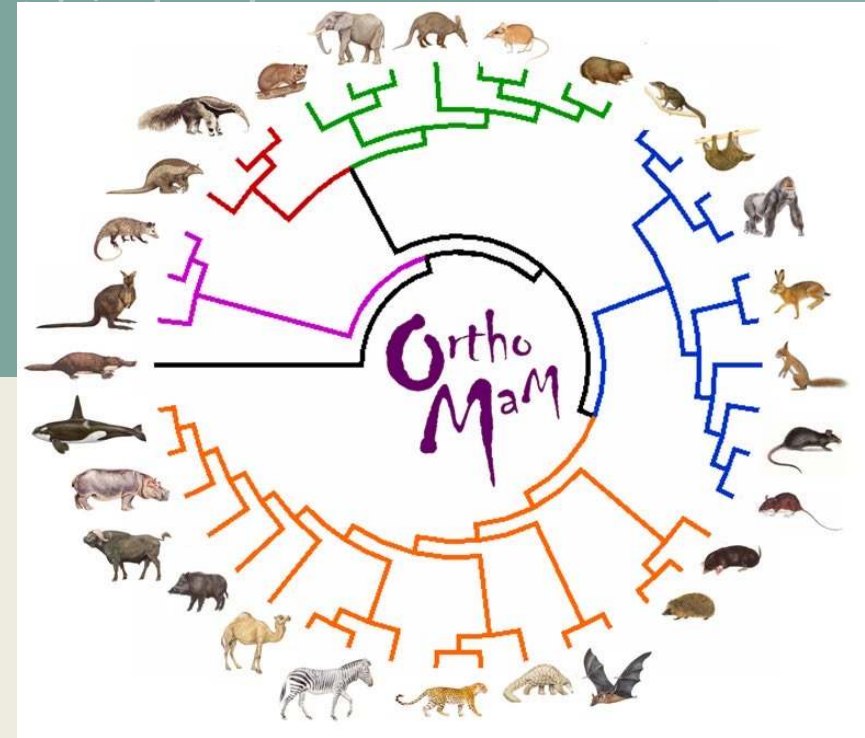


PhyloGenomics Lab

Finding genes involved in a trait
using comparative genomics

Prof. Nathan Clark
University of Pittsburgh



About me...

B.S. in Organic Chemistry

- University of Texas at Austin

PhD in Genome Sciences

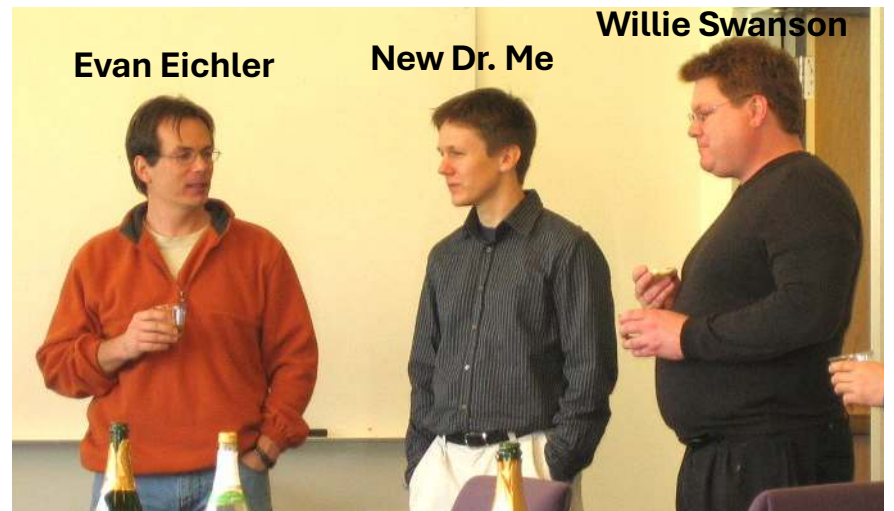
- University of Washington – Seattle
- Willie Swanson's lab

Postdoc

- Cornell with Chip Aquadro

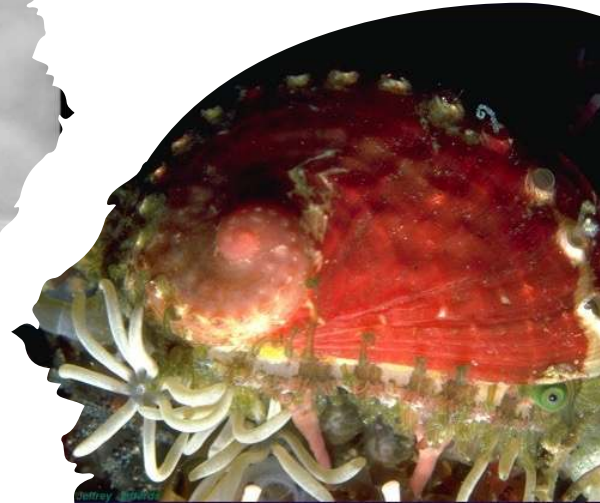
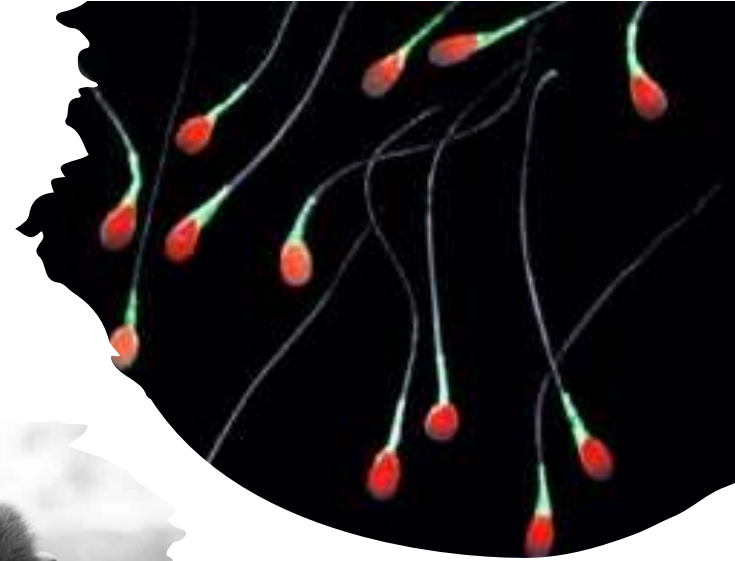
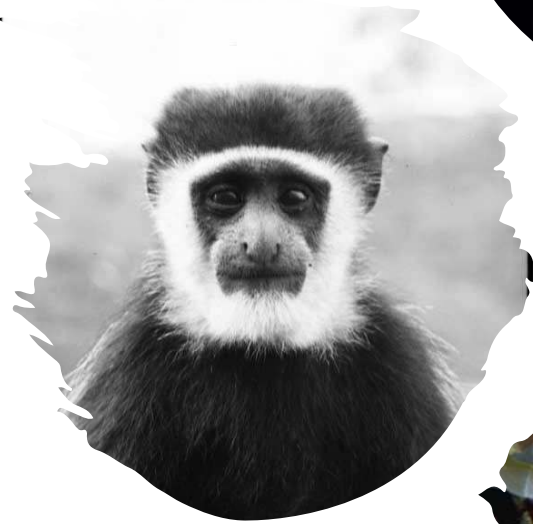
Professorship

- University of Pittsburgh



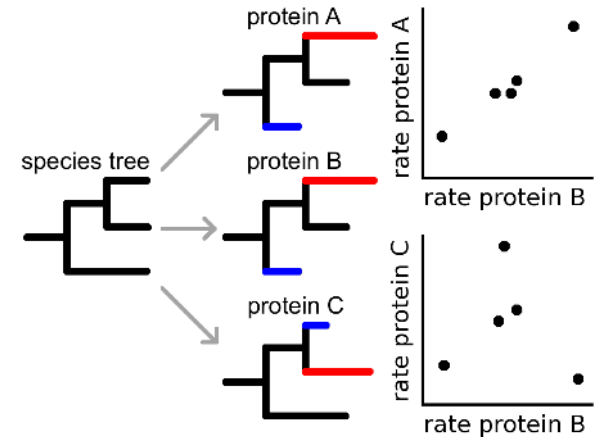
Early studies in the adaptive evolution of reproductive proteins

- Cooperation Conflict and Competition within and between the sexes
- Abalone, butterflies, primates
- This was all in early genomic era so often Sanger sequencing to compare to a reference species
- Then, more and more species genomes led to growth of comparative genomics...



Known for phylogenetic comparative methods and applications

- Linking phenotypes to genotypes through comparative species analysis
 - RERconverge
 - BUSTED-PH
- Connecting genes into functional networks with evolutionary patterns
 - Evolutionary Rate Covariation (ERC)



Evolutionary Adaptations



- Adaptive traits teach us how organisms function.
- How do we assign genetic regions to phenotypes?

Outline

Today

- Understanding phylogenetic trees
- Measuring molecular divergence
- Inferring phylogenies
- Natural Selection
- Methods to infer adaptation
- Lab structure

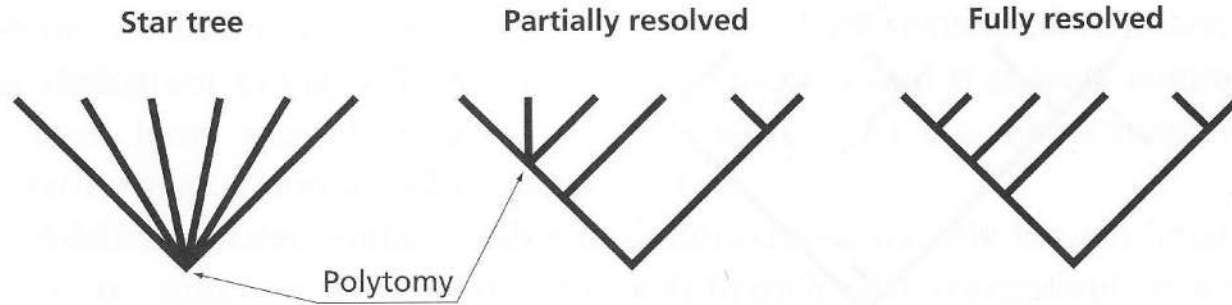
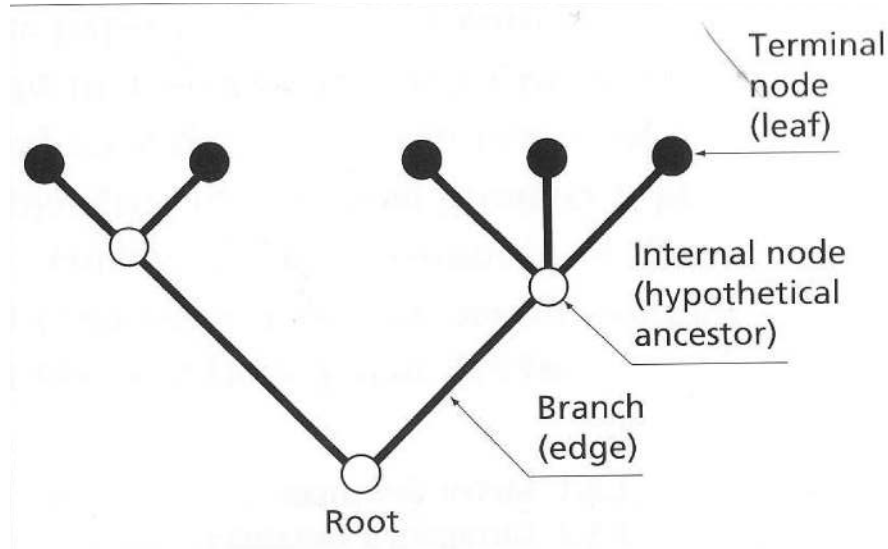
On Friday

- How to begin comparative genomics studies
- Finding and making multiple sequence alignments of orthologs
- Many many more comparative genomics approaches

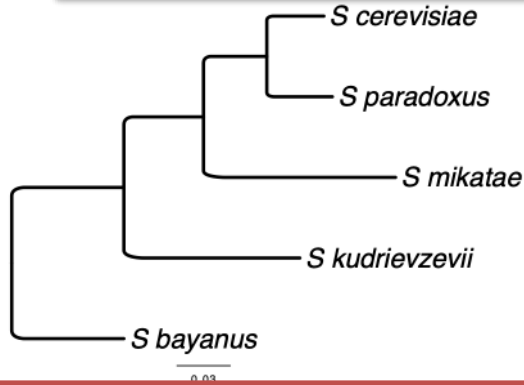
Phylogenetic Trees

- Phylogenetics is a powerful set of methods to describe change between species or genes.
 - Relationships between species or genes are inferred.
 - Specific hypotheses about origin and function can be tested using phylogenetics.

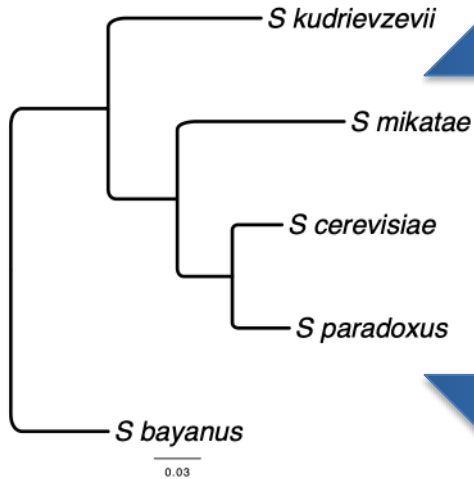
Tree Terminology



Rectangular trees

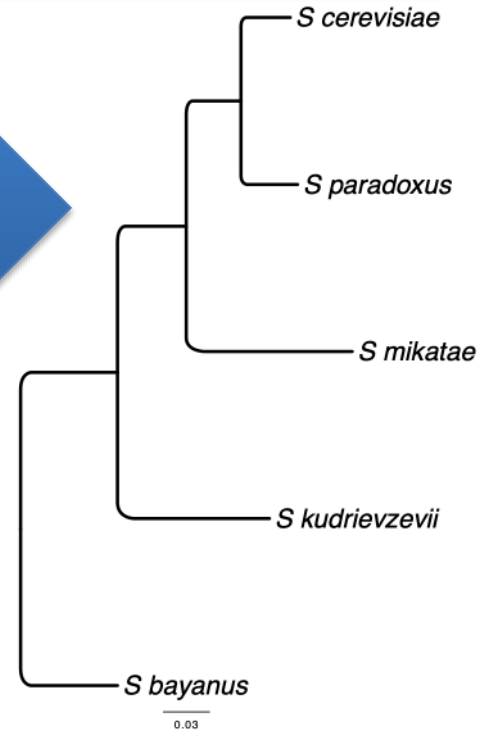


Only horizontal spacing indicates time/genetic distance.

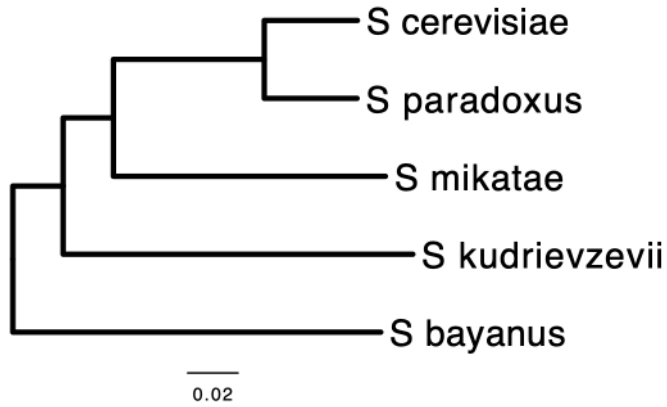


Vertical arrangement of leaves has no effect.

Vertical spacing is meaningless.



Rooted *versus* Unrooted

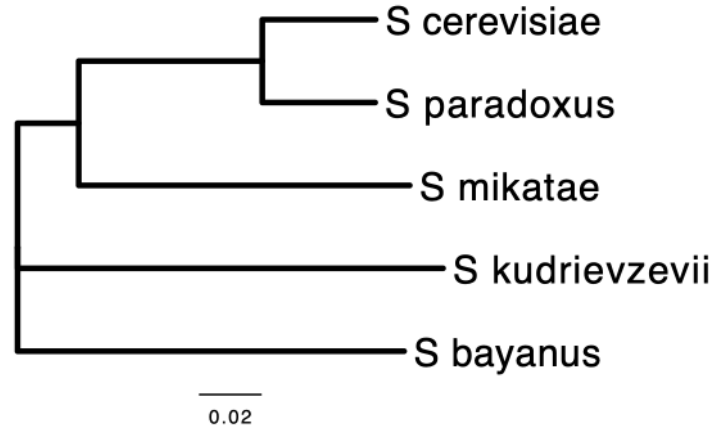


Rooted

N species

$2N - 2$ branches

$N - 1$ internal nodes



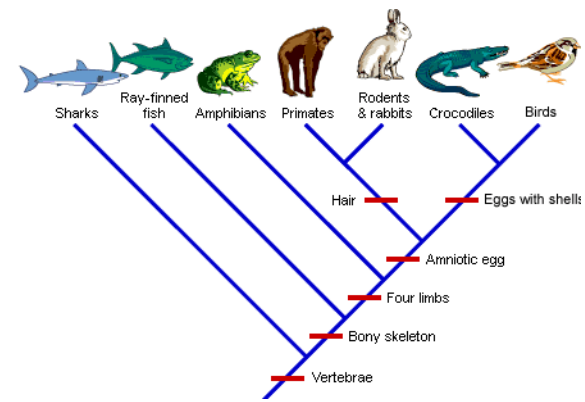
Unrooted

N species

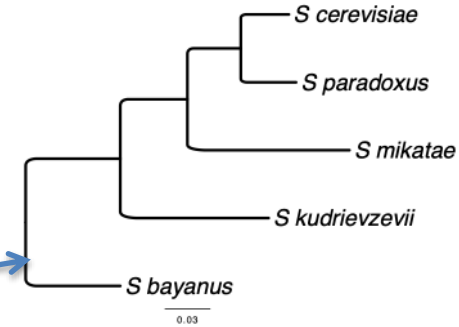
$2N - 3$ branches

$N - 2$ internal nodes

Cladogram – only intended to show connectivity, branch lengths do not reflect amount of divergence



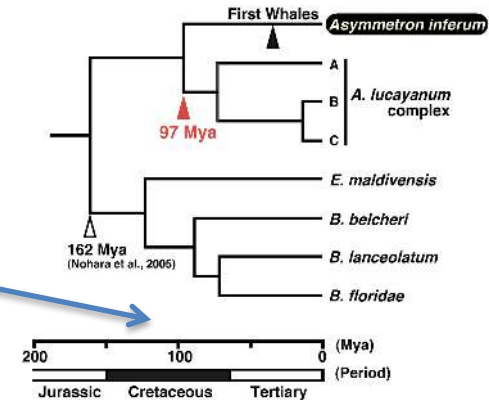
Phylogram – both connectivity and branch lengths are represented. Branch length could be genetic distance



or

Time (ultrametric tree)

Tips are always aligned.



Writing trees in Newick format

Parentheses surround
clades

Commas separate taxa

:numbers are branch
lengths

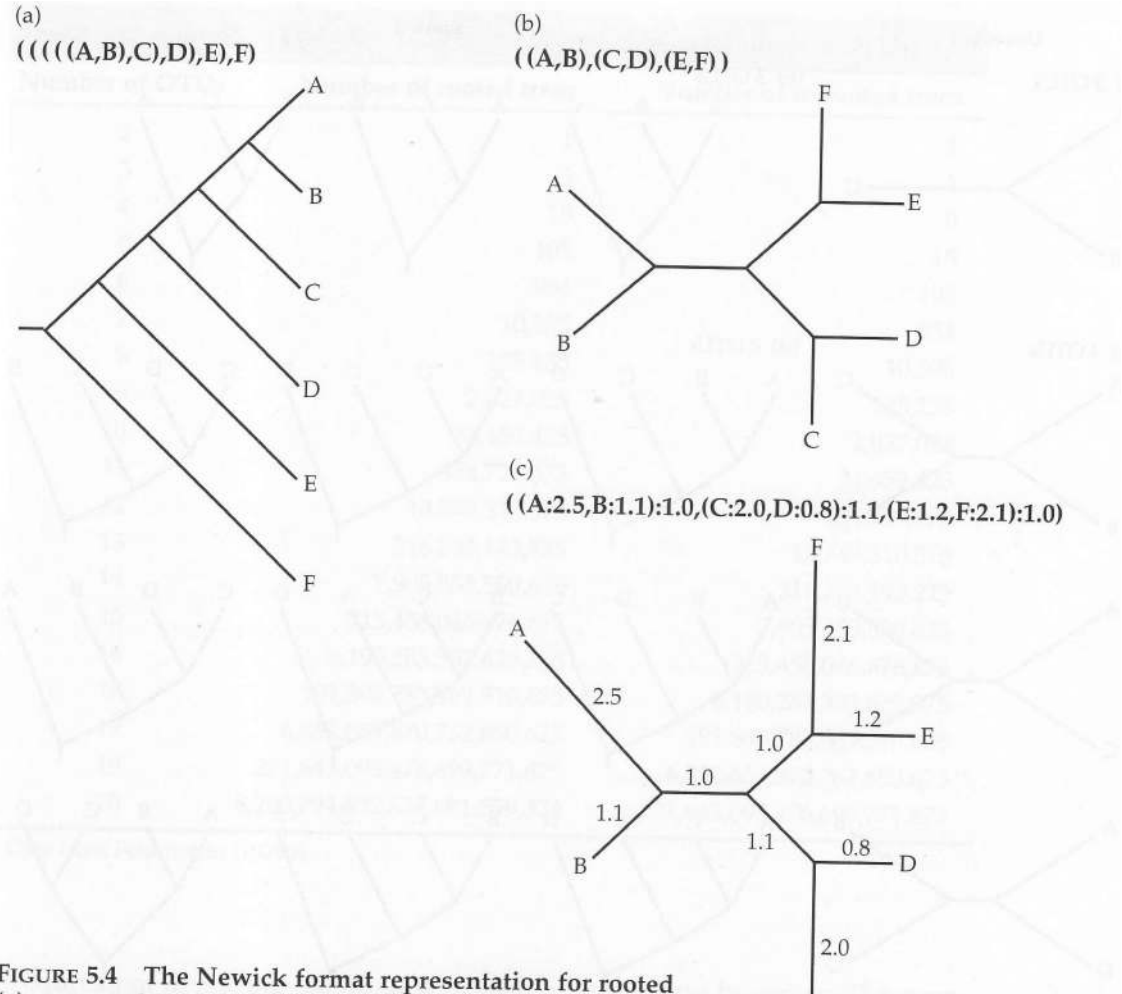
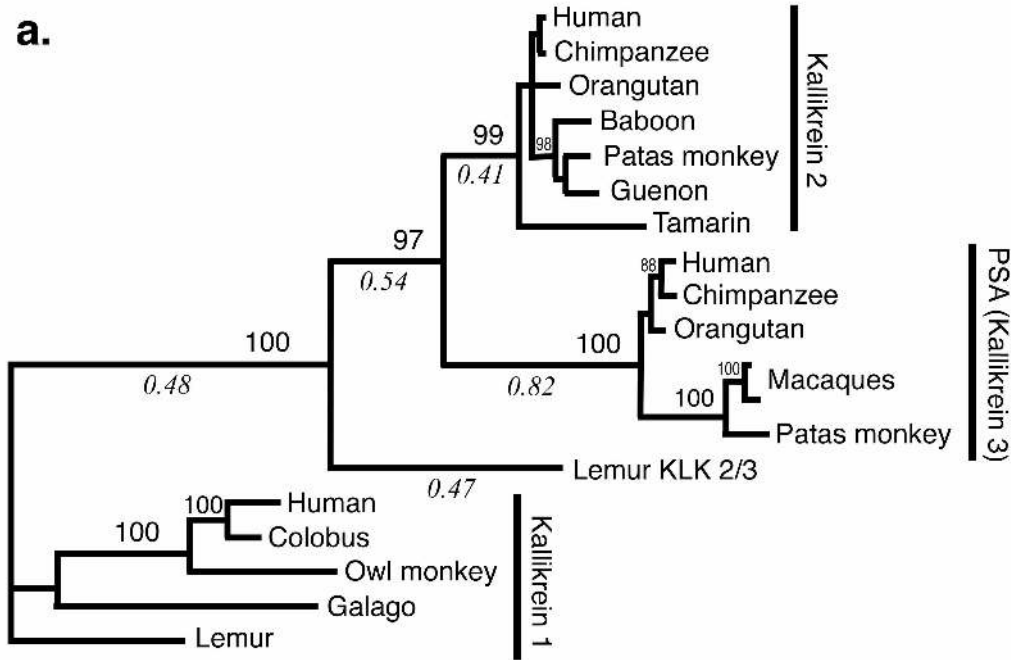


FIGURE 5.4 The Newick format representation for rooted

Orthology and Paralogy



Orthologous genes are those that diverged due to a **speciation event**.

- Orthologous groups will tend to “recreate” the species tree.

Paralogs are those that diverge due to **gene duplication**.

Measuring Molecular Divergence

Genetic distance = metric of sequence evolution.

Evolutionary rate = metric of change per unit time.

Why measure divergence?

Rate reveals functional clues via conservation and adaptation.

Rate is crucial for phylogenetic inference

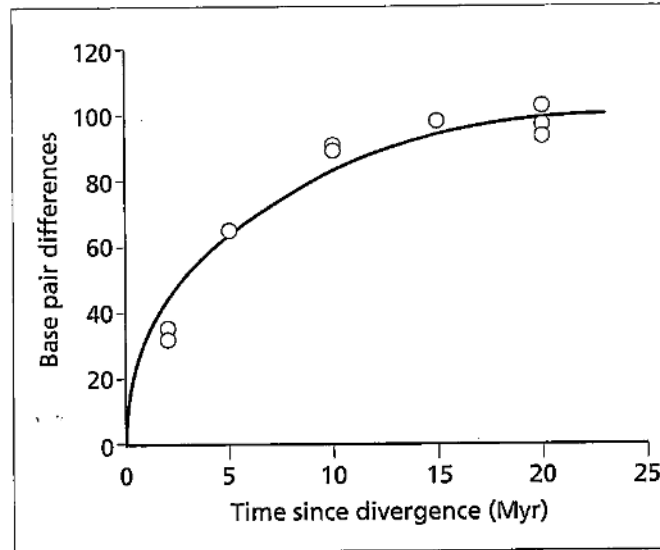
- determining species relationships
- dating evolutionary events with the
“molecular clock”

Percent divergence (%) is the simplest metric of divergence

It's intuitive and good for broad audiences.

But the **major problem** is that it underestimates divergence after only a moderate amount of change.

Fig. 5.11 Number of nucleotide substitutions between pairs of bovid mammal mitochondrial sequences (684 basepairs from the *COII* gene) against estimated time of divergence. Notice that the observed number of substitutions is not linear with time but curvilinear. Data from Janecek *et al.* (1996).



← Saturation

The bending of this curve

Saturation is when divergence reaches a level that observed differences will be lower than the actual number of changes.

Saturation bias occurs because some types of substitutions over branches are “invisible” when simply studying extant sequences.

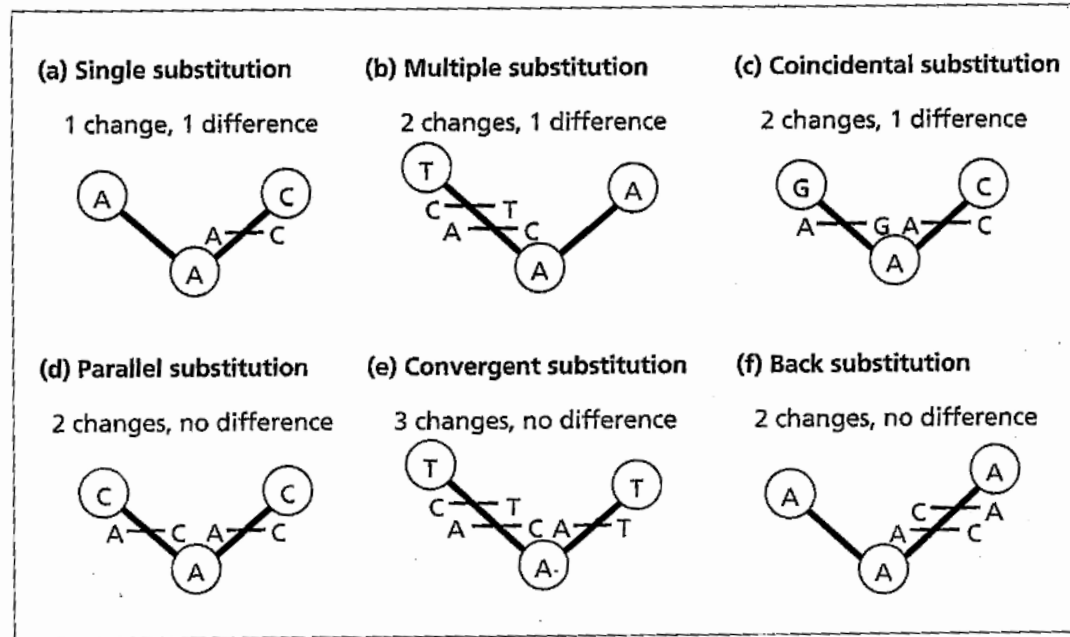


Fig. 5.9 Six kinds of nucleotide substitution. In each case the ancestral nucleotide was A. In all except the case of a single substitution, the number of substitutions that actually occurred is greater than would be counted if we just compared the two descendant sequences. In the lower three cases the nucleotides are identical in both descendant sequences, but this similarity has not been directly inherited from the ancestral sequence. Such similarity is termed ‘homoplasious’.

Saturation

Saturation causes a systematic underestimating bias when measuring molecular divergence.

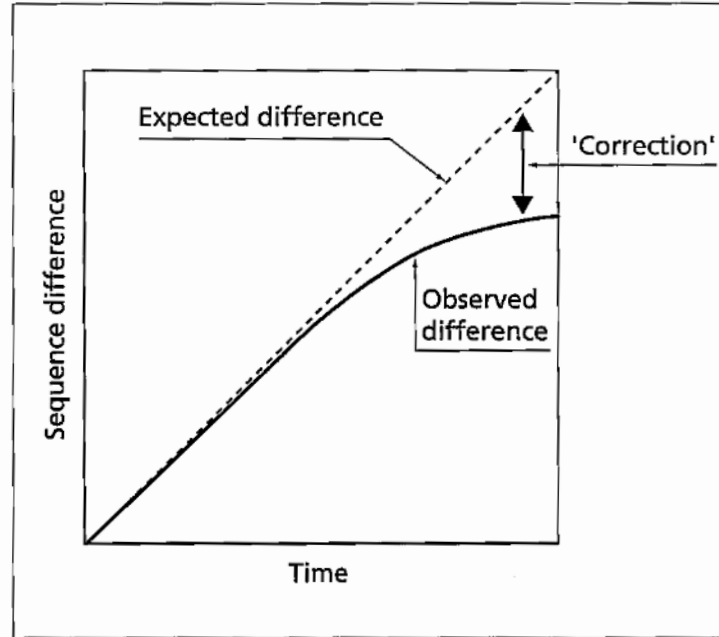


Fig. 5.12 The need to correct observed sequence differences. The extent of observed differences between two sequences is not linear with time (as we would expect if the rate of molecular evolution is approximately constant) but curvilinear due to multiple hits. The goal of distance correction methods is to recover the amount of evolutionary change that the multiple hits have overprinted and to 'correct' the distances for unobserved hits. In effect, the methods seek to 'straighten out' the line representing observed differences.

Nucleotide Substitution Models

- To make functional inferences we typically don't model insertions and deletions, large or small, because of the difficulty in assigning homology.
- We model nucleotide substitutions under the assumptions:
 - They occur as single, independent events
 - They don't affect substitutions at other sites
- More general models account for natural differences in rates between nucleotides

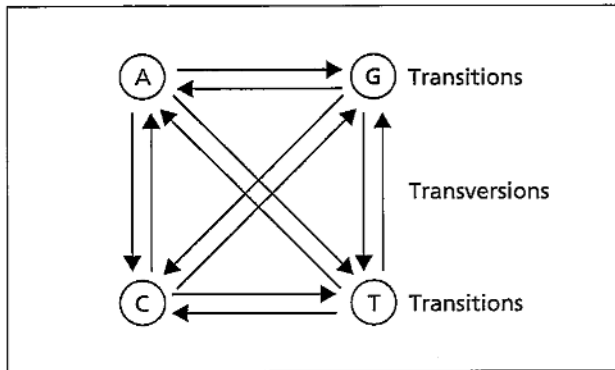
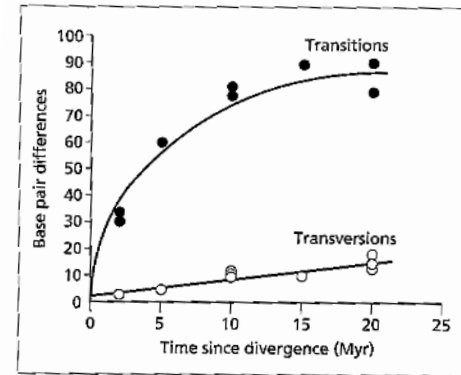
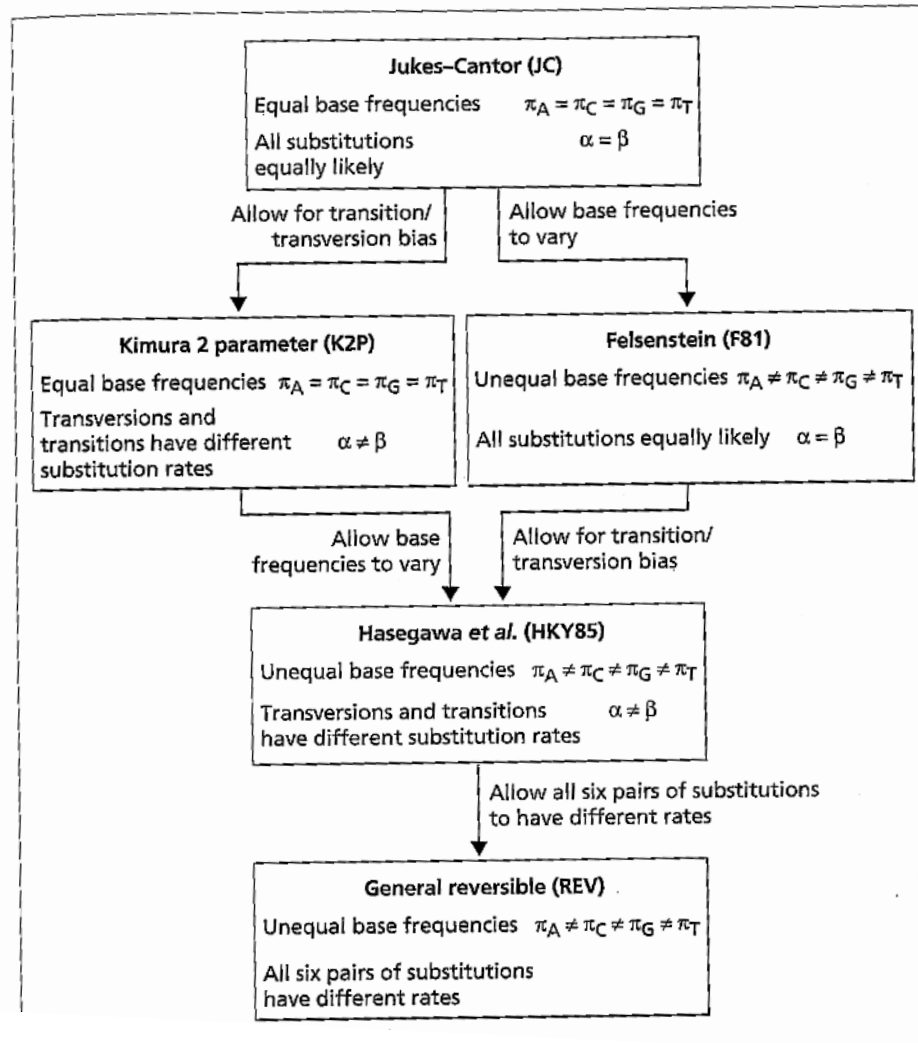


Fig. 5.10 The possible substitutions among the four nucleotides.



Overview of Models



Few parameters
+ Fast
- Less realistic



Parameter-rich
+ more realistic
- slow
- could overfit

Overview of Models

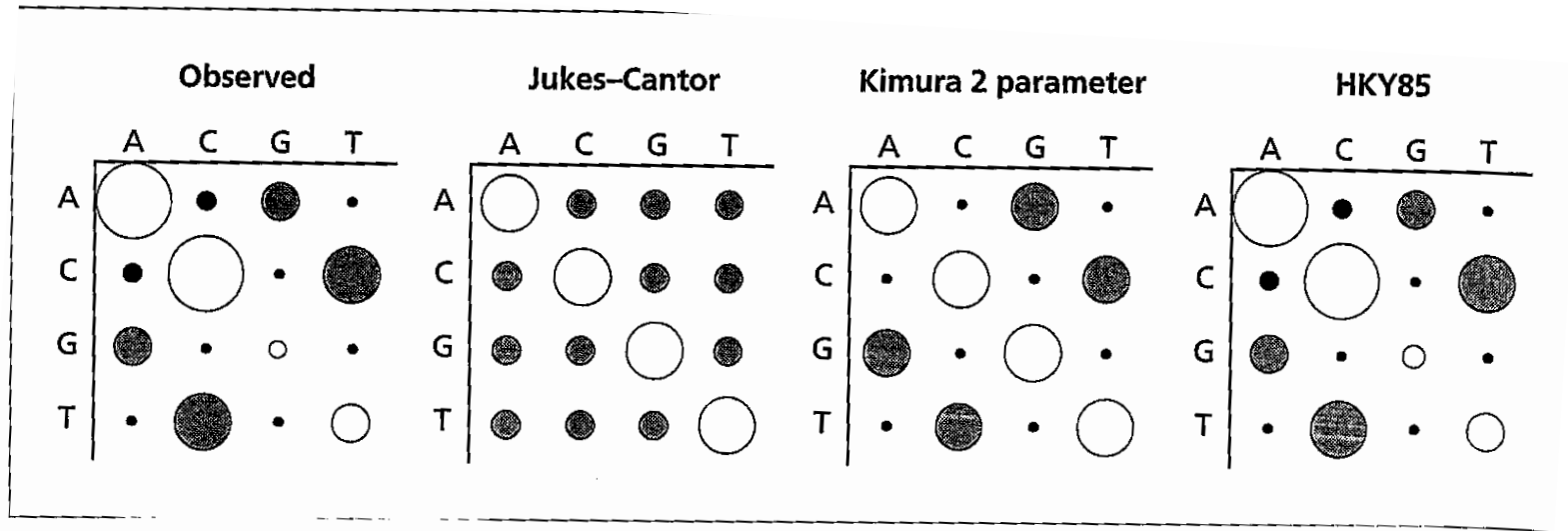


Fig. 5.15 Observed and expected numbers of nucleotide pairs between human and chimpanzee mtDNA sequences for three different models. As the models add parameters they more closely approximate the observed pattern. Data from Tamura (1994).

Protein evolution models

- Protein evolutionary rates range about 3 orders of magnitude.
- Proteins are easier to align because of homologous amino acid positions.
 - This allows study of longer timescales over which nucleotides have been long saturated.
- However, the natural rates of change between which amino acids vary greatly
 - The codon table governs exchanges
 - Physical and chemical differences are important and acted upon by natural selection. (Some are more similar than others.)
 - Leucine<-->Valine != Arginine<-->Lysine

Amino Acid Substitution Models

Rate Matrices model the expected substitution rates for proteins

$$R = \begin{pmatrix} A & C & D & E & F & G & H & I & K & L & M & N & P & Q & R & S & T & V & W & Y \\ - & .029 & .019 & .056 & .022 & .106 & .011 & .032 & .049 & .088 & .020 & .005 & .045 & .037 & .055 & .238 & .089 & .138 & .003 & .016 \\ .142 & - & .021 & .001 & .024 & .020 & .008 & .056 & .017 & .089 & .028 & .013 & .016 & .012 & .018 & .077 & .058 & .060 & .008 & .019 \\ .026 & .006 & - & .233 & .014 & .052 & .015 & .024 & .034 & .006 & .005 & .100 & .038 & .048 & .013 & .102 & .045 & .014 & .002 & .007 \\ .061 & .000 & .189 & - & .011 & .023 & .030 & .009 & .136 & .018 & .006 & .049 & .035 & .181 & .061 & .096 & .040 & .040 & .003 & .013 \\ .045 & .010 & .022 & .021 & - & .027 & .019 & .111 & .017 & .150 & .051 & .013 & .008 & .006 & .017 & .049 & .033 & .039 & .027 & .209 \\ .113 & .004 & .042 & .023 & .014 & - & .010 & .005 & .024 & .011 & .007 & .061 & .022 & .017 & .025 & .102 & .020 & .020 & .007 & .009 \\ .037 & .006 & .039 & .093 & .031 & .032 & - & .018 & .039 & .022 & .014 & .114 & .025 & .063 & .084 & .060 & .023 & .014 & .005 & .125 \\ .044 & .015 & .024 & .012 & .074 & .006 & .007 & - & .002 & .465 & .054 & .016 & .011 & - .002 & .017 & .050 & .032 & .692 & .002 & .023 \\ .059 & .004 & .030 & .150 & .010 & .027 & .014 & .002 & - & .049 & .025 & .059 & .040 & .100 & .222 & .113 & .041 & .041 & .003 & .015 \\ .072 & .015 & .004 & .014 & .060 & .008 & .005 & .282 & .033 & - & .138 & .012 & .013 & .021 & .031 & .030 & .053 & .113 & .006 & .025 \\ .069 & .019 & .012 & .020 & .084 & .022 & .014 & .135 & .070 & .566 & - & .026 & .033 & .127 & .070 & .101 & .050 & .239 & .015 & .026 \\ .008 & .004 & .115 & .069 & .010 & .088 & .053 & .018 & .076 & .022 & .012 & - & .018 & .055 & .075 & .191 & .082 & .012 & .001 & .014 \\ .065 & .005 & .041 & .046 & .005 & .031 & .011 & .012 & .048 & .024 & .014 & .017 & - & .034 & .022 & .057 & .047 & .037 & .002 & .009 \\ .068 & .005 & .065 & .307 & .006 & .030 & .035 & - .002 & .153 & .048 & .070 & .066 & .043 & - & .153 & .124 & .046 & .040 & .008 & .037 \\ .085 & .005 & .015 & .086 & .013 & .036 & .038 & .020 & .282 & .058 & .032 & .075 & .023 & .127 & - & .050 & .059 & .003 & .004 & .017 \\ .233 & .015 & .074 & .087 & .024 & .094 & .018 & .036 & .092 & .036 & .030 & .121 & .039 & .066 & .032 & - & .164 & .005 & .003 & .014 \\ .113 & .015 & .043 & .046 & .021 & .024 & .009 & .030 & .043 & .081 & .019 & .067 & .041 & .032 & .049 & .212 & - & .126 & .007 & .014 \\ .161 & .014 & .012 & .043 & .022 & .022 & .005 & .595 & .040 & .161 & .083 & .009 & .029 & .025 & .002 & .006 & .116 & .003 & .003 & .031 \\ .020 & .009 & .008 & .018 & .077 & .037 & .008 & .009 & .017 & .043 & .026 & .004 & .008 & .024 & .016 & .018 & .033 & .014 & - & .094 \\ .037 & .009 & .012 & .029 & .241 & .019 & .088 & .040 & .030 & .071 & .018 & .021 & .015 & .047 & .026 & .034 & .027 & .063 & .037 & - \end{pmatrix}$$

Margaret Dayhoff

Amino Acid Substitution Models

Rate Matrices model the expected substitution rates for proteins

$$R = \begin{pmatrix} \begin{matrix} A & C & D & E & F & G & H & I & K & L & M & N & P & Q & R & S & T & V & W & Y \end{matrix} \\ \begin{matrix} - & .029 & .019 & .056 & .022 & .106 & .011 & .032 & .049 & .088 & .020 & .005 & .045 & .037 & .055 & .238 & .089 & .138 & .003 & .016 \\ .142 & - & .021 & .001 & .024 & .020 & .008 & .056 & .017 & .089 & .028 & .013 & .016 & .012 & .018 & .077 & .058 & .060 & .008 & .019 \\ .026 & .006 & - & .233 & .014 & .052 & .015 & .024 & .034 & .006 & .005 & .100 & .038 & .048 & .013 & .102 & .045 & .014 & .002 & .007 \\ .061 & .000 & .189 & - & .011 & .023 & .030 & .009 & .136 & .018 & .006 & .049 & .035 & .181 & .061 & .096 & .040 & .040 & .003 & .013 \\ .045 & .010 & .022 & .021 & - & .027 & .019 & .111 & .017 & .150 & .051 & .013 & .008 & .006 & .017 & .049 & .033 & .039 & .027 & .209 \\ .113 & .004 & .042 & .023 & .014 & - & .010 & .005 & .024 & .011 & .007 & .061 & .022 & .017 & .025 & .102 & .020 & .020 & .007 & .009 \\ .037 & .006 & .039 & .093 & .031 & .032 & - & .018 & .039 & .022 & .014 & .114 & .025 & .063 & .084 & .060 & .023 & .014 & .005 & .125 \\ .044 & .015 & .024 & .012 & .074 & .006 & .007 & - & .002 & .465 & .054 & .016 & .011 & -.002 & .017 & .050 & .032 & .692 & .002 & .023 \\ .059 & .004 & .030 & .150 & .010 & .027 & .014 & .002 & - & .049 & .025 & .059 & .040 & .100 & .222 & .113 & .041 & .041 & .003 & .015 \\ .072 & .015 & .004 & .014 & .060 & .008 & .005 & .282 & .033 & - & .138 & .012 & .013 & .021 & .031 & .030 & .053 & .113 & .006 & .025 \\ .069 & .019 & .012 & .020 & .084 & .022 & .014 & .135 & .070 & .566 & - & .026 & .033 & .127 & .070 & .101 & .050 & .239 & .015 & .026 \\ .008 & .004 & .115 & .069 & .010 & .088 & .053 & .018 & .076 & .022 & .012 & - & .018 & .055 & .075 & .191 & .082 & .012 & .001 & .014 \\ .065 & .005 & .041 & .046 & .005 & .031 & .011 & .012 & .048 & .024 & .014 & .017 & - & .034 & .022 & .057 & .047 & .037 & .002 & .009 \\ .068 & .005 & .065 & .307 & .006 & .030 & .035 & -.002 & .153 & .048 & .070 & .066 & .043 & - & .153 & .124 & .046 & .040 & .008 & .037 \\ .085 & .005 & .015 & .086 & .013 & .036 & .038 & .020 & .282 & .058 & .032 & .075 & .023 & .127 & - & .050 & .046 & .040 & .008 & .037 \end{matrix} \end{pmatrix}$$

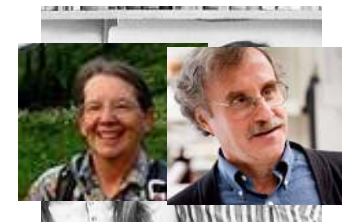
PAM – Accepted Point Mutation

Schwarz R & Dayhoff M (1979) Matrices for detecting distant relationships. In Dayhoff M, editor, Atlas of protein sequences, pages 353 - 58. National Biomedical Research Foundation.



BLOSUM

Henikoff and Henikoff (1992) Amino acid substitution matrices from protein blocks. PNAS 89: 10915-19.



Inference of Phylogenies

Phylogeny is inferred through distance data or discrete characters

Distance – all pairwise distances are computed from characters.

- Neighbor-Joining is widely used distance algorithm
- FAST-ME 2 is newer and faster

	hg19	panTro2	gorGor1	ponAbe2	rheMac2	papHam1	calJac1
hg19	0.000000	0.012640	0.017859	0.015237	0.052272	0.049648	0.063510
panTro2	0.012640	0.000000	0.020448	0.017809	0.054991	0.052361	0.066286
gorGor1	0.017859	0.020448	0.000000	0.023110	0.047165	0.049920	0.066730
ponAbe2	0.015237	0.017809	0.023110	0.000000	0.052298	0.049673	0.057901
rheMac2	0.052272	0.054991	0.047165	0.052298	0.000000	0.007565	0.080637
papHam1	0.049648	0.052361	0.049920	0.049673	0.007565	0.000000	0.083558
calJac1	0.063510	0.066286	0.066730	0.057901	0.080637	0.083558	0.000000

Discrete character methods use sequences directly during inference.

- Maximum parsimony, **Maximum likelihood**, and **Bayesian methods**

...	10	399
hg19	ATGGGATCTTCTGGACTTTTGAGCCTCCTGGTGC	
panTro2	ATGGGATCTTCTGGACTTTTGAGCCTCCTGGTGC	
gorGor1	ATGGGATCTTCTGGACTTTTGAGCCTCCTGGTGC	
ponAbe2	ATGGGATCTTCTGGACTTTTGAGCCTCCTGGTGC	
rheMac2	ATGGGATCTTCTGGACTTTTGAGCCTCCTGGTGC	
papHam1	ATGGGATCTTCTGGACTTTTGAGCCTCCTGGTGC	
calJac1	ATGGGATCTTCTGGACTTTTGAGCCTCCTGGTGC	
tarSyr1	ATGGAATCATCTAACTTTTGAGCCTCCTGGTGC	
micMur1	ATGGAATATTCCGGACTTTTGAGCCTCCTGGTGC	
tupBel1	ATGGAATCTTCTGGACTTCTGAGCATCGTGGTGT	

Distance Method: Neighbor-Joining

Genetic distances are first calculated between all pairs of sequences in the alignment.

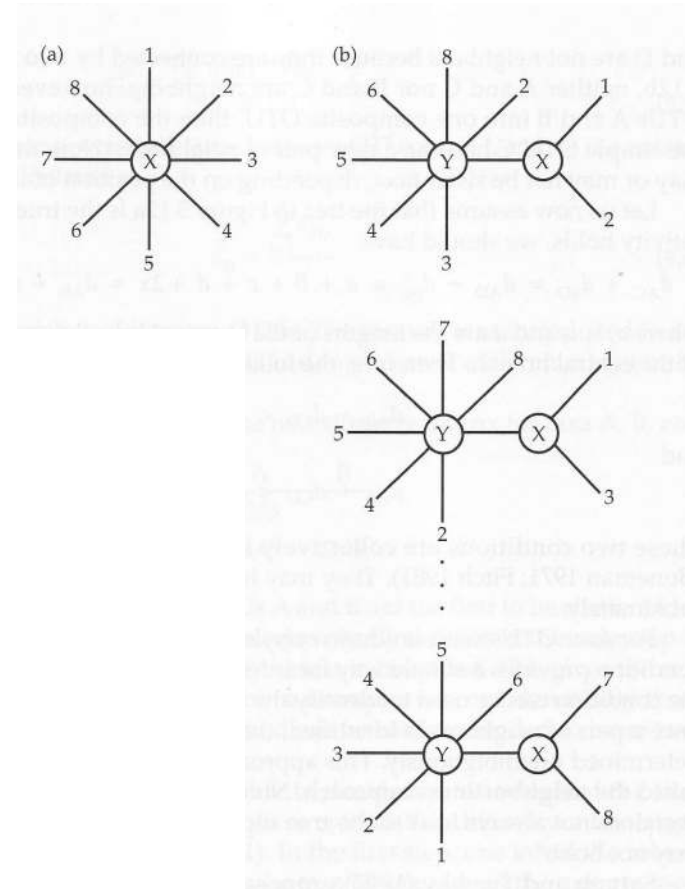
Distance methods are **very fast** and often a good choice, especially when there are a large number of sequences (> ~100).

Neighbor-Joining: Saitou and **Nei**. (1987) *MBE*
Clustering method that quickly generates a single tree reproducibly.

Begin with all taxa in a star-tree – one node polytomy.

$$S_0 = 1/(N-1) * \sum_{(i<j)} D_{ij}$$

Sequentially add nodes to pairs of taxa that minimize the total tree length.



Statistical models in phylogenetics

Likelihood and Bayesian methods discern between evolutionary models using probability.

- Probability provides a criterion to select the best tree topology and parameter estimates.
- Can test hypotheses using parameters such as rate of evolution (K , d_N/d_S , divergence times...)

Evolutionary models include:

- Substitution-related parameters
 - Branch-specific substitution rates
 - Transition/transversion rate ratio
 - Equilibrium frequencies for nucleotides/amino acids
 - Rate classes, which allow sites to have different rates
- Tree topology
 - The pattern of connections between sequences
 - Topology is a strange parameter space, and is harder to search than a continuous numerical parameter such as a rate or the transition/transversion ratio.

These methods are the **most accurate**, but are computationally expensive and **slower**.

Maximizing likelihood numerically

Complicated models must be maximized through a guided trial-and-error, “hill climbing” algorithm.

1. Set initial parameter values and tree.
2. Calculate likelihood.
3. Propose new parameter value or tree.
4. Calculate likelihood.
5. Decide whether to accept the new value.
6. Repeat steps 3-5 until changes no longer improve likelihood.



Because local maxima can trap this algorithm below the best model, must try multiple initial parameter values.

This is computationally expensive but allows us to determine the maximum likelihood model in many cases.

If the algorithm converges on the same parameter estimates after starting from several different initial values, it is a sign that the algorithm is converging on the maximum likelihood model.

Examples of likelihoods for different ape tree topologies.

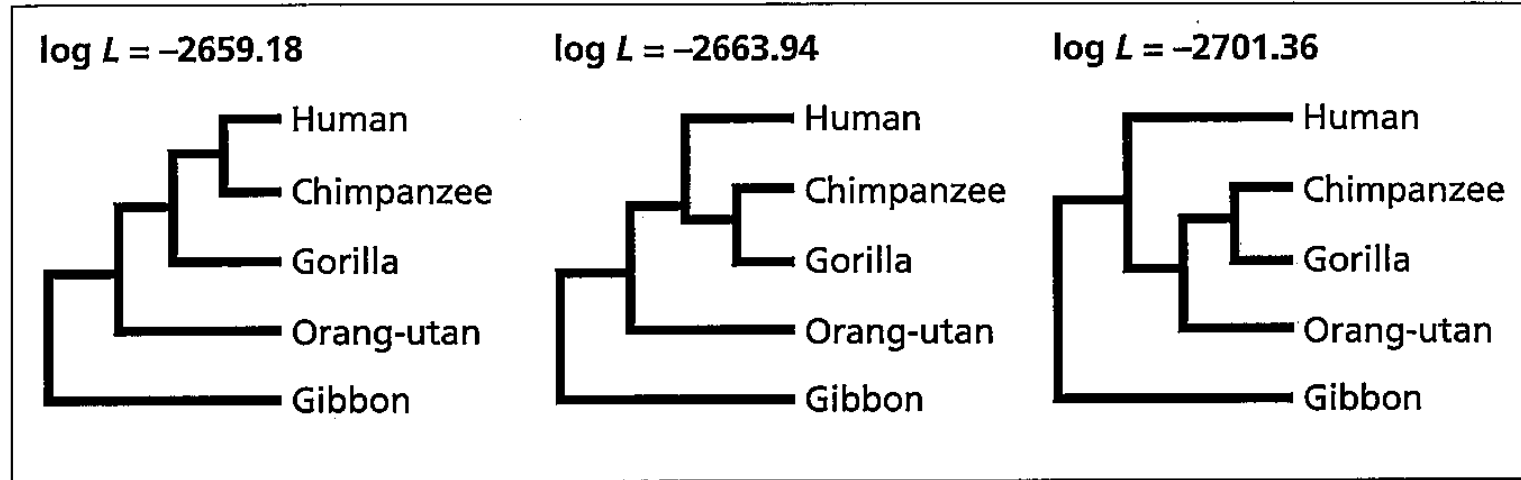


Fig. 6.19 Three different hypotheses of relationship among the hominoids and the likelihoods that each tree has given rise to the observed data.

- Do these represent all possible topologies?
- Which is most likely?

Phylogenetic software

- Distance
 - Command-line: FastME 2.0 (Fast Minimum Evolution)
 - <http://www.atgc-montpellier.fr/fastme/>
 - Graphic user interface: Seaview <https://doua.prabi.fr/software/seaview>
- Maximum Likelihood
 - PhyML <http://www.atgc-montpellier.fr/phyml/>
 - RAxML-NG <https://github.com/amkozlov/raxml-ng>
 - IQ-TREE 2 <https://github.com/iqtree/iqtree2>
 - Phangorn package in R (nice in R but slower) <https://klausvigo.github.io/phangorn/>
- Bayesian
 - BEAST 2 / StarBeast3 <https://www.beast2.org/>
 - RevBayes <https://revbayes.github.io/>

Forms of Natural Selection

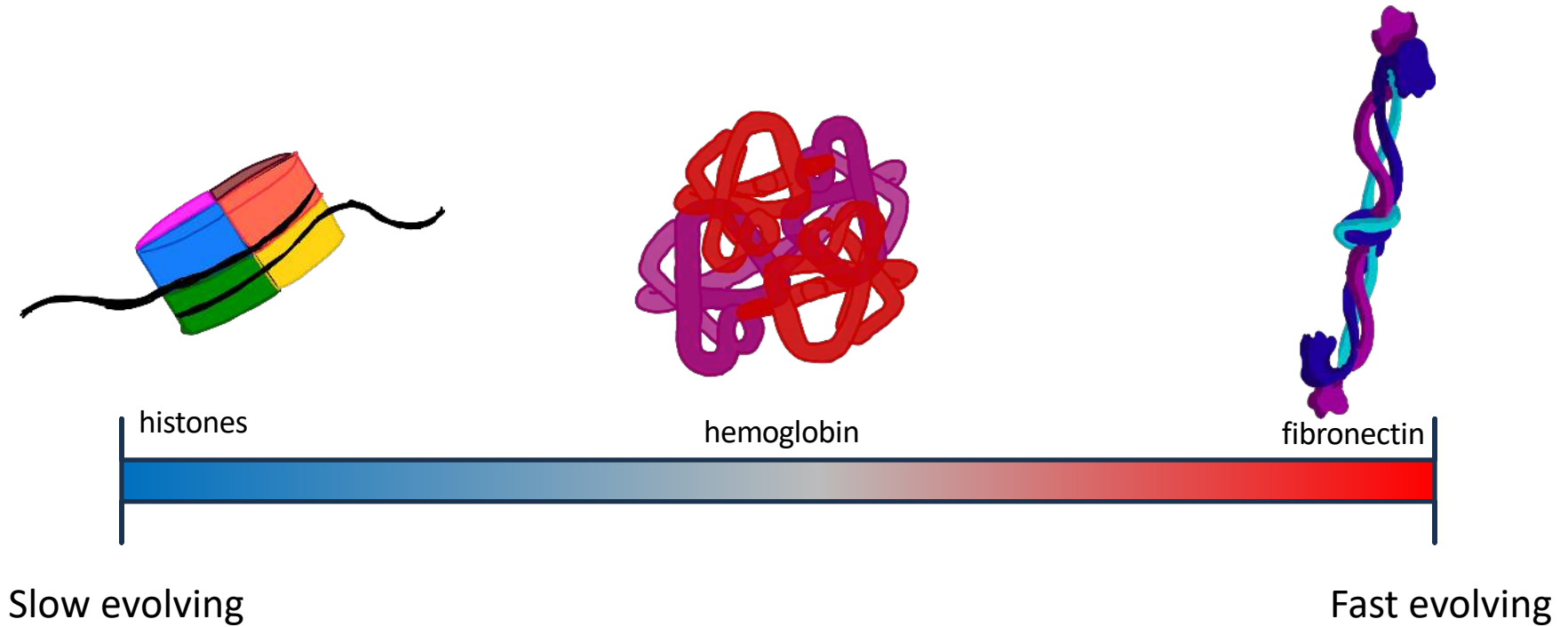
- **Positive selection**

- Beneficial alleles increase in frequency
- Over phylogenetic timescales, leads to **increased divergence** of nucleotide and/or protein sequences.
- “Diversifying selection”

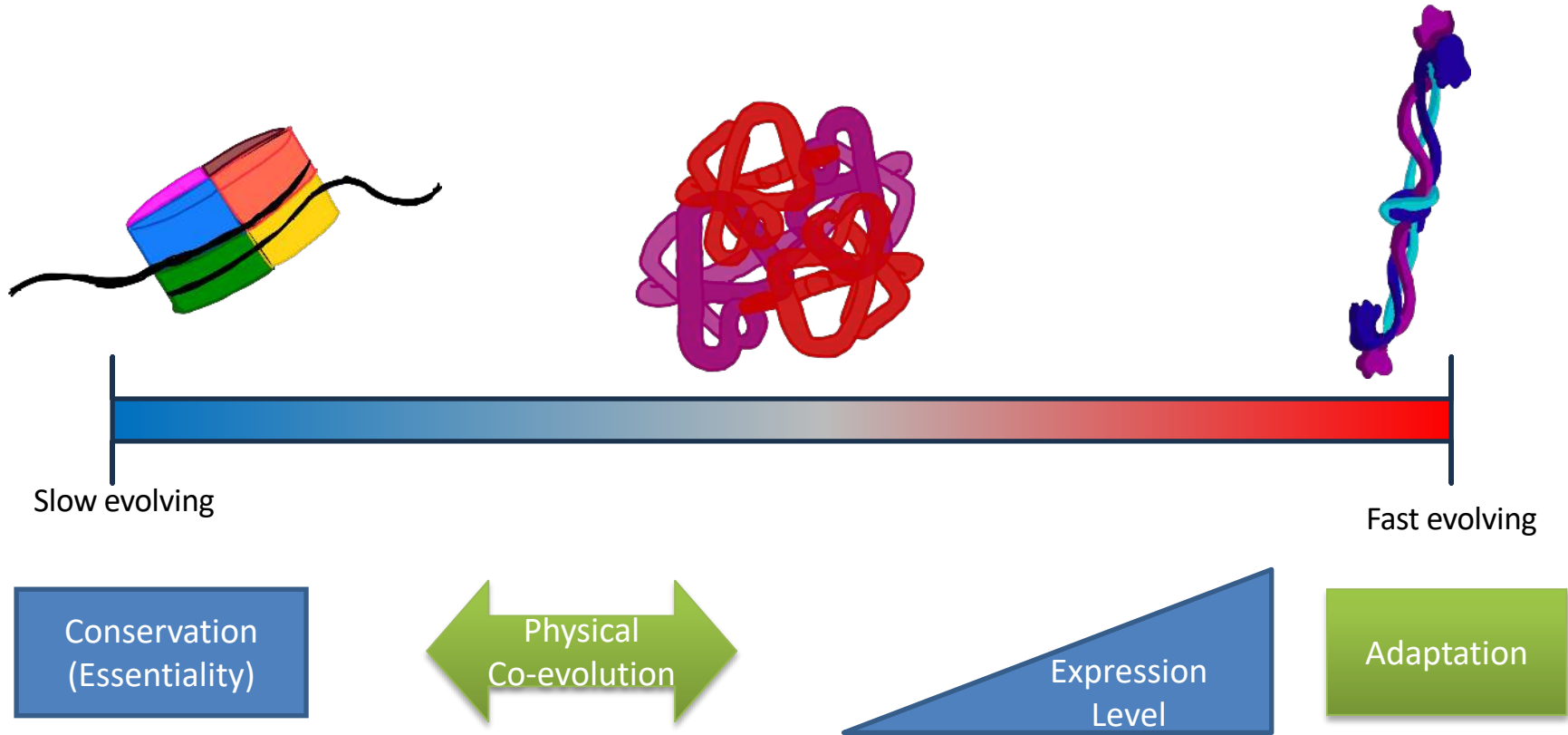
- **Negative selection**

- Deleterious alleles decrease in frequency
- On phylogenetic scales, leads to **conservation** of sequences and overall slower divergence.
- “Purifying selection”

Genes/proteins evolve at different average rates

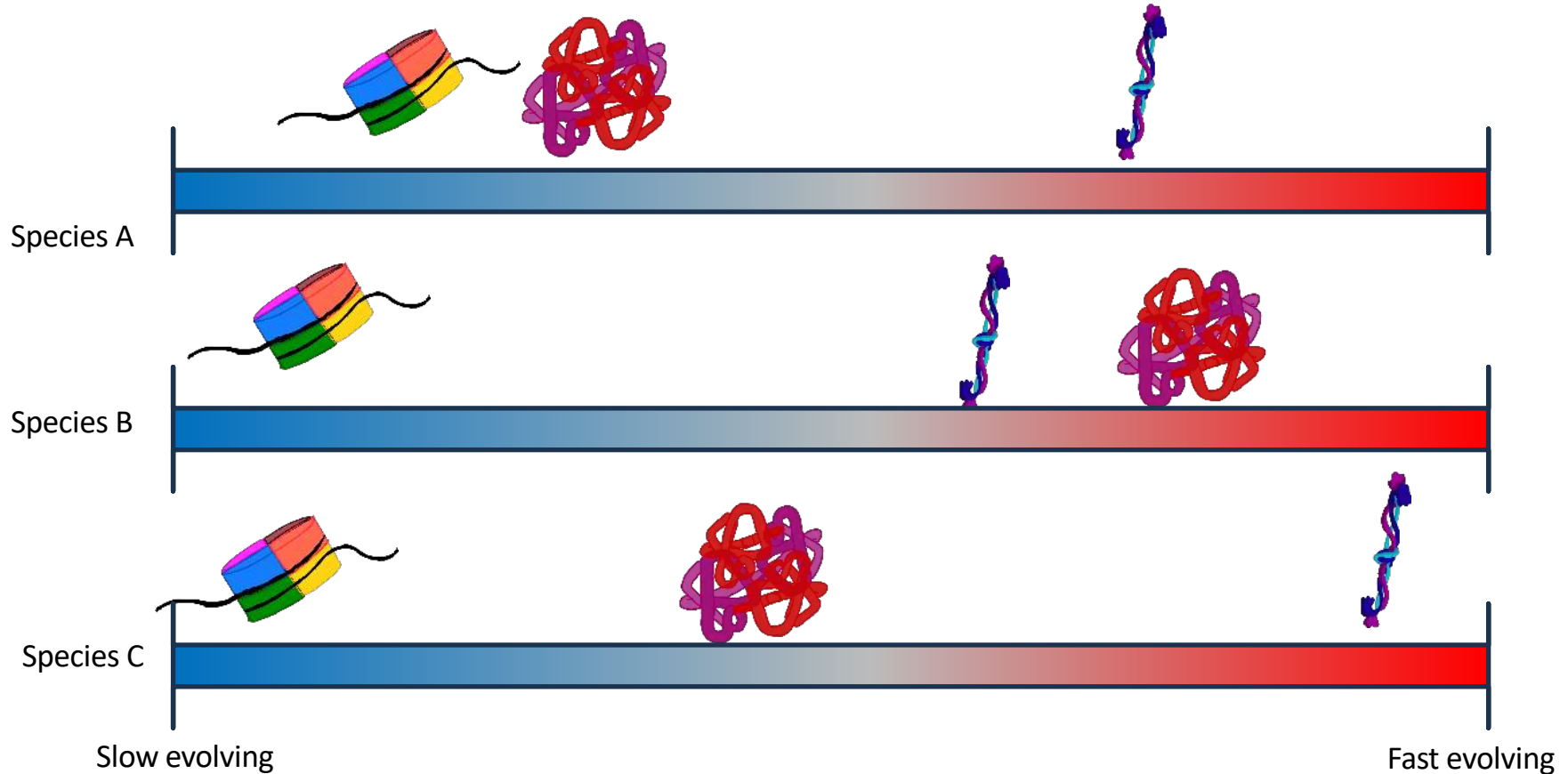


Which forces control evolutionary rate?



- Rates vary over time due to fluctuation in these forces

And... the evolutionary rate of any specific gene/protein changes over time (between species)



Comparative Approaches to Map Adaptive Phenotypes to Genotypes

Genetic crosses between populations or species.

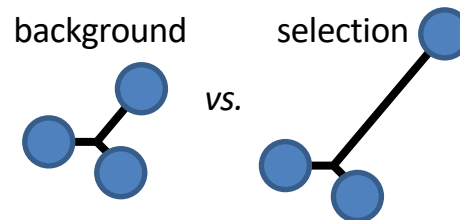
- Genetic mapping
- *Peromyscus*, blind cavefish



Linnen et al *Science* 2009

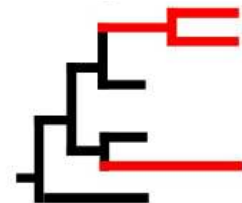
Positively selected regions leading to trait

- Population Genetics (F_{ST})
- Phylogenetics (d_N/d_S)
- High altitude populations and HIF1a



PhyloG2P – phylogenetic genotype to phenotype

Coincident evolution of genetic change and a **convergent** trait (i.e., repeatedly evolved trait)



Rate acceleration
correlated with
convergent trait

PhyloG2P

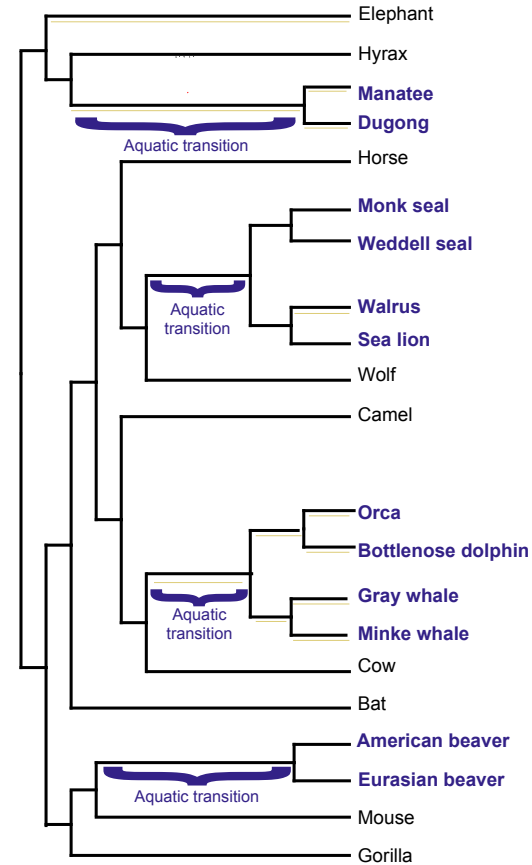
Phylogenetic Genotype to Phenotype

Smith et al. *TREE* 2020

Which genetic events are associated with a trait?

Genetic events include:

- Rate changes
- Positive selection
- Gene loss/ pseudogenization
- Gene family expansion/contraction



PhyloG2P

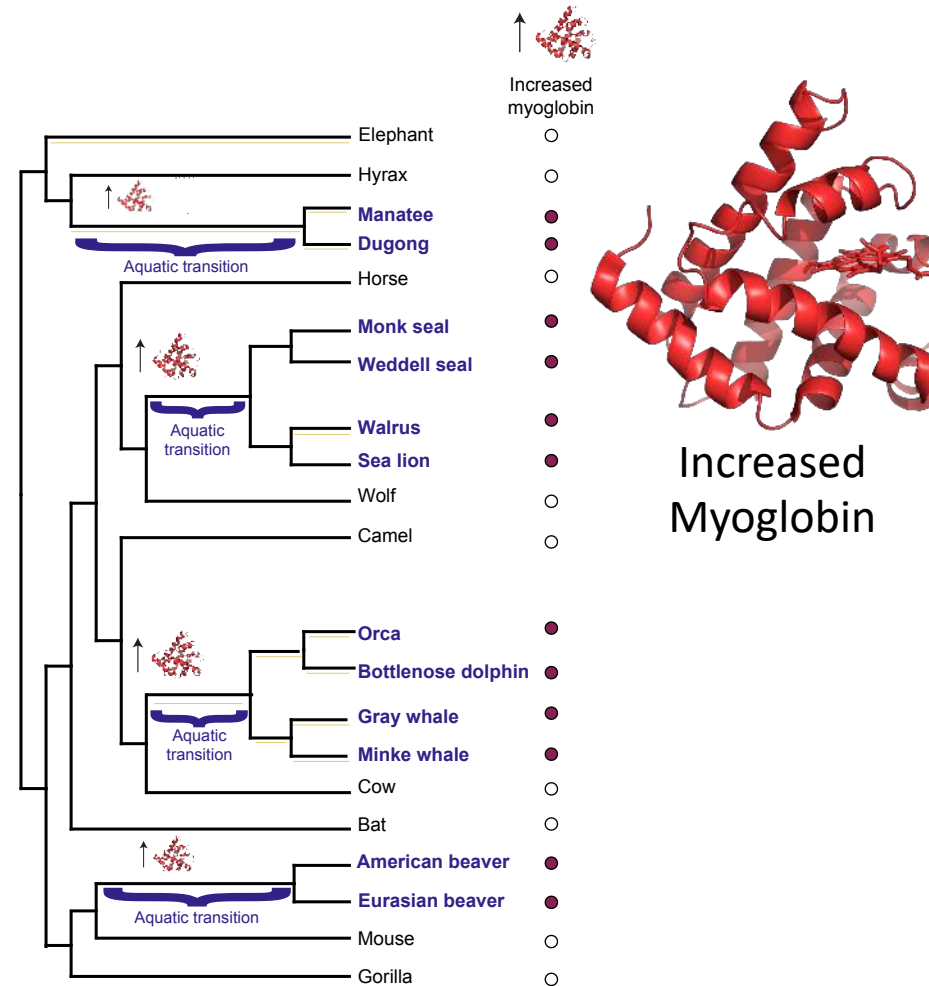
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Novel traits produce **transient genetic signals**.



PhyloG2P

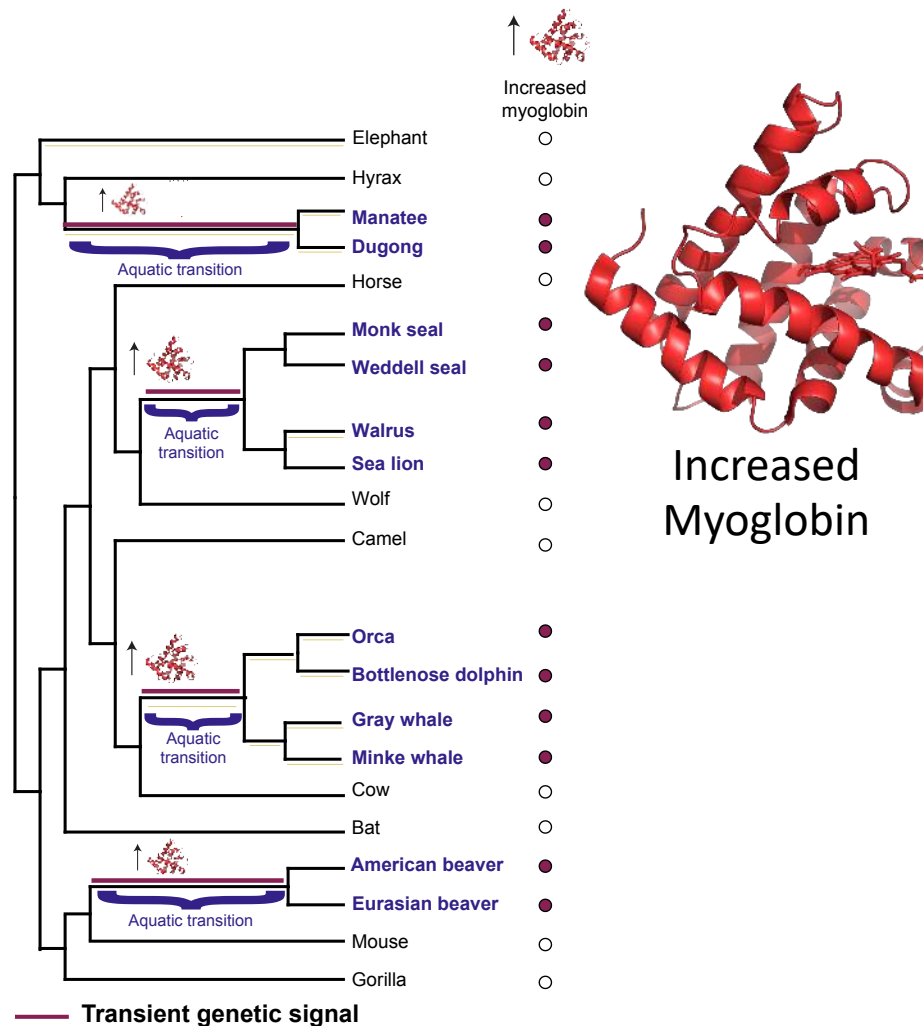
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PhyloG2P

Phylogenetic Genotype to Phenotype

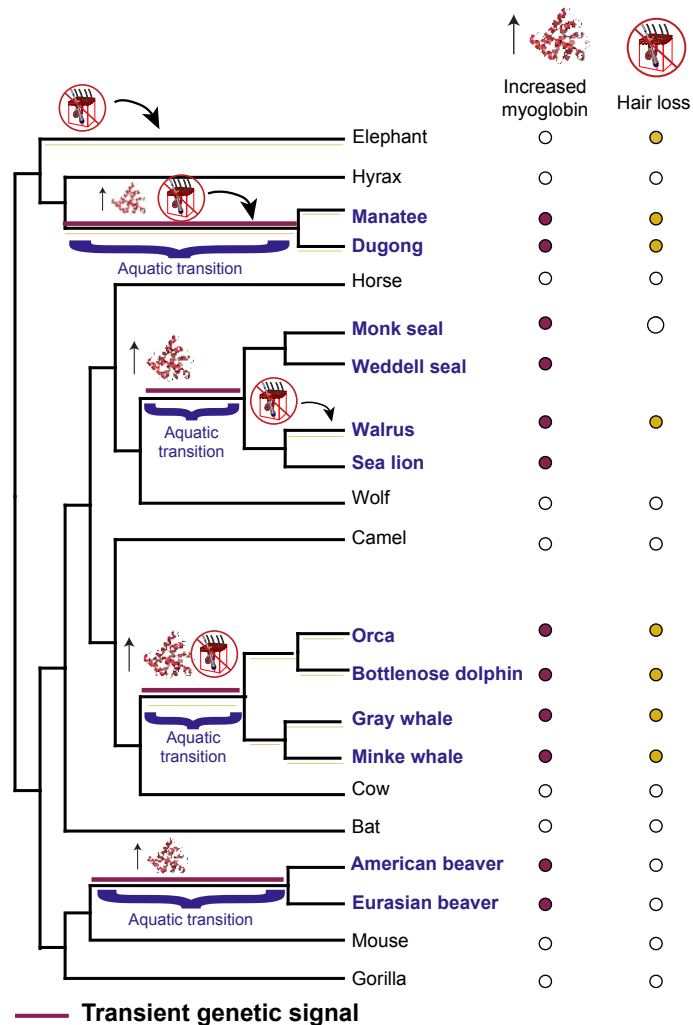
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Novel traits produce **transient genetic signals**.

Trait loss leads to **persistent genetic signals**.



Indicates putative branches on which pictured phenotypes first appeared

PhyloG2P

Phylogenetic Genotype to Phenotype

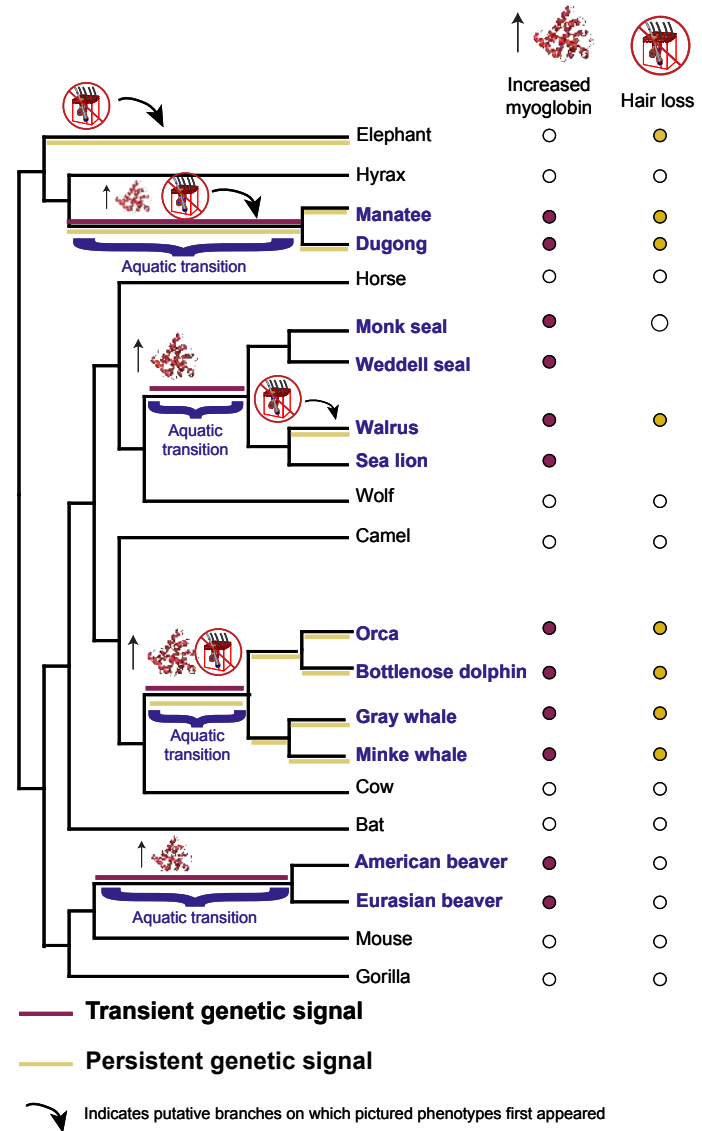
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Trait loss leads to **persistent genetic signals**.



RERconverge package, available on GitHub

<https://github.com/nclark-lab/RERconverge>

RERconverge: an R package for associating evolutionary rates with convergent traits

Amanda Kowalczyk, Wynn K Meyer, Raghavendran Partha, Weiguang Mao,

Nathan L Clark, Maria Chikina ✉ [Author Notes](#)

Bioinformatics, Volume 35, Issue 22, 15 November

Robust Method for Detecting Convergent Shifts in Evolutionary Rates

Raghavendran Partha, Amanda Kowalczyk, Nathan L Clark, Maria Chikina ✉

Molecular Biology and Evolution, Volume 36, Issue 8, August 2019, Pages 1817–18

RERconverge Expansion: Using Relative Evolutionary Rates to Study Complex Categorical Trait Evolution

Ruby Redlich,  Amanda Kowalczyk, Michael Tene, Heather H. Sestili, Kathleen Foley,  Elysia Saputra, Nathan Clark,  Maria Chikina,  Wynn K. Meyer,  Andreas Pfenning

Guillermo
Hoffman
Meyer



Maria Chikina Amanda Kowalczyk Wayne Mao

Wynn Meyer Raghav Partha

Joe Robinson

Elysia Saputra

Ruby Redlich

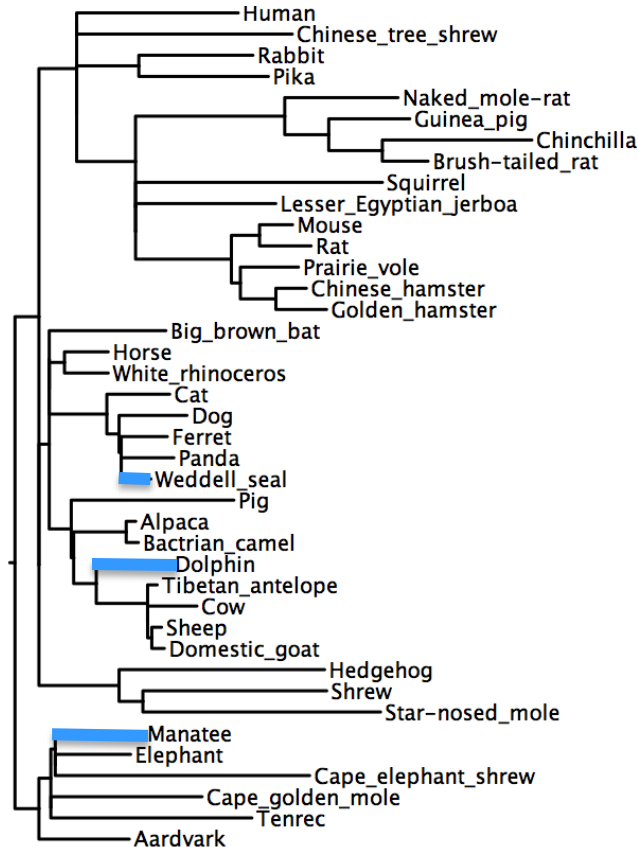
Andreas Pfenning

RERCONVERGE is widely used in comparative genomics

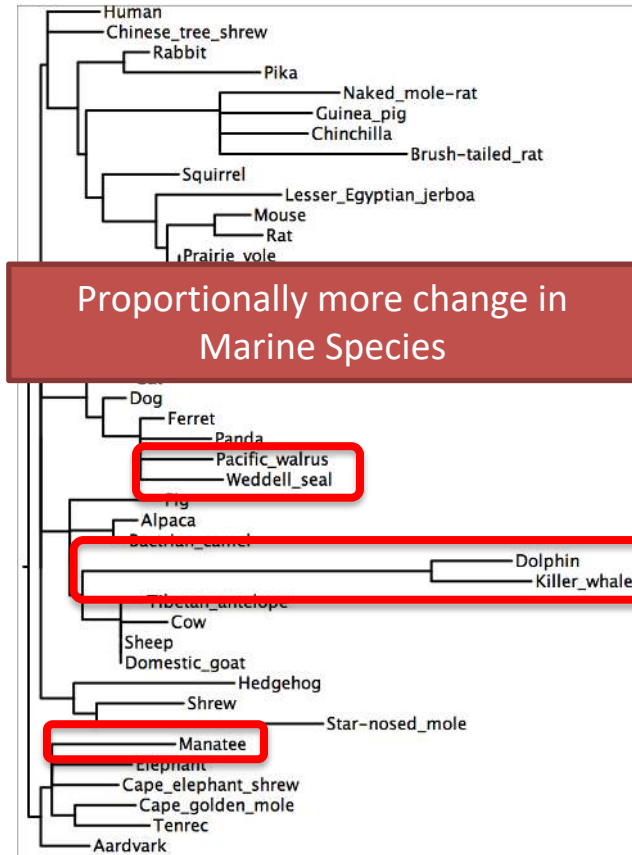


Gene evolutionary rates vary across species

Average Evolutionary Rate



Gustatory G-protein (GNAT3)

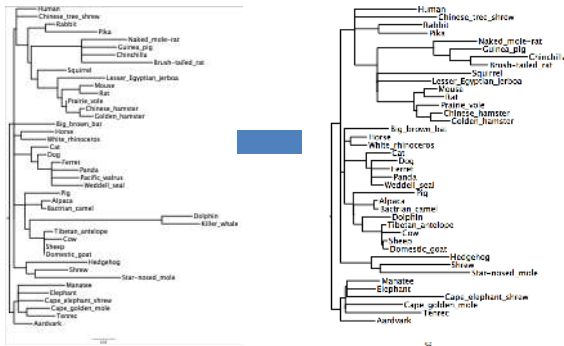


Proportionally more change in Marine Species

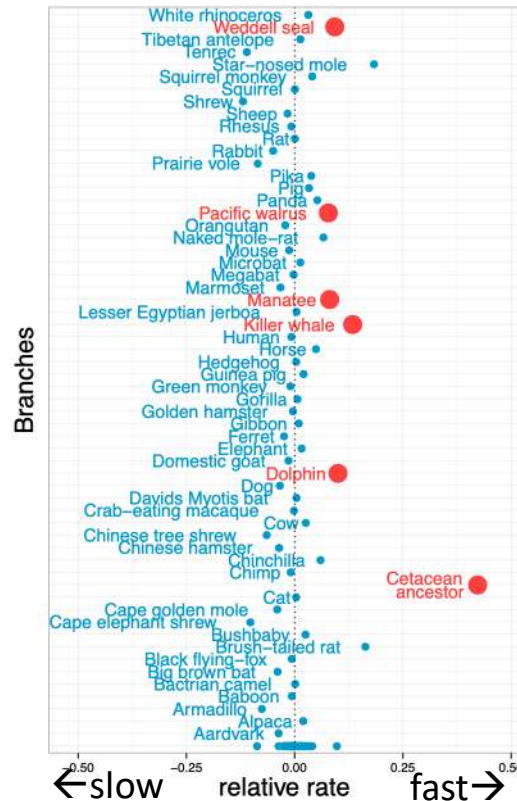
[illegible]

Relative evolutionary rates (RER) a method to study convergence

“Subtract” average tree from gene tree



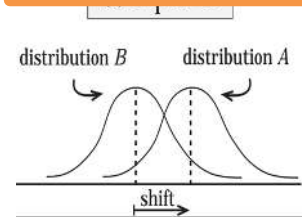
Relative Evolutionary Rates
(RERs) of *GNAT3*



Hypothesis test:
RER acceleration in marine
vs terrestrial species

$$P = 0.00008$$

hence
convergent
marine
acceleration

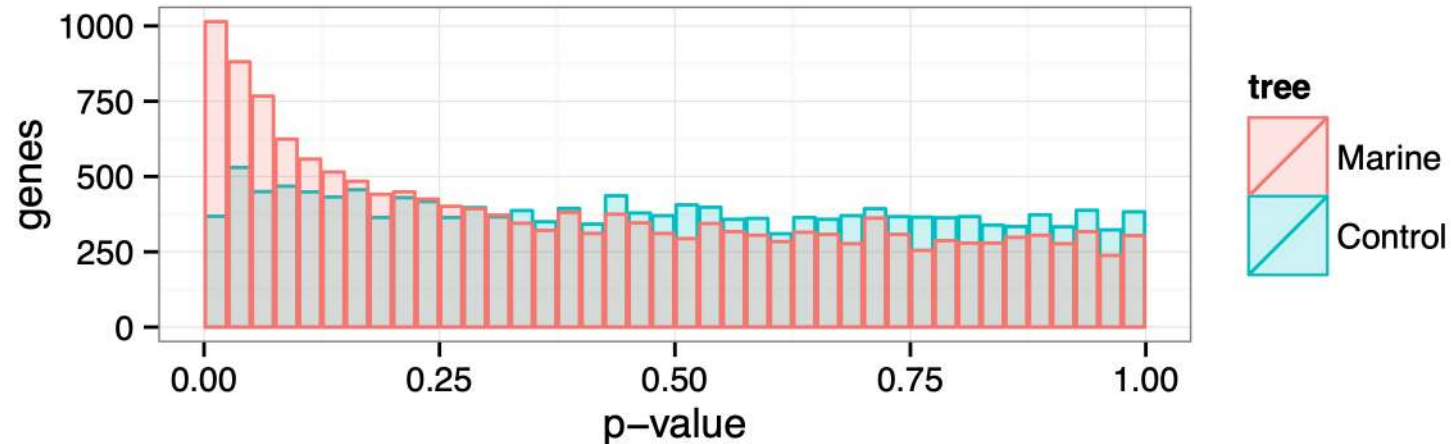


Actually, we take residuals
from a weighted regression

Marine-accelerated genes are in statistical excess

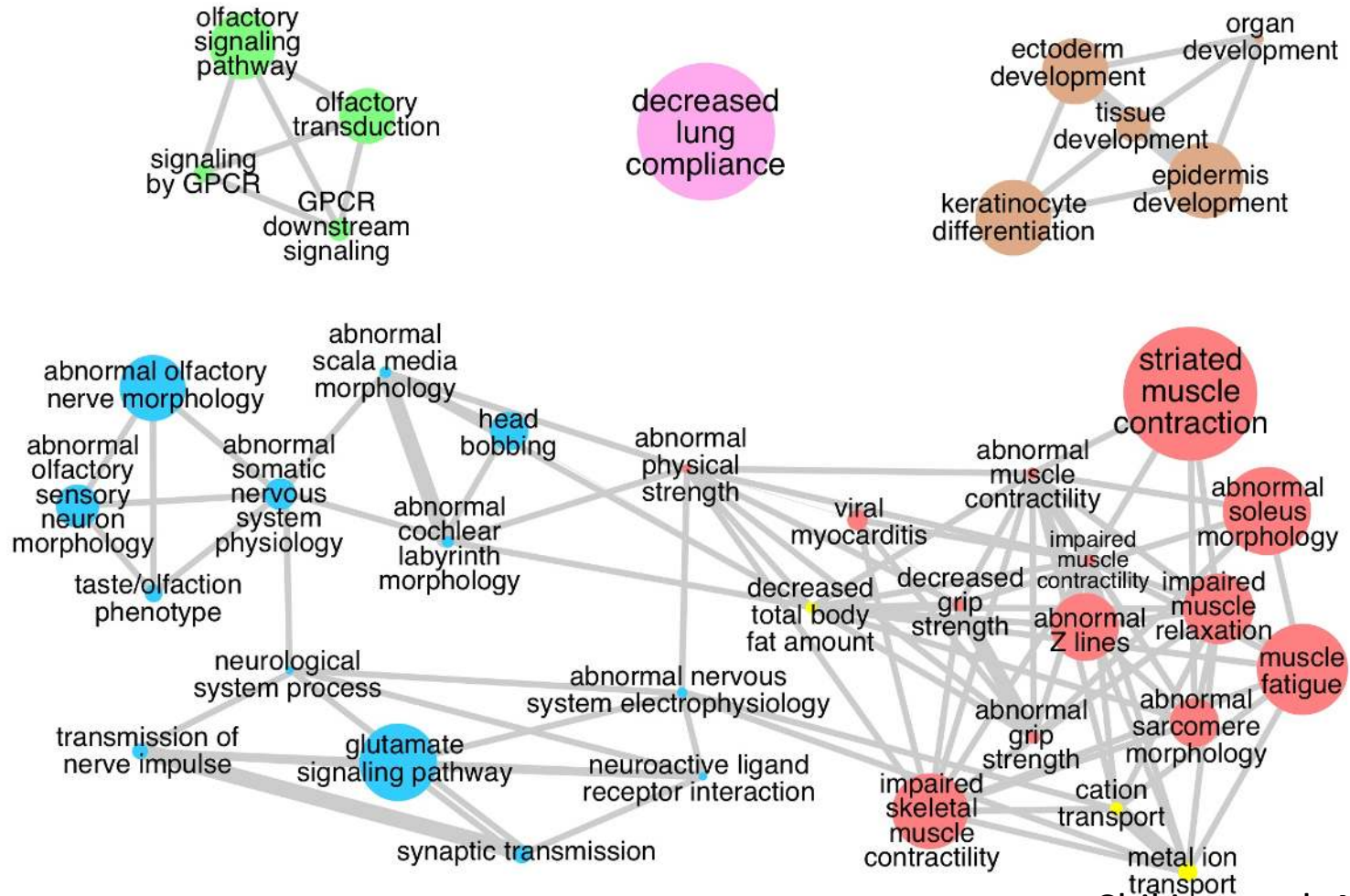
Table 1 | Top marine-accelerated genes

Gene	P-value	Description	Function	Evolutionary Mode
GNAT3	0.00008	G protein subunit in bitter, sweet, and umami taste transduction	Taste	Relaxed
OR6B1	0.00014	olfactory receptor	Olfaction	Relaxed
CALHM1	0.00014	ion channel required for sweet, bitter and umami tastes	Taste	Relaxed
SSTR4	0.00016	somatostatin receptor 4		Relaxed
COL9A2	0.00023	collagen, type IX, alpha 2; hyaline joint cartilage protein	Cartilage	Pos. selection
FGF11	0.00033	fibroblast growth factor. Nervous system development		Relaxed
HMGCS2	0.00042	catalyzes ketogenesis, which provides lipid-derived energy	Lipid metabolism	Relaxed
CLSTN2	0.00046	calsyntenin 2	Nervous system	Relaxed
PERP	0.00049	component of intercellular desmosome junctions in epithelia	Skin	Pos. selection
S100A5	0.00059	S100 calcium binding protein A5		Relaxed



RNF222	0.00154	ring finger protein 222		Relaxed
OR10Z1	0.00164	olfactory receptor, family 10, subfamily Z, member 1	Olfaction	Relaxed
TGM3	0.00166	transglutaminase 3, cell envelope formation in the epidermis and hair follicle	Skin, Hair	Pos. selection
SFTPB	0.00168	Lung-specific surfactant protein B	Lung	Relaxed

Functions enriched among marine-accelerated genes



Lab Structure

Convergent evolution provides a powerful natural experiment for identifying the genetic basis of complex traits.

- Goal: Use a PhyloG2P method to study a convergent trait
- Learning Objectives
 - Understand and view multiple sequence alignments
 - Infer trees
 - Use a relative rates to study a convergent trait
 - Find functional enrichments among gene sets

Lab Structure

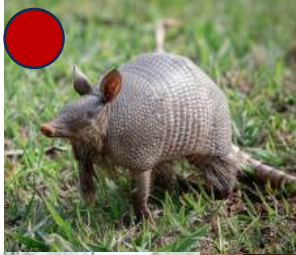
Activity: Use RERconverge to find genes whose rates associate with the loss of teeth and/or tooth enamel.

Steps

1. Make gene trees for sample genes in 108 mammal species
2. Read in large number of orthologous gene trees
3. Calculate Relative Evolutionary Rates (RERs)
4. Encode a convergent trait in a phylogenetic tree
5. Correlate RERs with the convergent trait
6. Study top correlated genes in with faster RERs and slower RERs in the convergent species
7. Calculate enrichments of functional annotations in top genes

There are mammals that have convergently lost their enamel and/or teeth

Xenarthra



Aardvarks



Pangolins



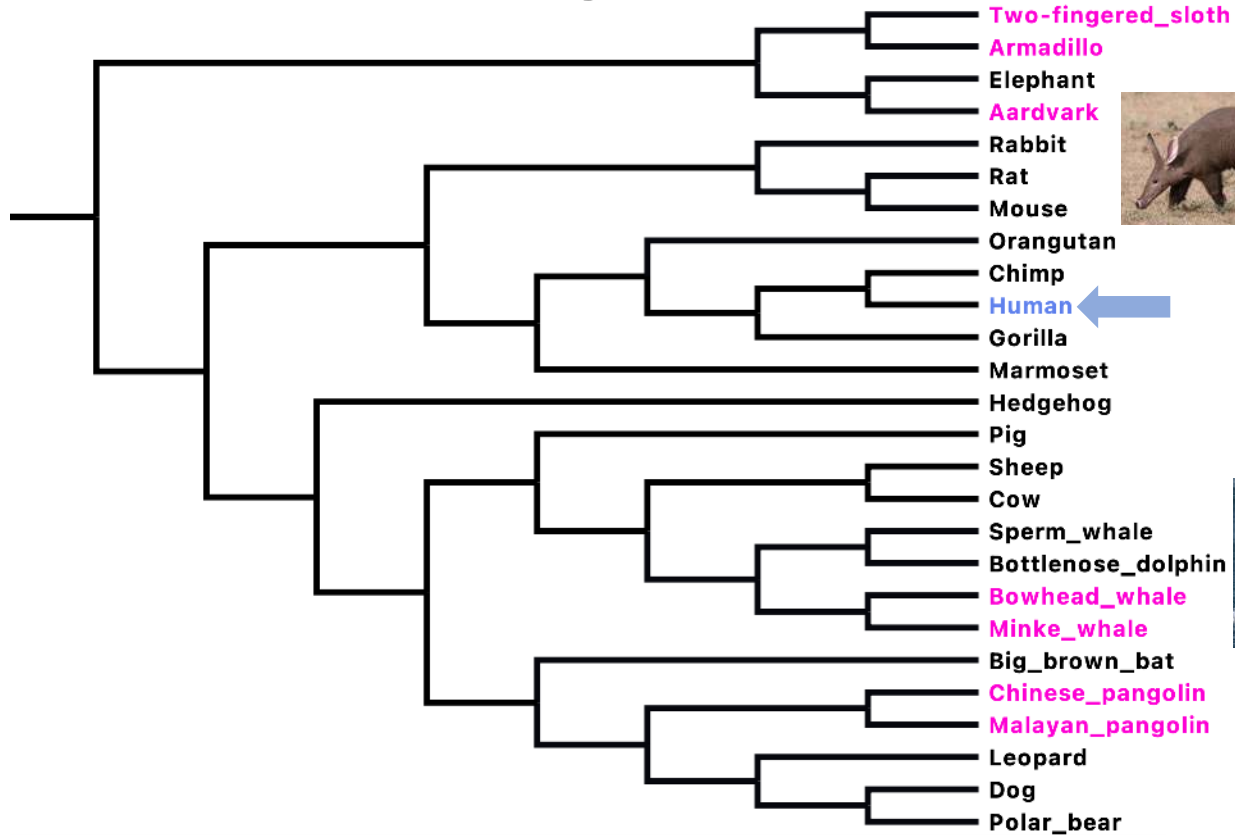
Baleen whales



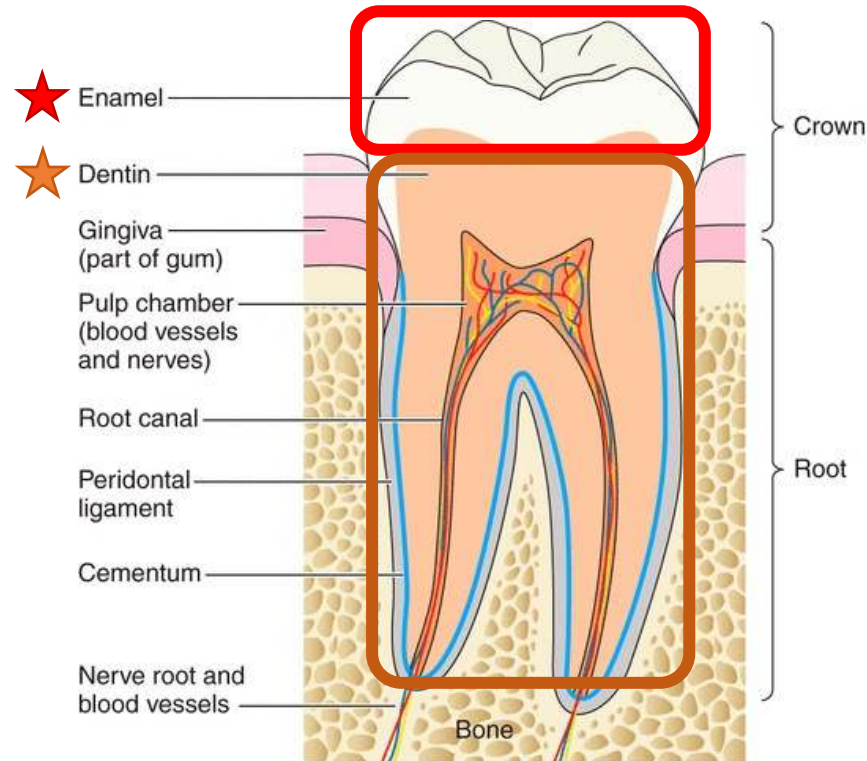
 Enamelless only

 Enamelless and toothless

Tooth/enamel loss has occurred in several mammalian lineages

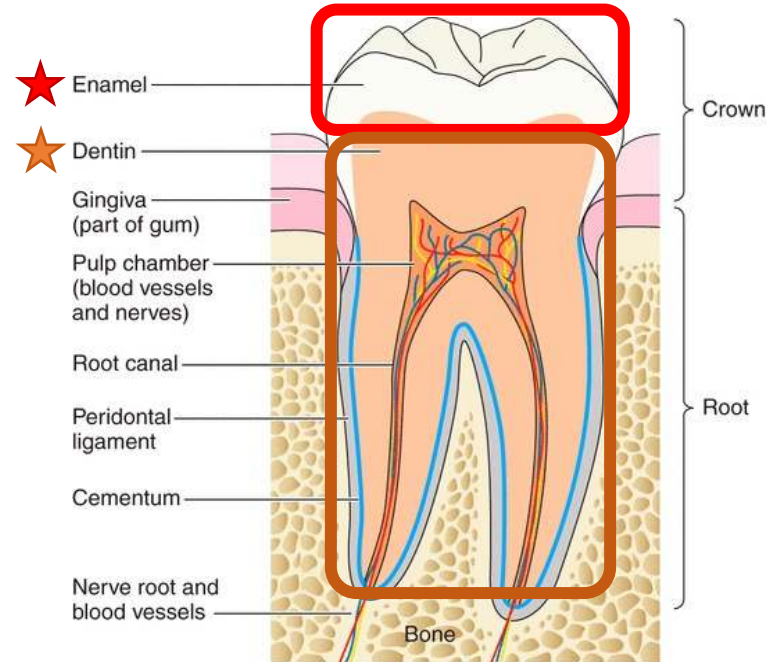


Teeth have two main parts: the crown and root



There are several genes that contribute to tooth development... or tooth loss

*ACP4, AMBN, AMEL, AMTN,
ODAPH, DSPP, ENAM, KLK4,
MMP20, ODAM, DMP1, MEPE*



Lab Structure

Activity: Use RERconverge to find genes whose rates associate with the loss of teeth and/or tooth enamel.

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Terminology

- Relative evolutionary rate (RER) – rate of change for 1 gene over 1 branch, normalized by the expected genome-wide and tree-wide rates
- Trait tree – a phylogenetic tree encoding which branches saw trait change, and their relative weighting
- Rho – estimate of correlation coefficient, sign indicates direction (+ or -) of relationship, range -1 to 1
- signedLogP / stat [RERconverge output] – the $\log_{10}$ of the P-value, carrying the sign of Rho.
 - Top accelerated genes have extreme high values.
 - Top decelerated genes have low values.
- Stat [enrichment analysis] – statistic of the strength of enrichment of an annotation in a gene ranking