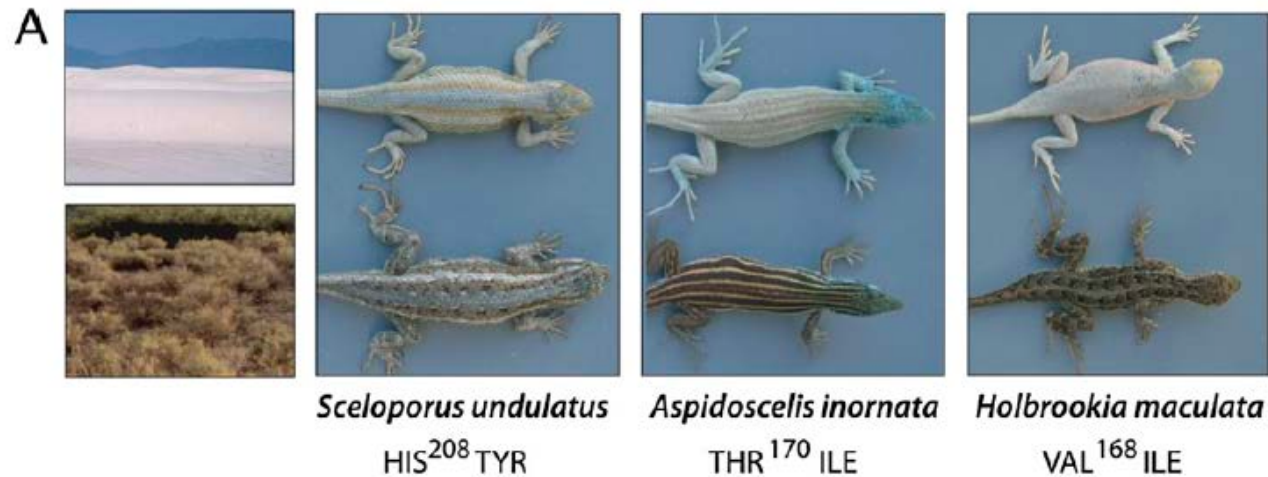


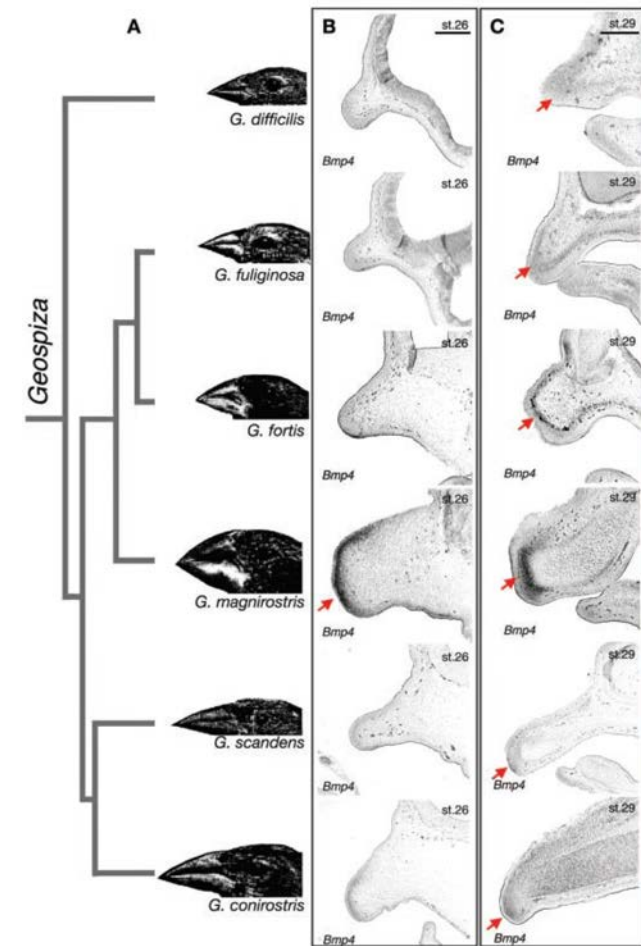
# Comparative Methods

(most figures are from Harmon 2019)

# Evolution is happening all around us



Rosenblum, E. B., H. Römpler, T. Schöneberg, and H. E. Hoekstra. 2010. Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *Proc. Natl. Acad. Sci. U. S. A.* 107:2113–2117

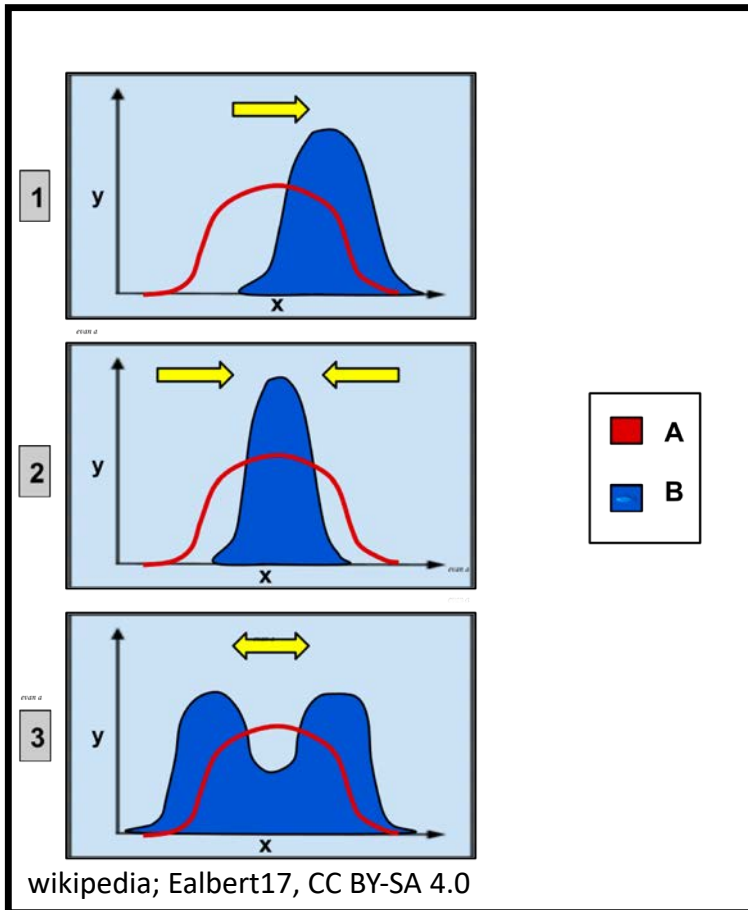


Abzhanov, A., M. Protas, B. R. Grant, P. R. Grant, and C. J. Tabin. 2004. *Bmp4* and morphological variation of beaks in Darwin's finches. *Science* 305:1462–1465.

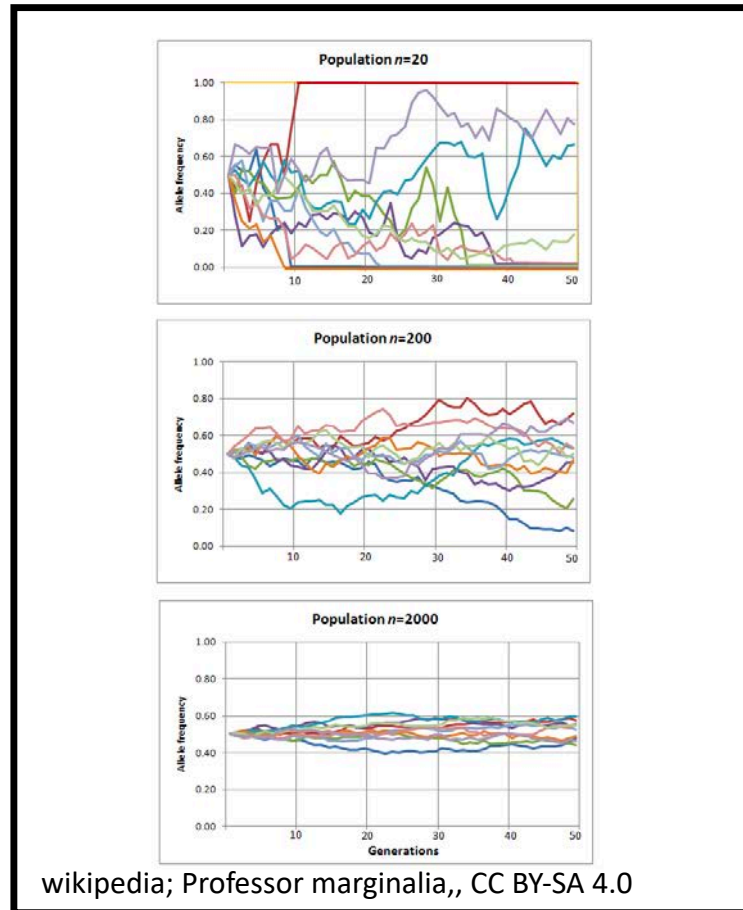
# The Tree of Life

# Evolutionary Processes in Populations

## Natural Selection



## Genetic Drift



*Also migration, sexual selection*

*These have been occurring for 4 billion years*

***How do these processes connect with broad-scale patterns?***

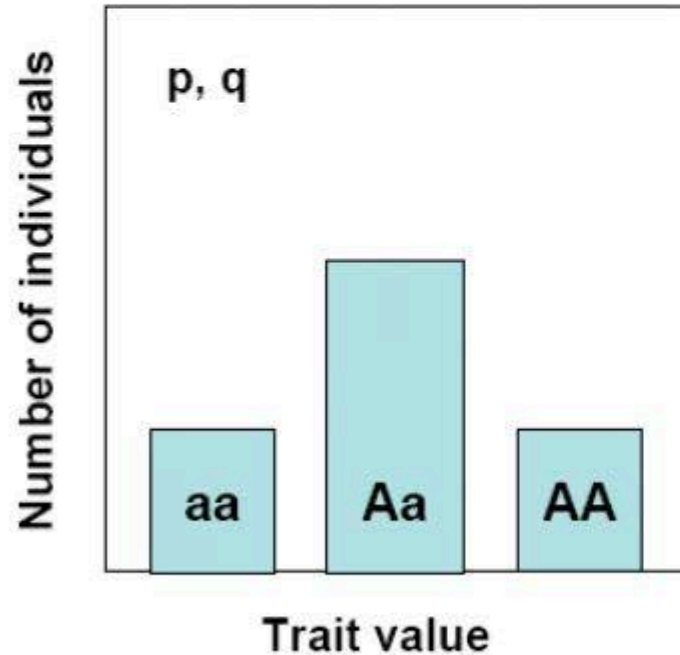
# Comparative Methods

- Connect evolutionary processes to broad scale patterns in the tree of life
  - *Why did whales get so big?*
- combine biology, mathematics, and computer science
- Uses phylogenetic trees and other data
- Brings together three fields:
  1. Population and Quantitative Genetics
  2. Paleobiology
  3. Phylogenetics

# Population and Quantitative Genetics

## ***Population genetics***

Mendelian traits:  
discrete variation



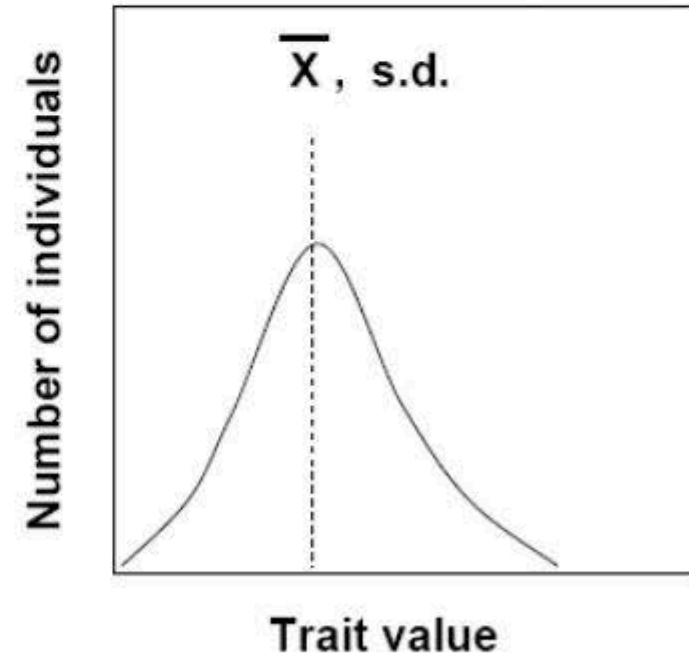
focuses on allele frequencies

***Genomics  
blurs this  
distinction***

***Both focus  
on change  
over a few  
generations***

## ***Quantitative genetics***

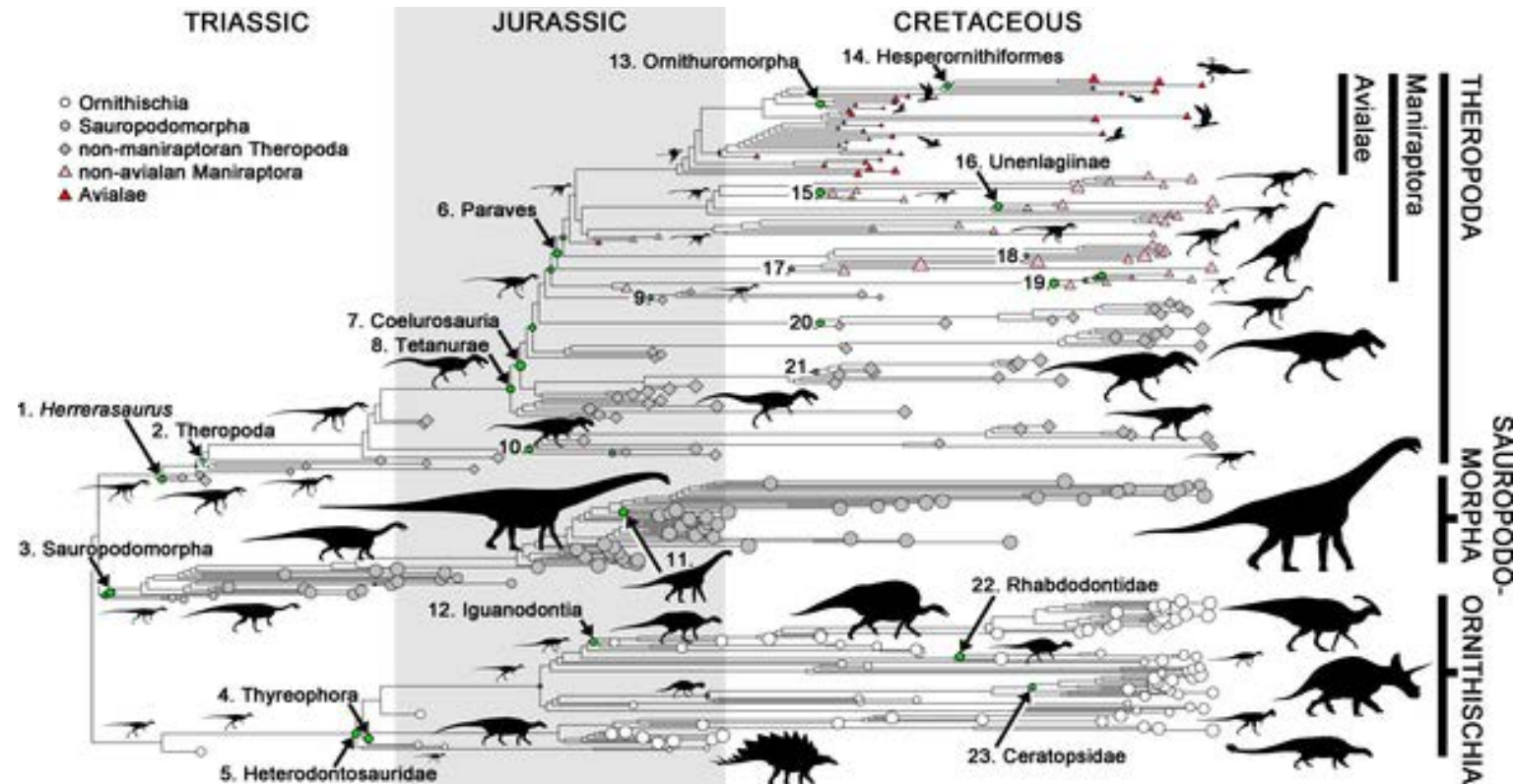
Quantitative traits:  
continuous variation



focuses on traits and heritability

# Paleontology

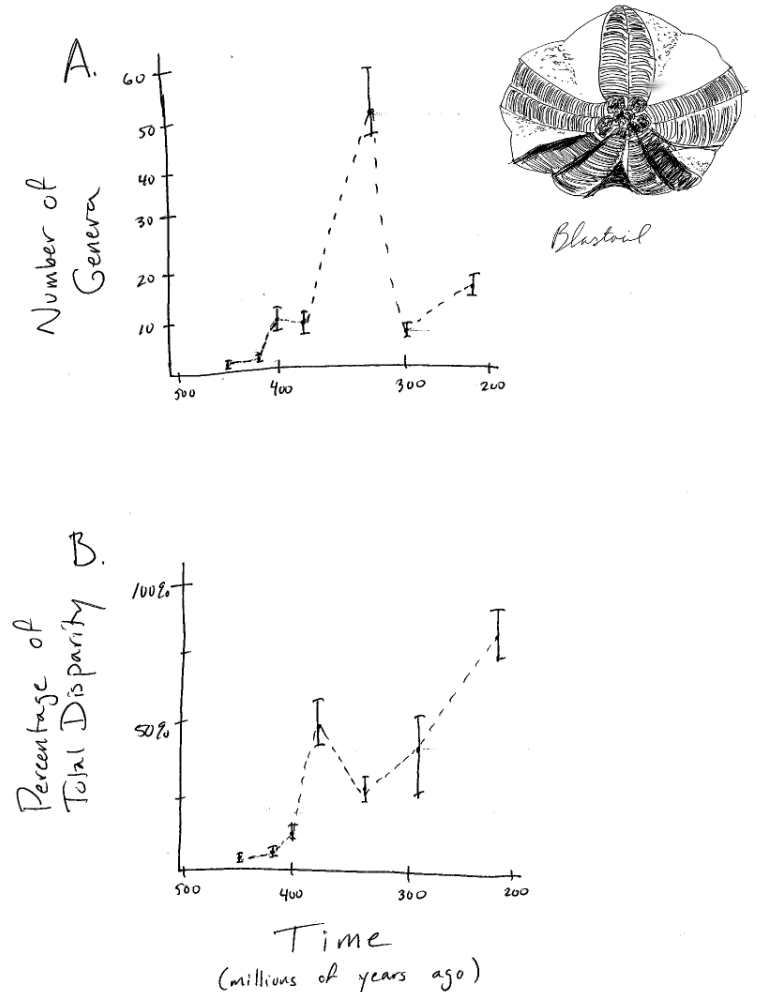
*Uses macroevolutionary models to explain the distribution of species and traits in the fossil record*



Benson et al. 2014. *PLoS Biology*

# Macroevolution

- using fossils to quantify rates and patterns of evolution
- fossils are good:
  - showing character change through time
- But:
  - fossil record is incomplete
  - in ways that hamper the ability to understand how diversification works
  - for instance, anoles and Darwin's finches lack many fossils even though they are adaptive radiations
  - age and taxonomy not always perfect





# This is where phylogenetic trees come in

- phylogenies are complementary to fossils
  - you can include all living species of a clade in a phylogeny, theoretically
- can use informed taxonomic decisions – unlike fossils which are often ambiguous
- phylogenies have different limitations than fossils
  - reconstructing traits at ancestral nodes
  - tempo of evolution can change through time
  - debate on whether extinction rates can be detected

# Phylogenetics

Independent contrasts Felsenstein (1981)

think about the branches in the phylogenetic tree as the historical “pathways” of evolution.

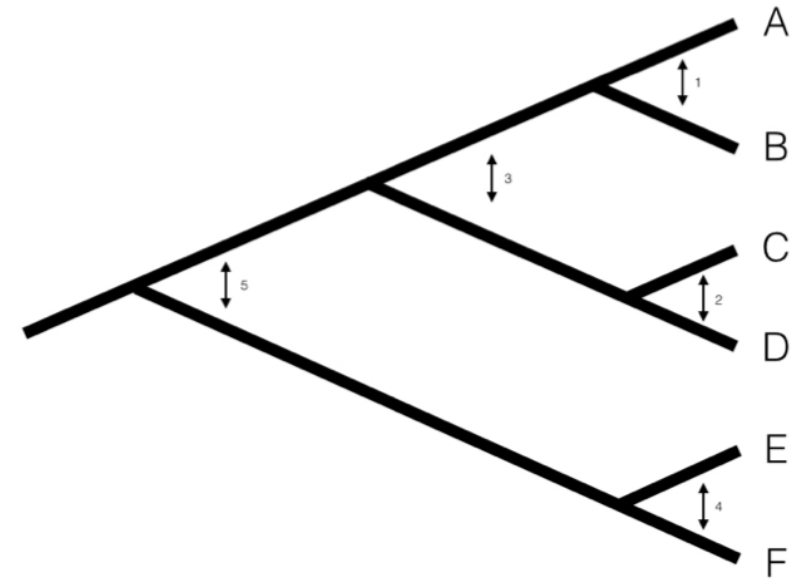
Each branch on the tree represents a lineage that was alive at some time in the history of the Earth, and during that time experienced some amount of evolutionary change

We can imagine trying to measure that change initially by comparing sister taxa.

We can compare the trait values of the two sister taxa by finding the difference in their trait values, and then compare that to the total amount of time they have had to evolve that difference.

“pruning algorithm,” chopping off pairs of sister taxa to create a smaller tree

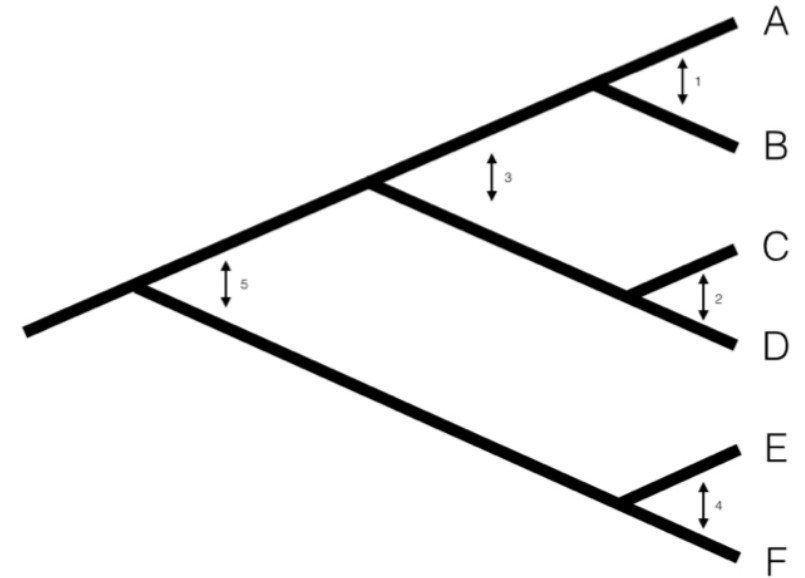
generalize the approach of comparing sister taxa so that we can quantify the rate of evolution throughout the whole tree.



# Phylogenetics

## ***Independent Contrasts:***

1. Presented the necessity of placing comparative work in a phylogenetic context
2. IC is computationally and easy to interpret
3. connects quantitative genetics and paleobiology using math

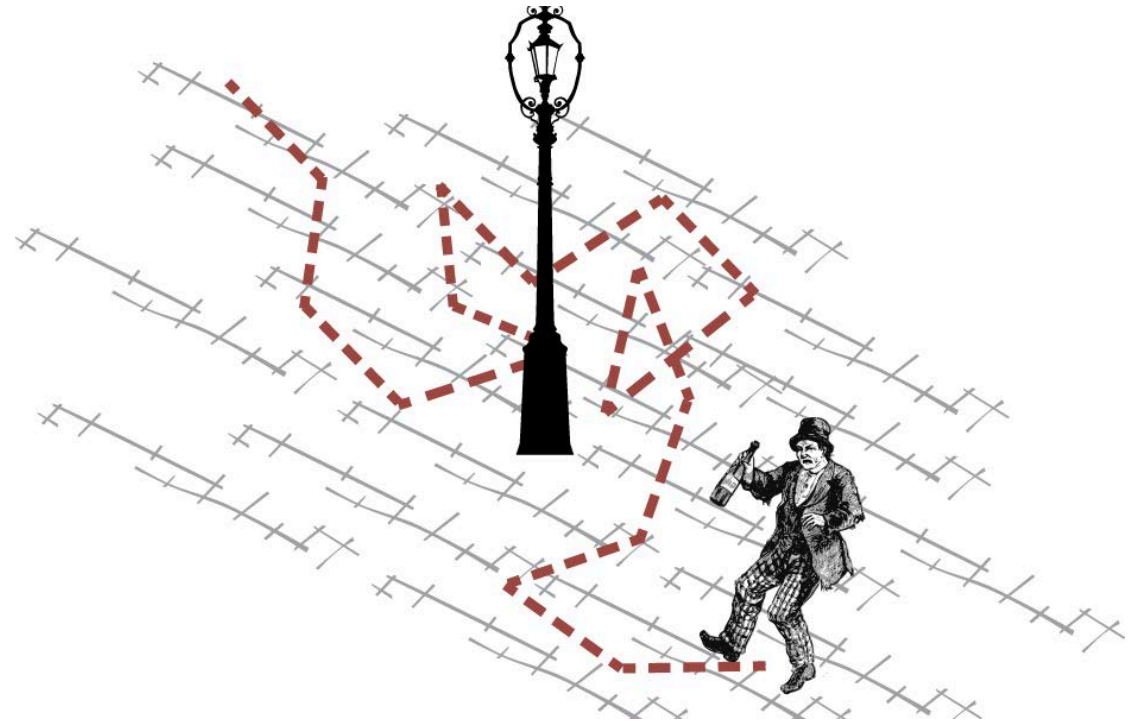


# Statistical approaches to understand how traits change through time

- requires a mathematical specification of how evolution takes place
  - Model 1: trait starts with a certain value, then has a probability of changing in any unit of time
  - Model 2: considers a large set of individuals in a population, their genotypes, and allow evolution through reproduction with selection
- Brownian motion is more similar to the first

# Brownian Motion

- Models the evolution of a continuous trait through time
- Continuous trait: a distribution of phenotypes along a continuum
  - examples: height, weight, reflectance
- “Random walk”
  - **Uncorrelated** means the direction of movement is completely independent of the previous directions taken.
  - **Unbiased** means there is no preferred direction, the direction moved at each step is completely random.



simple simulations to show how the Brownian motion model behaves.

- mean character value, which we will denote as  $\bar{z}$ , in a population
  - you can measure a sample of the individuals in a population and estimate the mean average trait value
- We will denote the mean trait value at some time  $t$  as  $\bar{z}(t)$
- We can model the mean trait value through time
- two parameters:
  - starting value of the population mean trait,  $\bar{z}(0)$
  - the evolutionary rate parameter,  $\sigma^2$
  - This parameter determines how fast traits will randomly walk through time.

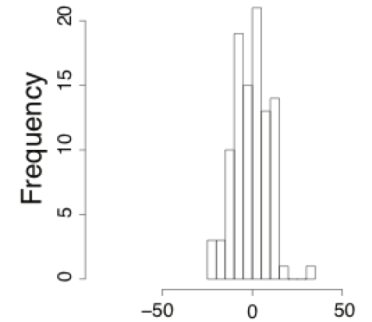
# simple simulations to show how the Brownian motion model behaves.

- At the core of Brownian motion is the normal distribution
  - can be described by two parameters, the mean and variance
- changes in trait values over any interval of time are always drawn from a normal distribution with mean 0 and variance proportional to the product of the rate of evolution and the length of time (variance =  $\sigma^2 t$ ).
- normal distributions for expected changes have a mean of zero and get wider as the time interval we consider gets longer

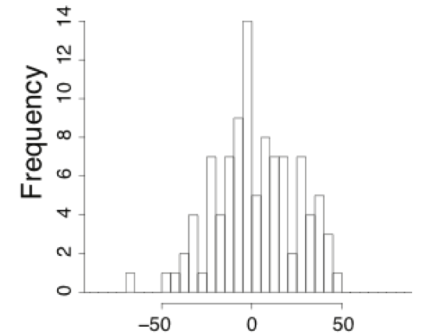
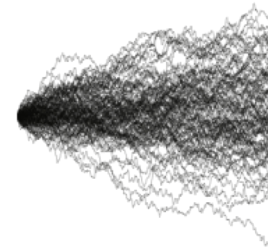
# simple simulations to show how the Brownian motion model behaves.

- sets of Brownian motion run over three different time periods
- You can see that the tip values look like normal distributions
- the variance among separate runs of the process increases linearly with time

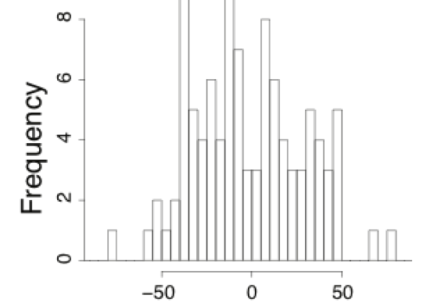
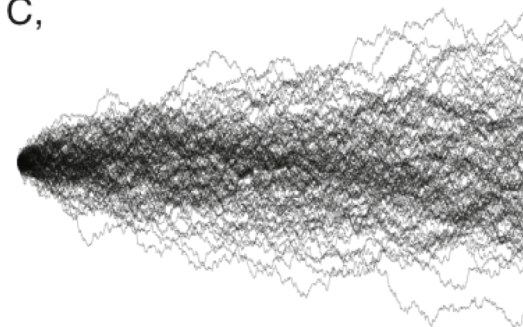
A,



B,



C,

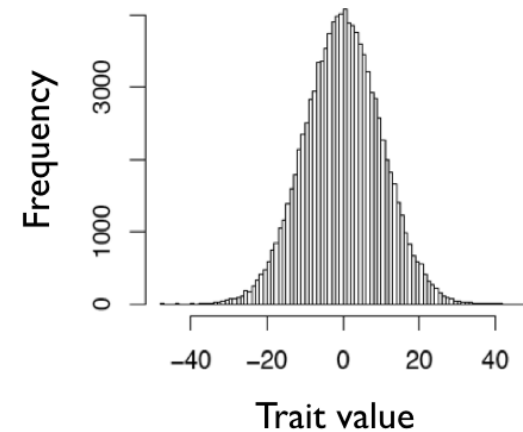




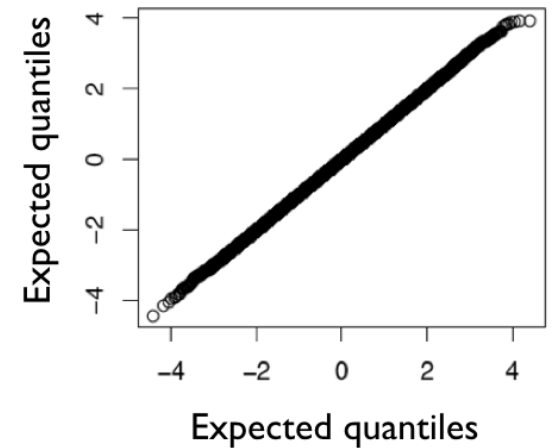
# simple simulations to show how the Brownian motion model behaves.

1. run a Brownian motion process over a given time interval many times
2. save the trait values at the end of each of these simulations
3. the distributions of possible character states at any time point in a Brownian walk is normal

A.

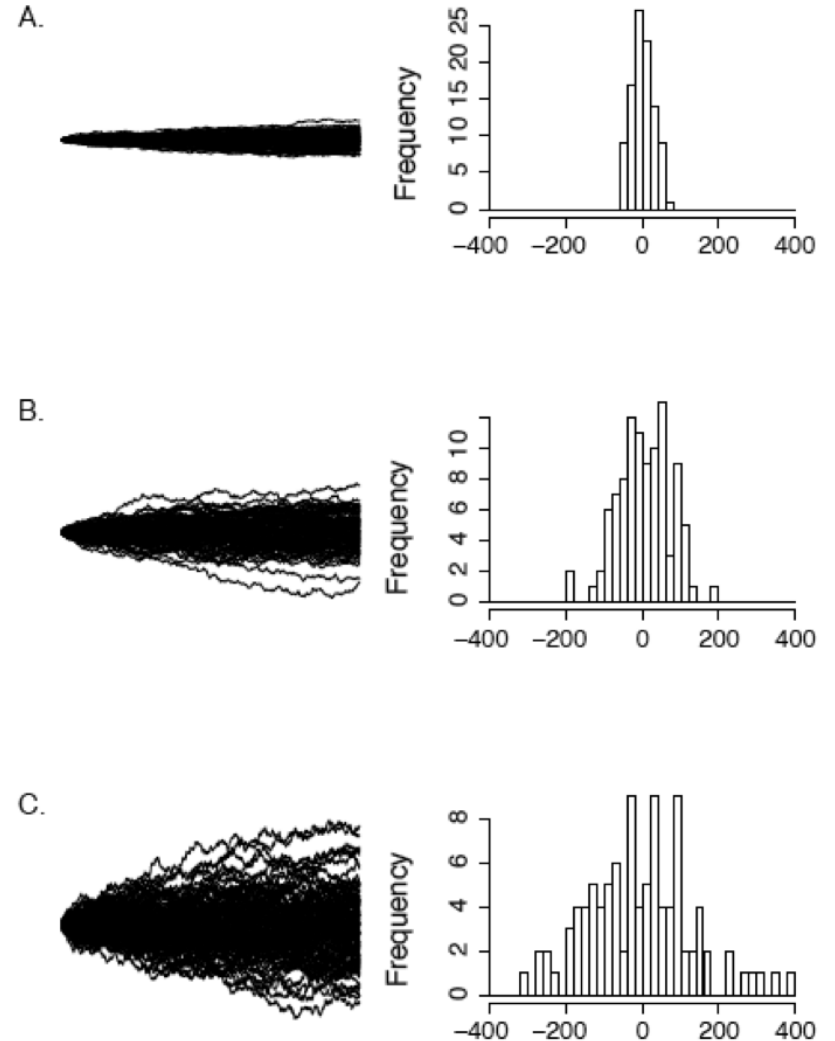


B.



# how rate parameter $\sigma^2$ affects the rate of spread of Brownian walks

*simulations with a higher rate parameter create a larger spread of trait values among simulations over the same amount of time.*



# The 1<sup>st</sup> main property of Brownian motion

- the expected value of the character at any time  $t$  is equal to the value of the character at time zero
- Brownian motion has no “trends,” and wanders equally in both positive and negative directions
- If you take the mean of a large number of simulations of Brownian motion over any time interval, you will likely get a value close to  $\bar{z}(0)$ ; as you increase the sample size, this mean will tend to get closer and closer to  $\bar{z}(0)$ .

# The 2<sup>nd</sup> main property of Brownian motion

- each successive interval of the “walk” is independent
- if you sample the process from time 0 to time  $t$ , and then again at time  $t + \Delta t$ , the change that occurs over these two intervals will be independent of one another
- It is worth noting that only the changes are independent, and that the value of the walk at time  $t + \Delta t$  is not independent of the value of the walk at time  $t$ ,  $\bar{z}(t)$

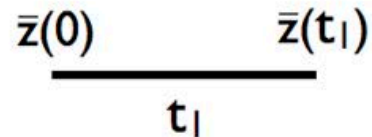
# The 3<sup>rd</sup> main property of Brownian motion

- the value of  $\bar{z}(t)$  is drawn from a normal distribution with mean  $\bar{z}(0)$  and variance  $\sigma^2 t$
- the parameter  $\sigma^2$  is important for Brownian motion models, as it describes the rate at which the process wanders through trait space
- The overall variance of the process is that rate times the amount of time that has elapsed

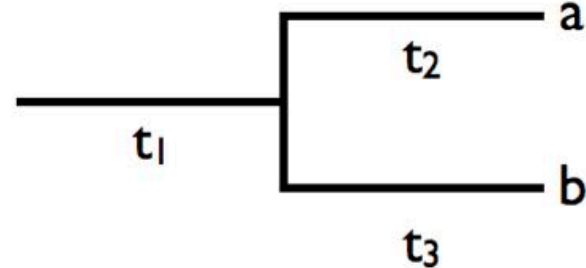
# Brownian motion on a phylogenetic tree

- consider evolution along a single branch with length  $t_1$
- we can model simple Brownian motion over time  $t_1$  and denote the starting value as  $\bar{z}(0)$
- Now consider a small section of a phylogenetic tree including two species and an ancestral stem branch
- Assume a character evolves on that tree under Brownian motion, again with starting value  $\bar{z}(0)$  and rate parameter  $\sigma_B^2$
- The mean trait in that species  $\bar{x}_a$  evolves under Brownian motion from the ancestor to species a over a total time of  $t_1 + t_2$
- For species  $\bar{x}_b$  the mean trait evolves over a total time of  $t_1 + t_3$

A.



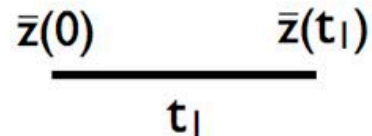
B.



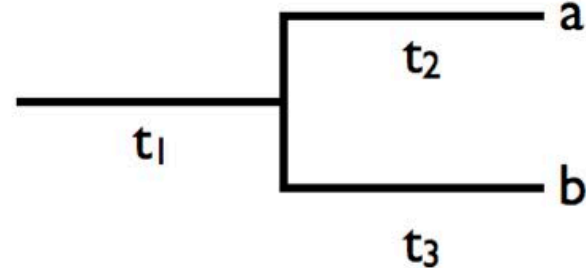
# Brownian motion on a phylogenetic tree

- However,  $\bar{x}_a$  and  $\bar{x}_b$  are not independent of each other.
- the two species share one branch in common
- Each tip trait value can be thought of as an ancestral value plus the sum of two evolutionary changes:
  - one (from branch 1) that is shared between the two species
  - one that is unique (branch 2 for species a and branch 3 for species b)
- mean trait values  $\bar{x}_a$  and  $\bar{x}_b$  will share similarity due to their shared evolutionary history
- ***(they covary!!!)***

A.

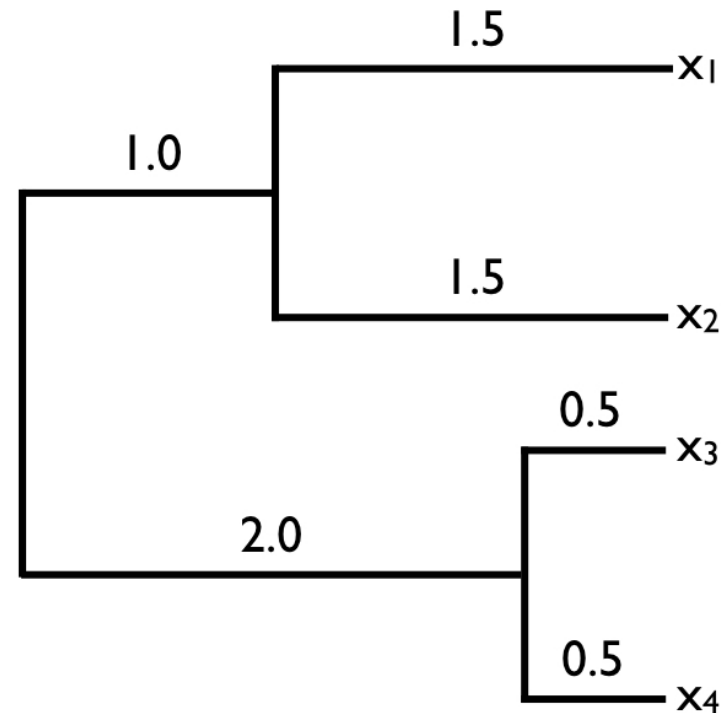


B.



# the phylogenetic variance-covariance matrix

- For phylogenetic trees with  $n$  species, this is an  $n \times n$  matrix
  - each row and column corresponding to one of the  $n$  taxa in the tree
- Along the diagonal are the total distances of each taxon from the root of the tree
- the off-diagonal elements are the total branch lengths shared by particular pairs of taxa

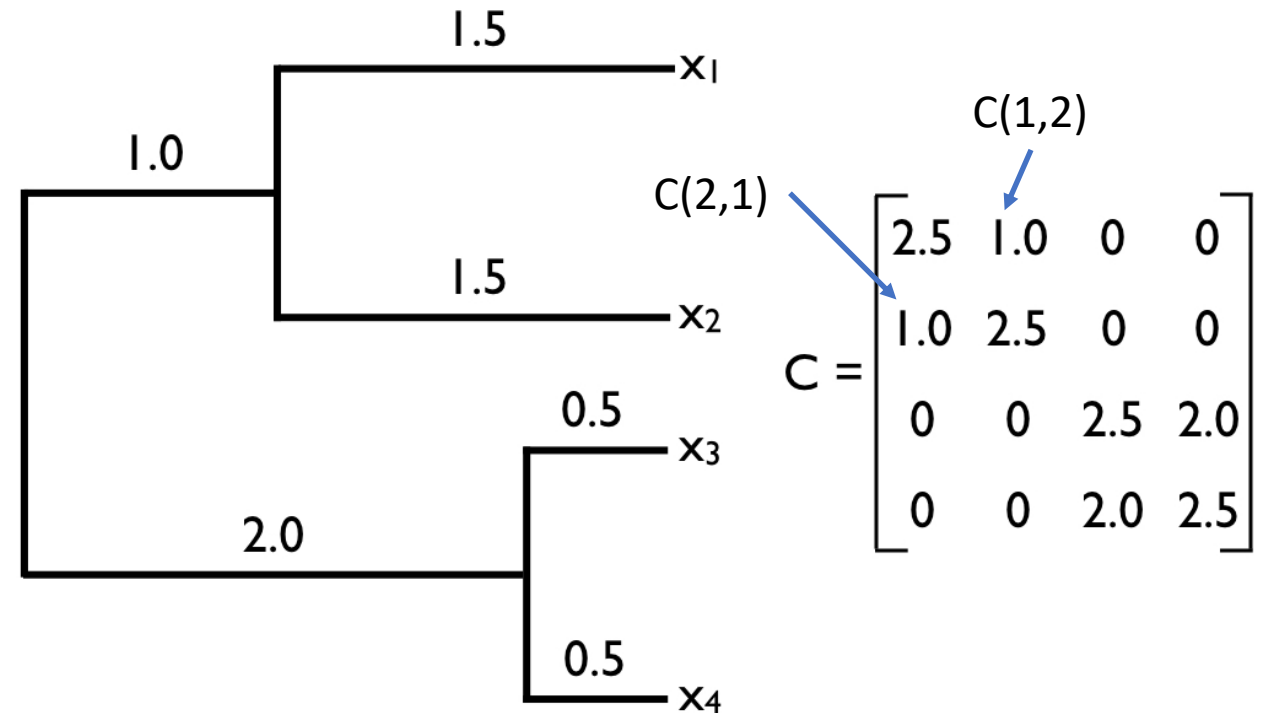


$$C = \begin{bmatrix} 2.5 & 1.0 & 0 & 0 \\ 1.0 & 2.5 & 0 & 0 \\ 0 & 0 & 2.5 & 2.0 \\ 0 & 0 & 2.0 & 2.5 \end{bmatrix}$$



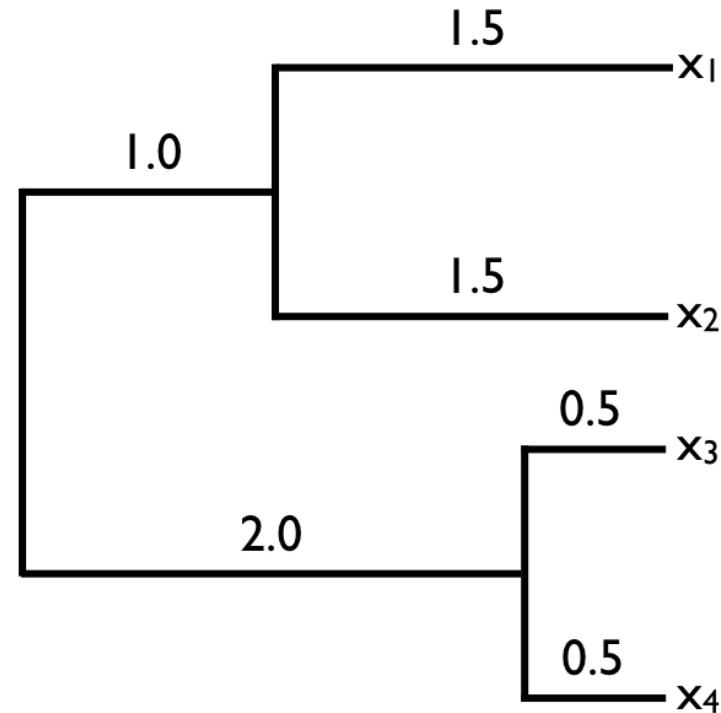
# the phylogenetic variance-covariance matrix

- $\mathbf{C}(1, 2)$  and  $\mathbf{C}(2, 1)$  is the shared phylogenetic path length between the species in the first row and the species in the second row
- Under Brownian motion, these shared path lengths are proportional to the phylogenetic covariances of trait values.



# the phylogenetic variance-covariance matrix

- This multivariate normal distribution completely describes the expected statistical distribution of traits on the tips of a phylogenetic tree if the traits evolve according to a Brownian motion model.



$$C = \begin{bmatrix} 2.5 & 1.0 & 0 & 0 \\ 1.0 & 2.5 & 0 & 0 \\ 0 & 0 & 2.5 & 2.0 \\ 0 & 0 & 2.0 & 2.5 \end{bmatrix}$$

# Models of discrete character evolution

*Squamates have lost their limbs over and over again through their history*

Limbllessness is an example of a ***discrete trait***

Analyzing the evolution of ***discrete traits*** requires a different modeling approach than what we used for ***continuous traits***



# Modeling the evolution of discrete states

For limblessness in squamates, each species is either legless (state 0) or not (state 1)

how many times this character has changed in the evolutionary history of squamates?

How often does limblessness evolve?

Do limbs ever re-evolve?

Is the evolution of limblessness related to some other aspect of the lives of these reptiles?

# Modeling the evolution of discrete states

Discrete characters: each species might exhibit one of  $k$  states. (In the limbless example above,  $k = 2$ )

Ordered characters can be placed in an order so that transitions only occur between adjacent states.

for example, the “mermaid skinks” (*Sirenoscincus*) from Madagascar, so called because they lack hind limbs

For unordered characters, any state can change into any other state



# Modeling the evolution of discrete states

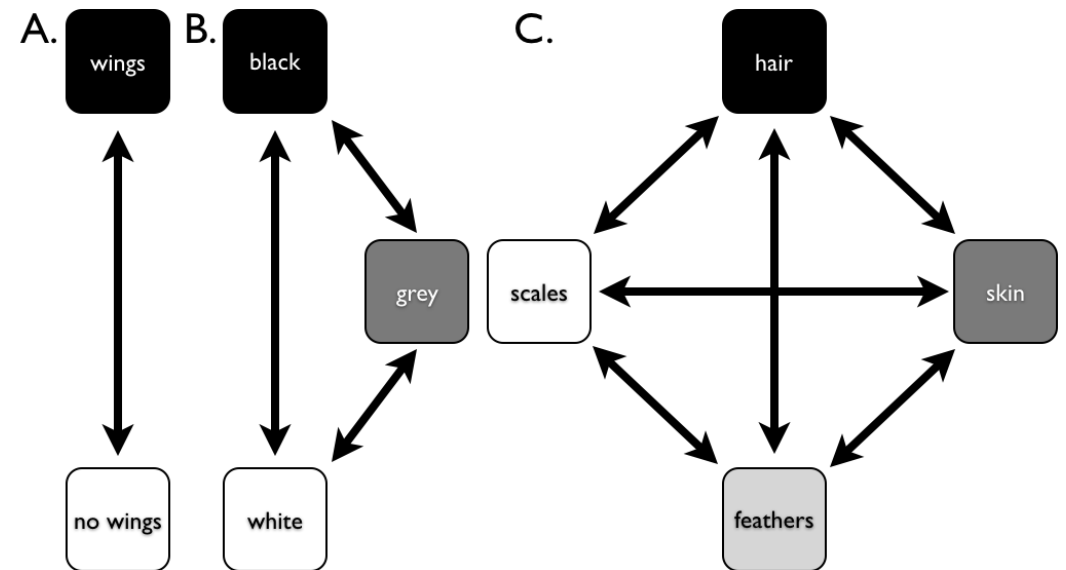
Most work on the evolution of discrete characters on phylogenetic trees has focused on the evolution of gene or protein sequences

Models of sequence evolution allow transitions among all four character states at certain rates



# Modeling the evolution of discrete states

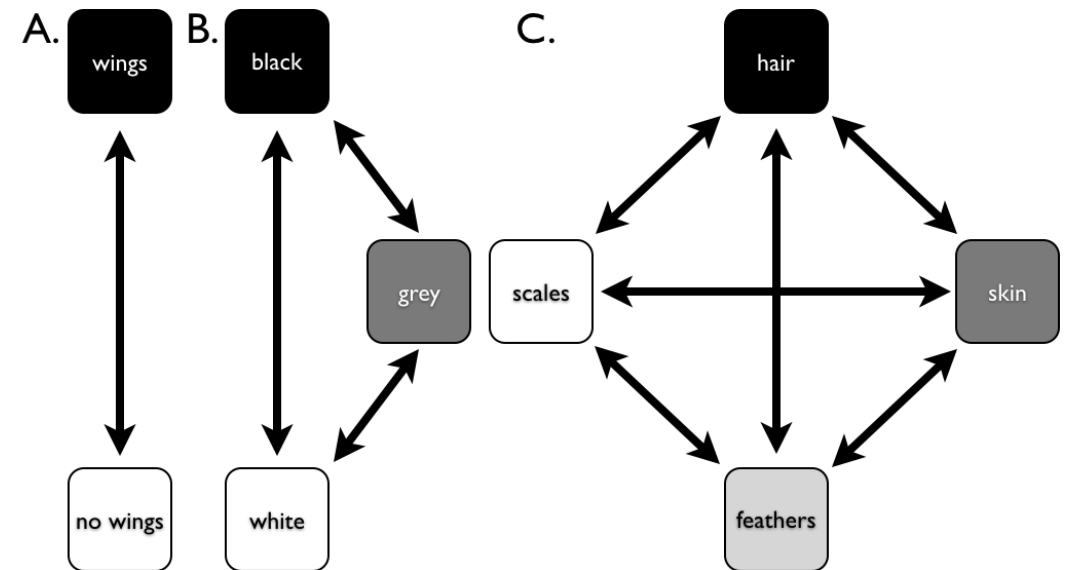
- The Mk model is a direct analogue of the Jukes-Cantor (JC) model for sequence evolution.
- The model applies to a discrete character having  $k$  unordered states.
- Evolution involves changing between these  $k$  states



Examples of discrete characters with (A)  $k = 2$ , (B)  $k = 3$ , and (C)  $k = 4$  states.

# Modeling the evolution of discrete states

- transitions among these states follow a Markov process
- the probability of changing from one state to another depends only on the current state
- For the basic Mk model, we can denote the instantaneous rate of change between states using the parameter  $q$ .
- In general,  $q_{ij}$  is called the instantaneous rate between character states  $i$  and  $j$ .
- for the basic Mk model, instantaneous rates between all pairs of characters are equal.



Examples of discrete characters with (A)  $k = 2$ , (B)  $k = 3$ , and (C)  $k = 4$  states.



# Modeling the evolution of discrete states

We can summarize general Markov models for discrete characters using a transition rate matrix where  $k$  is the number of states.

In a two-state Mk model ( $k = 2$ ), rates are symmetric so that  $q_{12} = q_{21}$ .

$$Q = \begin{bmatrix} -q & q \\ q & -q \end{bmatrix}$$

Once we have this transition rate matrix, we can calculate the probability distribution of trait states after any time interval  $t$  using the equation

$$P(t) = e^{Qt}$$

raising  $e$  to a power defined by a matrix, so the result is a matrix.

Each element in the matrix gives the probability that starting in state  $i$  you will end up in state  $j$  over time interval  $t$ .

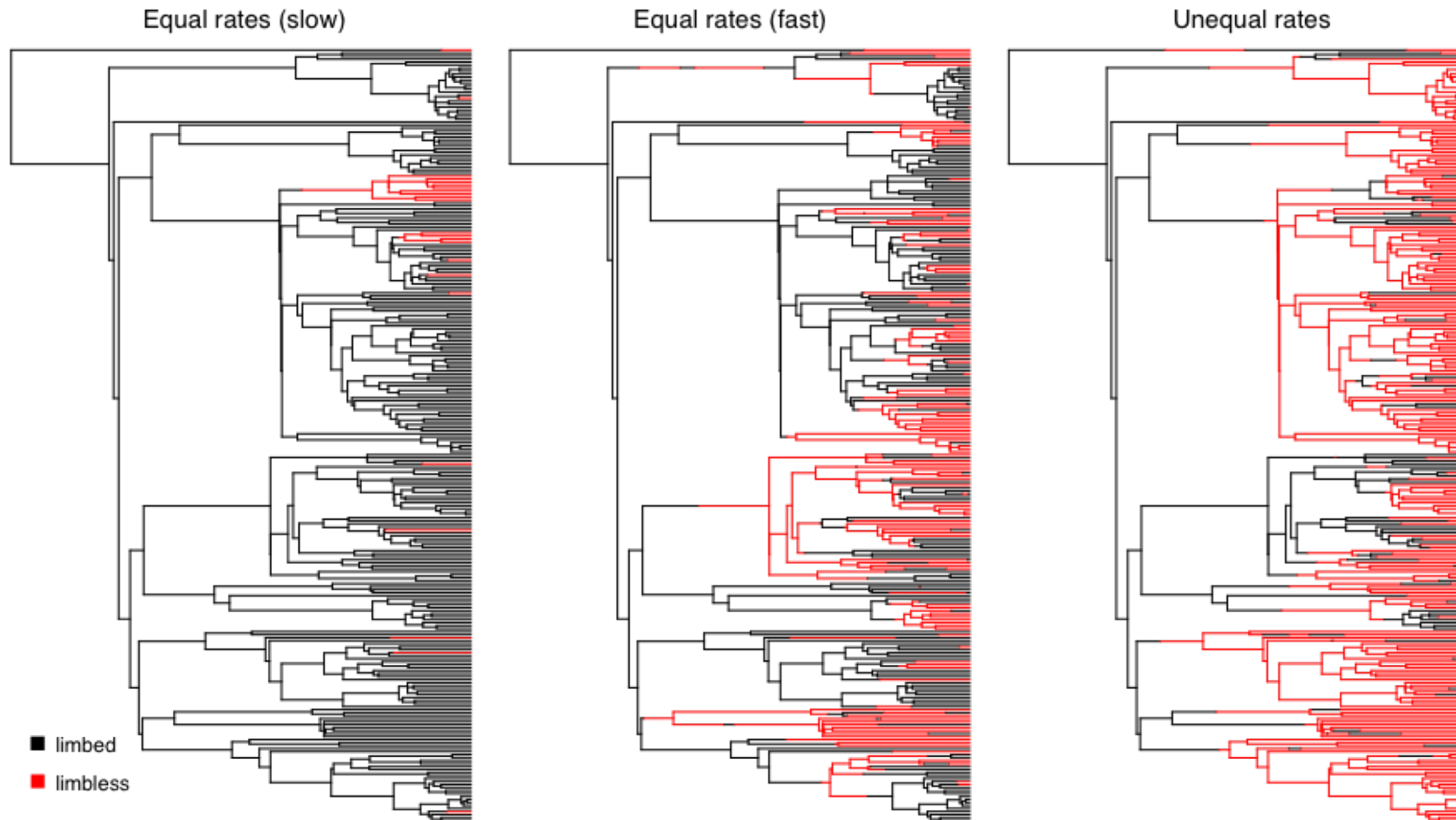
# Simulating the Mk model on a tree

- simulate character evolution on each branch of the tree, starting at the root and progressing towards the tips
- At speciation, we assume that both daughter species inherit the character state of their parental species immediately following speciation, and then evolve independently after that.
- At the end of the simulation, we will obtain a set of character states, one for each tip in the tree.
- The distribution of character states will depend on the shape of the phylogenetic tree (both its topology and branch lengths) along with the parameters of our model of character evolution.

# Simulating the Mk model on a tree

- draw a beginning character state at the root of the tree
  - For example, if we are simulating evolution under Mk with  $k = 2$ , then state 0 and 1 each have a probability of  $1/2$  at the root
  - We can draw the root state from a binomial distribution with  $p_{state0} = 0.5$
- we then simulate evolution along each branch in the tree.
  - We start with the (usually two) branches descending from the root.
  - We then proceed up the tree, branch by branch, until we get to the tips.

# Simulating the Mk model on a tree



Baleen Whales  
(Mysticetes)  
facing right →

# WHALES of the WORLD

The Order of Cetacea

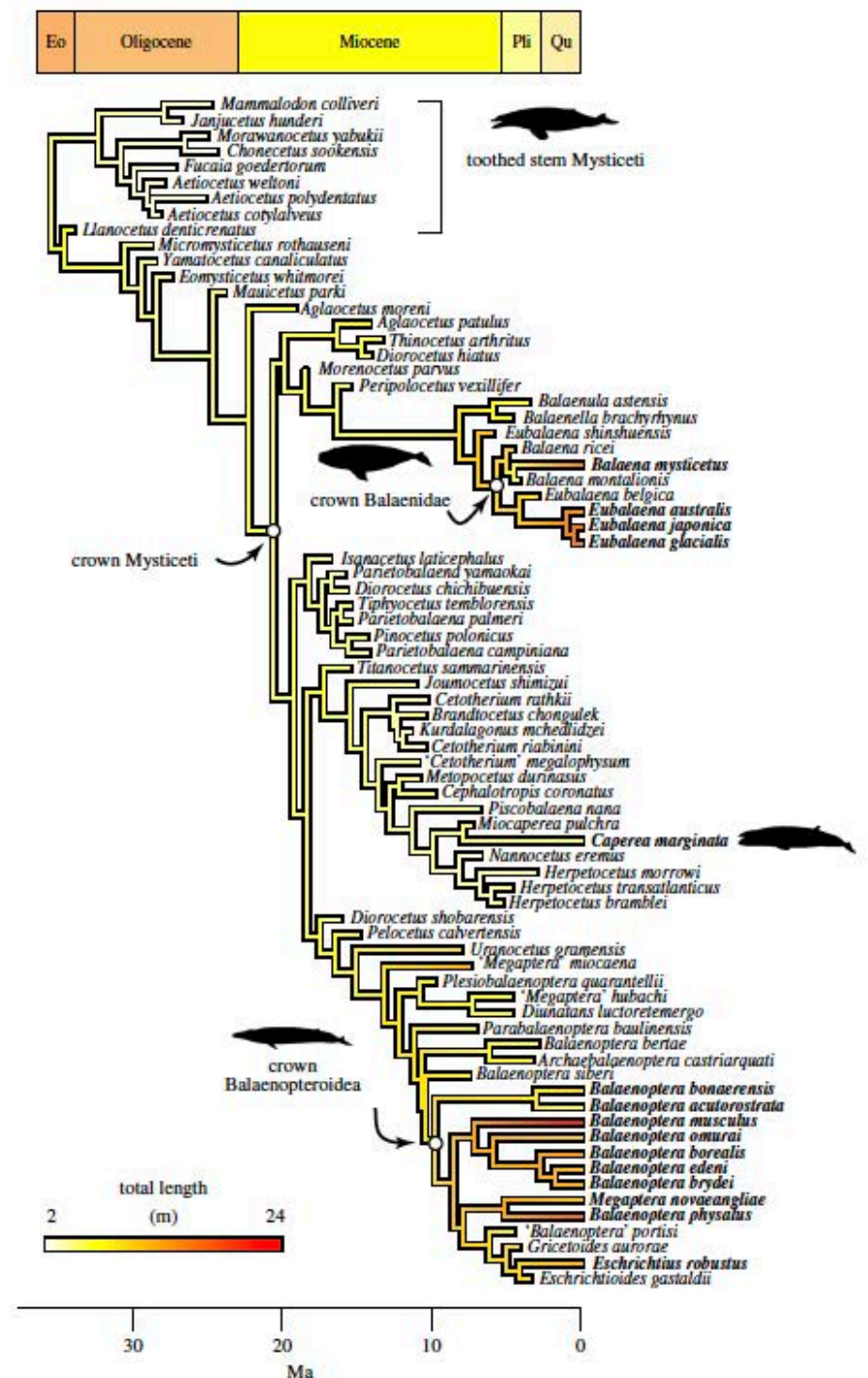
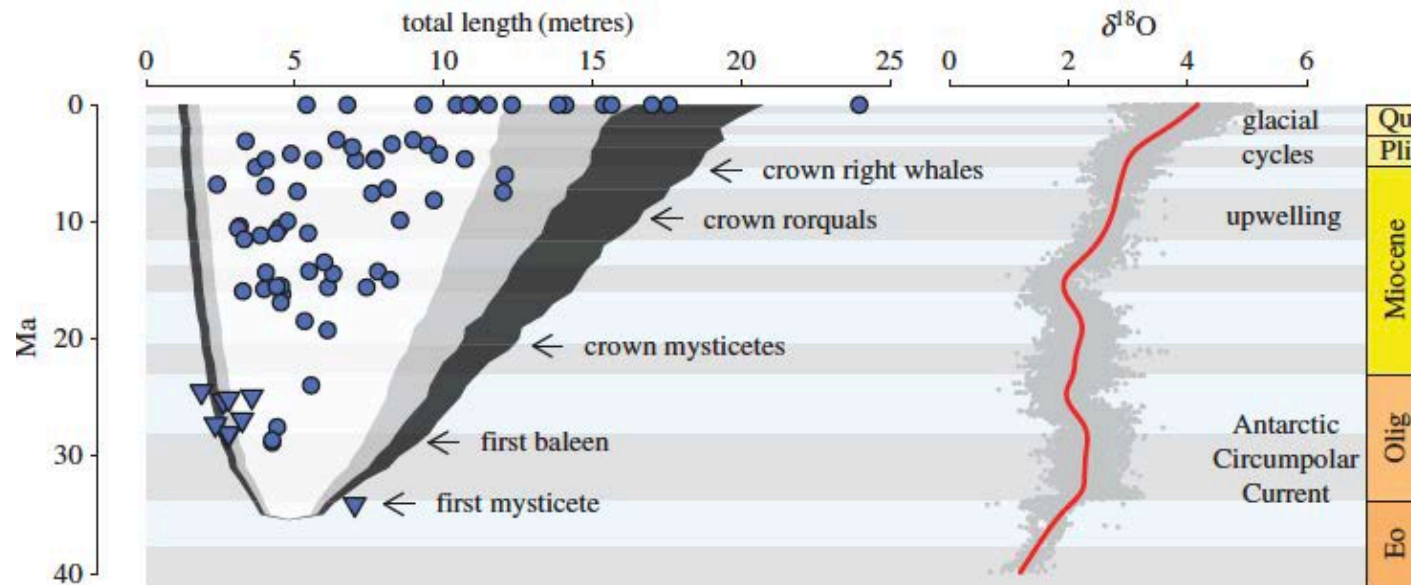
→ Toothed Whales  
(Odontocetes)  
← facing left





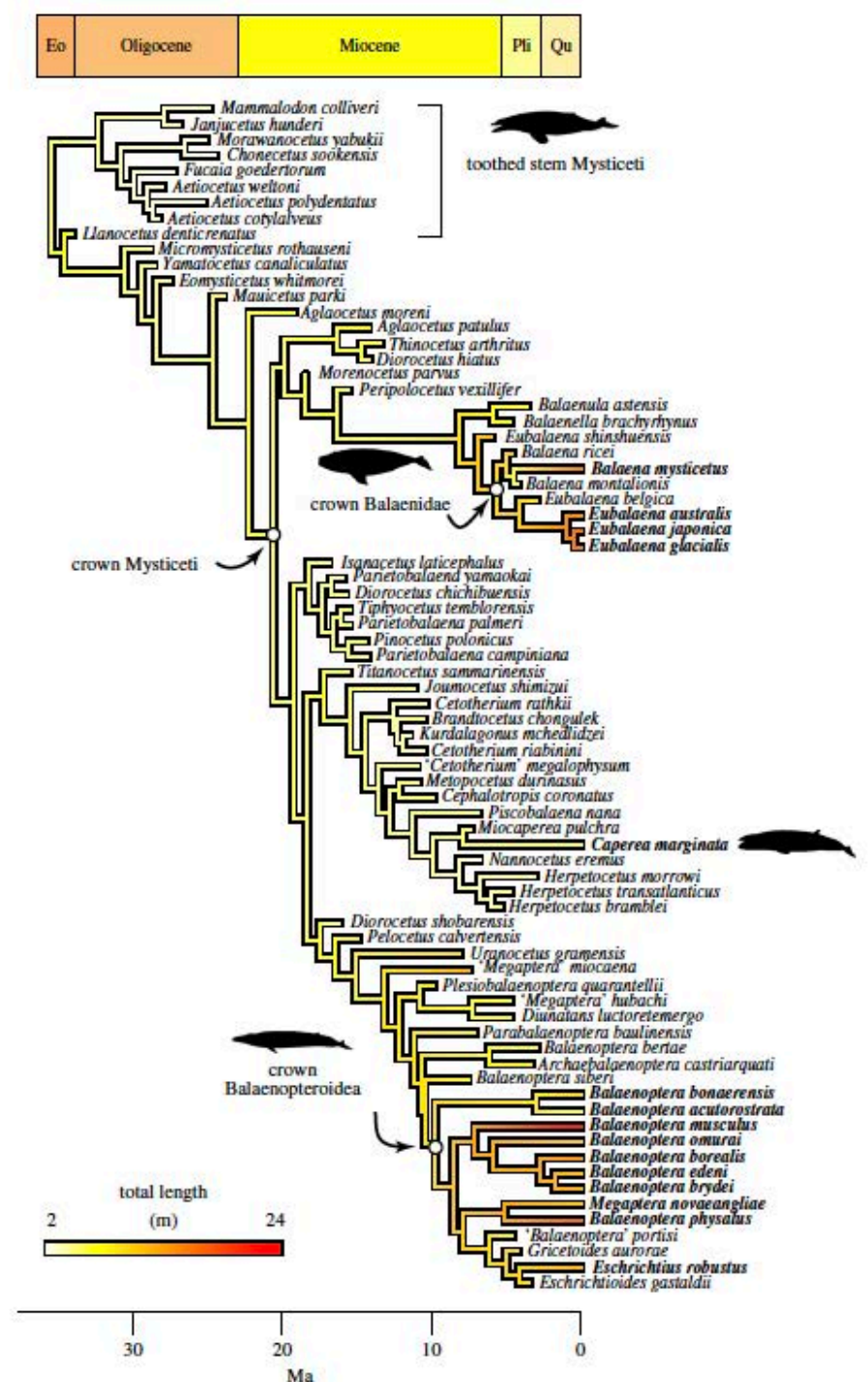
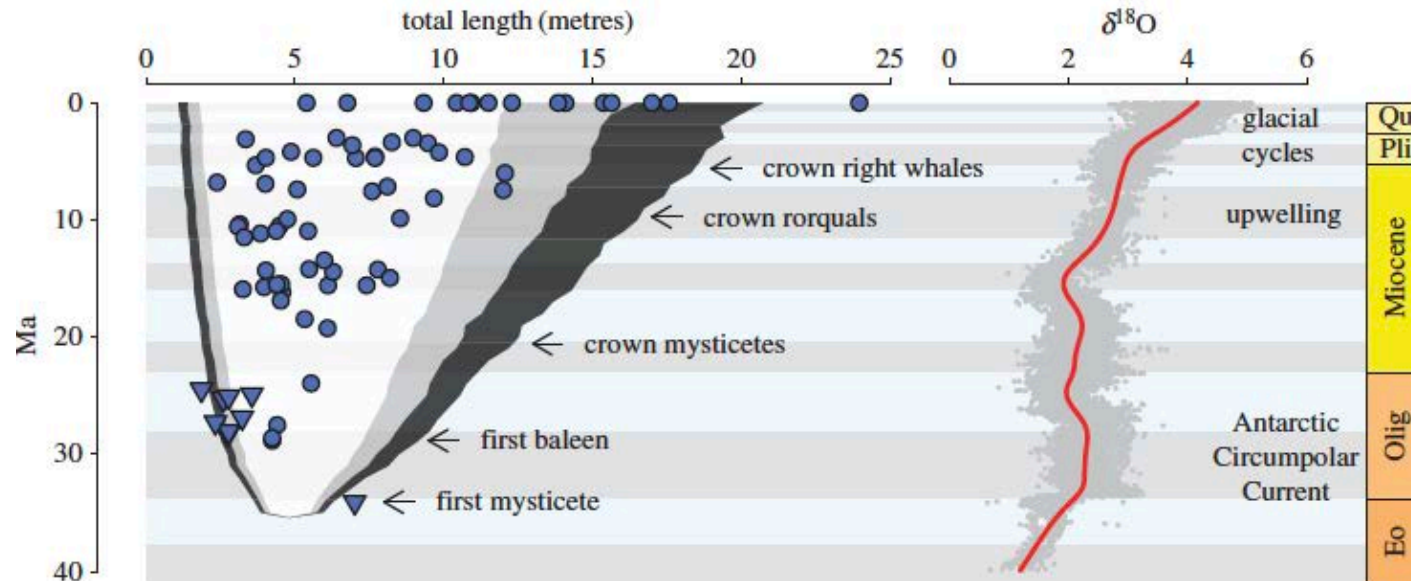
**Slater et al. 2017. Independent evolution of baleen whale gigantism linked to Plio-Pleistocene ocean dynamics. Proc Roy Sci B**

- Combined body mass data, fossils, and molecular phylogenies
- Tested a model where certain branches moved from Brownian motion to a more trended walk



**Slater et al. 2017. Independent evolution of baleen whale gigantism linked to Plio-Pleistocene ocean dynamics. Proc Roy Sci B**

- Determined that all rate shifts in body size evolution towards gigantism occurred during the Plio-Pleistocene.
- This means:
  1. Hyper gigantism evolved **multiple times** in baleen whales.
  2. This evolution was governed by recent climate shifts



# Summary

## ***Comparative Methods can:***

- Help us determine which processes must have been common, and which rare, across clades in the tree of life;
- whether evolution has proceeded differently in some lineages compared to others;
- whether the evolutionary potential that we see playing out in real time is sufficient to explain the diversity of life on earth,
- or whether we might need additional processes that may come into play only very rarely or over very long timescales, like adaptive radiation or species selection



# Simulating the Mk model on a tree

- For each branch, we first calculate  $\mathbf{P}(t)$ , the transition probability matrix, given the length of the branch and our model of evolution as summarized by  $\mathbf{Q}$  and the branch length  $t$
- We then focus on the row of  $\mathbf{P}(t)$  that corresponds to the character state at the beginning of the branch.
- For example, let's consider a basic two-state Mk model with  $q = 0.5$ .
- We can calculate  $\mathbf{P}(t)$  for a branch with length  $t = 3$  as:

$$\mathbf{P}(t) = e^{\mathbf{Q}t} = \exp\left(\begin{bmatrix} -0.5 & 0.5 \\ 0.5 & -0.5 \end{bmatrix} * 3\right) = \begin{bmatrix} 0.525 & 0.475 \\ 0.475 & 0.525 \end{bmatrix}$$

# Simulating the Mk model on a tree

$$\mathbf{P}(t) = e^{\mathbf{Q}t} = \exp\left(\begin{bmatrix} -0.5 & 0.5 \\ 0.5 & -0.5 \end{bmatrix} * 3\right) = \begin{bmatrix} 0.525 & 0.475 \\ 0.475 & 0.525 \end{bmatrix}$$

- If we had started with character state 0 at the beginning of this branch, we would focus on the first row of this matrix.
- We want to end up at state 0 with probability 0.525 and change to state 1 with probability 0.475.
- We again draw a uniform random deviate  $u$ , and choose state 0 if  $0 \leq u < 0.525$  and state 1 if  $0.525 \leq u < 1$ .
- If we started with a different character state, we would use a different row in the matrix.
- If this is an internal branch in the tree, then both daughter species inherit the character state that we chose immediately following speciation
- By repeating this along every branch in the tree, we obtain a set of character states at the tips of the tree.