
....depending upon how effectively they represent  
our expert knowledge of evolution

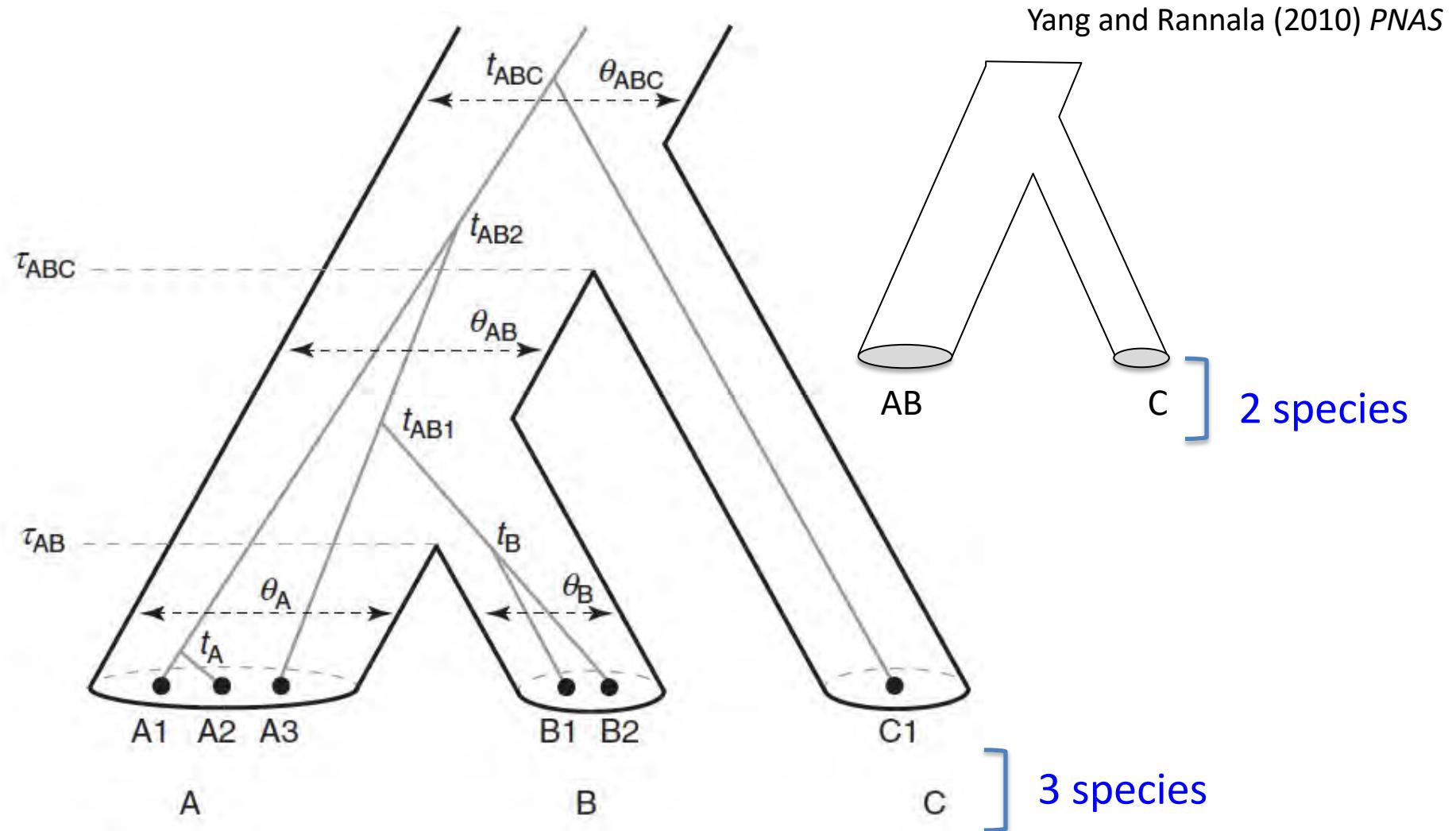
# Transformative potential of model-based analyses:

- Codon substitution and analysis of natural selection
- Adaptive molecular evolution
- Divergence time estimation and biogeographic analysis
- Phylogenetic inference
- Species delimitation
- Demographic inference  
(e.g., time of divergence)



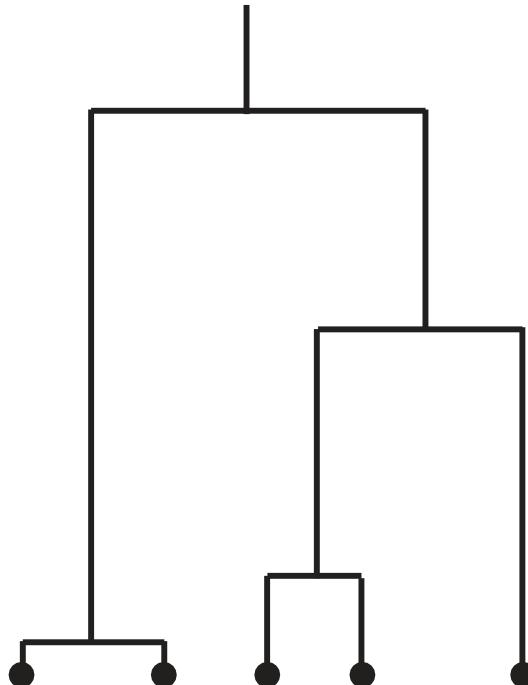
- All models are flawed..., some are more or less useful  
....depending upon how effectively they represent  
our expert knowledge of evolution

# Multispecies coalescent (MSC) model used to evaluate different species delimitation hypotheses



Different species delimitation hypotheses are formulated as competing statistical models and inferred from genetic data through Bayesian model selection (i.e., through calculation of posterior probabilities of a model), as in the popular program bpp

# Delimitation with the Coalescent

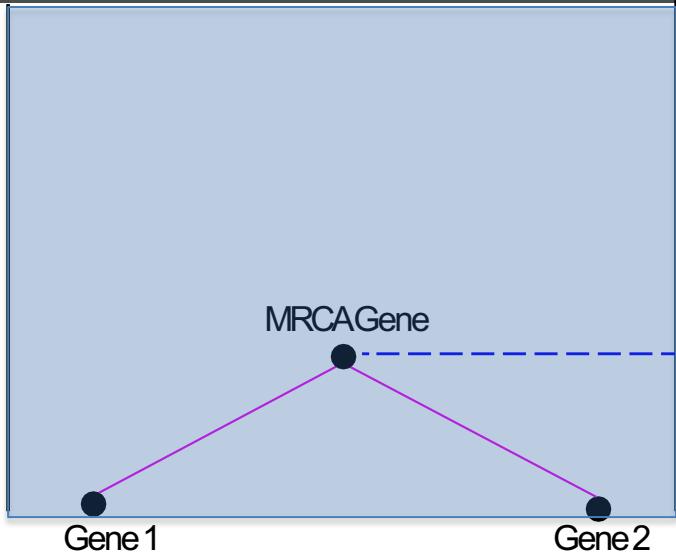


- Have a gene tree

# Coalescent Theory Applications in a Nutshell

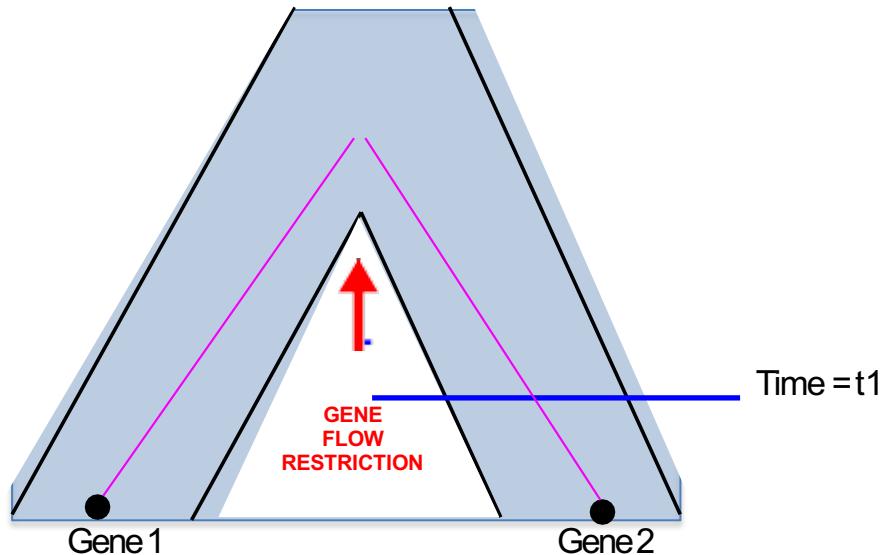
- Makes predictions about the *waiting time* between coalescence events based on population size and sample size.
- “coalescence events” (backward-time) == “divergence events” (forward-time)
- Predictions are based on assumptions of particular properties of the population that the genes (or individuals having those genes) are evolving.
- Deviances in observed waiting times from that predicted can be used to make inferences about deviances in actual population properties from assumed Wright-Fisher panmictic population

# How Does Structuring Change the Coalescent Times?



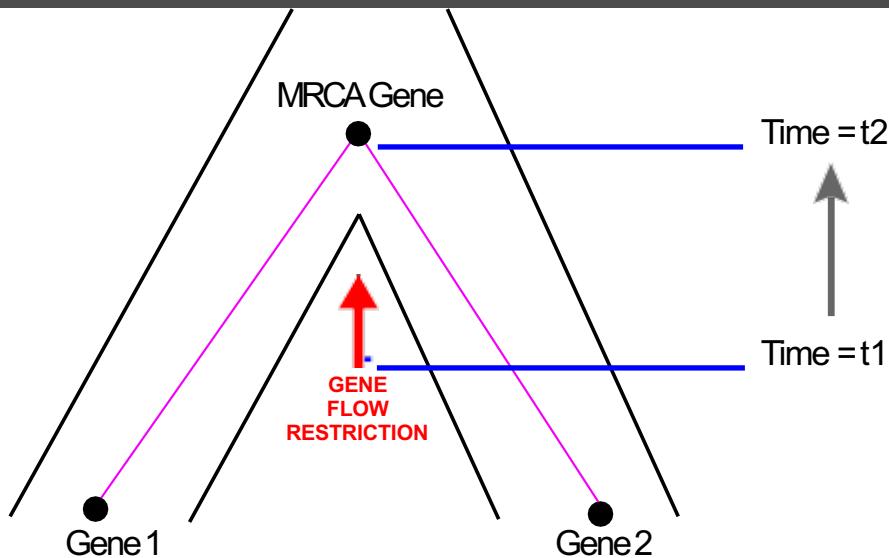
- Recall that the coalescent makes predictions about the timings to coalescence for genes sampled at random from a panmictic population.

# How Does Structuring Change the Coalescent Times?



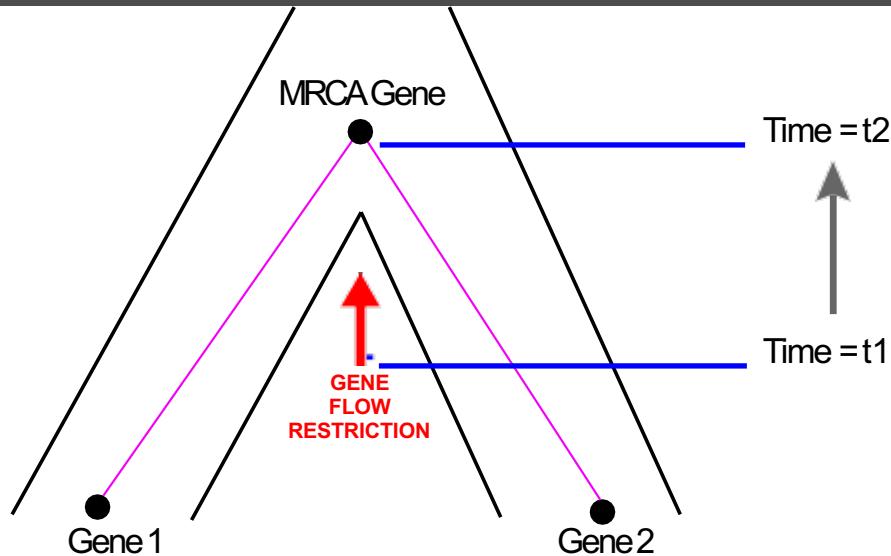
- Recall that the coalescent makes predictions about the timings to coalescence for genes sampled at random from a panmictic population.
- What happens if there are restrictions to panmixia?

# How Does Structuring Change the Coalescent Times?



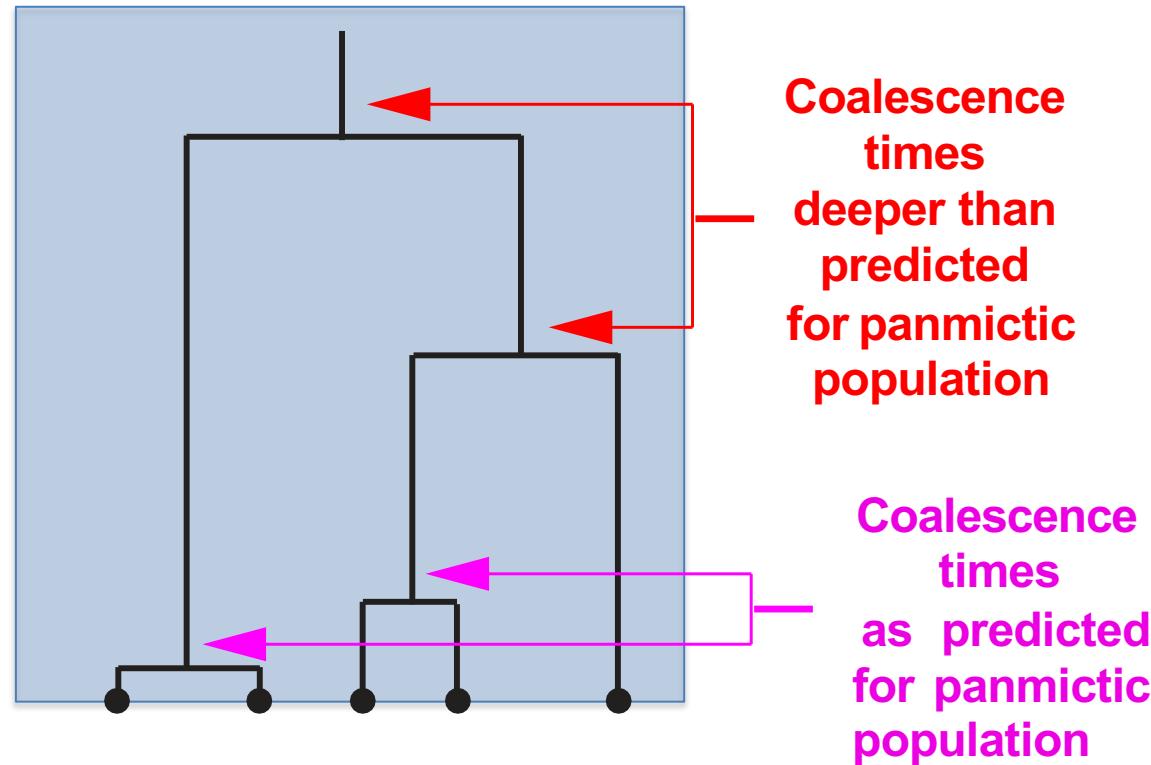
- Recall that the coalescent makes predictions about the timings to coalescence for genes sampled at random from a panmictic population.
- What happens if there are restrictions to panmixia?
- Then the timings to coalescent get *extended*

# How Does Structuring Change the Coalescent Times?

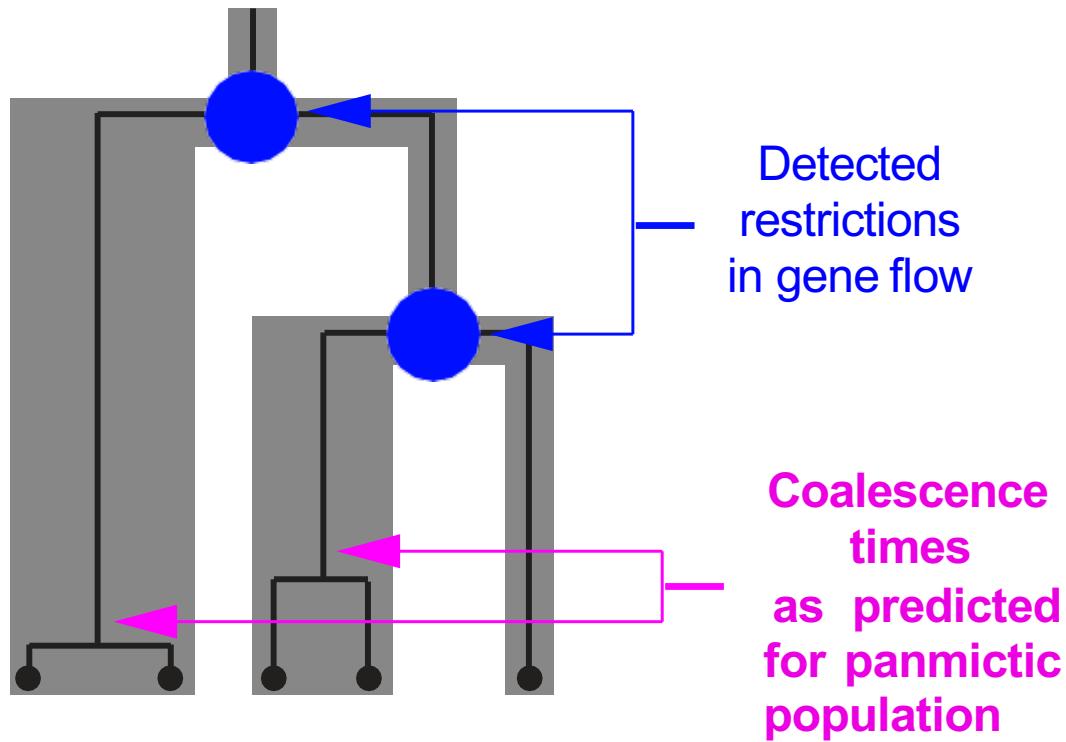


- Recall that the coalescent makes predictions about the timings to coalescence for genes sampled at random from a panmictic population.
- What happens if there are restrictions to panmixia?
- Then the timings to coalescent get *extended*
- This is the basis of the multispecies coalescent, MSC

# Delimiting Units with the MSC



# Delimiting Units with the MSC



- the MSC models the extensions in timings of coalescent events as disruptions of Wright-Fisher panmixia.
- It fits a “containing tree” to these disruptions (i.e., 3 species in this example)

# Explosion of applications using the MSC for delimitation

## Bayesian species delimitation usin

Received: 28 July 2017 | Revised: 12 December 2017 | Accepted: 13 December 2017

Received: 15 September 2017 | Revised: 30 March 2018 | Accepted: 3 April 2018

DOI: 10.1111/mec.12887

Zihl  
DOI: 10.1111/mec.14486

### INVITED REVIEWS AND SYNTHESES

## Cryptic species as a window into the paradigm shift of the species concept

CC

AC Cene Fiser<sup>1</sup> | Christopher T. Robinson<sup>2,3</sup> | Florian Malard<sup>4</sup>

### EMPIRICAL EXAMPLE WITH LIZARDS OF THE *LIOLAEMUS DARWINII* COMPLEX (SQUAMATA: LIOLAEMIDAE)

Arley Camargo,<sup>1,2</sup> Mariana Morando,<sup>3</sup> Luciano J. Avila,<sup>3</sup> and Jack W. Sites, Jr.<sup>1</sup>

<sup>1</sup>Department of Biology & Monte L. Bean Museum, Brigham Young University, Provo, Utah 84602

E-mail: arley.camargo@gmail.com

<sup>2</sup>CONICET-CENPAT Boulevard Almirante Brown 2915, 19120ACD, Puerto Madryn, Chubut, Argentina

Syst. Biol. 0(0):1–13, 2018

© The Author(s) 2018. Published by Oxford University Press, on behalf of the Society of Systematic Biologists.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits

non-commercial reuse, distribution, and reproduction in any medium, provided the original work is properly cited. For Permissions, please email: journals.permissions@oup.com

DOI:10.1093/sysbio/syy011

## Comparison of Methods for Molecular Species Delimitation Across a Range of Speciation Scenarios

ARONG LUO<sup>1,2,\*</sup>, CHENG LING<sup>3</sup>, SIMON Y. W. HO<sup>2</sup>, AND CHAO-DONG ZHU<sup>1,4</sup>

<sup>1</sup>Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China;

<sup>2</sup>School of Life and Environmental Sciences, University of Sydney, Sydney, New South Wales 2006, Australia; <sup>3</sup>Department of Computer Science and Technology, College of Information Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China; and

<sup>4</sup>College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

\*Correspondence to be sent to: Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China;  
E-mail: luoar@ioz.ac.cn

Simon Y. W. Ho and Chao-Dong Zhu contributed equally to this article.

E-mail: jacksonN@njhealth.org.

WILEY MOLECULAR ECOLOGY RESOURCES

chine learning method for n genetic data

<sup>3</sup> | Yufeng Wu<sup>1</sup> ID

WILEY MOLECULAR ECOLOGY

Bayesian species identification under the multispecies coalescent provides significant improvements to DNA barcoding analyses

ZIHENG YANG<sup>\*</sup> ID and BRUCE RANNALA<sup>†‡</sup> ID

<sup>\*</sup>Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK,

<sup>†</sup>College of Life Sciences, Beijing Normal University, Beijing 100875, China, <sup>‡</sup>Department of Evolution and Ecology, University

998

770

Advance Access Publication Date: 23 November 2014

Original Paper

ment-free Bayesian for species delimitation species coalescent

lin<sup>1,2</sup> and Bengt Oxelman<sup>1,\*</sup>

al Sciences, University of Gothenburg, Box 461, SE 405 30 Göteborg, Sweden; <sup>2</sup>Department of Biology, Faculty of Sciences, University of Dicle, 21280 Diyarbakir, Turkey

# Pros of species delimitation under MSC

- Can delimit species before reciprocal monophyly of alleles or fixed differences

Knowles & Carstens (2007) *Syst. Biol.*

- Still detects lineages under low gene flow

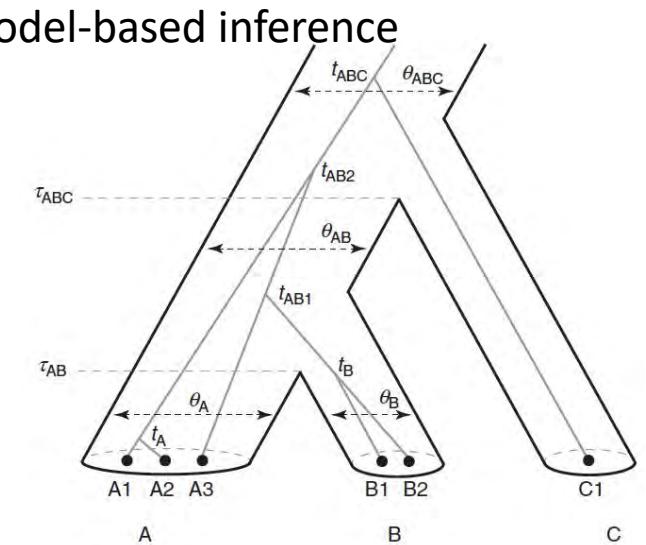
Zhang et al. (2011) *Syst. Biol.*

- Accuracy of species delimitation to sampling can be evaluated (i.e., will more data change status)

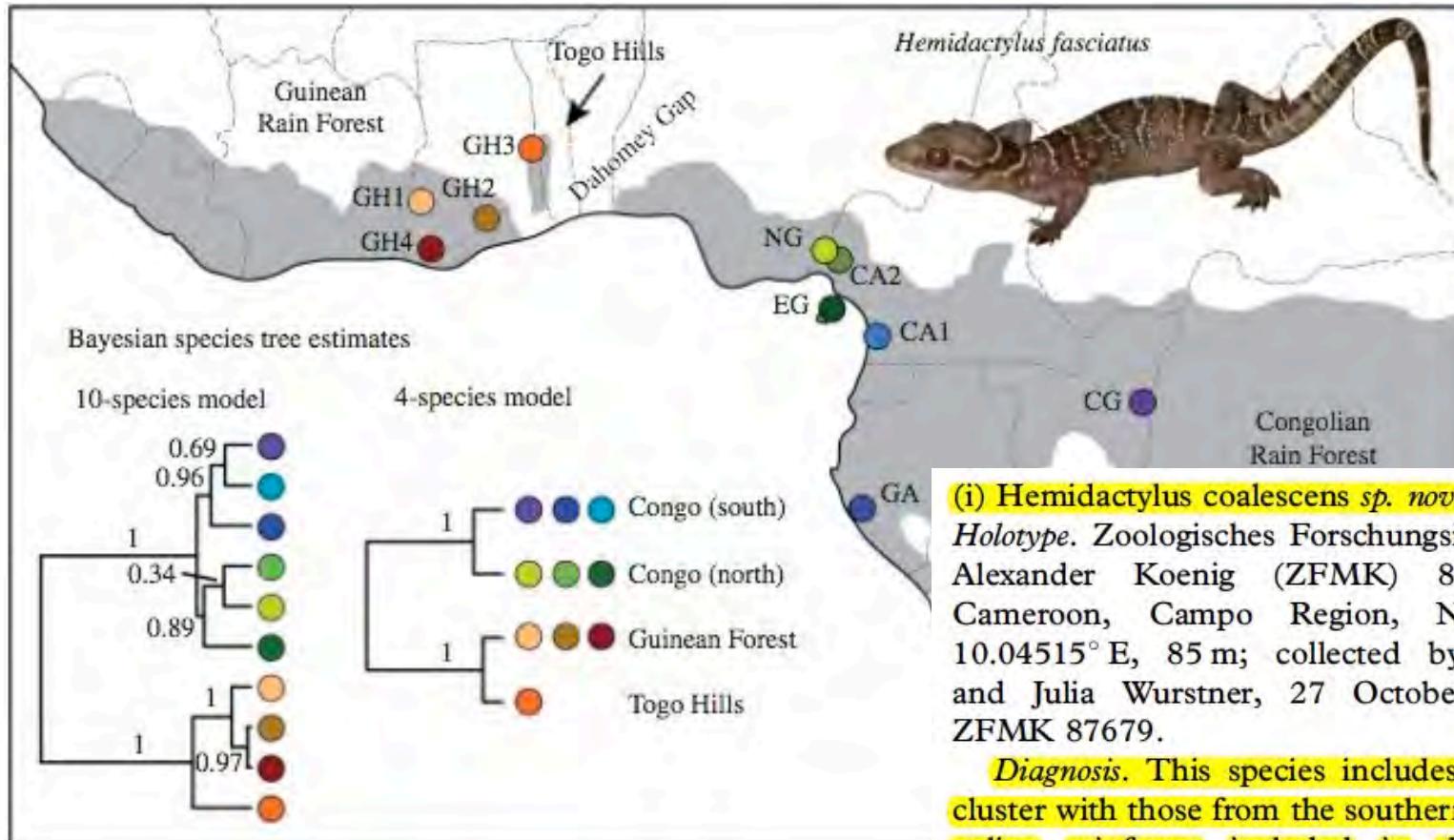
- De facto standardization for objectively delimiting taxa (i.e., data treated equally among all living things and avoid subjectiveness of what characters to measure) Fujita et al. (2012) *TREE*

- Can take into account uncertainty in gene trees

Yang & Rannala 2010

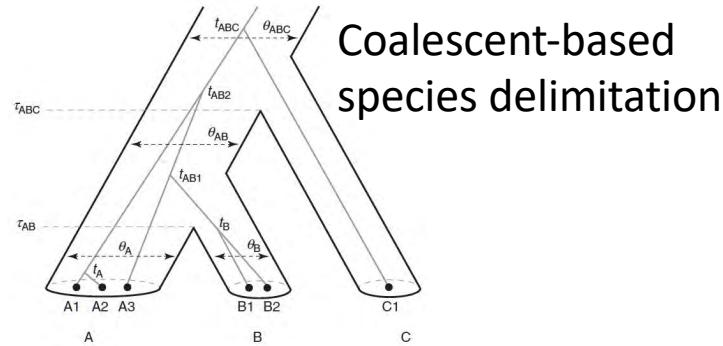


# Model-based inference: probability of different hypotheses about species boundaries based on genetic data alone!



Leache & Fujita (2010) *Proc. R. Soc. B.*

Data-informed summary suggests problems.....



Most newly discovered species go undescribed.

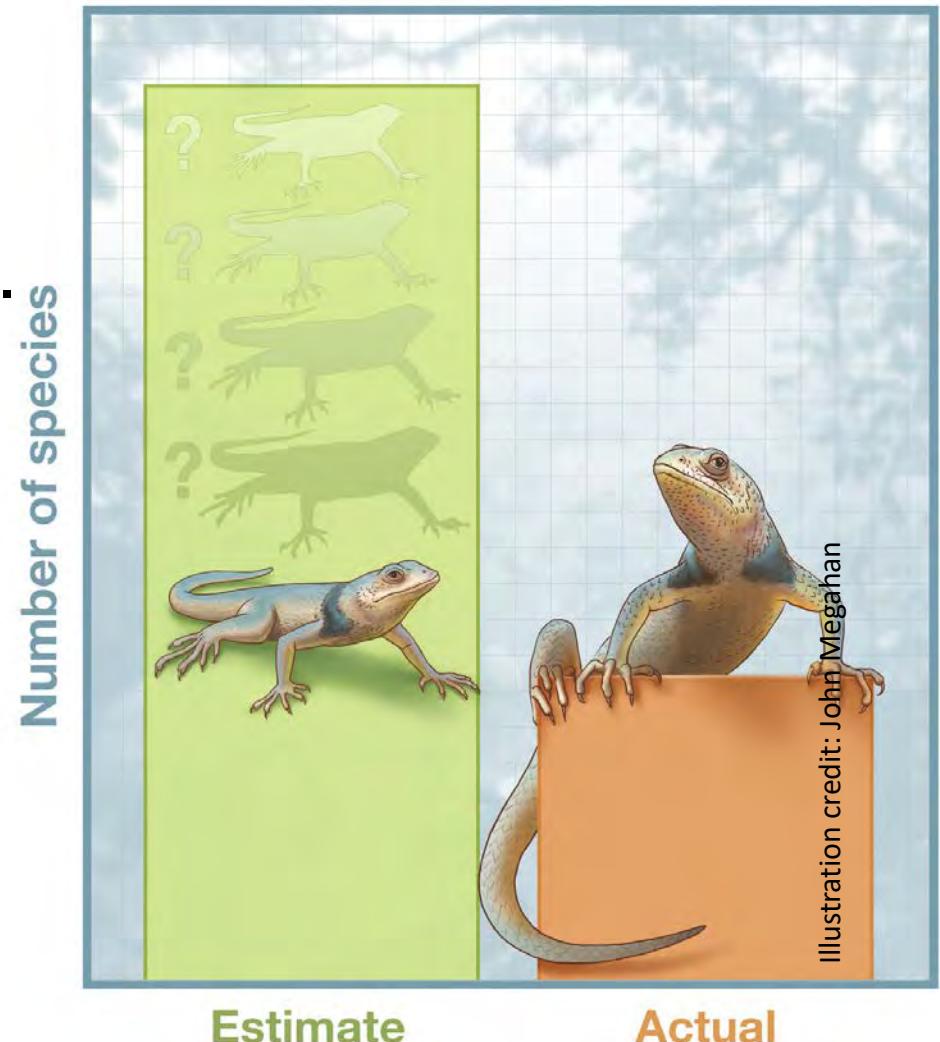
- Less than 30% of researchers applying MSC models made taxonomic recommendations!
- Less than 25% of researchers applying MSC models actually use results to describe new species!

Carstens et al. 2013



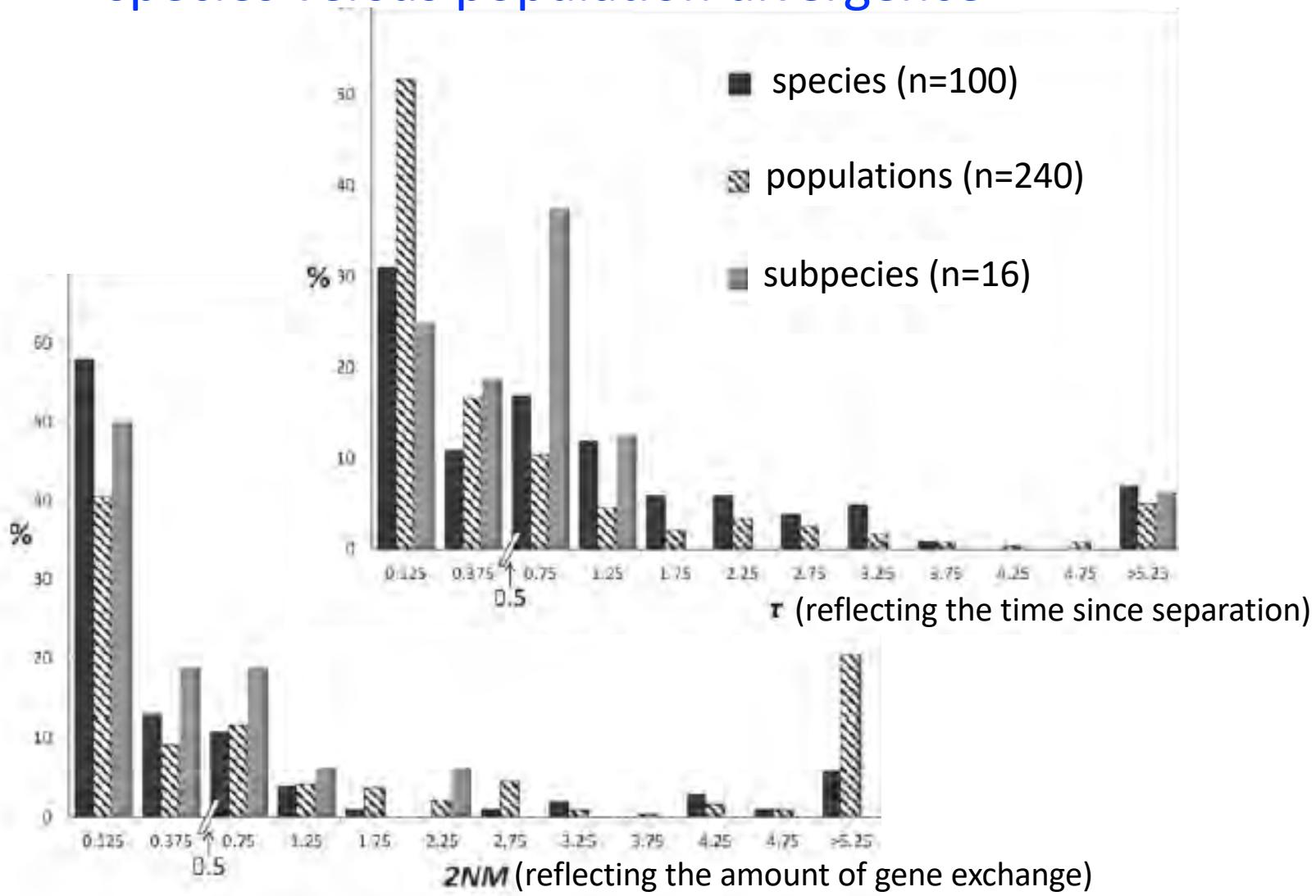
## the multispecies coalescent for delimiting species

- All models are flawed...  
some are more or less useful.



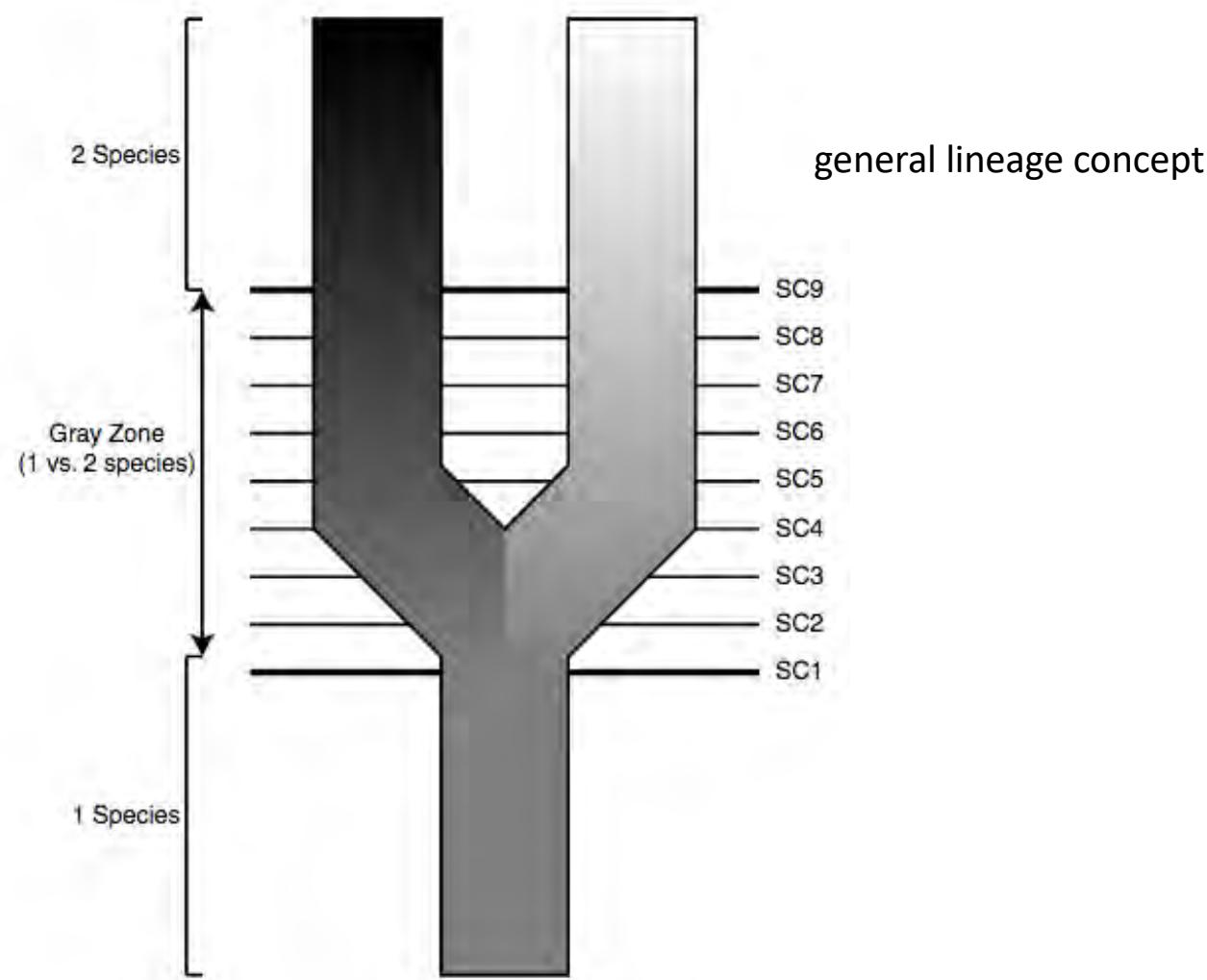
Sukumaran & Knowles (2017) PNAS

## No genetic distinction that separates species versus population divergence



Pinho and Hey(2010) *Evolution*

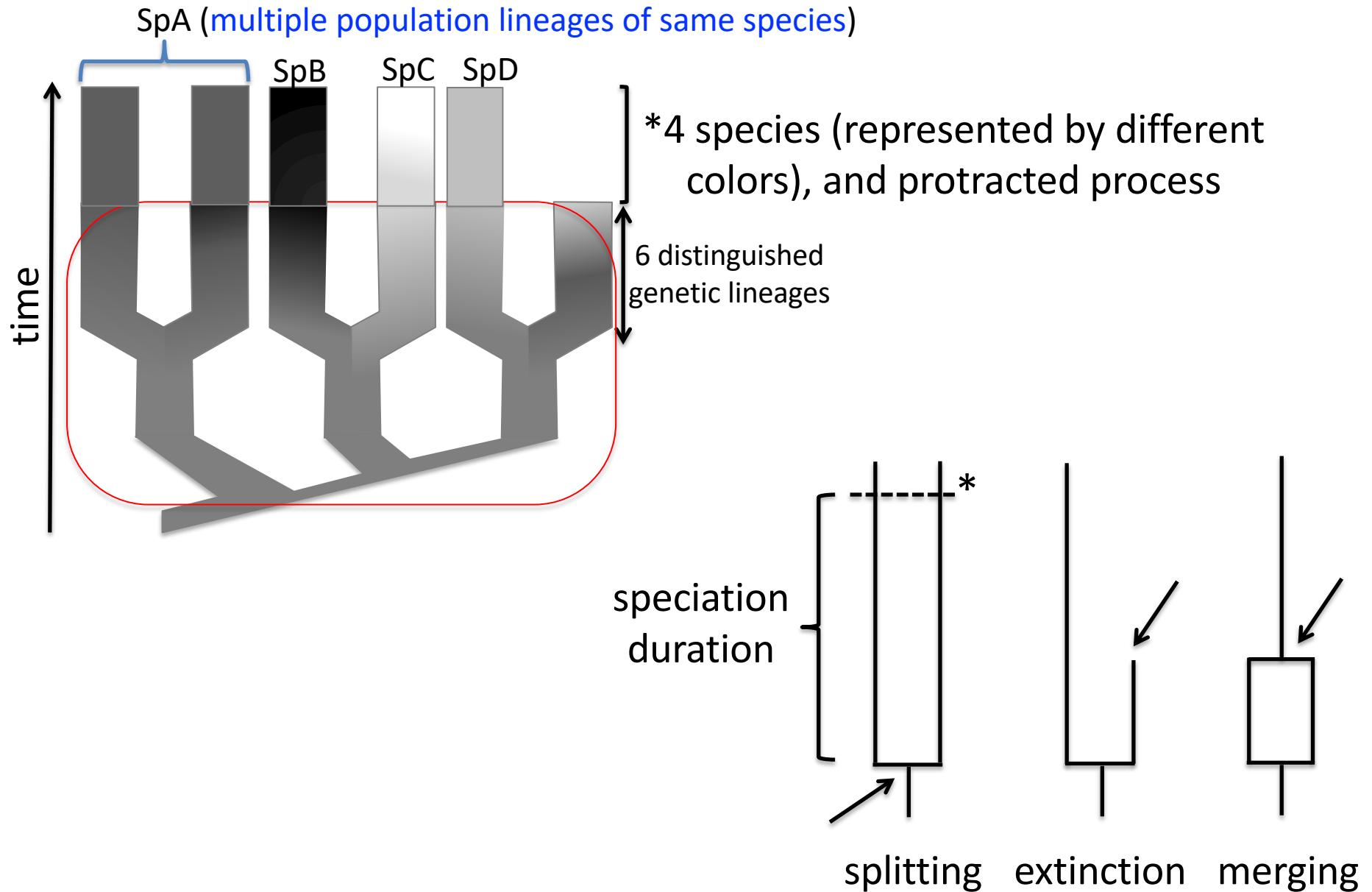
Eventually all species concepts agree...so no big deal right?!?



de Querroz 2005, 2007

\* Not all lineages become species!

And multiple population lineages of the same species!



The MSC dominates the field...

# How bad is the confounding of population verus species divergence?

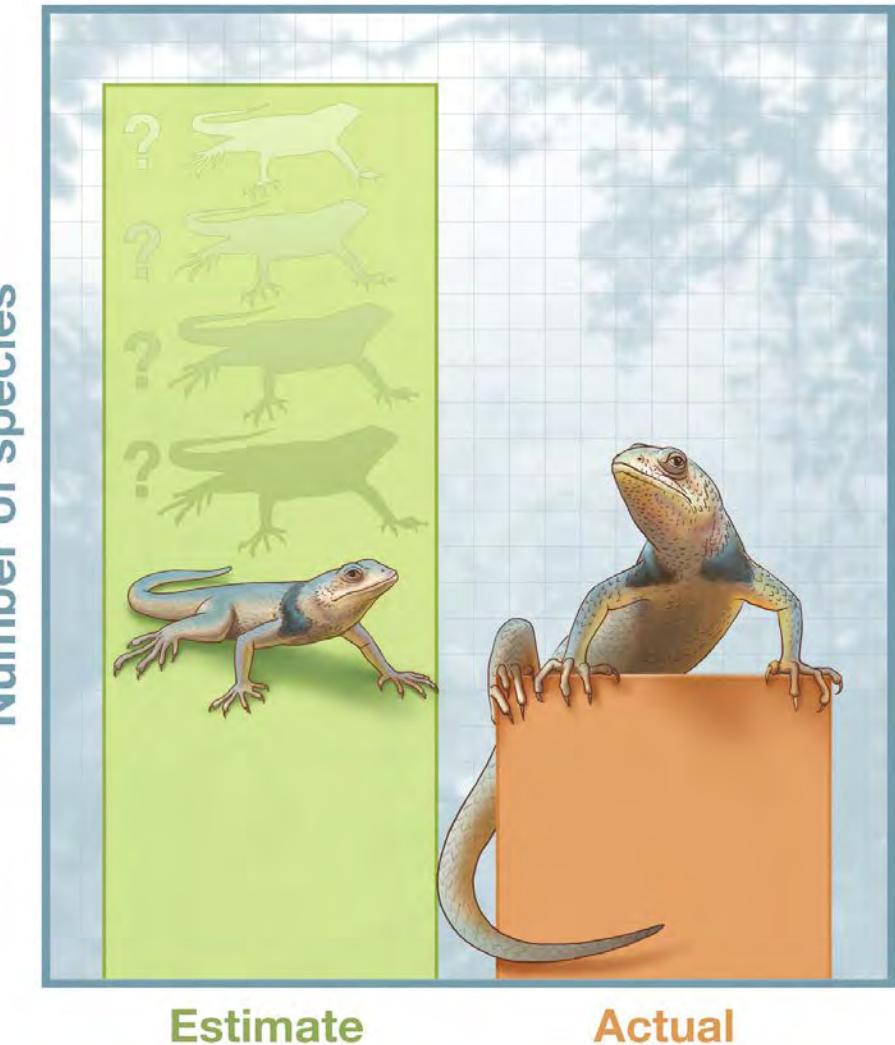
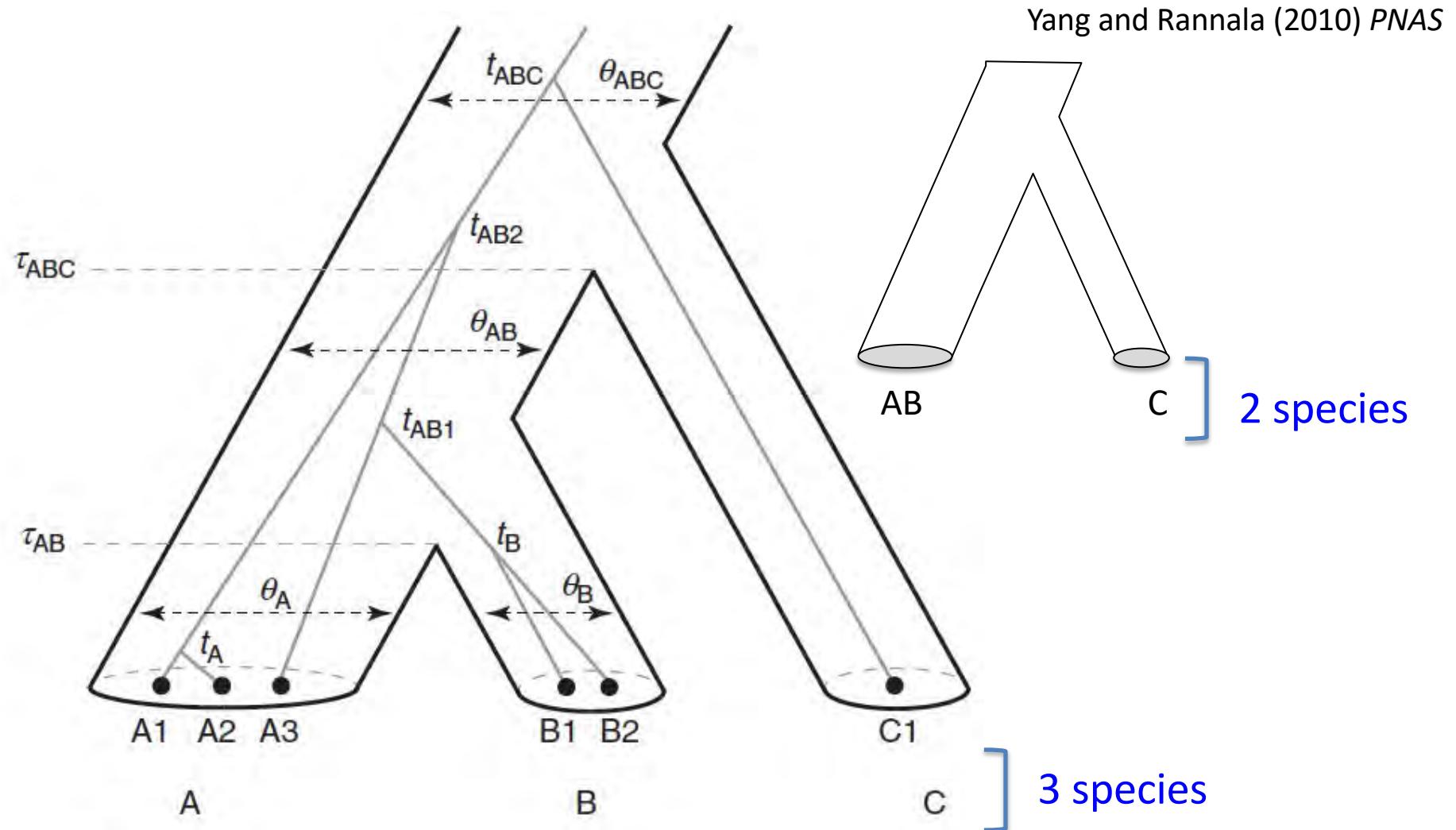


Illustration credit: John Megahan

Sukumaran & Knowles (2017) PNAS

# Multispecies coalescent (MSC) model used to evaluate different species delimitation hypotheses

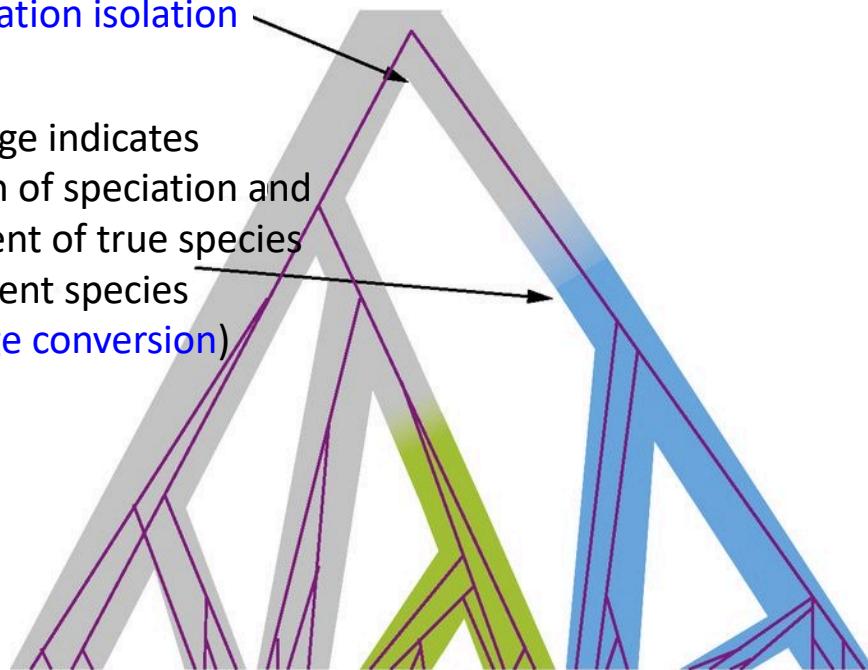


Different species delimitation hypotheses are formulated as competing statistical models and inferred from genetic data through Bayesian model selection (i.e., through calculation of posterior probabilities of a model), as in the popular program bpp

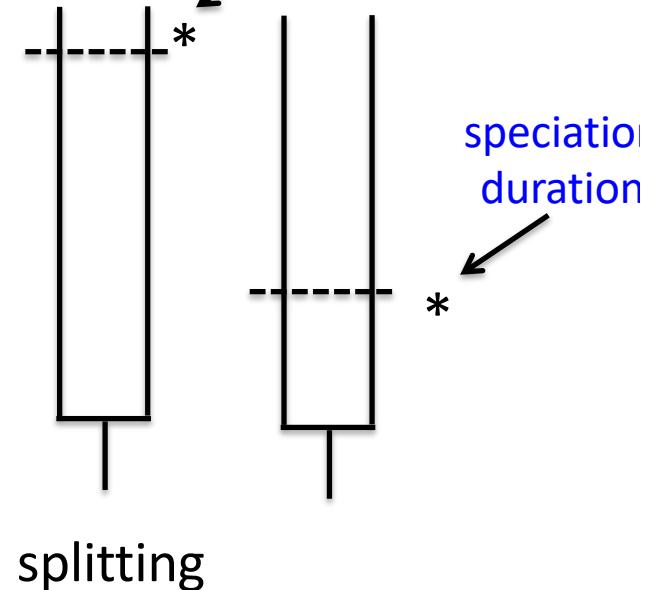
# Simulate data to account for differences in speciation duration (i.e., speciation is not instantaneous)

Splitting events such as this are initiation of speciation through, e.g., population isolation

Color change indicates completion of speciation and development of true species from incipient species (i.e., lineage conversion)



Sukumaran & Knowles (2017) PNAS



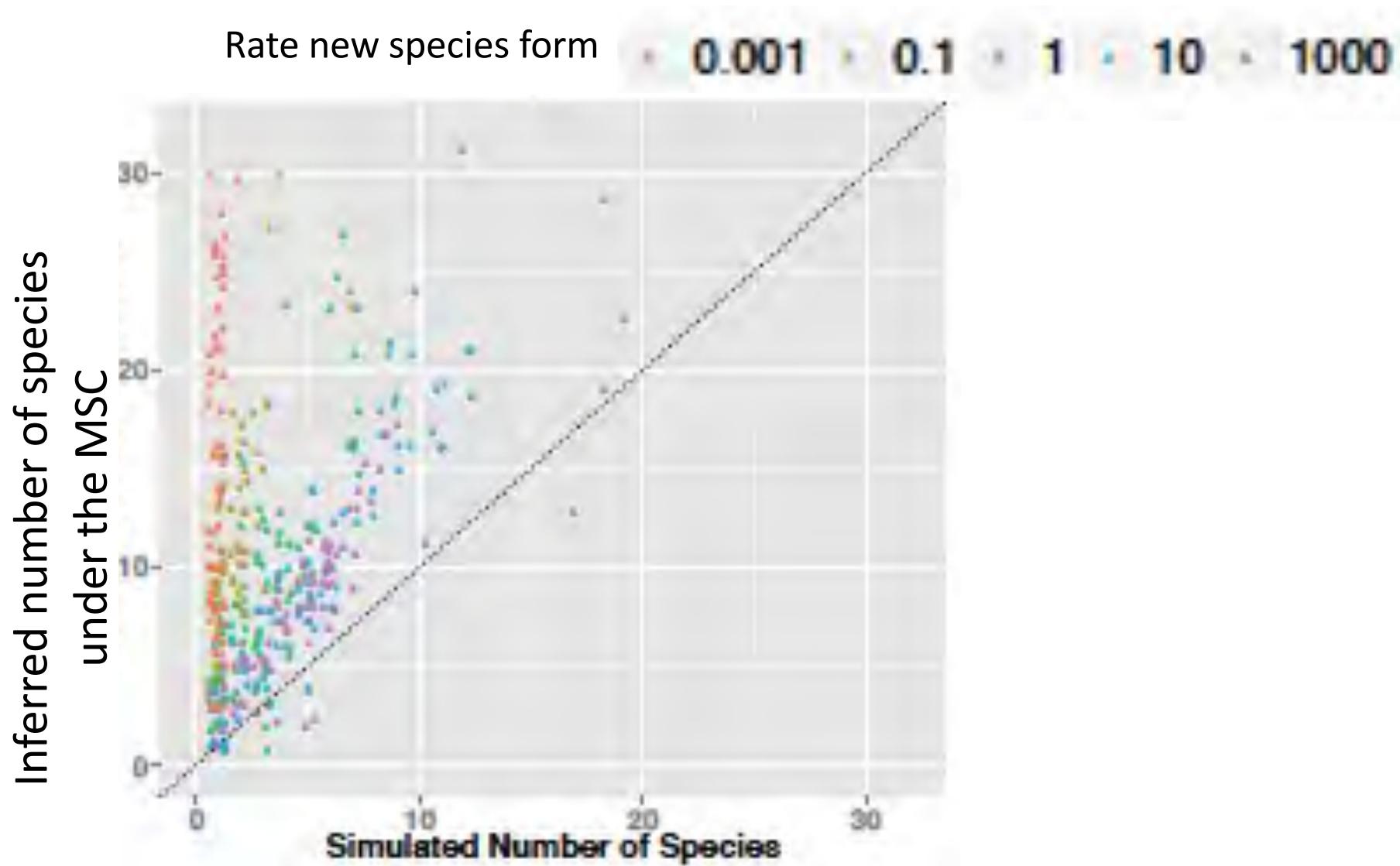
Does the MSC accurately  
delimit species?



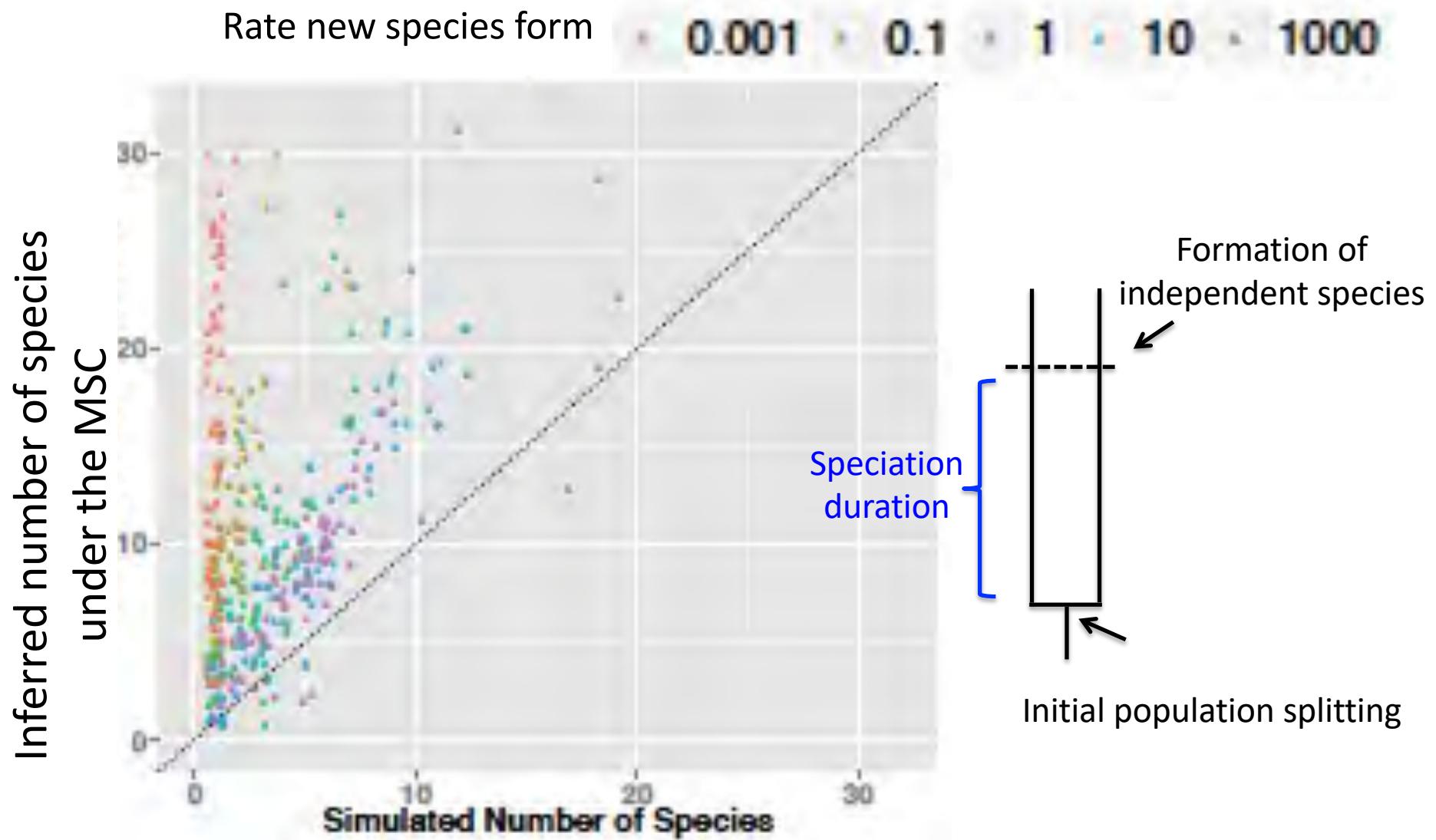
Model  
with  
8 vs 3  
species



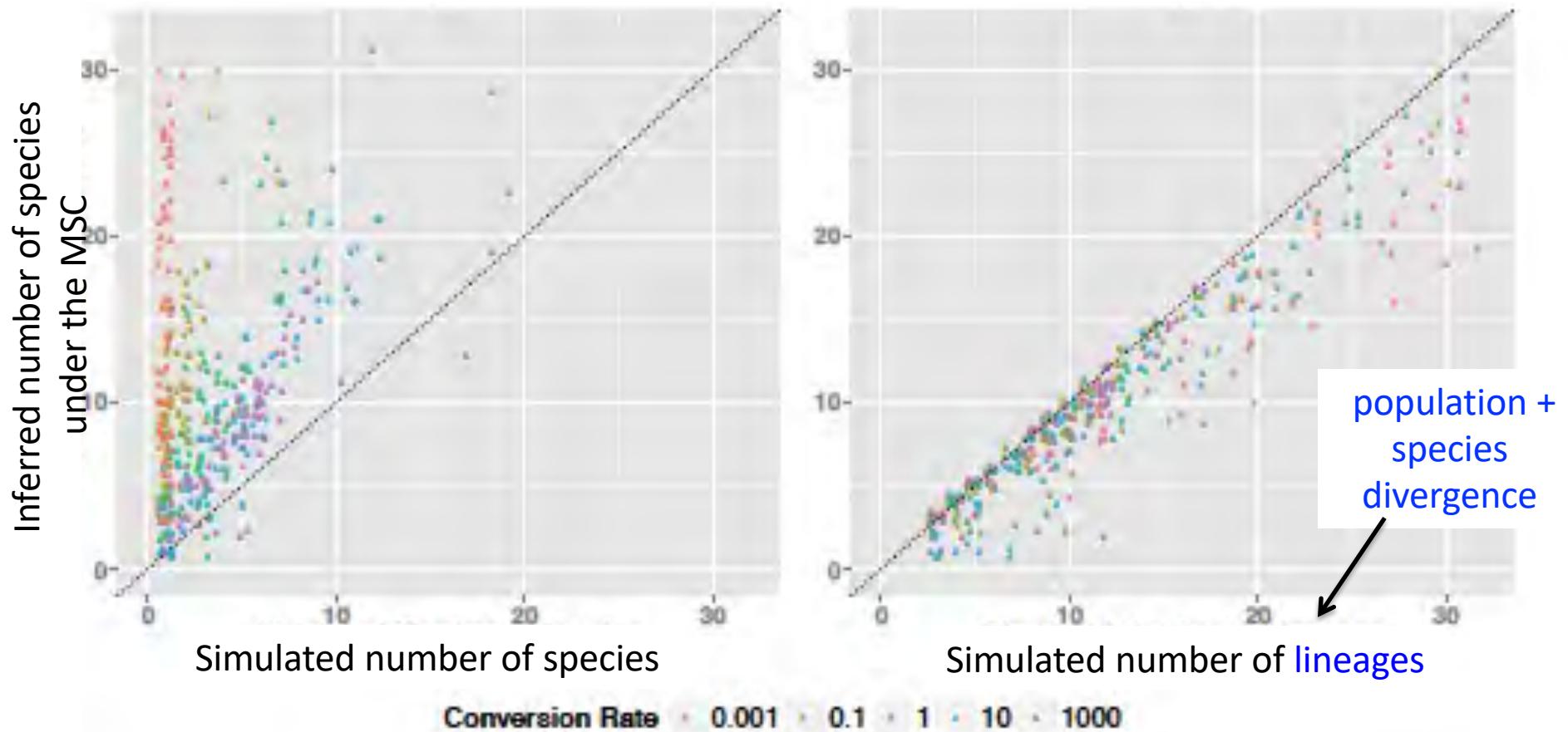
## Degree of overestimation of species richness under the MSC



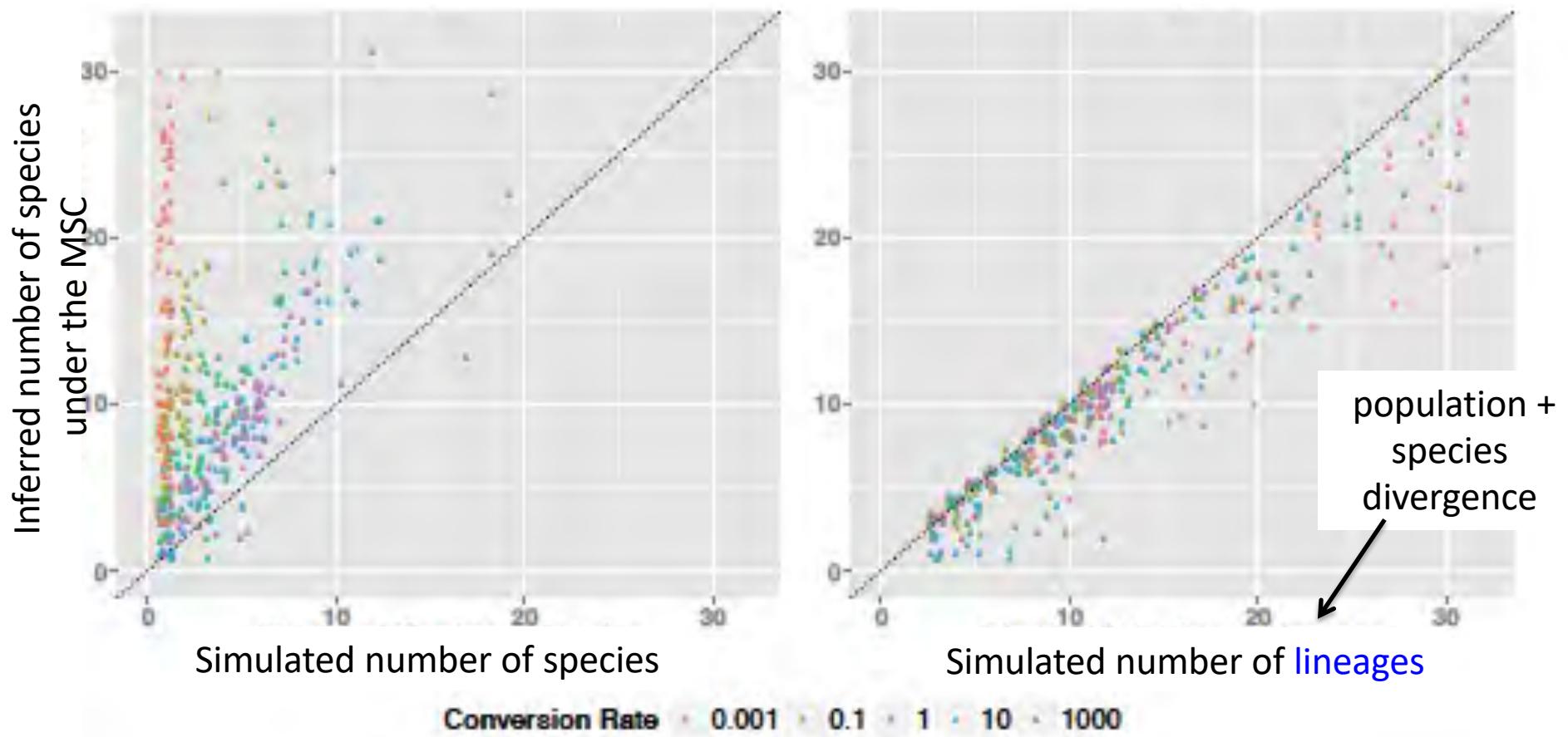
# Degree of overestimation of species richness under the MSC depends on the speciation duration



# MSC powerful model for detecting genetic structure



## MSC powerful model for detecting genetic structure



HOWEVER, the MSC is not capable of distinguishing genetic structure due to population versus species divergence

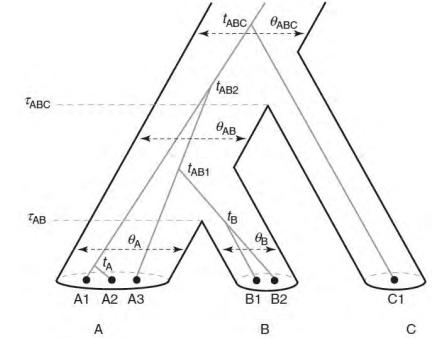
# Problems with species delimitation under the MSC

- MSC detects structure – not species

Sukumaran & Knowles (2017) *PNAS*

(different statistical delimitation methods all based on the MSC, which also means seeking consensus across methods is not a good way to fail)

See Rannala (2015) *Current Zoology* 61, 846-853



- “Robustness” to lineage detection with low levels of gene flow is not the same as accurate species delimitation

- Sensitivity to sampling (e.g., sparse geographic coverage over-splits species)

Chambers & Hillis (2020) *Syst. Biol.*

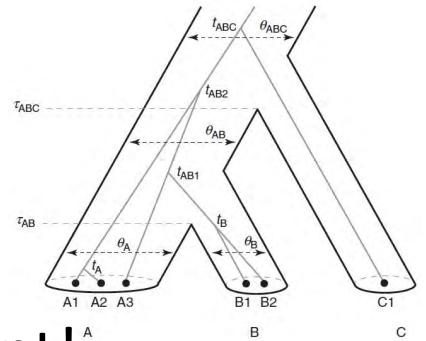
- MSC is not a de facto standardization for delimiting taxa: degree of over estimation varies depending on speciation process

Sukumaran & Knowles (2017) *PNAS*

# Accurate species delimitation cannot be achieved with current models based on MSC

Delimitation under the MSC:  
genetic structure = species

- Don't run MSC and add a caveat – what's the point!
- STOP reporting about all this “cryptic” diversity



# Model-based delimitation:

- Erroneous species boundaries are inferred from current model-based genetic approaches

Delimitation under the MSC:  
• genetic structure = species

- Relying on heuristics to interpret results from current genetic methods (e.g., bpp) is not the answer; does not validate the MSC for species delimitation



Ad hoc heuristics to interpret results from MSC-based models for delimitation

- Genealogical sorting index\*:  $2T/\theta$   
(i.e., population divergence time relative to the population size)  
Cummings et al. (2008) Evolution 62-9: 2411–2422

- use population divergence parameters (e.g., distantly related species, lots of migration)\*

\*Jackson et al. (2018) Syst. Biol.

\*Leache et al. (2018) Syst. Biol.

These heuristics do not validate  
the MSC for species delimitation

Using diverse sources of data for inferring species boundaries has a long systematic tradition, but not with model-based inference.

# Joint analysis of morphology and genetic data!

Solis-Lemus C, Knowles LL, Ané C (2014) Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* 69:492-507.

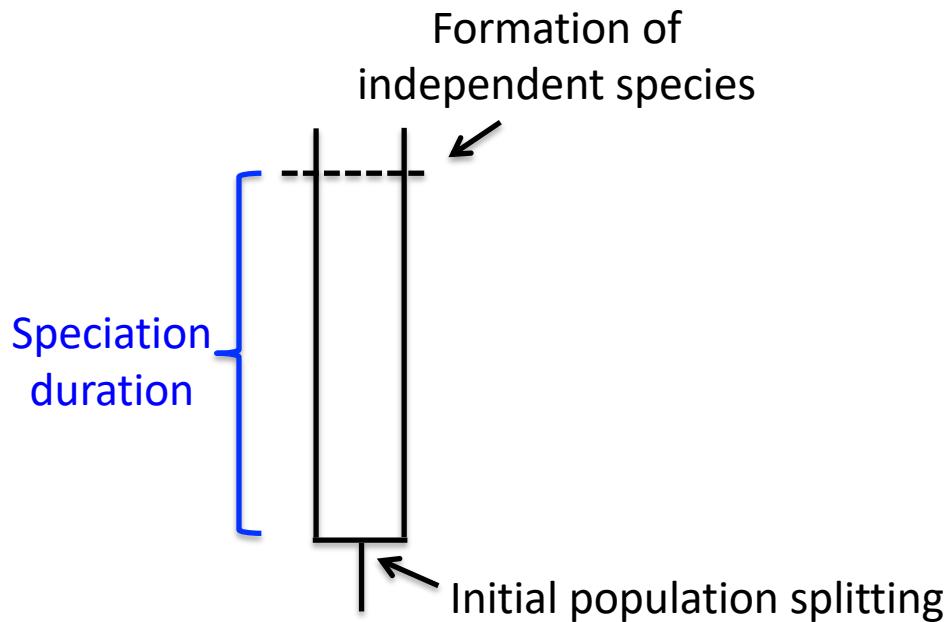
# Hypotheses about species boundaries



# Incorporating the speciation process into species delimitation

Jeet Sukumaran<sup>1</sup>\*, Mark T. Holder<sup>2</sup>, L. Lacey Knowles<sup>3</sup>

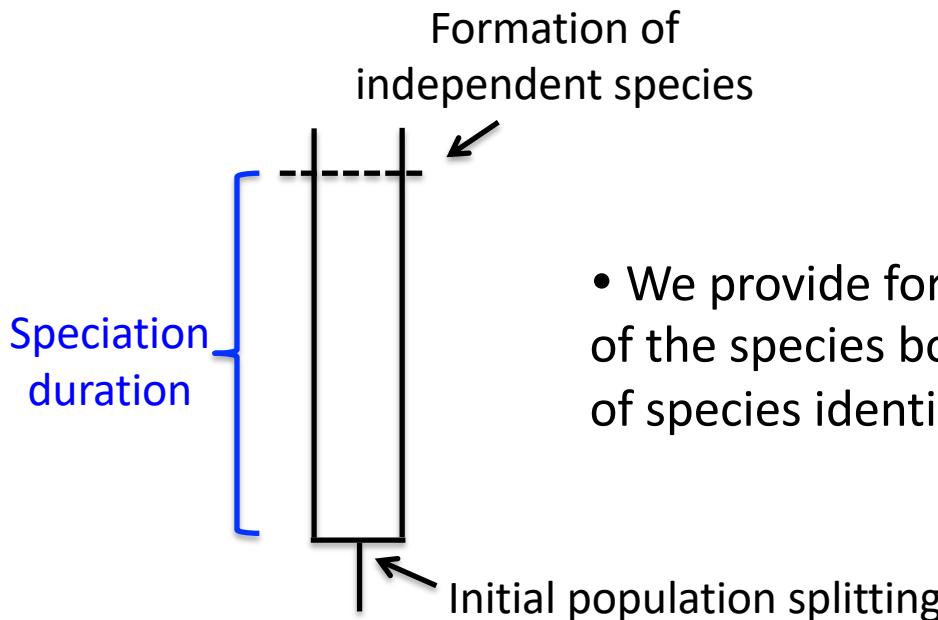
- We model the formation of new population lineages and their subsequent development into independent species modeled as separate processes



# Incorporating the speciation process into species delimitation

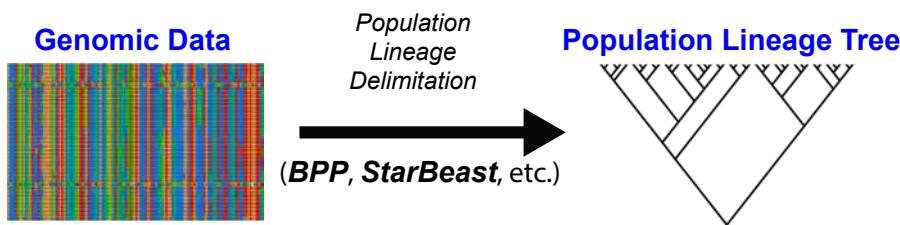
Jeet Sukumaran<sup>1</sup>\*, Mark T. Holder<sup>2</sup>, L. Lacey Knowles<sup>3</sup>

- We model the formation of new population lineages and their subsequent development into independent species modeled as separate processes



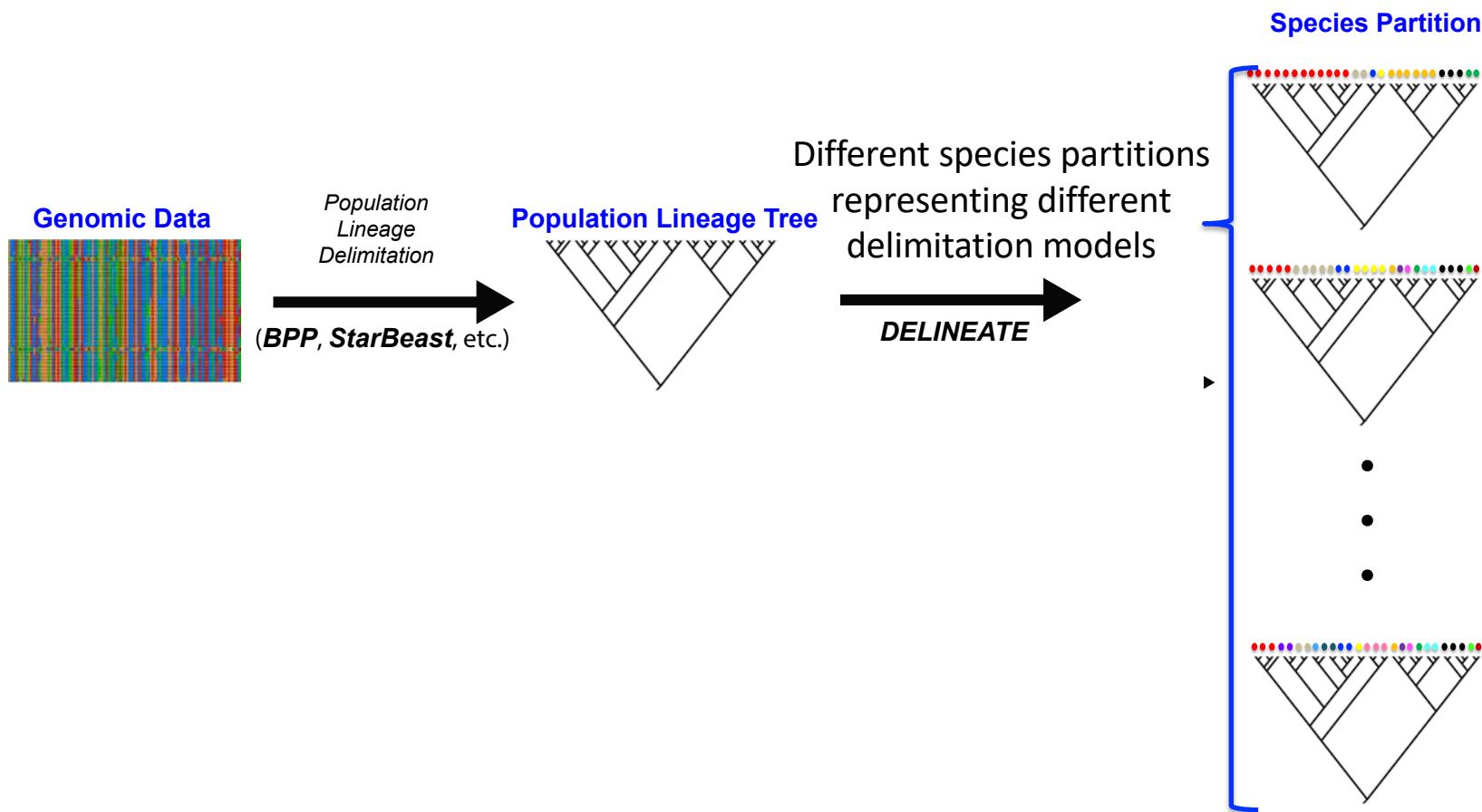
- We provide for a way to incorporate current understanding of the species boundaries in the system through specification of species identities for a subset of population lineages

**DELINATE**: a species delimitation method which makes probabilistic statements about whether or not distinct lineages are members of the same species



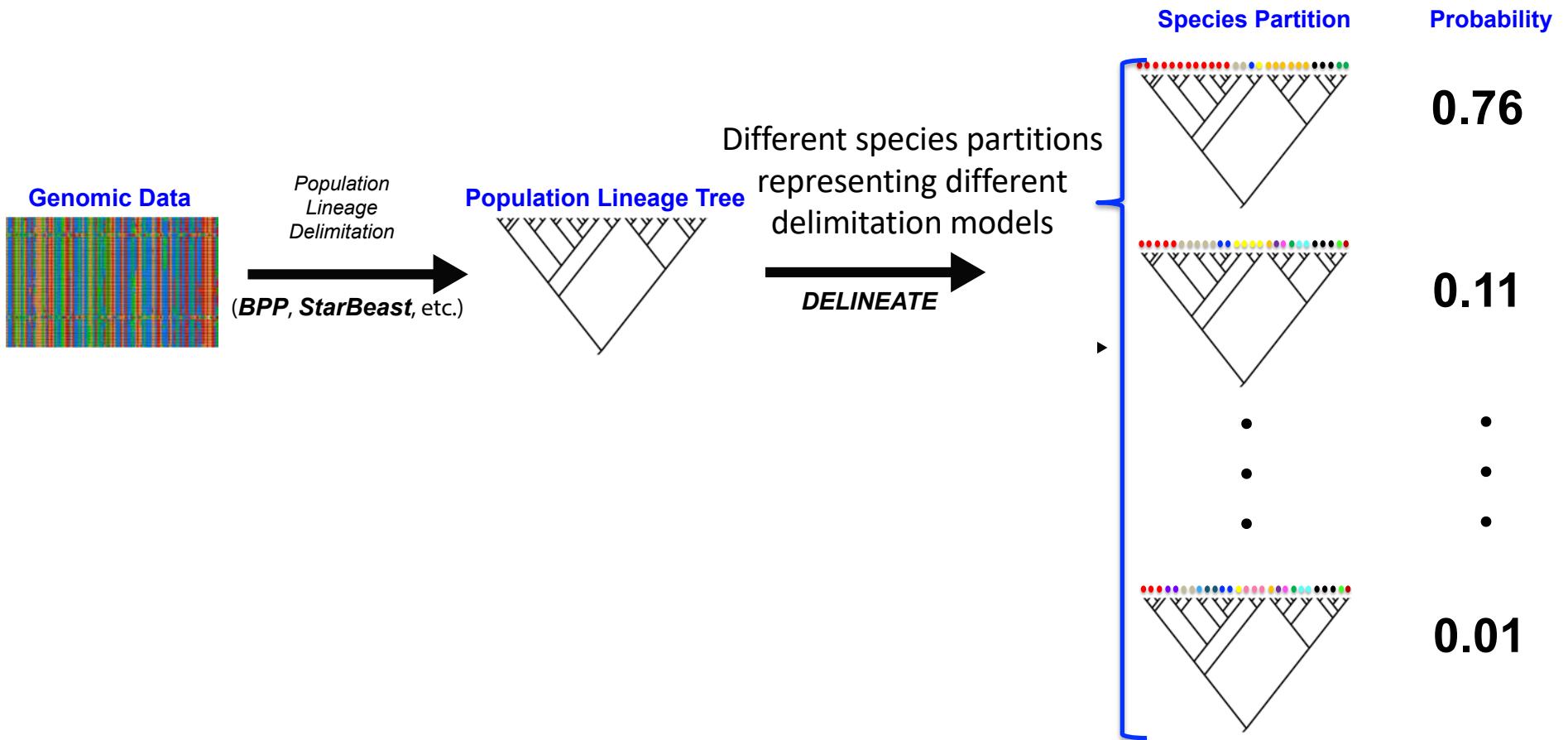
- Lineages are Wright-Fisher populations within which the neutral coalescent process dominates
- Boundaries between lineages are structure imposed by ancestral population splitting or isolation

# DELINATE



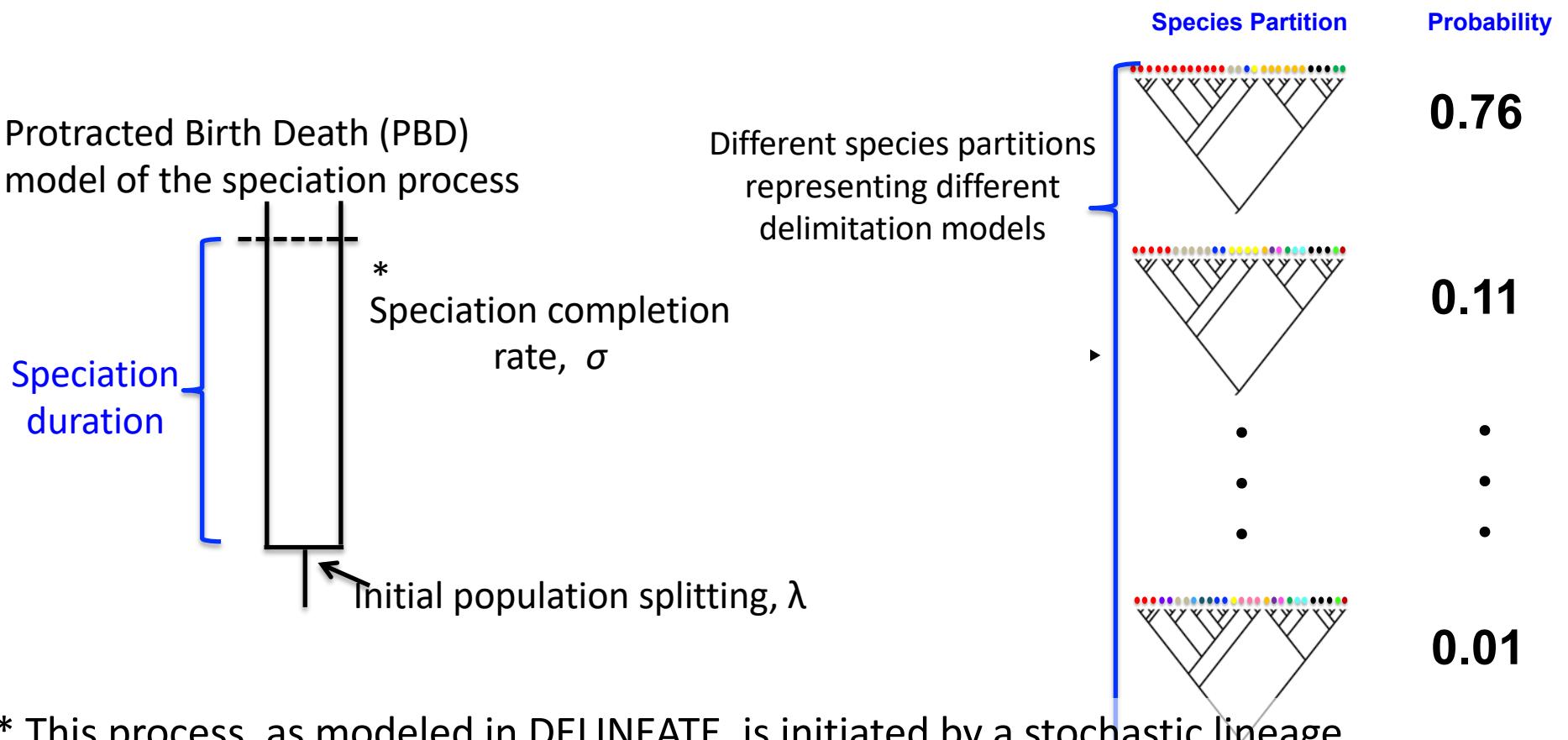
# DELINATE

- probabilities of different *partitions* are calculated conditional on the lineage tree and the speciation dynamic parameters that capture the tempo of speciation



# DELINATE

- probabilities of different *partitions* are calculated conditional on the lineage tree and the speciation dynamic parameters that capture the tempo of speciation

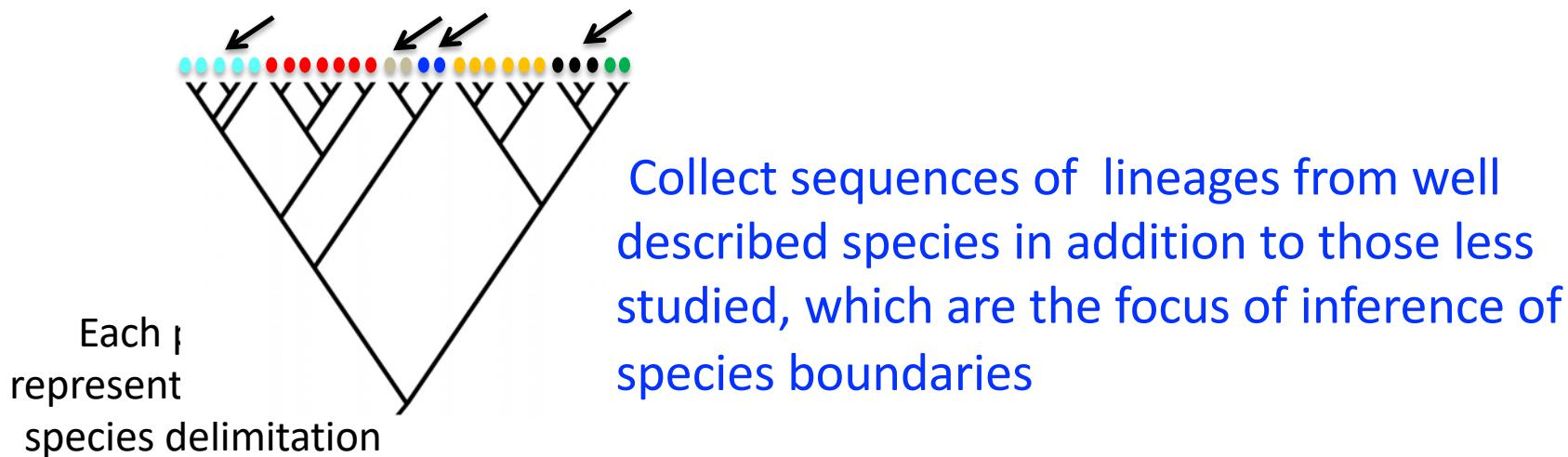


\* This process, as modeled in DELINATE, is initiated by a stochastic lineage splitting process that extends over a duration of time that is determined stochastically by a speciation completion rate parameter

# Incorporating the speciation process into species delimitation

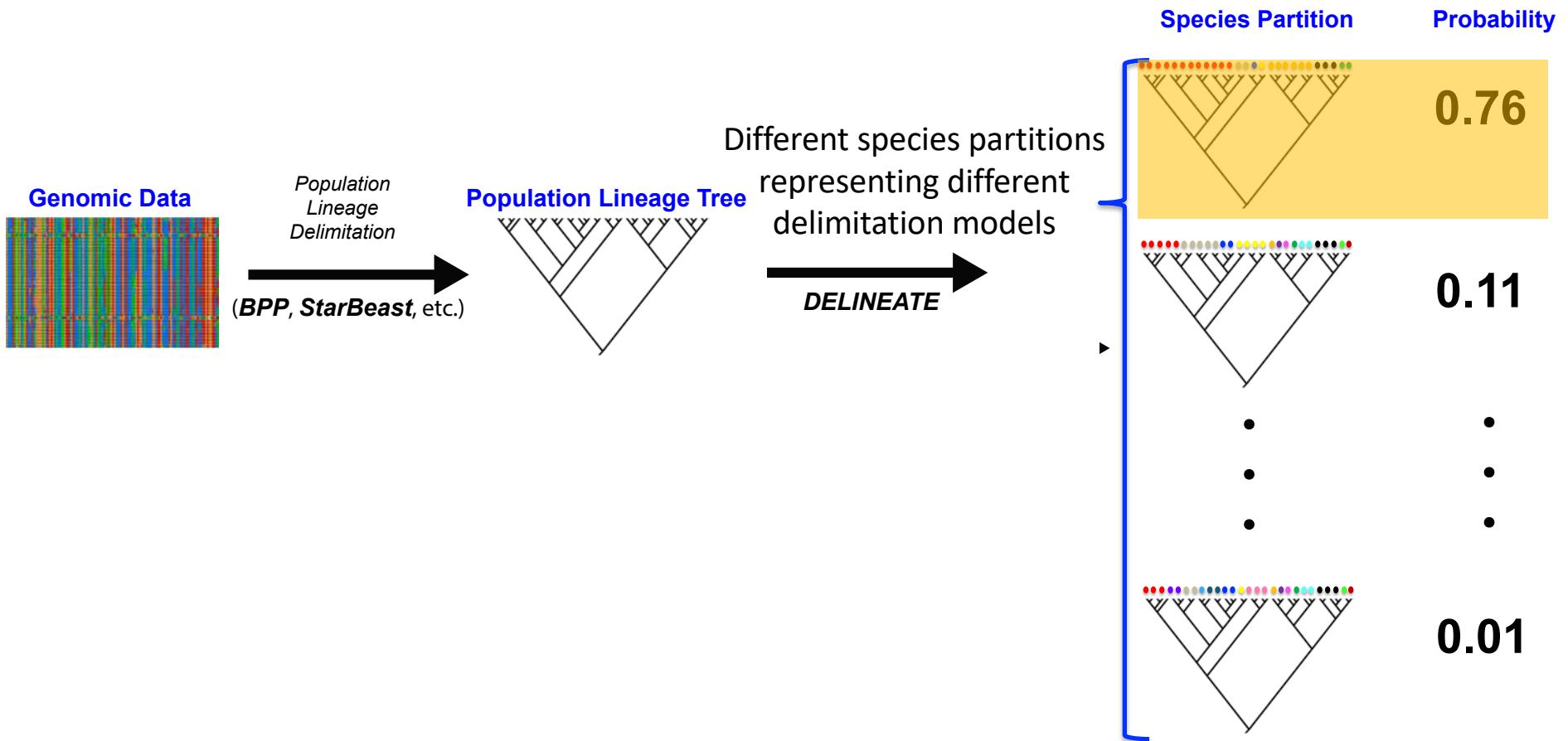
Jeet Sukumaran<sup>1</sup>✉\*, Mark T. Holder<sup>2</sup>, L. Lacey Knowles<sup>3</sup>

- We retain the rational of a comparative context in our computational framework: specification of species identities of a subset of population lineages that are well studied

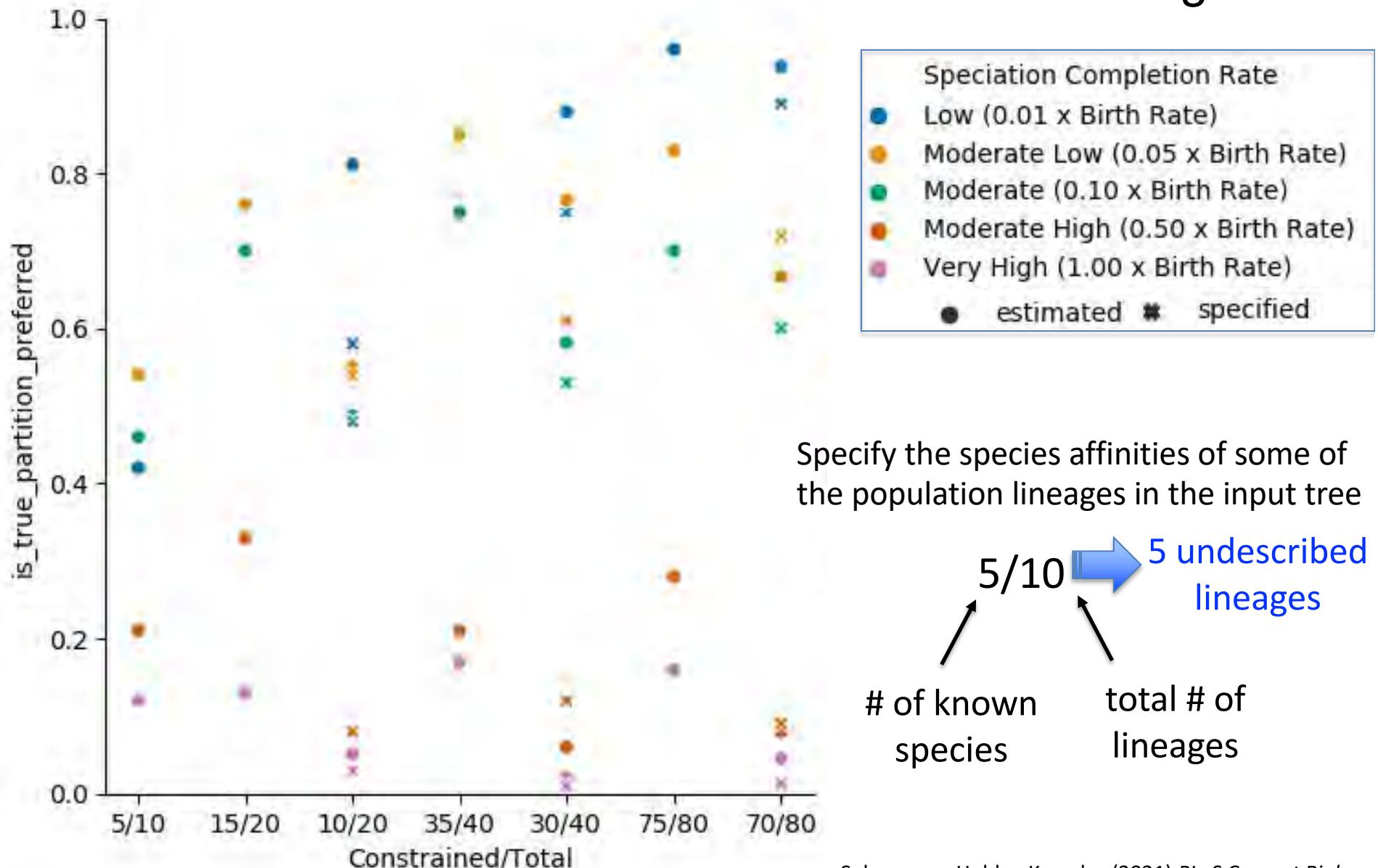


# DELINATE

- Preferred partition with highest probability

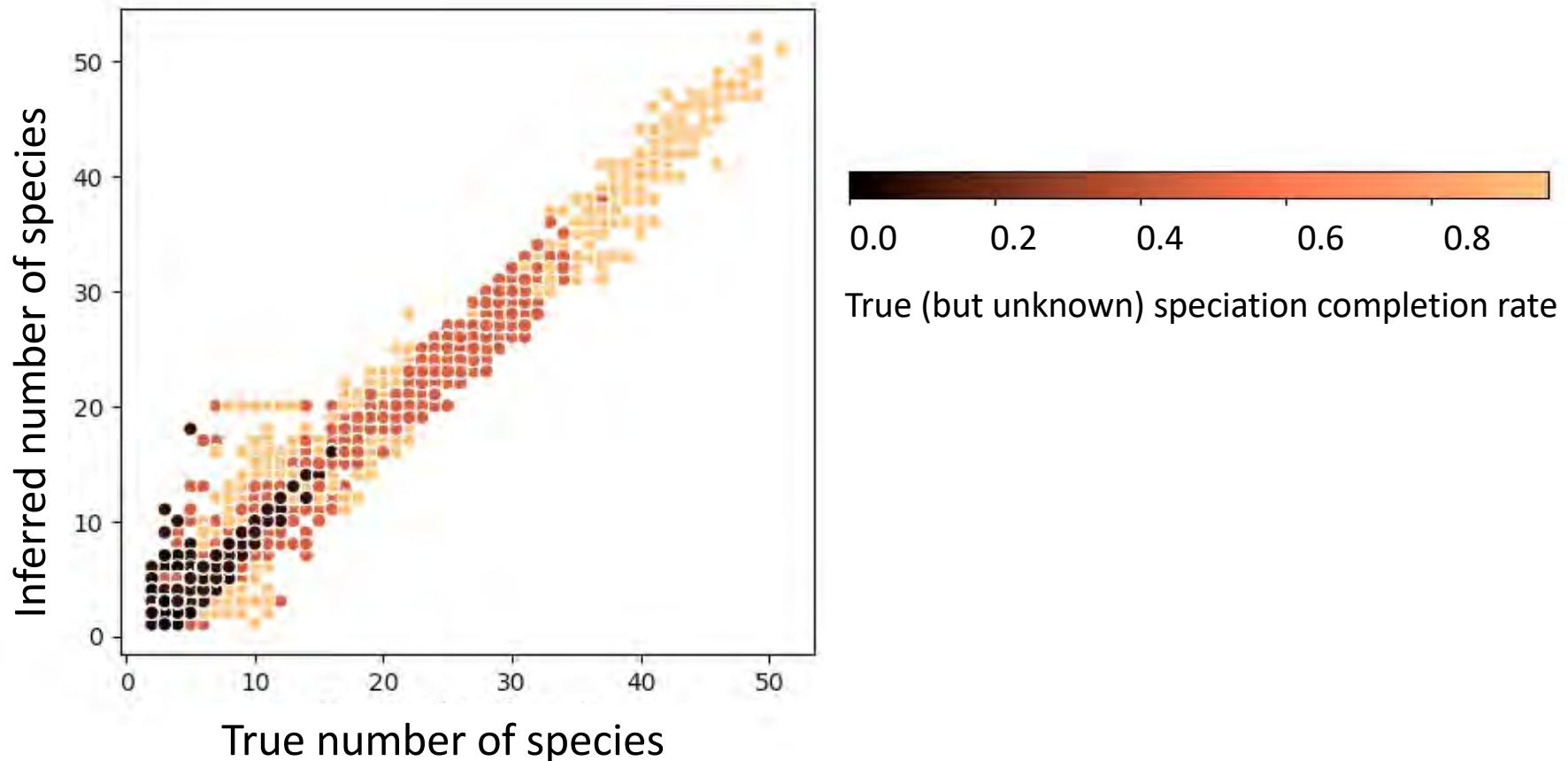


# Accuracy: recovery of true species partition for different sized trees with different numbers of undescribed lineages

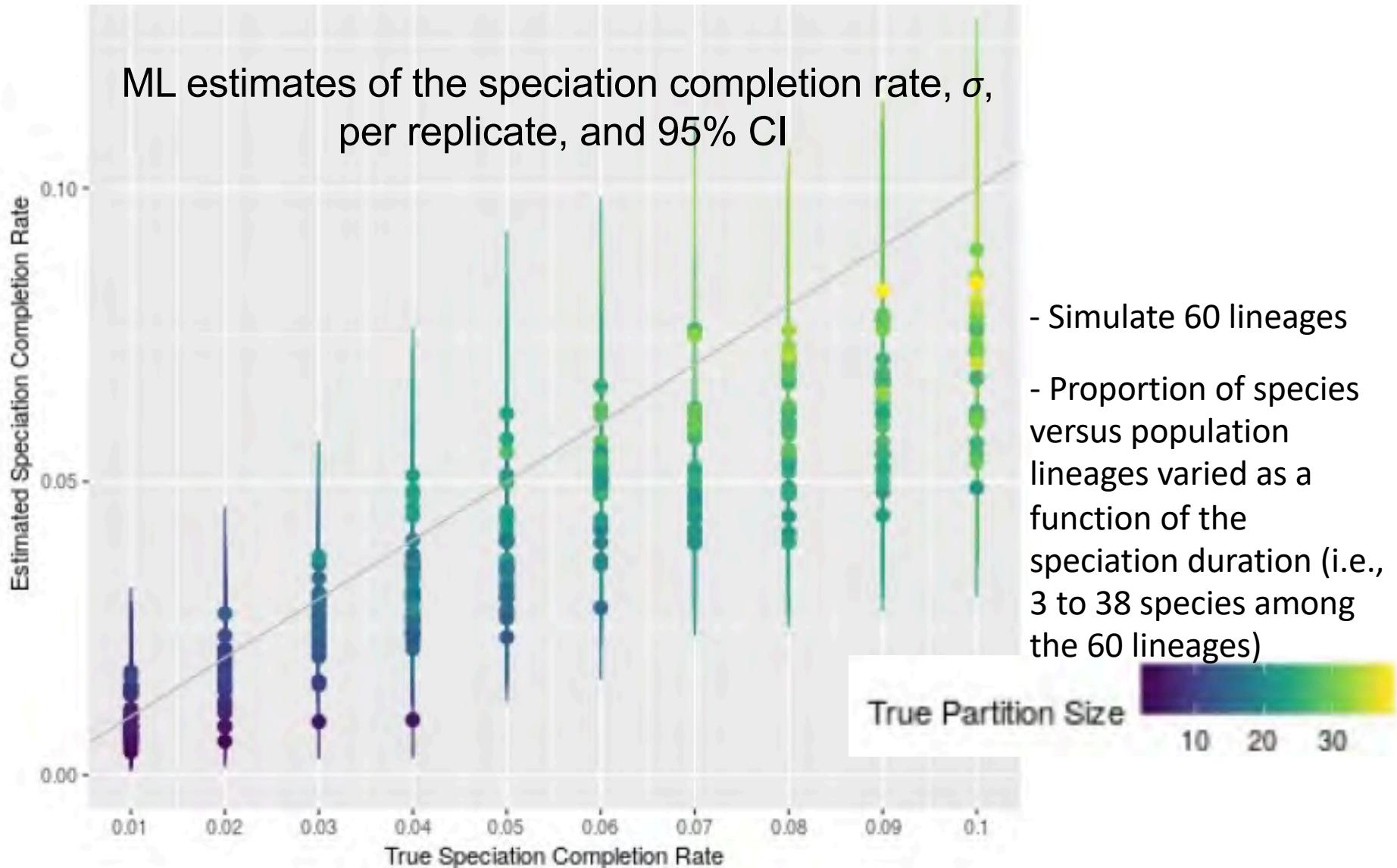


## Other implementations/applications of DELINEATE

Quantification of biodiversity with a model of an extended speciation process

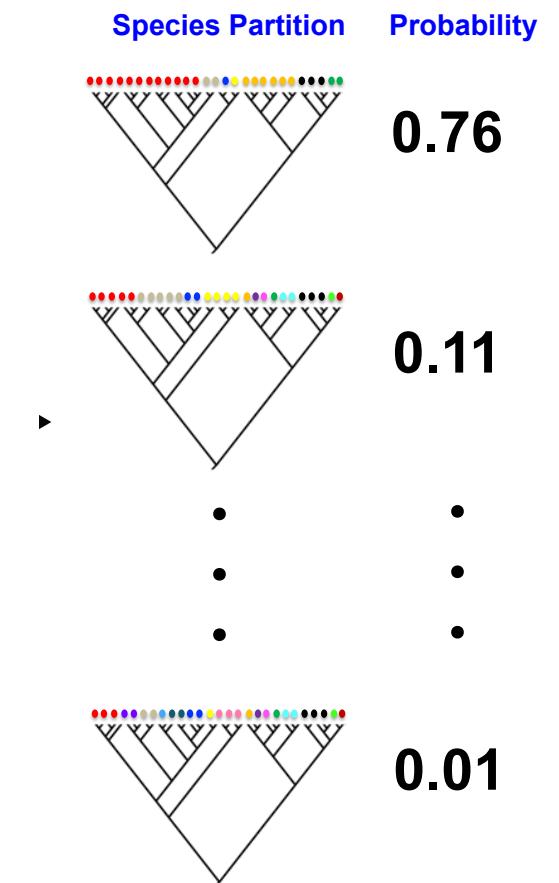


Can estimate the speciation completion rate,  $\sigma$ , if input data contains at least one con-specific statement and one hetero-specific statement



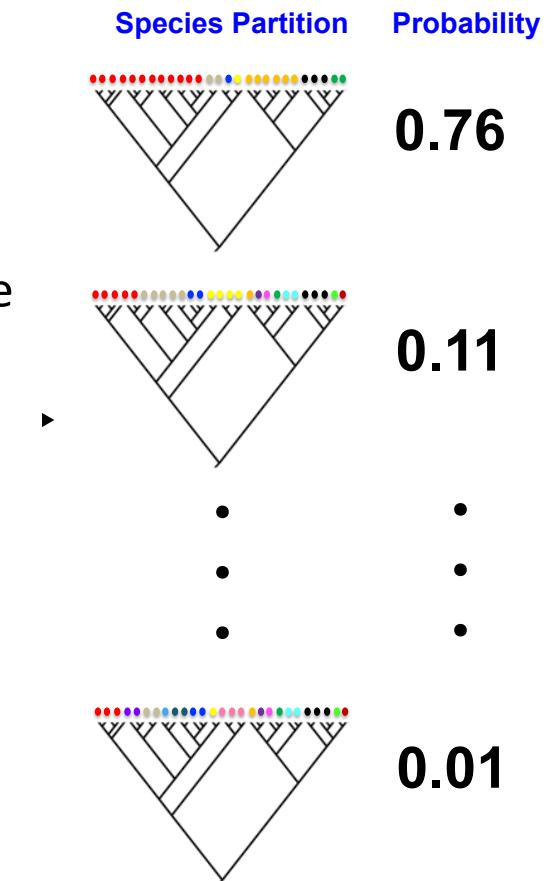
## Other implementations/applications of DELINEATE

- Integrate across partitions to determine if target populations are new species or belong to previously described species



## Other implementations/applications of DELINEATE

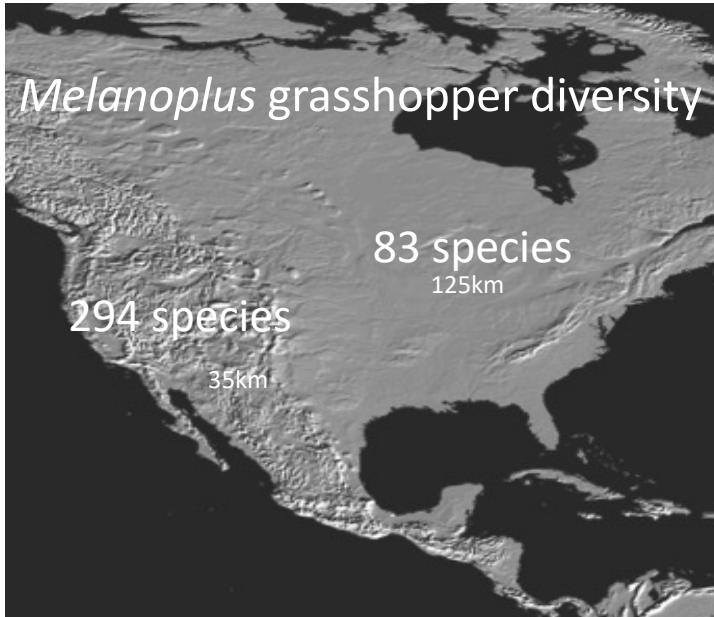
- Integrate across partitions to determine if target populations are new species or belong to previously described species



## **DELINATE:** a new class of delimitation models that incorporate the speciation process

- address the proliferation of artifactual species that results as within-species population lineages, detected due to restrictions in gene flow, are mis-identified as distinct species (as under the MSC)
- can assign probabilistically lineages of unknown affinities to pre-existing or new species
- we are able to learn not only about species boundaries, but also about the tempo of the speciation process itself

# DELINATE: the process of population splitting and species conversion are decoupled



We can ask whether the higher diversity reflects:

- higher rates of population isolation (perhaps due to landscape complexity or dynamic geographies), or
- higher rates of development of speciation isolating mechanisms

- Big Data: Between and within species genetic structure; specifically, target capture of 15,000 loci developed from RADseq across 30,000 individuals across the ranges of 352 species



## **DELINATE:** a new class of delimitation models that incorporate the speciation process

- addresses the proliferation of artifactual species that results as within-species population lineages, detected due to restrictions in gene flow, are identified as distinct species
- can assign probabilistically lineages of unknown affinities to pre-existing or new species
- we are able to learn not only about species boundaries, but also about the tempo of the speciation process itself
- By explicitly accounting for restrictions in gene flow not only between, but also within species, we also address the limits of genetic data for delimiting species.

## Limitations of genetic data alone for species delimitation

- Without any information about species affinities for a subset of taxa, or about speciation dynamics, accurate delimitation is not possible

## Limitations of DELINEATE

Sukumaran, Holder, Knowles (2021) *PLoS Comput Biol*

- Without any information about species affinities for a subset of taxa, or about speciation dynamics, accurate delimitation is not possible

That is, without incorporating independent information from other data sources, genetic data alone is not sufficient for accurate delimitation of species.

Software: *DELINEATE* <https://github.com/jeetsukumaran/delineate>

- Phylogenetic modeling approach that delineates species versus population lineages under a protracted speciation model

# Skeptical of statements that claim otherwise:

*Syst. Biol.* 68(1):168–181, 2019

© The Author(s) 2018. Published by Oxford University Press, on behalf of the Society of Systematic Biologists.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

DOI:10.1093/sysbio/syy051

Advance Access publication July 5, 2018

## The Spectre of Too Many Species

ADAM D. LEACHE<sup>1</sup>, TIANQI ZHU<sup>2,3</sup>, BRUCE RANNALA<sup>4</sup>, AND ZIHENG YANG<sup>2,5,6,\*</sup>

180

SYSTEMATIC BIOLOGY

VOL. 68

distinctness of the populations signifies the presence of reproductive barriers or isolation mechanisms. There seems to be no controversy in assigning species status to populations that exist in sympatry and are genetically distinct.

For heuristic delimitation of allopatric species, we suggest the use of Bayesian parameter estimation. The genomic data allows reliable estimation of population-divergence parameters ( $\theta_s$ ,  $\tau_s$ , and  $M_s$ ), which can then be used to apply a heuristic definition of species status.

### *Heuristic Criteria for Species Status*

The *gdi* attempts to use the overall genetic divergence between two populations affected by the combined effects of genetic isolation and gene flow. The index appears to have weaknesses. First, the criterion depends on the population divergence time relative to the

sequence data. There appears to be no controversy regarding the use of Bayesian model selection under MSC or BPP to identify morphologically cryptic species. For allopatric populations or species, the accurate estimation of important population parameters should allow one to apply any empirical criterion for defining species that the evolutionary biologist entertains. For these reasons, the MSC model and BPP will continue to be useful tools in the analysis of genomic data to better understand biodiversity despite the fact that the interpretation of these results in assessing species status may be debated.

### SUPPLEMENTARY MATERIAL

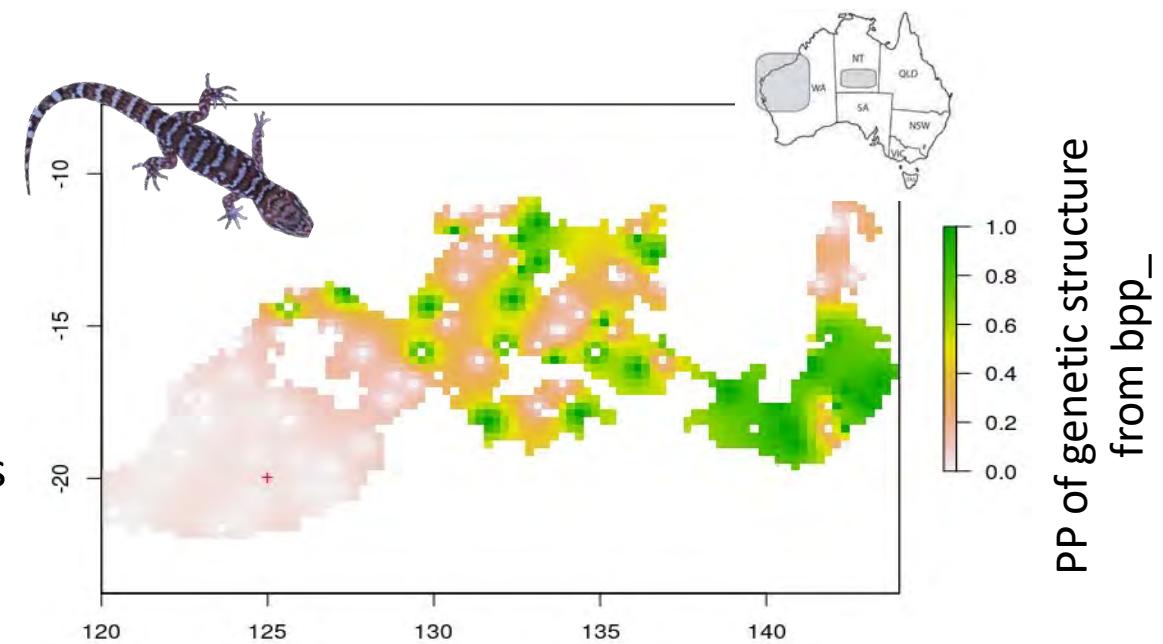
Data available from the Dryad Data Repository:  
<http://dx.doi.org/10.5061/dryad.t66gq81>.

## Landscapes, species delimitation and sampling schemes

- Model of the geography of genetic divergence under a spatially explicit coalescent to evaluate the robustness of the MSC

Software: *DECRIPT* <https://becheler.github.io/pages/applications.html>

Basically, simulate data under a single species demographic history and examine which populations are inferred to represent two species under the MSC when the actual history was simulated as a single species.



## General methodology

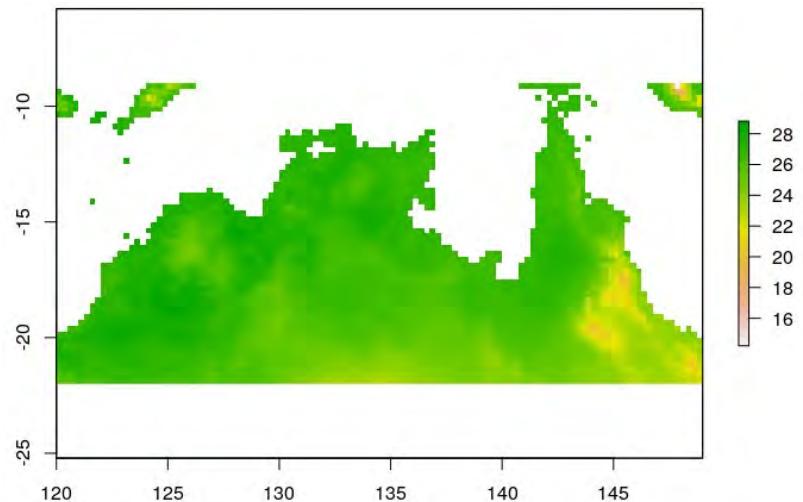
---

1. Simulate a spatially explicit demography in an heterogeneous landscape
2. Calibrate the simulation to reproduce gene flow reduction by:
  - geographic distance
  - environmental barriers (or shifting environmental conditions over time)
3. simulate many different spatial sampling schemes
4. simulate coalescent trees
5. simulate many genetic data
6. apply species delimitation method
7. understand how the number of detected species varies as a function of the sampling

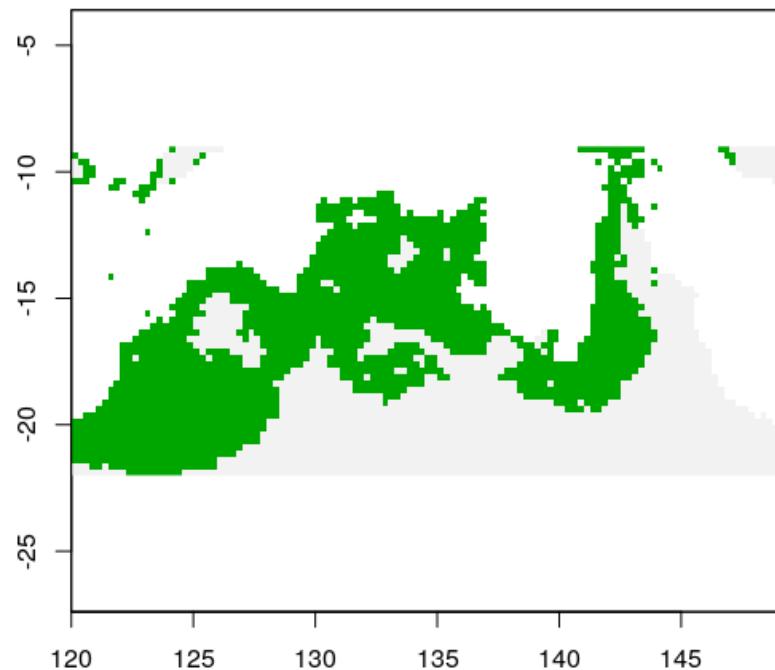
## Heterogeneous landscape

---

- Landscape is discretized in  $n$  demes (grid cells).
- Let be  $L = 1$  environmental variables.



**Figure 3:** Bio1. Annual Mean Temperature in  $C^\circ$ .



**Figure 4:** Locations where the Annual Mean Temperature is higher than  $26.4\text{ }C^\circ$  (in green).

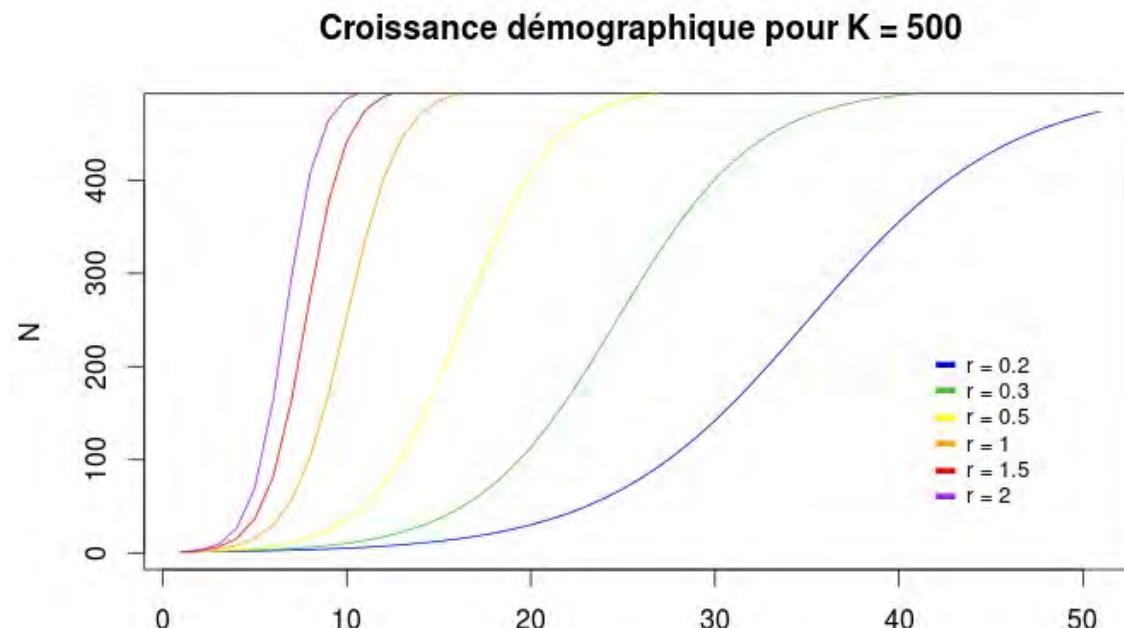
# Demographic growth

Number of children:  $\tilde{N}_x^t \sim \text{Poisson}(g(x, t))$

Logistic growth:

$$g : \begin{array}{ccc} X \times N & \xrightarrow{I} & \mathbb{R}^+ \\ x, t & \xrightarrow{I} & \frac{N_x^t \times (1 + r(x, t))}{1 + \frac{r(x, t) \times N_x^t}{k(x, t)}} \end{array}$$

- $r$ : growth rate constant
- $k$ : carrying capacity function of bio1



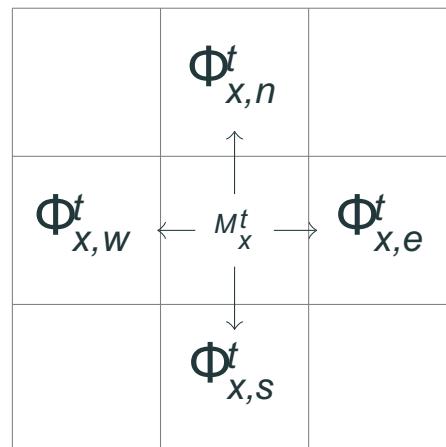
# Dispersal

---

Dispersal of children in deme  $x$  among the 4 neighboring cells:

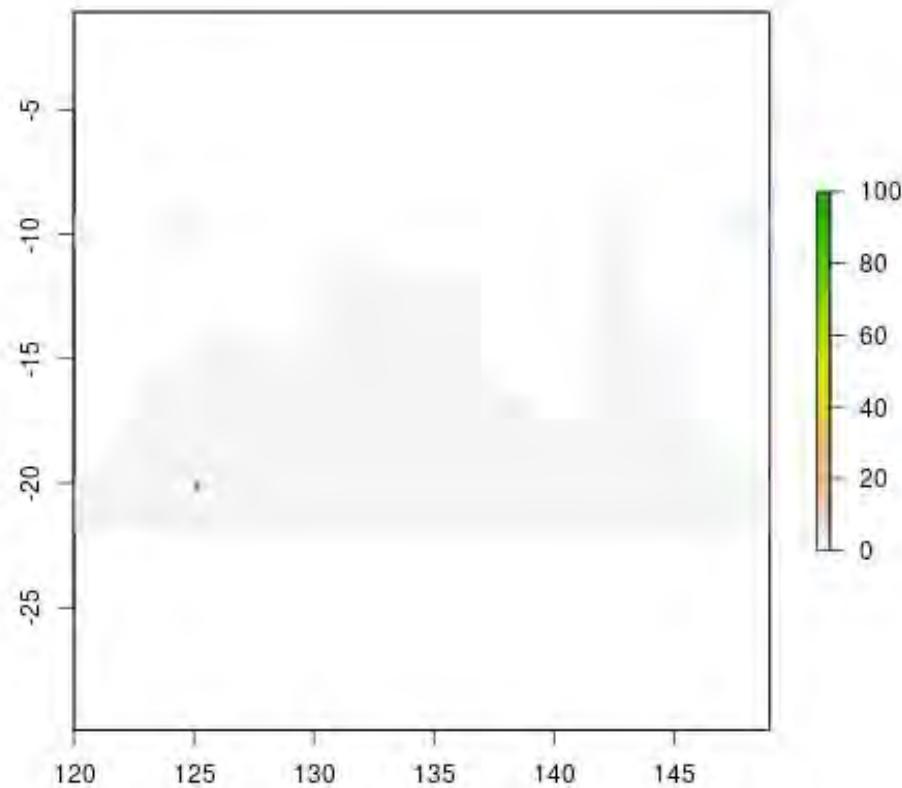
- emigration rate  $e$
- number of effective emigrants going out of deme  $x$ :  $M = e \times N_x^t$
- set of  $x$  neighbours (North, South, East, West):  $V_x$
- number of individuals going from  $x$  to  $y \in V_x$  at time  $t$ :  $\Phi_{x,y}^t$
- sampling emigrants destination in a multinomial law defines  $\Phi_{x,y}^t$ :

$$(\Phi_{x,y}^t)_{y \in V_x} \sim M(\tilde{N}_x^t, (p_{xy})_y).$$



## Demographic process

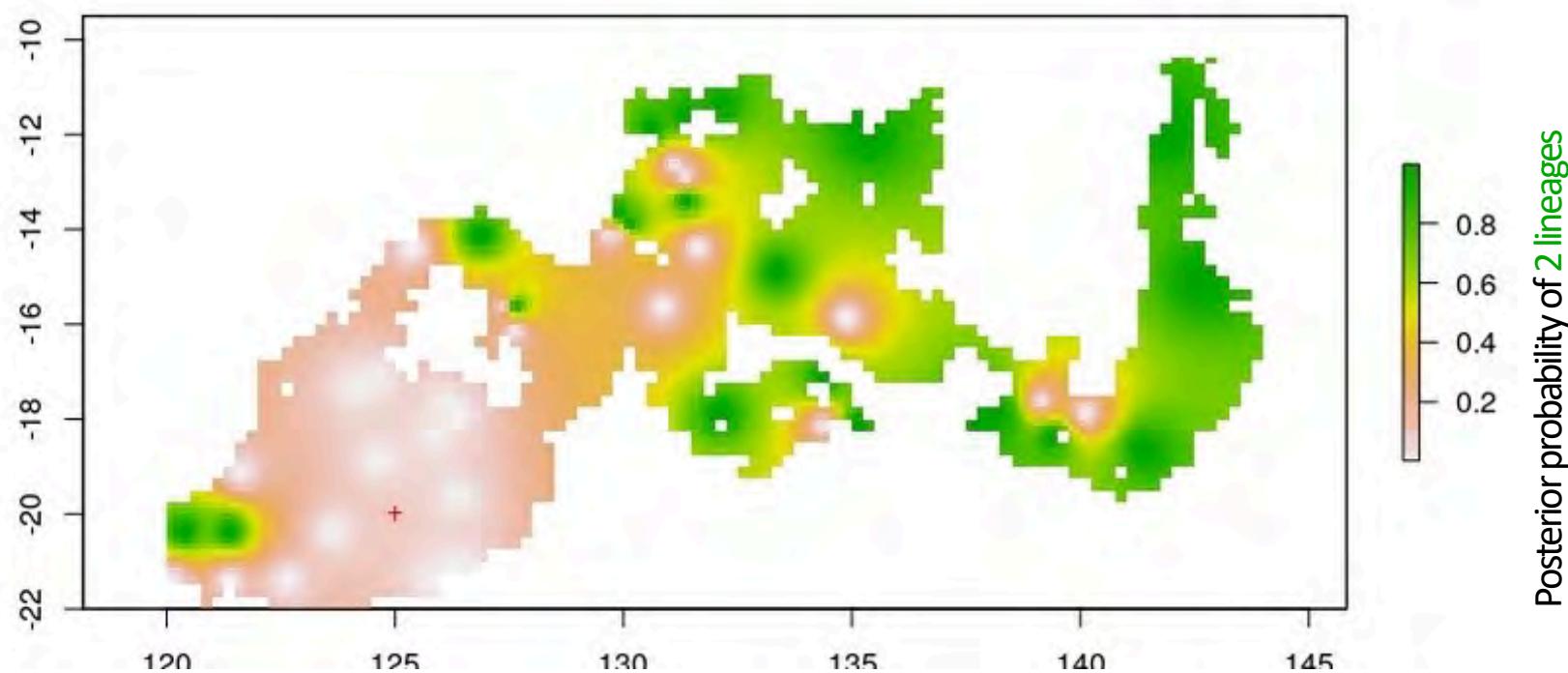
$$\text{Flux de propagules } \Phi : (\Phi_{x,y}^t)_{y \in V_x} \sim M(\tilde{N}_x^t, (p_{xy})_y) . \quad | \quad \text{Taille de population } N : N_j^{t+1} = \sum_{i \in X} \Phi_{i,j}^t$$



**Figure 5:**  
location of  
genetic

ed line: the  
or many

Results: multiple species will be inferred over some sampled populations even though genetic structure reflects nothing more than reductions in gene flow expected by geographic distance and environmental heterogeneity



# Thank you!

former Postdoctoral fellows:



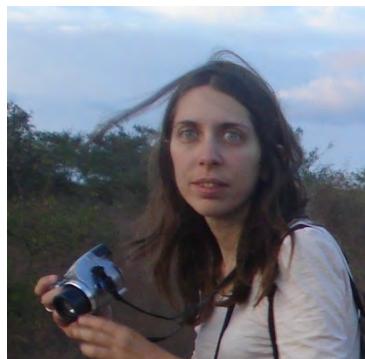
Jeet Sukumaran  
San Diego State Univ.

<https://github.com/jeetsukumaran/delineate>

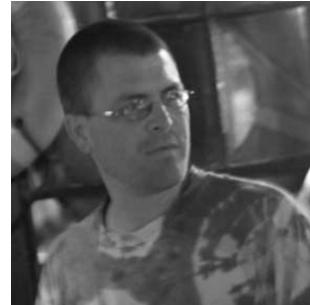


Arnaud Becheler  
University of Michigan

<https://becheler.github.io/pages/applications.html>



Anna Papadopoulou



Mark Holder  
Univ. of Kansas

Former UM  
Ph. D. students:  
Giorgia G. Auteri



Qixin He



Rob Massatti



support NSF & the UM  
[knowlesl@umich.edu](mailto:knowlesl@umich.edu)

