INTRODUCTION TO PHYLOGENOMICS

Rosa Fernández

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www.metazomics.com

A little bit about myself

Madrid (PhD)

⇩

Boston (1st postdoc)

⇩

Barcelona (2nd postdoc & my lab)

HARVARD UNIVERSITY

CRG Centre for Genomic Regulation

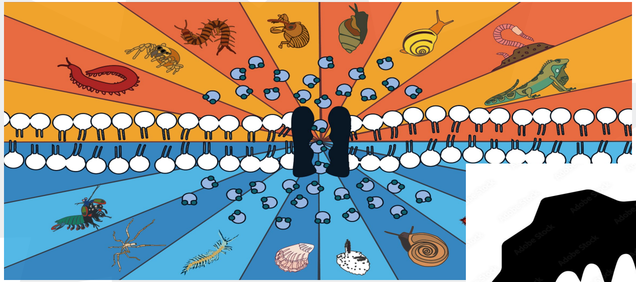
INSTITUT de BIOLOGIA EVOLUTIVA IBE CSIC upf.

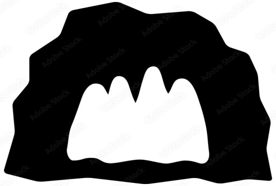
Metazoa Phylogenomics Lab

www.metazomics.com

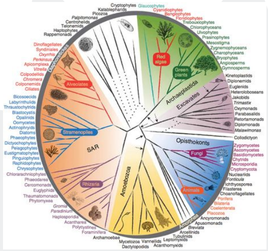
@rosafernandez.bsky.social

Main lines of research:





MACROevolution :-)



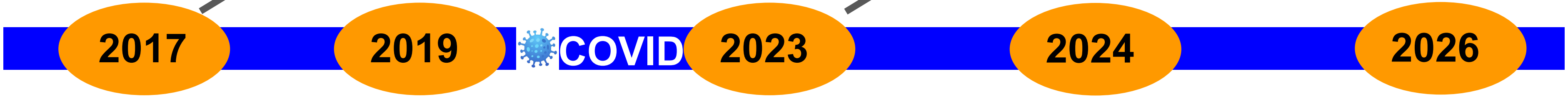
Fun Facts:

I'm a zoologist by training, I did not jump into the world of genomics until I was a postdoc

I did my PhD on earthworms

Mother of two amazing girls :-)

ME AND THE WORKSHOP(S)



2017

Workshop on Phylogenomics (1st edition), TA

2019

COVID

2023

Workshop on Genomics (Faculty & Scientific Advisory Board)

2024

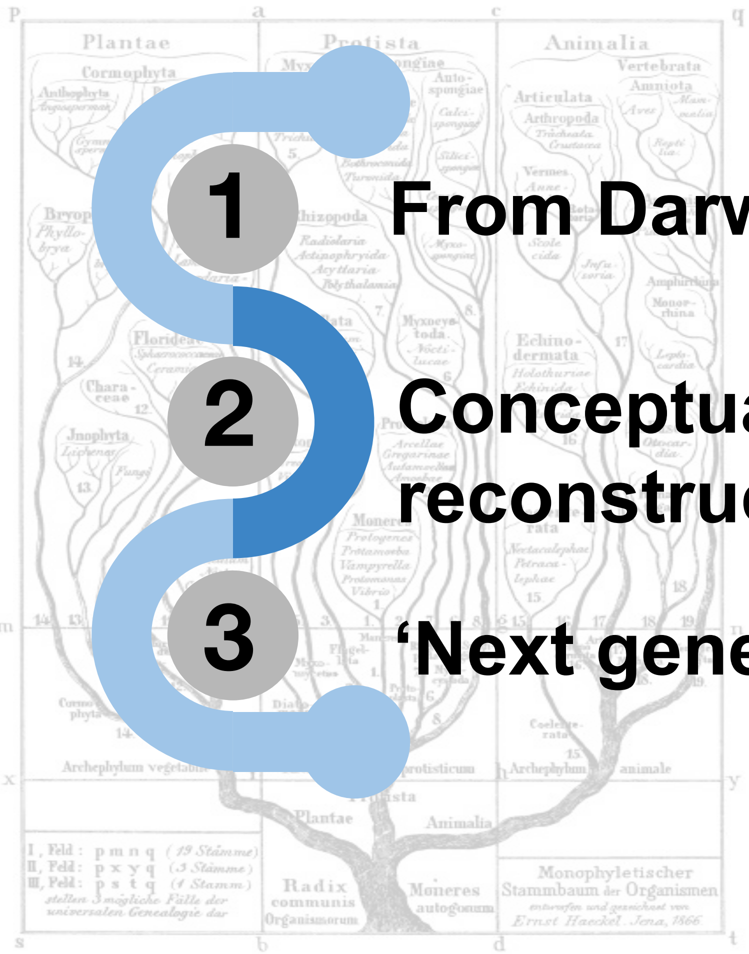
Workshop on Genomics (Faculty)

Workshop on Phylogenomics (3rd Edition), Co-Director

2026

Today :-)

Today’s menu

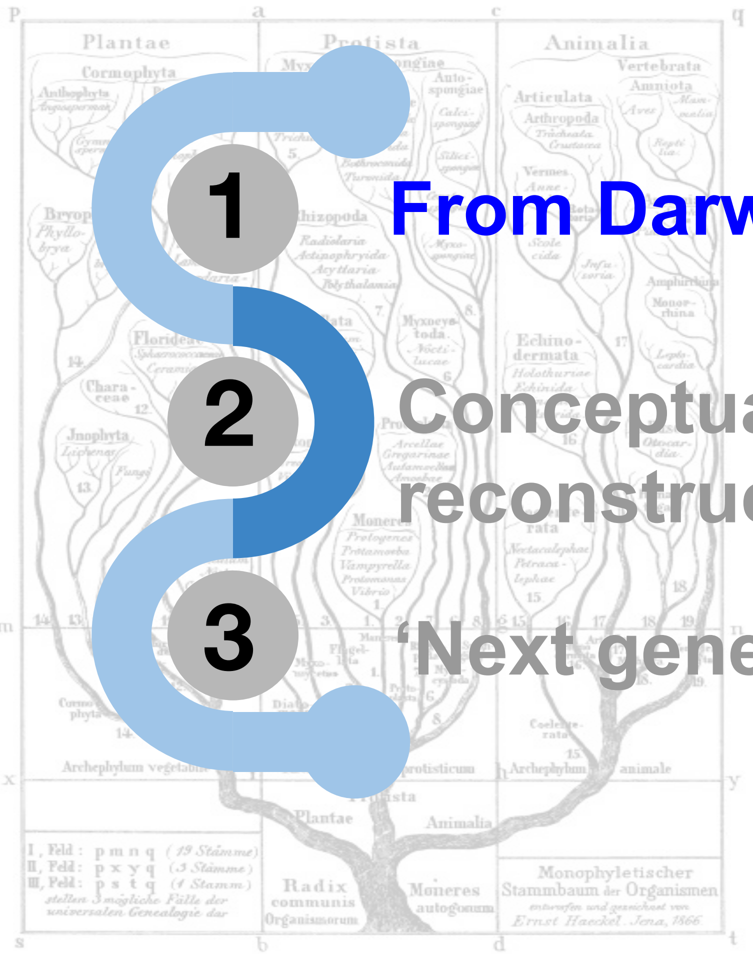


1 From Darwin to phylogenomics

2 Conceptual framework for phylogenomic reconstruction

3 ‘Next generation’ phylogenomics

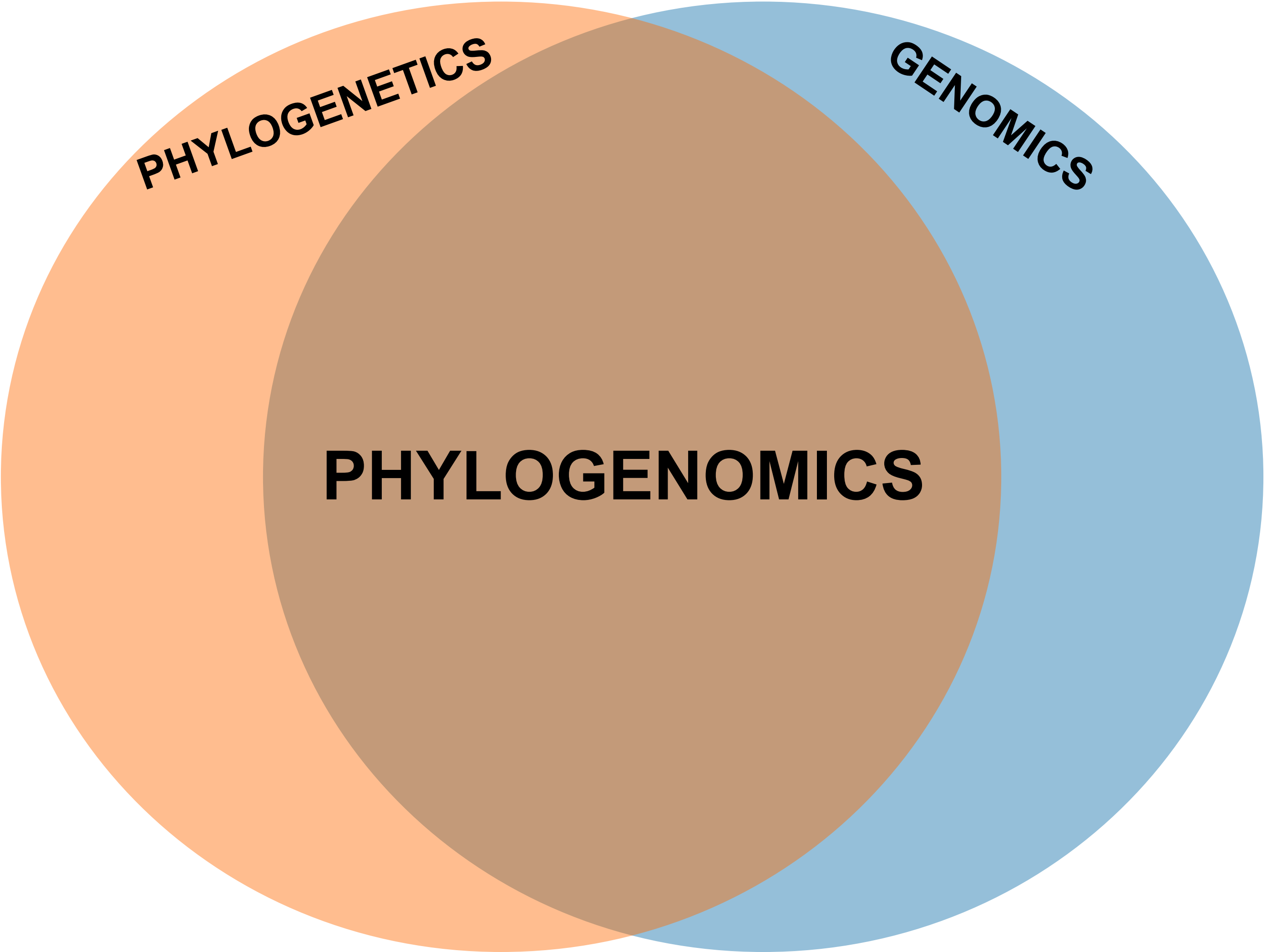
Today’s menu


Plantae
Protista
Animalia
Radix communis Organismorum
Moneres autogonum
Monophyletischer Stammbaum der Organismen
entworfen und gezeichnet von Ernst Haeckel. Jena, 1866

1 From Darwin to phylogenomics

2 Conceptual framework for phylogenomic reconstruction

3 ‘Next generation’ phylogenomics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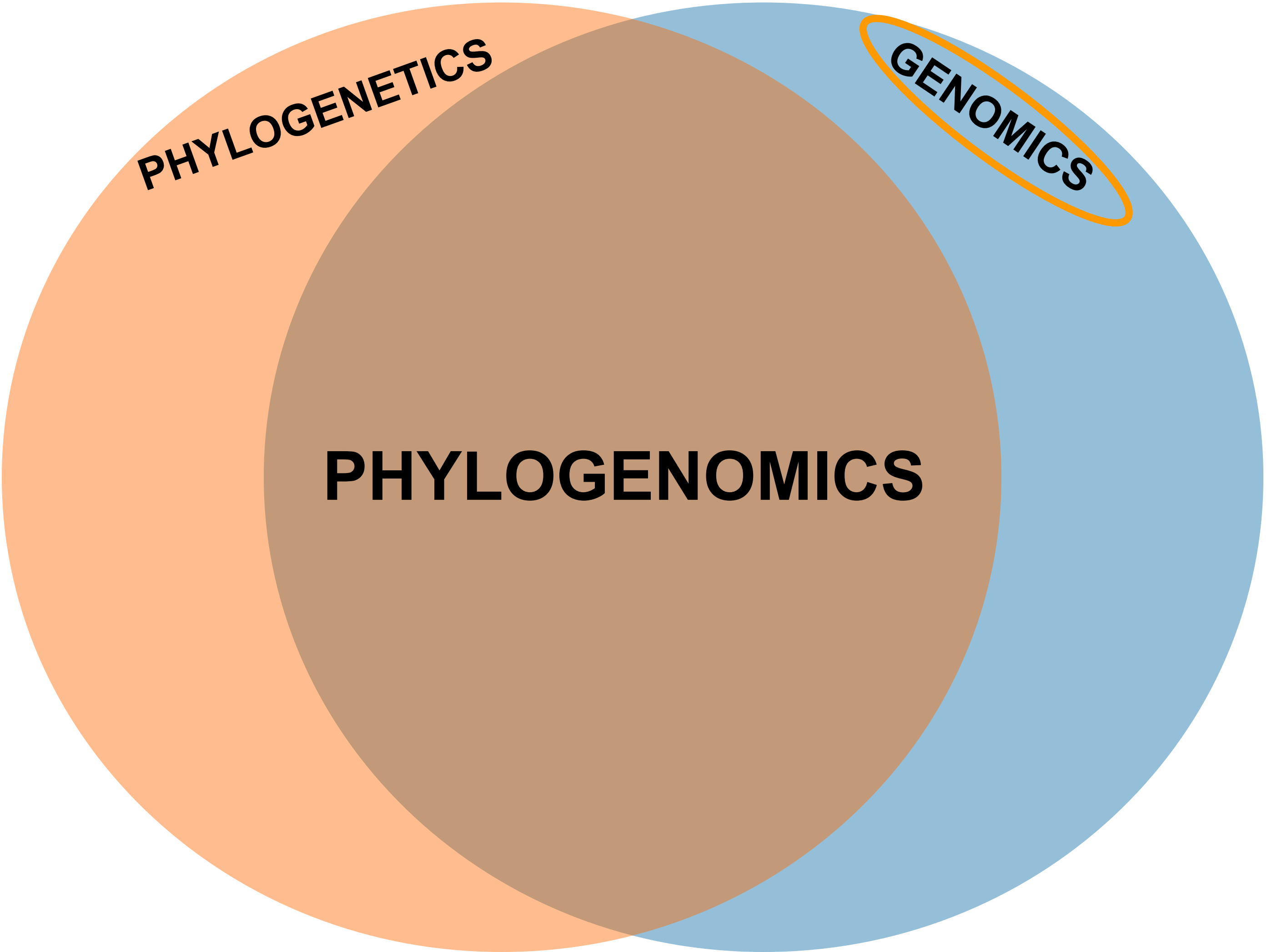
GENOMICS
PHYLOGENOMICS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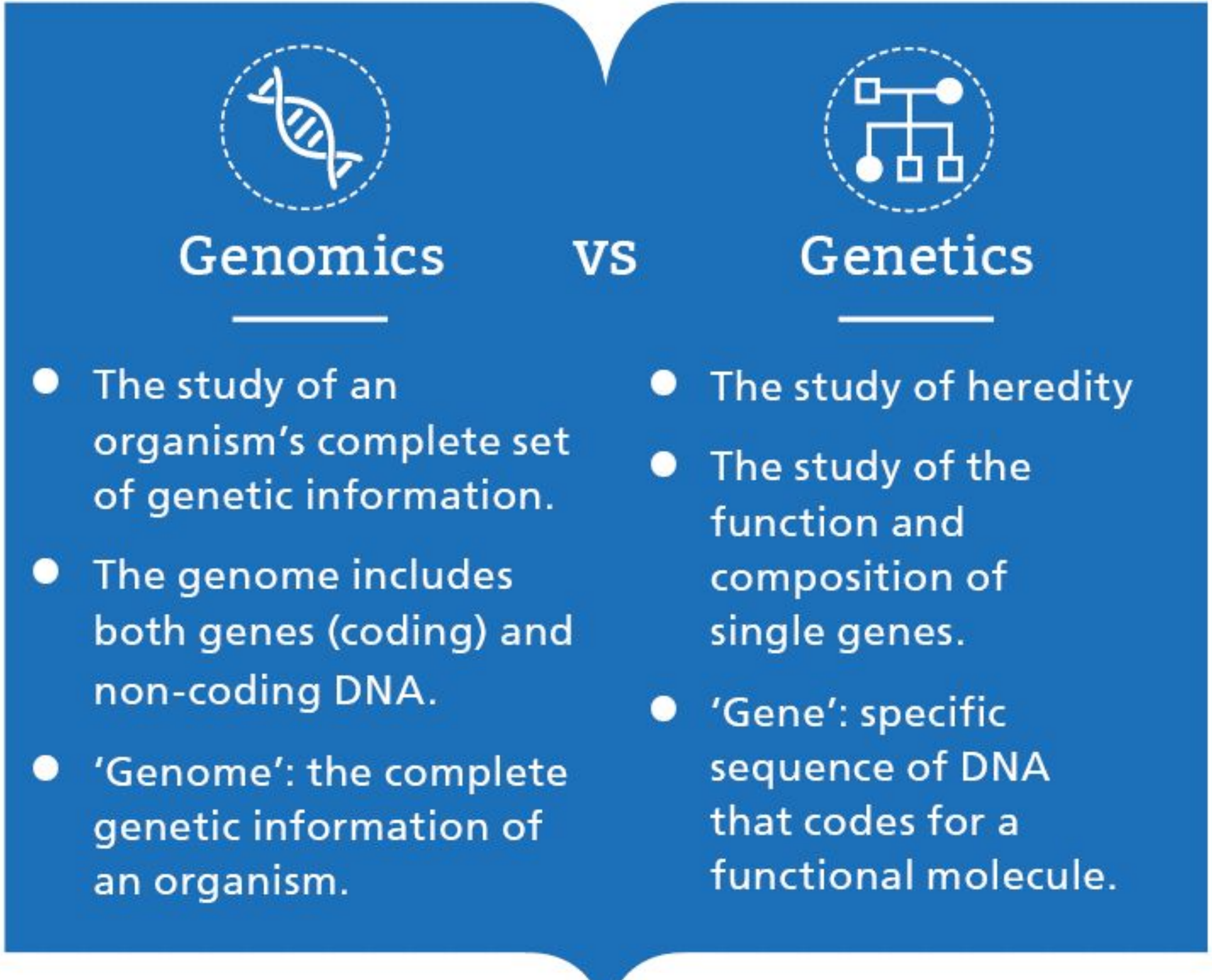


Genomics vs Genetics

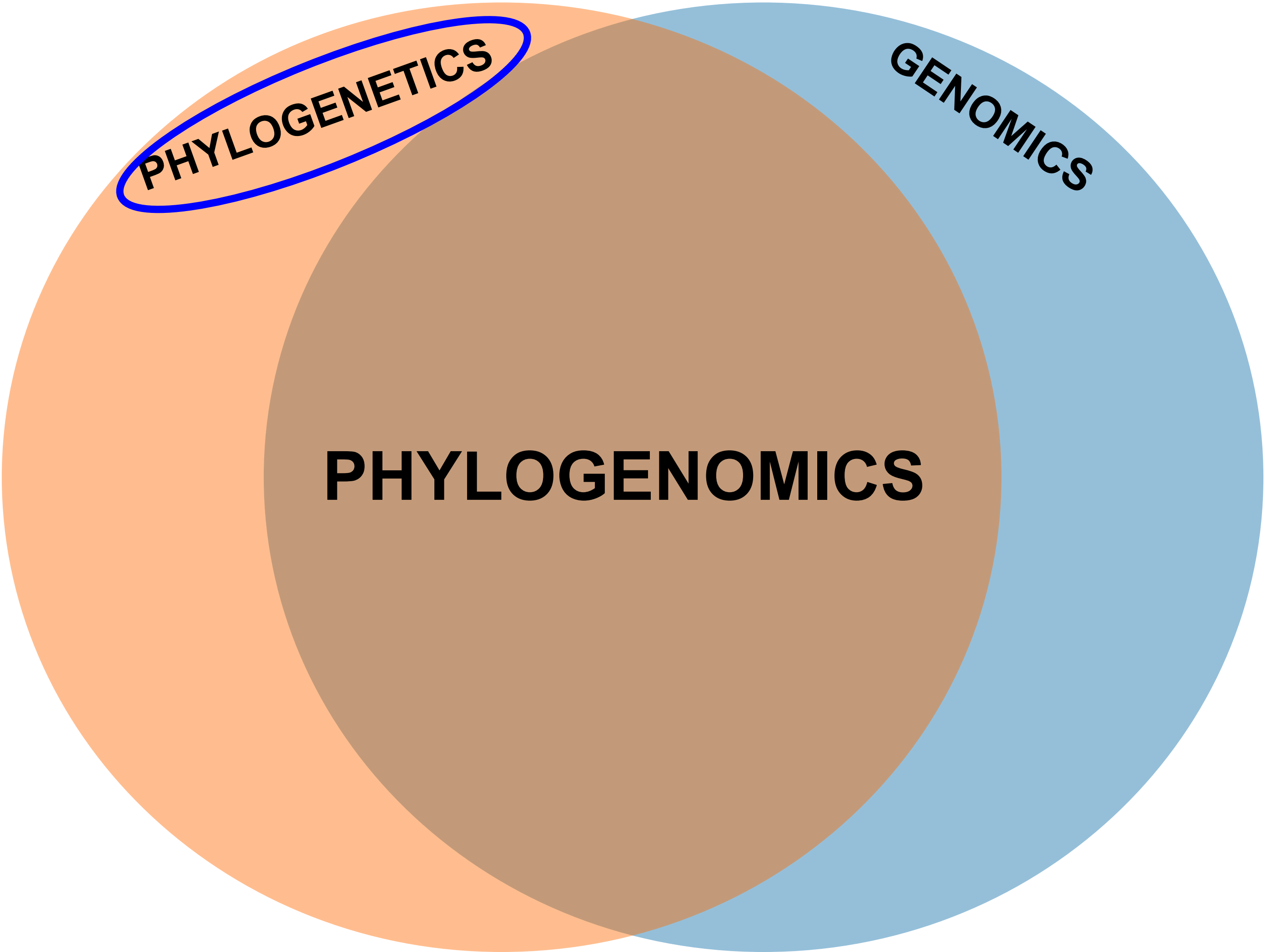
Genomics

- The study of an organism's complete set of genetic information.
- The genome includes both genes (coding) and non-coding DNA.
- 'Genome': the complete genetic information of an organism.

Genetics

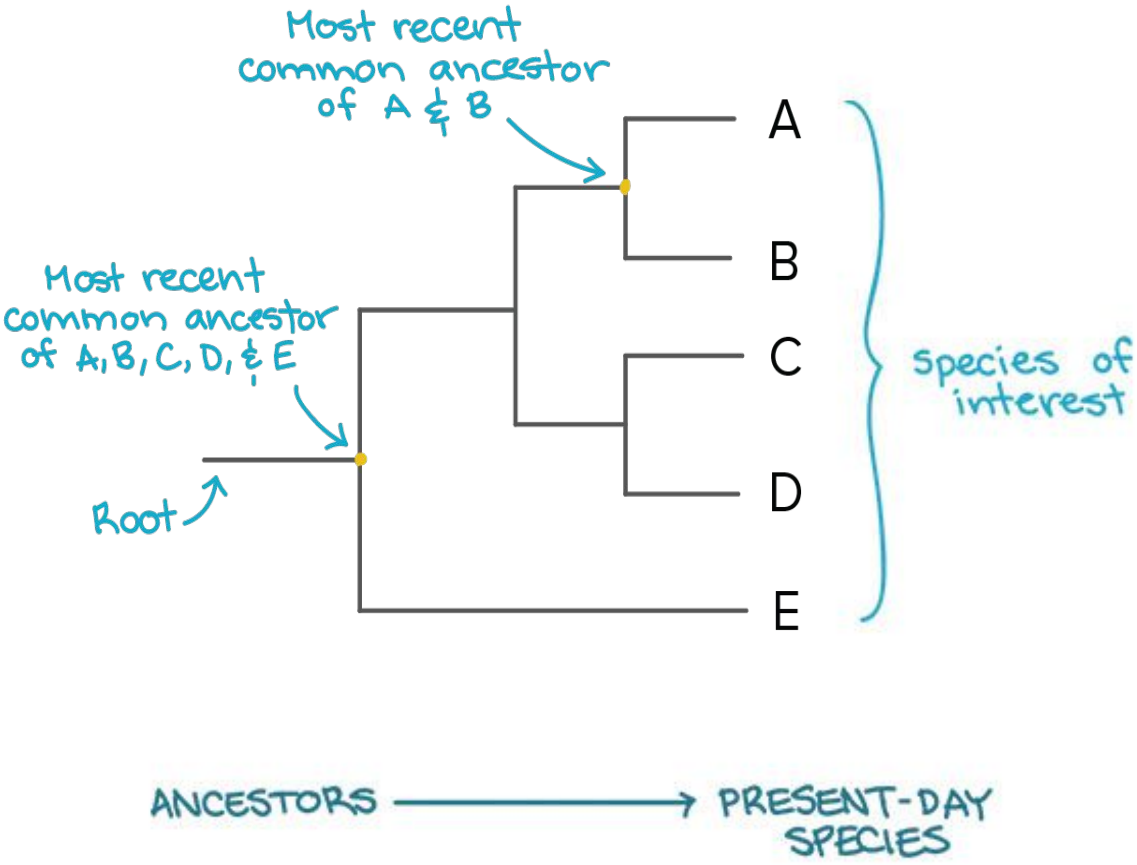
- The study of heredity
- The study of the function and composition of single genes.
- 'Gene': specific sequence of DNA that codes for a functional molecule.

https://www.genomicseducation.hee.nhs.uk/education/core-concepts/what-is-genomics/


PHYLOGENETICS
GENOMICS
PHYLOGENOMICS

What is a phylogeny…?

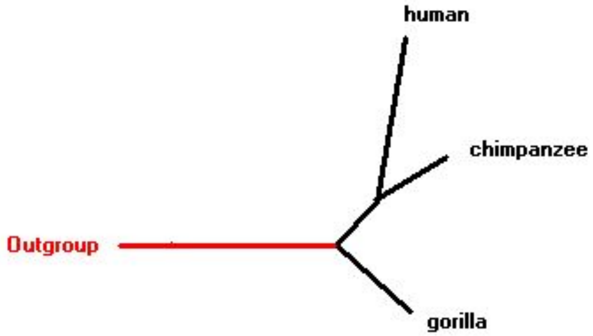
What is a phylogeny…?


Most recent common ancestor of A & B
A
B
Most recent common ancestor of A, B, C, D, & E
C
Species of interest
D
Root
E
ANCESTORS
PRESENT-DAY SPECIES

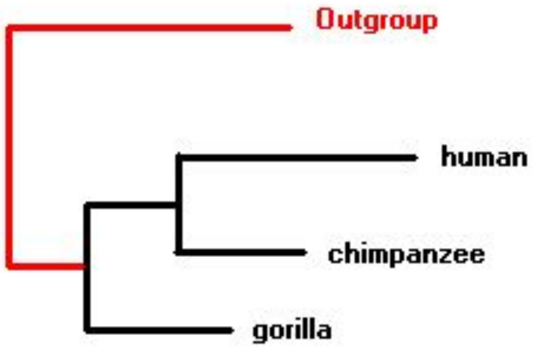
A **phylogenetic tree** is a hypothesis of how species or genes are related through evolution

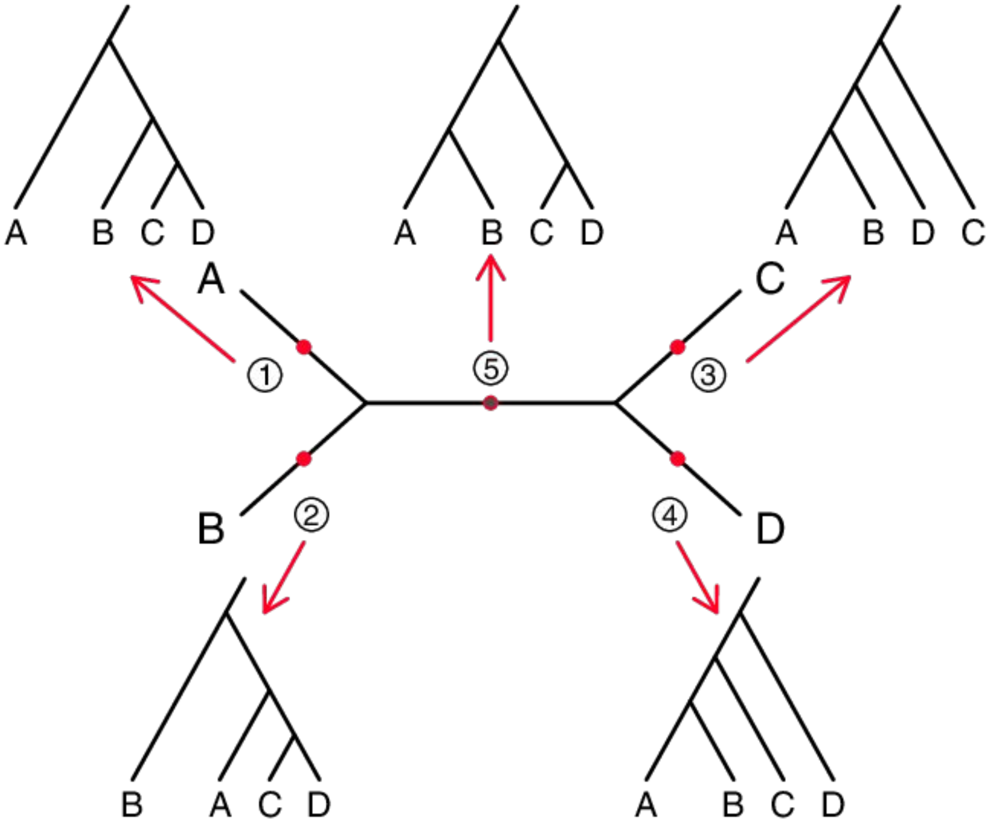
What is a phylogeny…?

Unrooted tree



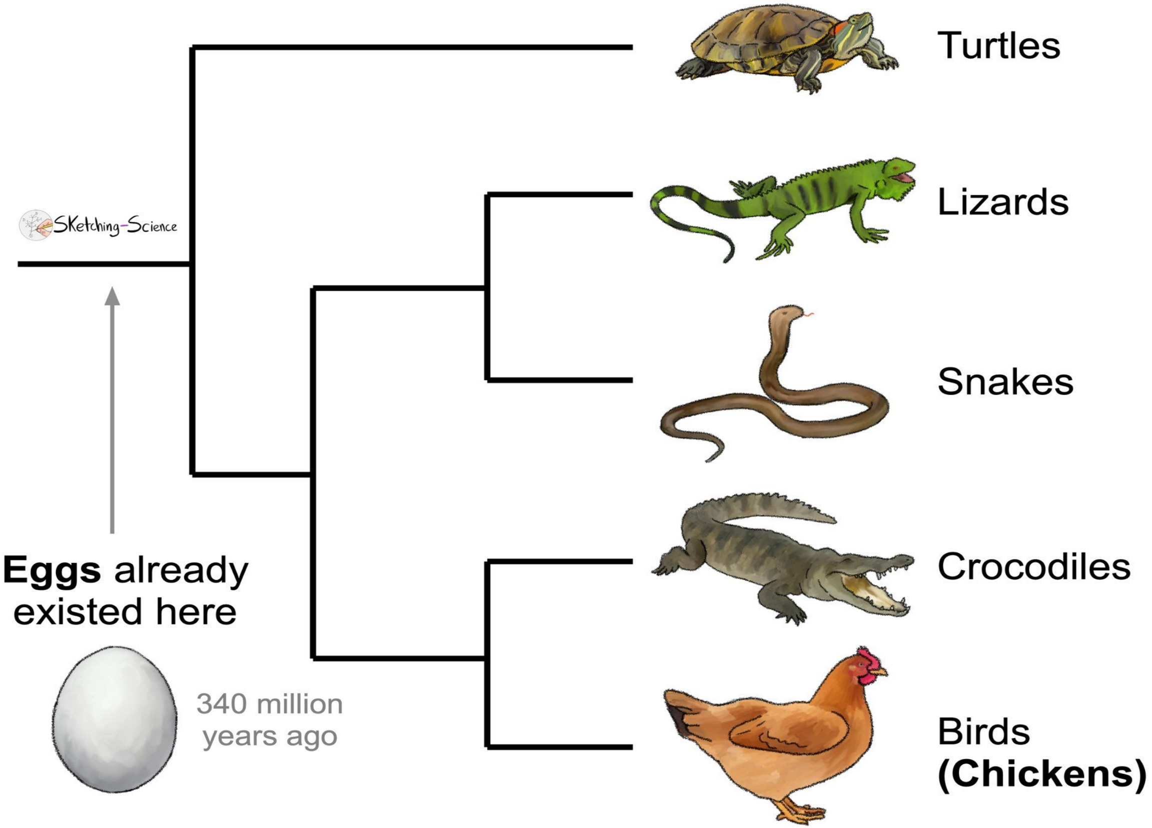
Rooted tree



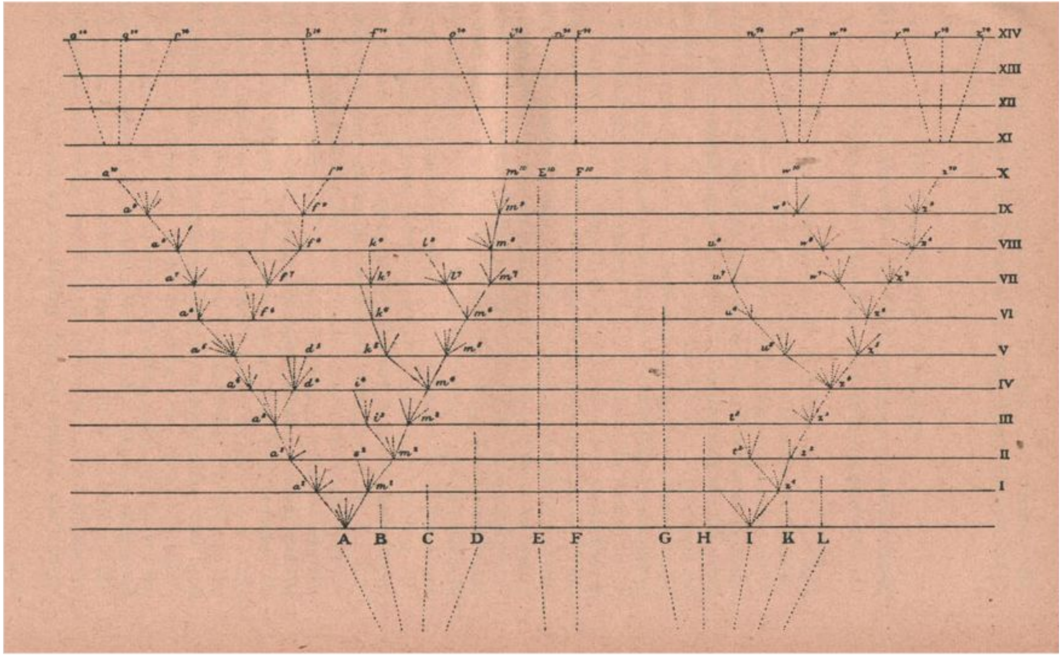


What is a phylogeny, why is it important…?

Which came first, the chicken or the egg?



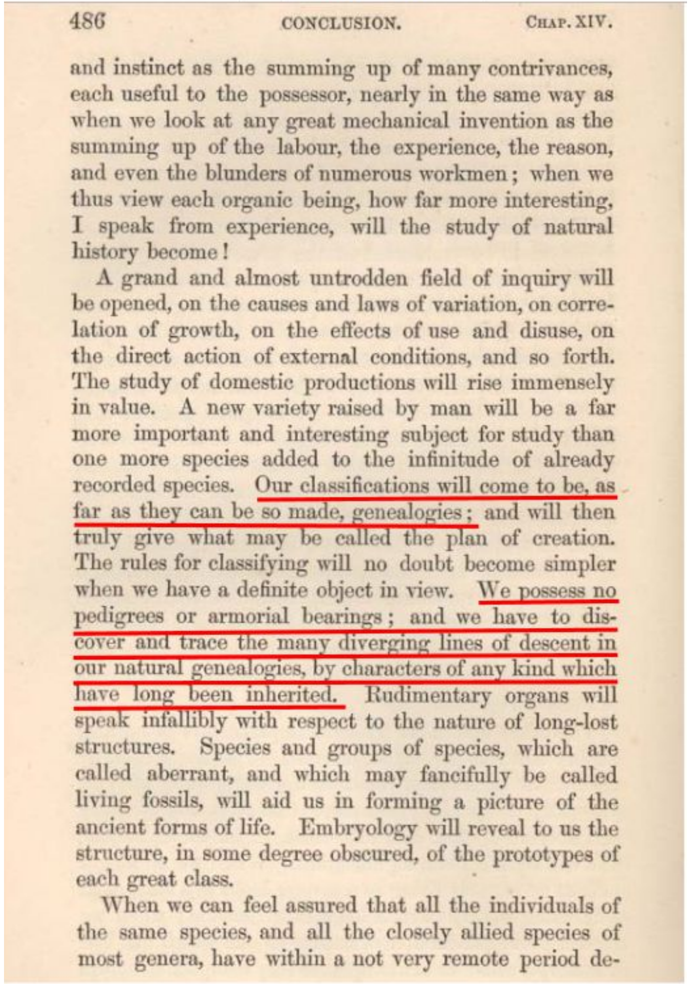
The first phylogenies



(Darwin 1859)

"As buds give rise by growth to fresh buds, and these, if vigorous, branch out and overtop on all sides many a feebler branch, so by generation I believe it has been with the great Tree of Life, which fills with its dead and broken branches the crust of the earth, and covers the surface with its ever branching and beautiful ramifications"

The first phylogenies



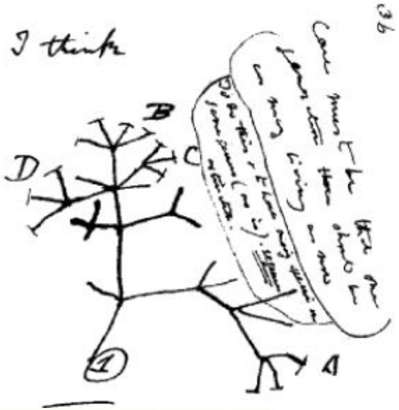
and instinct as the summing up of many contrivances, each useful to the possessor, nearly in the same way as when we look at any great mechanical invention as the summing up of the labour, the experience, the reason, and even the blunders of numerous workmen; when we thus view each organic being, how far more interesting, I speak from experience, will the study of natural history become!

A grand and almost untrodden field of inquiry will be opened, on the causes and laws of variation, on correlation of growth, on the effects of use and disuse, on the direct action of external conditions, and so forth. The study of domestic productions will rise immensely in value. A new variety raised by man will be a far more important and interesting subject for study than one more species added to the infinitude of already recorded species. Our classifications will come to be, as far as they can be so made, genealogies; and will then truly give what may be called the plan of creation. The rules for classifying will no doubt become simpler when we have a definite object in view. We possess no pedigrees or armorial bearings; and we have to discover and trace the many diverging lines of descent in our natural genealogies, by characters of any kind which have long been inherited. Rudimentary organs will speak infallibly with respect to the nature of long-lost structures. Species and groups of species, which are called aberrant, and which may fancifully be called living fossils, will aid us in forming a picture of the ancient forms of life. Embryology will reveal to us the structure, in some degree obscured, of the prototypes of each great class.

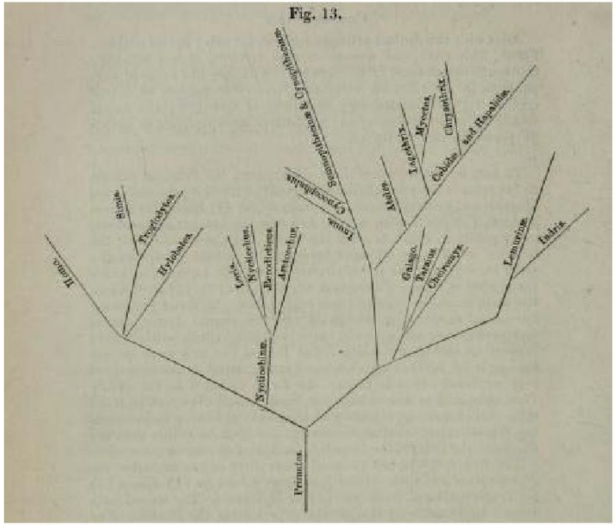
When we can feel assured that all the individuals of the same species, and all the closely allied species of most genera, have within a not very remote period de-

The first phylogenies

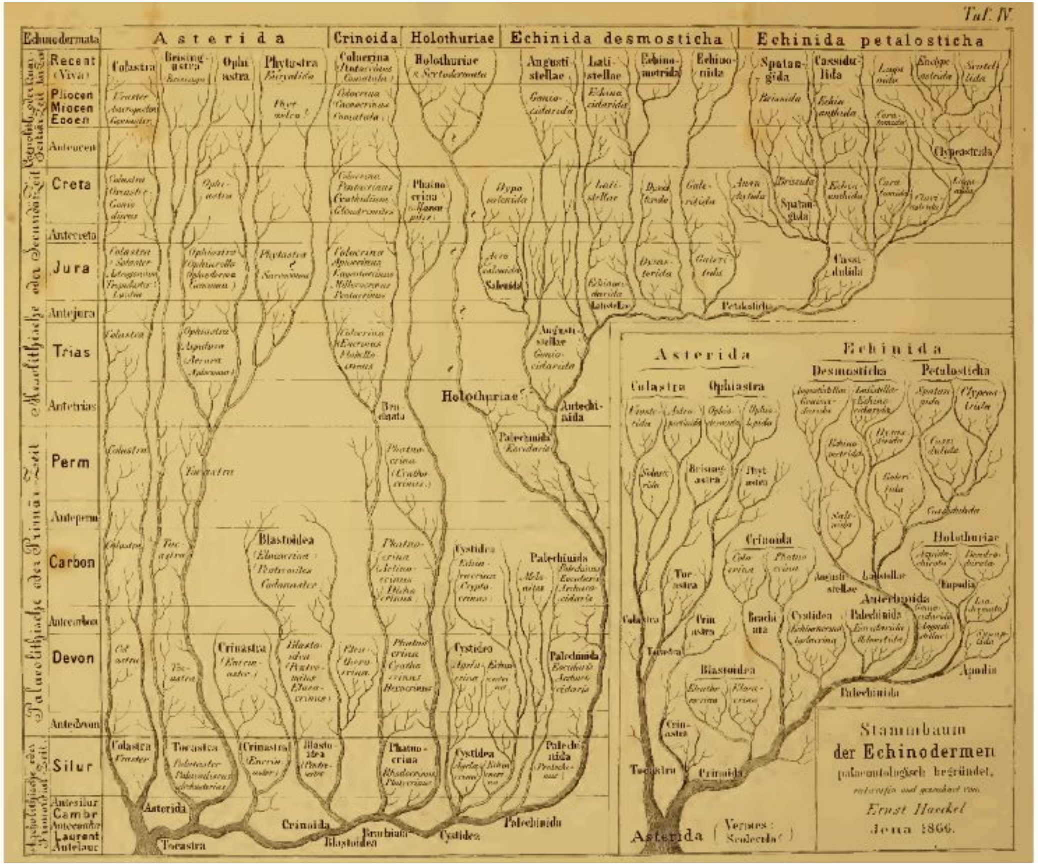
The concept: Darwin's 'I think' (1837)



36
I think
Case must be that one generation then should be as many living as now. To do this & to have many species in same genus (as is). requires extinction.
1
A
B
C
D



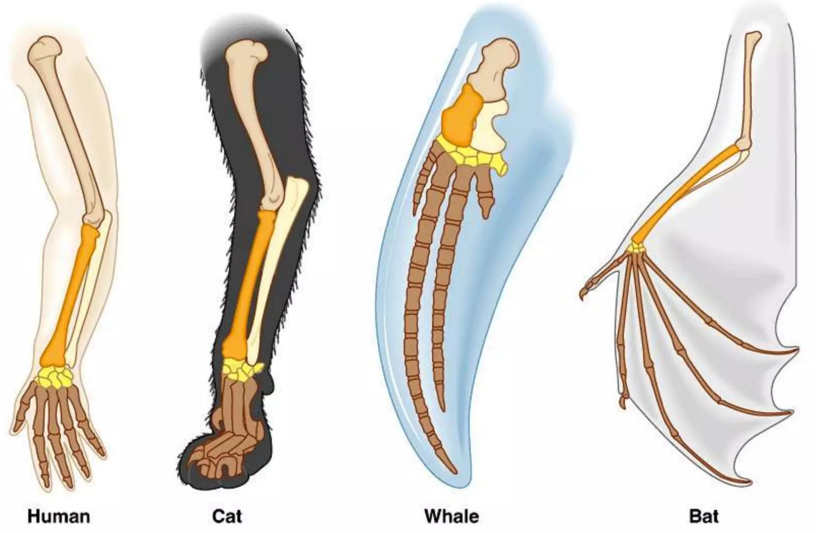
Mivart (1865) Proc. Zool. Soc. London



Haeckel (1866)

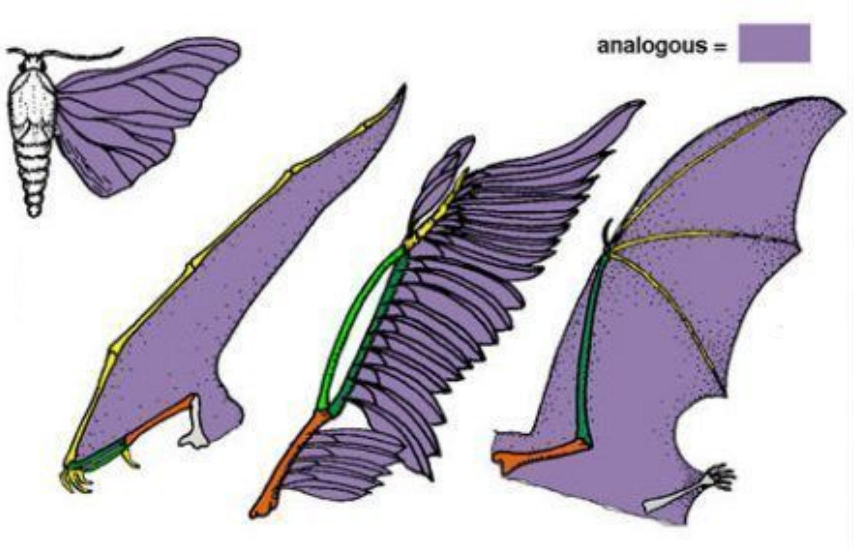
What is a phylogeny, why is it important… and how do you build one?

Homologous Structures

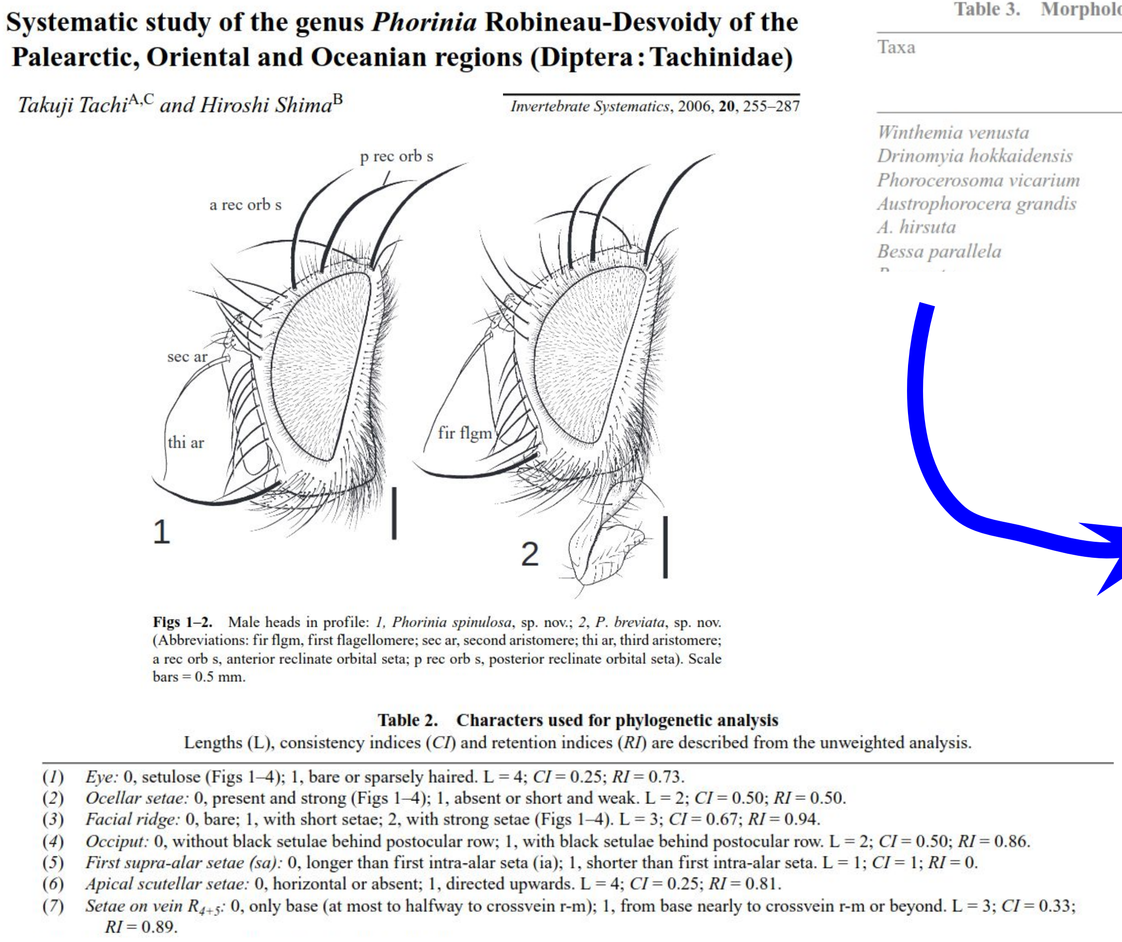


VS

Analogous Structures



What is a phylogeny, why is it important… and how do you build one?



Systematic study of the genus *Phorinia* Robineau-Desvoidy of the Palearctic, Oriental and Oceanian regions (Diptera : Tachinidae)

Takuji Tachi[A,C] *and Hiroshi Shima*[B]

Invertebrate Systematics, 2006, **20**, 255–287

p rec orb s
a rec orb s
sec ar
thi ar
1
fir flgm
2

Figs 1–2. Male heads in profile: *1, Phorinia spinulosa*, sp. nov.; *2, P. breviata*, sp. nov. (Abbreviations: fir flgm, first flagellomere; sec ar, second aristomere; thi ar, third aristomere; a rec orb s, anterior reclinate orbital seta; p rec orb s, posterior reclinate orbital seta). Scale bars = 0.5 mm.

Table 2. Characters used for phylogenetic analysis

Lengths (L), consistency indices (*CI*) and retention indices (*RI*) are described from the unweighted analysis.

(*1*) *Eye:* 0, setulose (Figs 1–4); 1, bare or sparsely haired. L = 4; *CI* = 0.25; *RI* = 0.73.

(*2*) *Ocellar setae:* 0, present and strong (Figs 1–4); 1, absent or short and weak. L = 2; *CI* = 0.50; *RI* = 0.50.

(*3*) *Facial ridge:* 0, bare; 1, with short setae; 2, with strong setae (Figs 1–4). L = 3; *CI* = 0.67; *RI* = 0.94.

(*4*) *Occiput:* 0, without black setulae behind postocular row; 1, with black setulae behind postocular row. L = 2; *CI* = 0.50; *RI* = 0.86.

(*5*) *First supra-alar setae (sa):* 0, longer than first intra-alar seta (ia); 1, shorter than first intra-alar seta. L = 1; *CI* = 1; *RI* = 0.

(*6*) *Apical scutellar setae:* 0, horizontal or absent; 1, directed upwards. L = 4; *CI* = 0.25; *RI* = 0.81.

(*7*) *Setae on vein R_{4+5}:* 0, only base (at most to halfway to crossvein r-m); 1, from base nearly to crossvein r-m or beyond. L = 3; *CI* = 0.33; *RI* = 0.89.

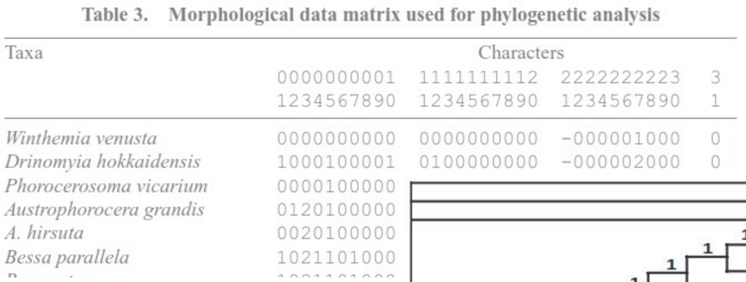


Table 3. Morphological data matrix used for phylogenetic analysis

Taxa	Characters			
	0000000001 1234567890	1111111112 1234567890	2222222223 1234567890	3 1
Winthemia venusta	0000000000	0000000000	-000001000	0
Drinomyia hokkaidensis	1000100001	0100000000	-000002000	0
Phorocerosoma vicarium	0000100000			
Austrophorocera grandis	0120100000			
A. hirsuta	0020100000			
Bessa parallela	1021101000			

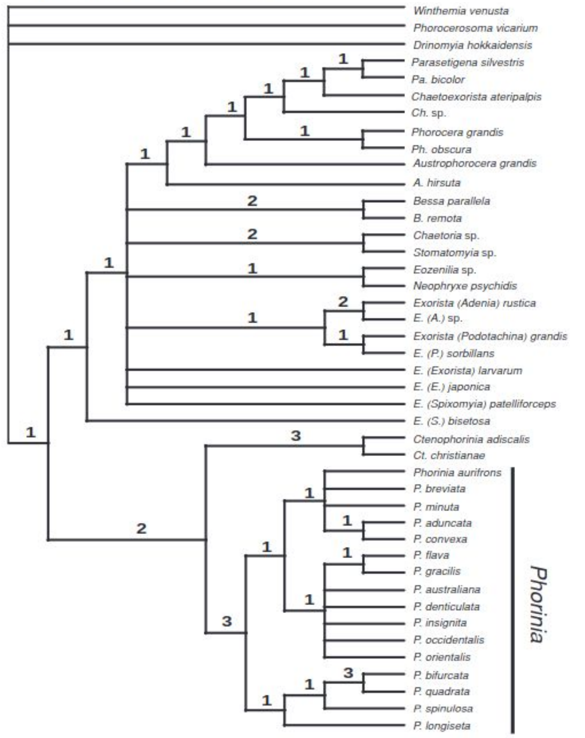
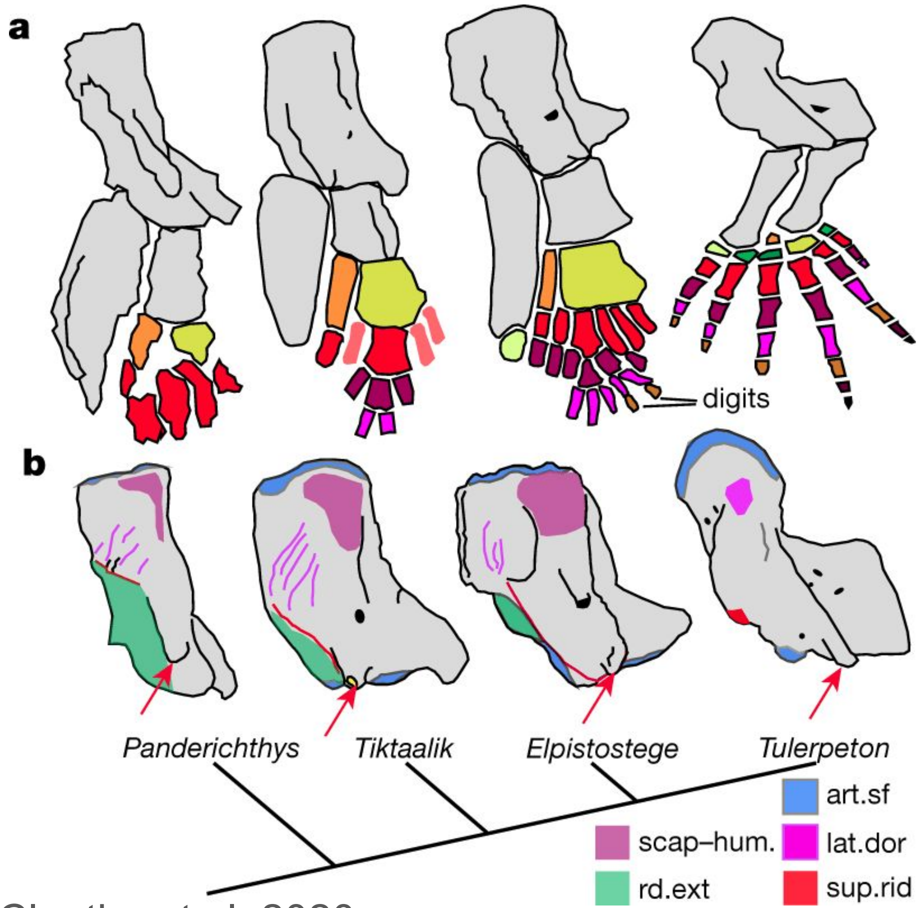
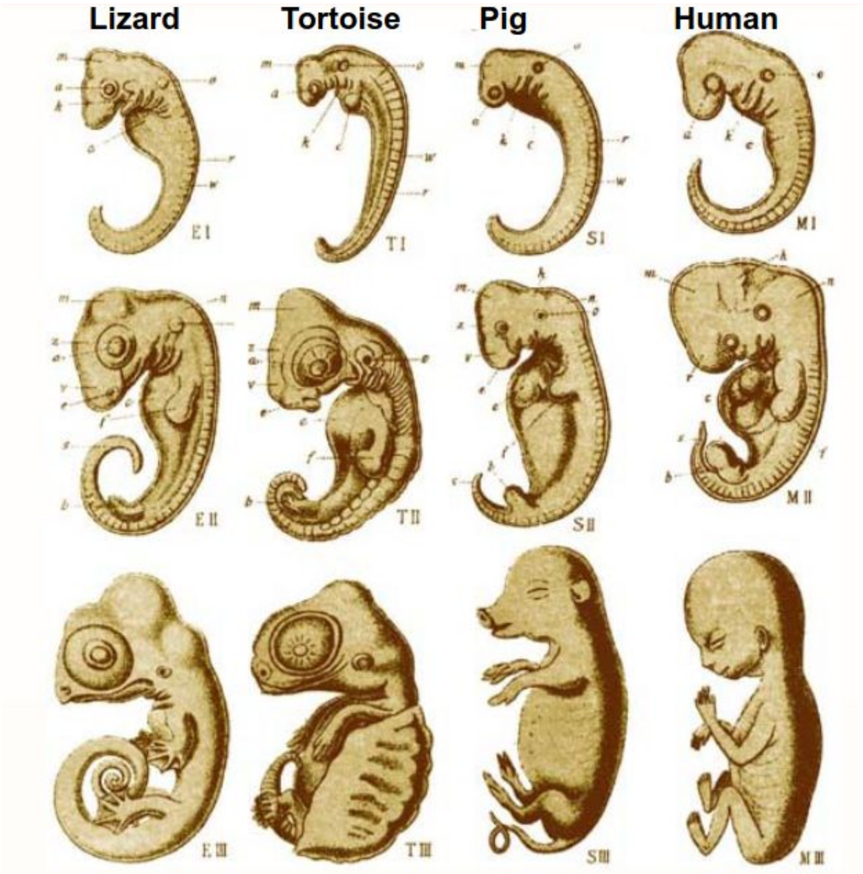


Fig. 79. Strict consensus of 186 equally most parsimonious cladograms (length = 66, consistency index (*CI*) = 0.530, rescaled consistency index (*RC*) = 0.462) generated from an analysis of thirty-one morphological characters. Bremer support values are given on the branches.

What is a phylogeny, why is it important… and how do you build one?

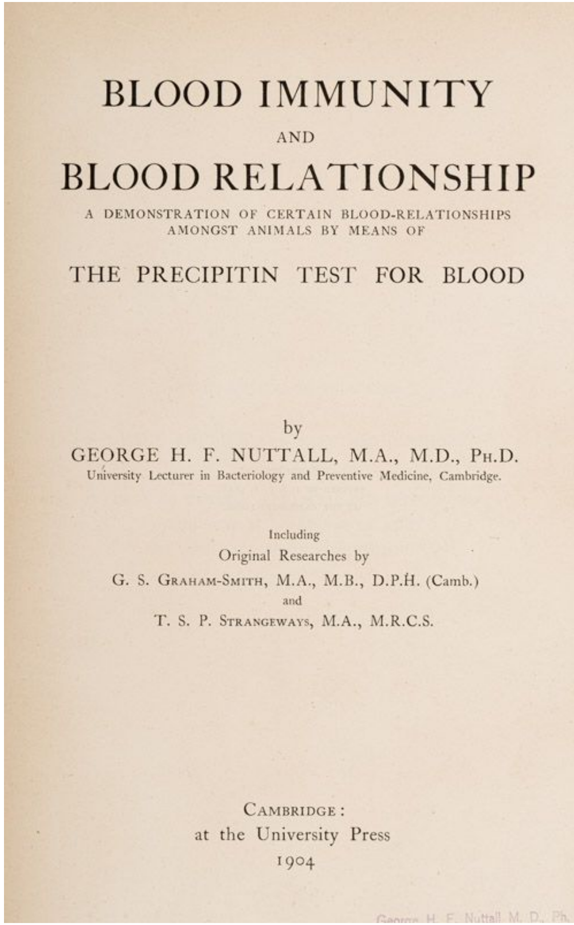


Cloutier et al. 2020



http://www.nature.com/nrg/journal/v7/n11/images/nrg1918-f2.jpg

The origin of molecular phylogenetics



BLOOD IMMUNITY

AND

BLOOD RELATIONSHIP

A DEMONSTRATION OF CERTAIN BLOOD-RELATIONSHIPS AMONGST ANIMALS BY MEANS OF

THE PRECIPITIN TEST FOR BLOOD

by

GEORGE H. F. NUTTALL, M.A., M.D., Ph.D.

University Lecturer in Bacteriology and Preventive Medicine, Cambridge.

Including

Original Researches by

G. S. Graham-Smith, M.A., M.B., D.P.H. (Camb.)

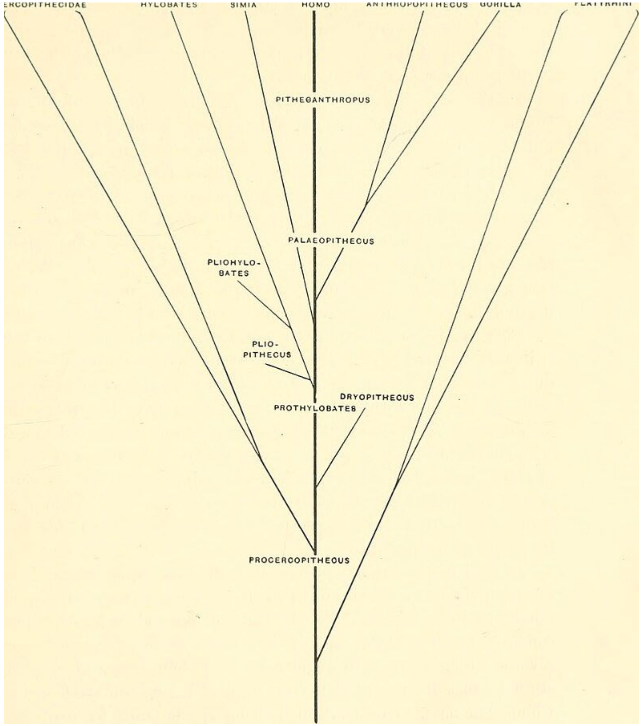
and

T. S. P. Strangeways, M.A., M.R.C.S.

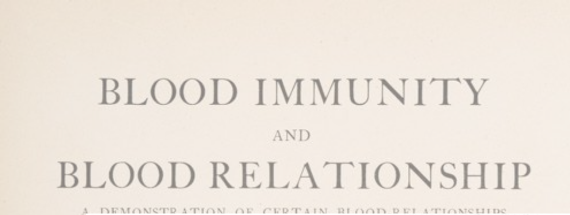
Cambridge:
at the University Press
1904

George H. F. Nuttall M. D., Ph.

Nuttal (1904) - serological cross-reactions were stronger for more closely related organisms -> phylogeny of apes



The origin of molecular phylogenetics



BLOOD IMMUNITY

AND

BLOOD RELATIONSHIP

A DEMONSTRATION OF CERTAIN BLOOD RELATIONSHIPS

Nuttal (1904) - serological cross-reactions were stronger for more closely related organisms -> phylogeny of apes

Dobzhansky & Sturtevant (1938) - genomic rearrangements in *Drosophila* as phylogenetic markers

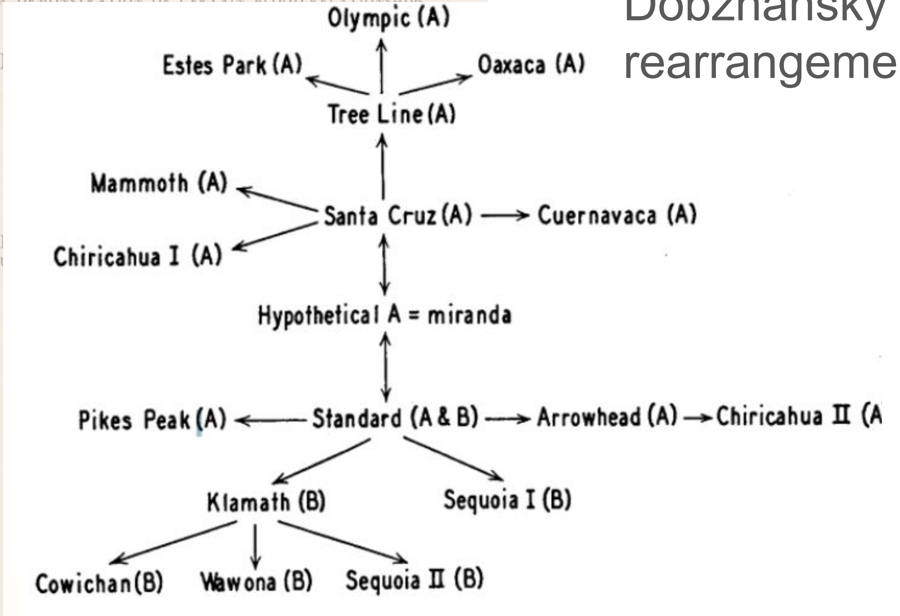
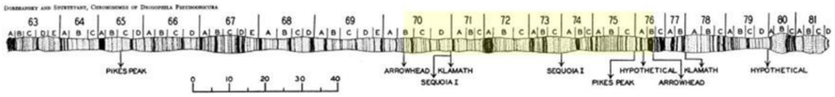
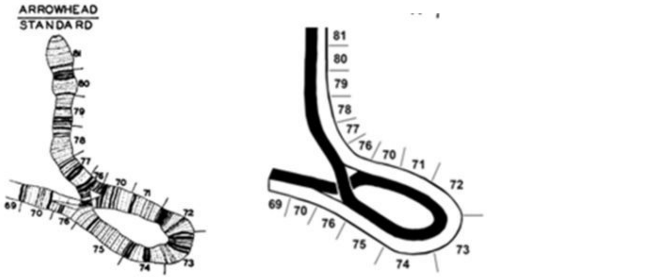


FIGURE 3.—Phylogeny of the gene arrangements in the third chromosome of *Drosophila pse doobscura*. Any two arrangements connected by an arrow in the diagram differ by a single i version. Further explanation in text.

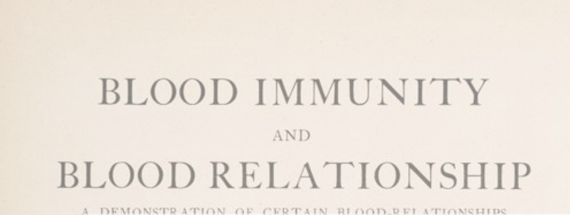


Chromosome 3 of *Drosophila pseudoobscura*



Standard and Arrowhead arrangements differ by an inversion from segments 70 to 76

The origin of molecular phylogenetics



BLOOD IMMUNITY

AND

BLOOD RELATIONSHIP

A DEMONSTRATION OF CERTAIN BLOOD-RELATIONSHIPS
AMONGST ANIMALS BY MEANS O

THE PRECIPIT OR BLOOD

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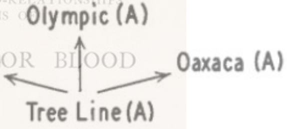
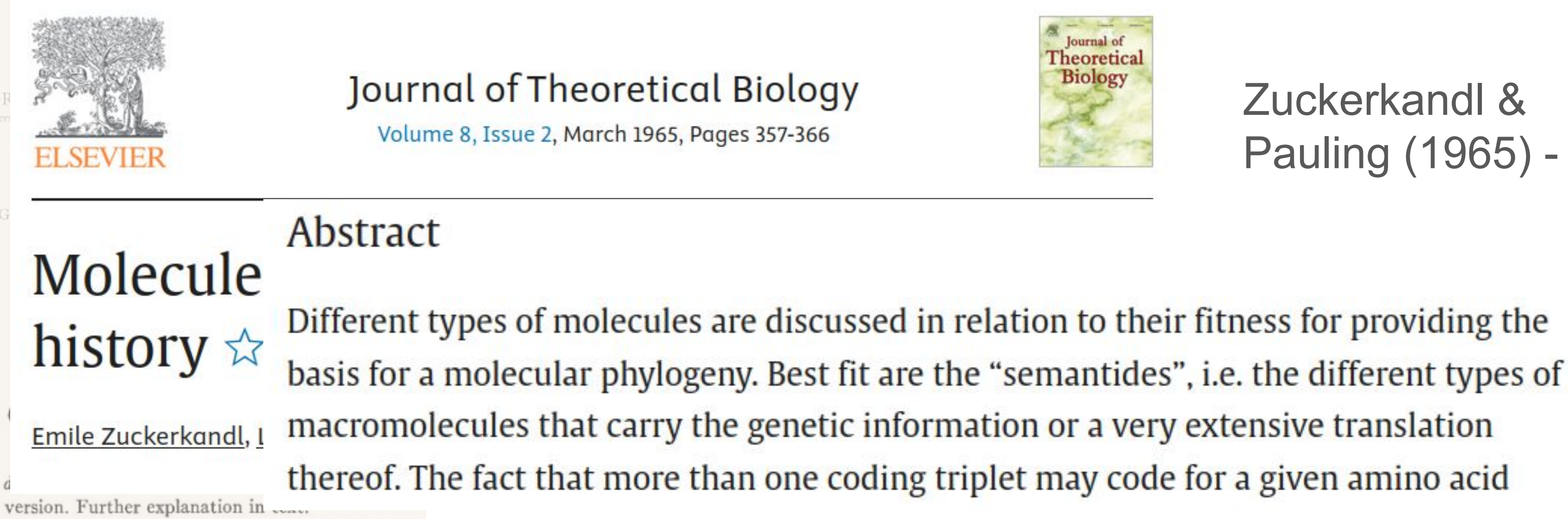
version. Further explanation in text.

Nuttal (1904) - serological cross-reactions were stronger for more closely related organisms -> phylogeny of apes



Dobzhansky & Sturtevant (1938) - genomic rearrangements in Drosophila as phylogenetic markers



Journal of Theoretical Biology
Volume 8, Issue 2, March 1965, Pages 357-366

Molecule history ☆

Emile Zuckerkandl, l

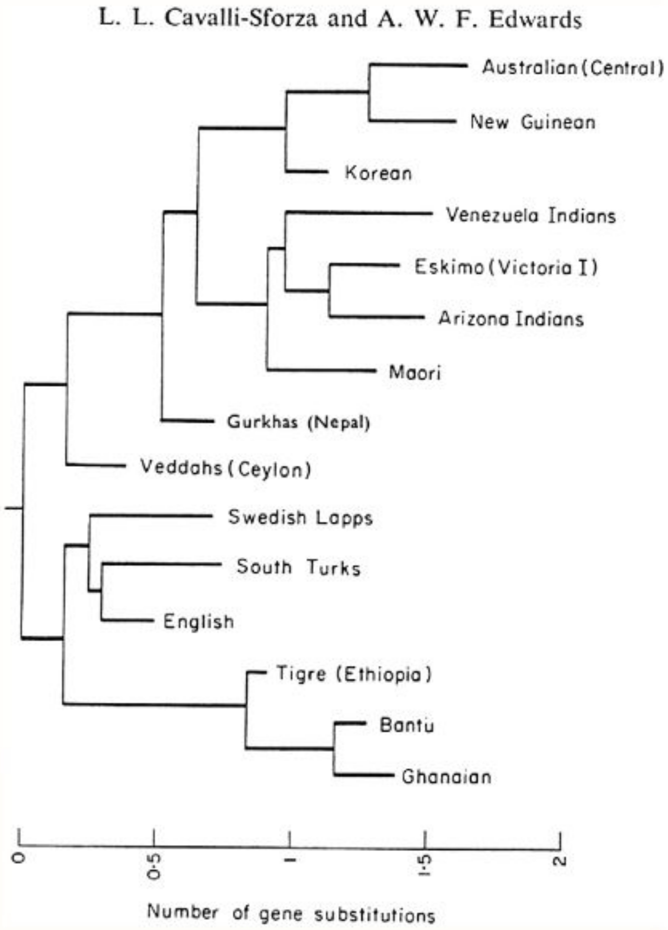
Abstract

Different types of molecules are discussed in relation to their fitness for providing the basis for a molecular phylogeny. Best fit are the “semantides”, i.e. the different types of macromolecules that carry the genetic information or a very extensive translation thereof. The fact that more than one coding triplet may code for a given amino acid

Zuckerkandl & Pauling (1965) -

Molecular phylogenetics: the new wave

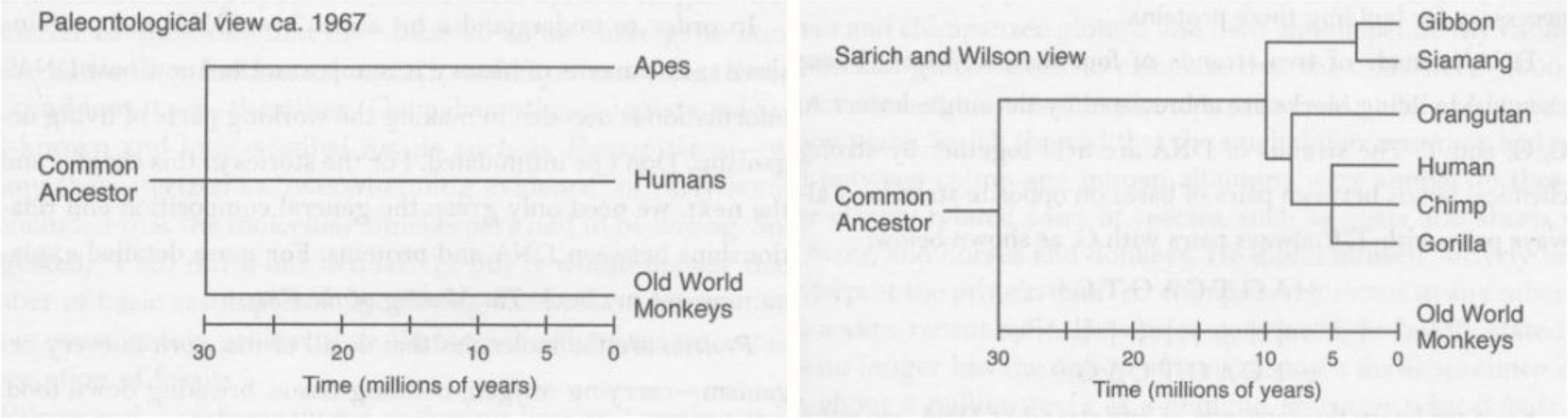
Phylogeny inferred from blood group allele frequencies from 15 populations



Cavalli-Sforza & Edwards (1965) in Genetics Today

Molecular phylogenetics: the new wave

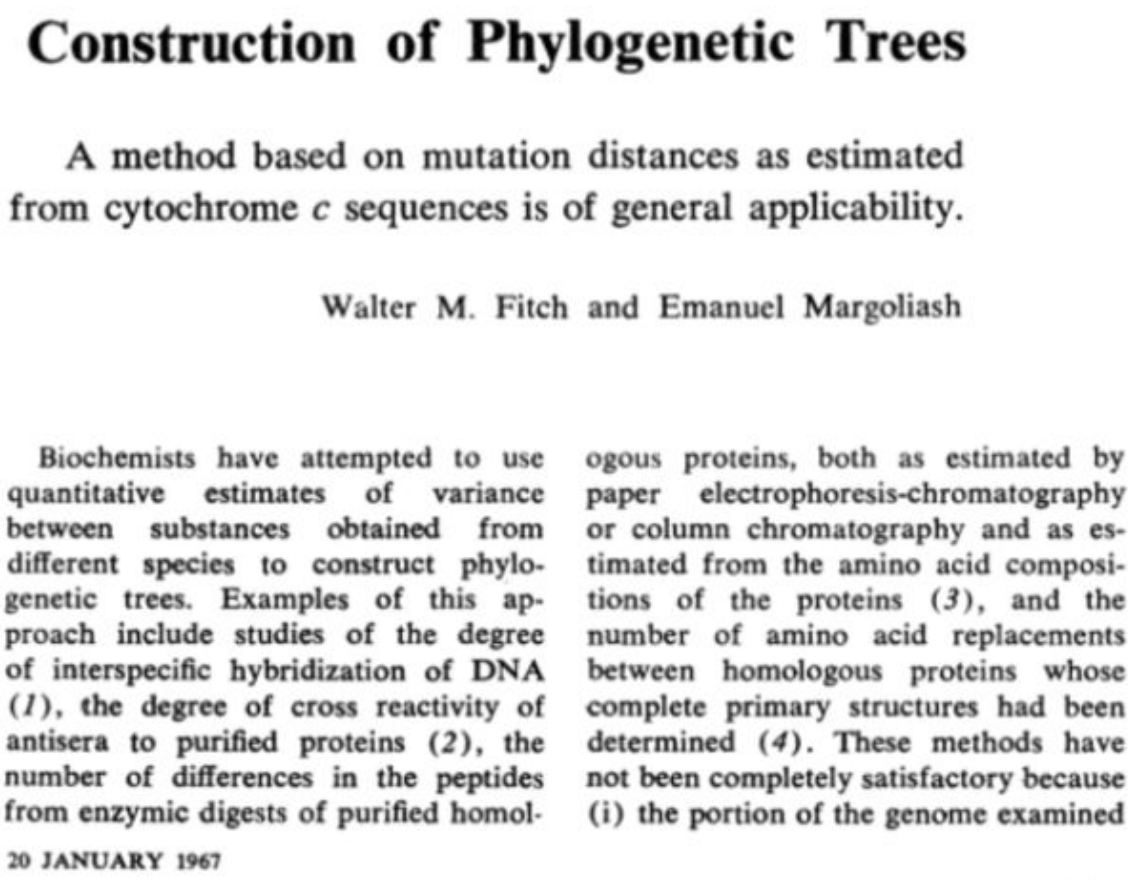
Divergence times were estimated by measuring the immunological cross-reaction of blood serum albumin between pairs of primates



“no fuss, no muss, no dishpan hands. Just throw some proteins into a laboratory apparatus, shake them up, and bingo! – we have an answer to questions that have puzzled us for three generations.”

Sarich & Wilson (1967) Science

Molecular phylogenetics: the new wave



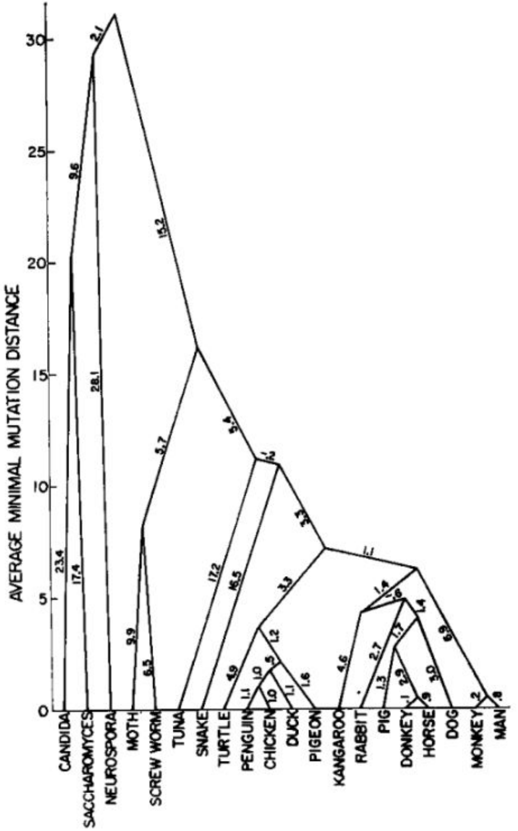
Construction of Phylogenetic Trees

A method based on mutation distances as estimated from cytochrome *c* sequences is of general applicability.

Walter M. Fitch and Emanuel Margoliash

Biochemists have attempted to use quantitative estimates of variance between substances obtained from different species to construct phylogenetic trees. Examples of this approach include studies of the degree of interspecific hybridization of DNA (*1*), the degree of cross reactivity of antisera to purified proteins (*2*), the number of differences in the peptides from enzymic digests of purified homologous proteins, both as estimated by paper electrophoresis-chromatography or column chromatography and as estimated from the amino acid compositions of the proteins (*3*), and the number of amino acid replacements between homologous proteins whose complete primary structures had been determined (*4*). These methods have not been completely satisfactory because (i) the portion of the genome examined

20 JANUARY 1967



Molecular phylogenetics: the new wave

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 11, pp. 5088–5090, November 1977
Evolution

Phylogenetic structure of the prokaryotic domain: The primary kingdoms

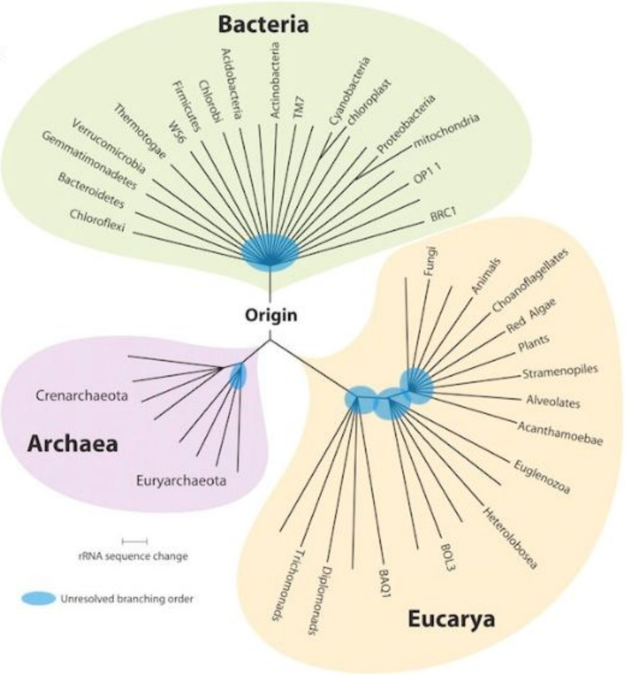
(archaebacteria/eubacteria/urkaryote/16S ribosomal RNA/molecular phylogeny)

CARL R. WOESE AND GEORGE E. FOX*

Department of Genetics and Development, University of Illinois, Urbana, Illinois

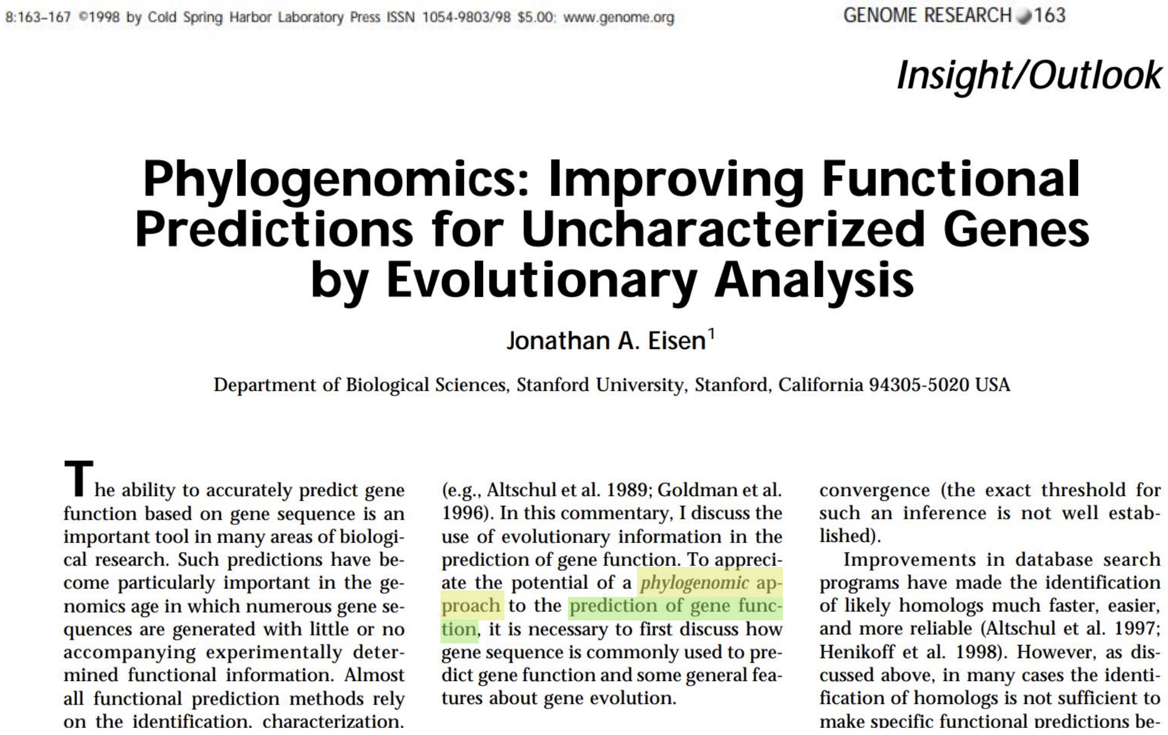
Communicated by T. M. Sonneborn, August 18, 1977

ABSTRACT A phylogenetic analysis based upon ribosomal RNA sequence characterization reveals that living systems represent one of three aboriginal lines of descent: (*i*) the eubacteria, comprising all typical bacteria; (*ii*) the archaebacteria, containing methanogenic bacteria; and (*iii*) the urkaryotes, now represented in the cytoplasmic component of eukaryotic cells.



The dawn of phylogenomics

The dawn of phylogenomics



8:163–167 ©1998 by Cold Spring Harbor Laboratory Press ISSN 1054-9803/98 $5.00; www.genome.org

GENOME RESEARCH 163

Insight/Outlook

Phylogenomics: Improving Functional Predictions for Uncharacterized Genes by Evolutionary Analysis

Jonathan A. Eisen[1]

Department of Biological Sciences, Stanford University, Stanford, California 94305-5020 USA

The ability to accurately predict gene function based on gene sequence is an important tool in many areas of biological research. Such predictions have become particularly important in the genomics age in which numerous gene sequences are generated with little or no accompanying experimentally determined functional information. Almost all functional prediction methods rely on the identification, characterization, (e.g., Altschul et al. 1989; Goldman et al. 1996). In this commentary, I discuss the use of evolutionary information in the prediction of gene function. To appreciate the potential of a *phylogenomic* approach to the prediction of gene function, it is necessary to first discuss how gene sequence is commonly used to predict gene function and some general features about gene evolution.

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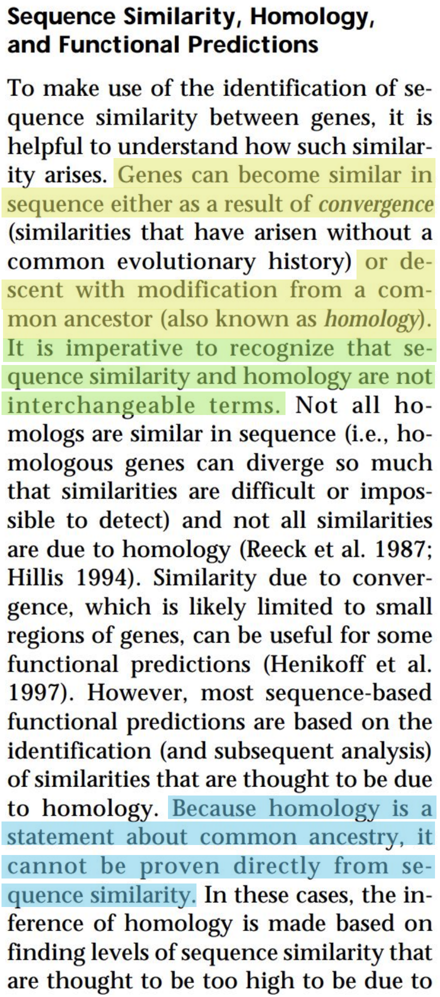
Improvements in database search programs have made the identification of likely homologs much faster, easier, and more reliable (Altschul et al. 1997; Henikoff et al. 1998). However, as discussed above, in many cases the identification of homologs is not sufficient to make specific functional predictions be-

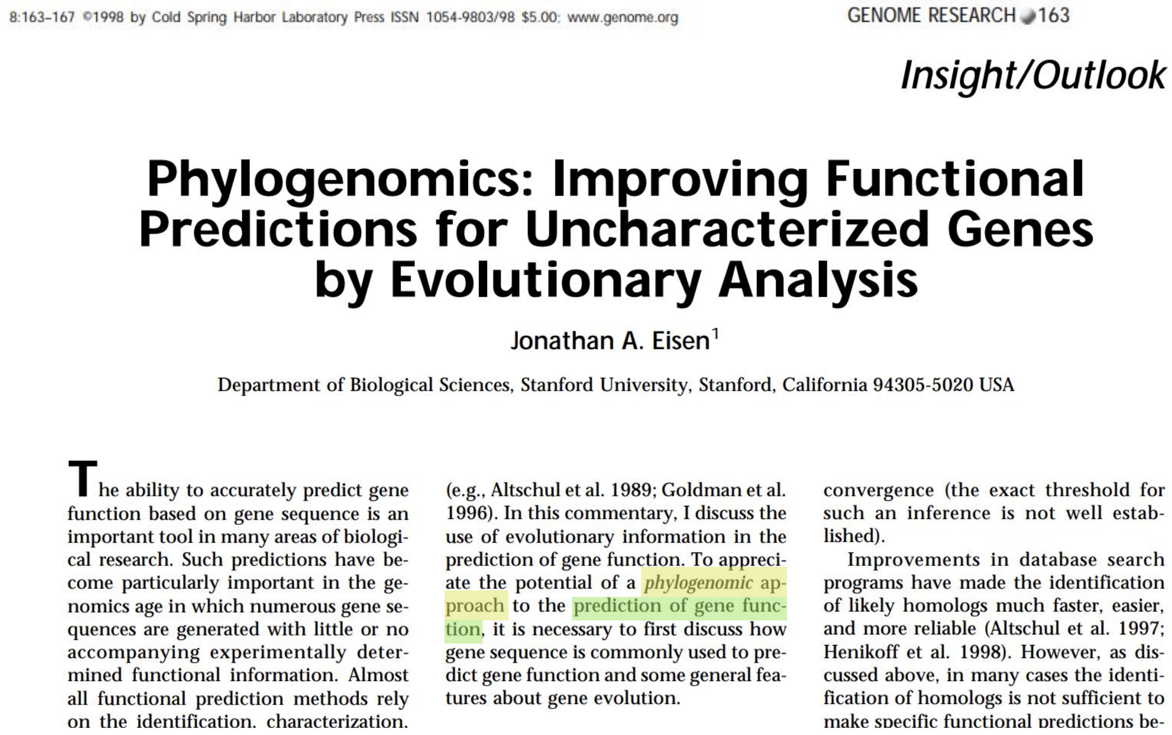
Phylogenomics: prediction of gene function and gene family evolution



Sequence Similarity, Homology, and Functional Predictions

To make use of the identification of sequence similarity between genes, it is helpful to understand how such similarity arises. Genes can become similar in sequence either as a result of *convergence* (similarities that have arisen without a common evolutionary history) or descent with modification from a common ancestor (also known as *homology*). It is imperative to recognize that sequence similarity and homology are not interchangeable terms. Not all homologs are similar in sequence (i.e., homologous genes can diverge so much that similarities are difficult or impossible to detect) and not all similarities are due to homology (Reeck et al. 1987; Hillis 1994). Similarity due to convergence, which is likely limited to small regions of genes, can be useful for some functional predictions (Henikoff et al. 1997). However, most sequence-based functional predictions are based on the identification (and subsequent analysis) of similarities that are thought to be due to homology. Because homology is a statement about common ancestry, it cannot be proven directly from sequence similarity. In these cases, the inference of homology is made based on finding levels of sequence similarity that are thought to be too high to be due to

The dawn of phylogenomics



8:163–167 ©1998 by Cold Spring Harbor Laboratory Press ISSN 1054-9803/98 $5.00; www.genome.org

Insight/Outlook

Phylogenomics: Improving Functional Predictions for Uncharacterized Genes by Evolutionary Analysis

Jonathan A. Eisen[1]

Department of Biological Sciences, Stanford University, Stanford, California 94305-5020 USA

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Phylogenomics: prediction of gene function and gene family evolution

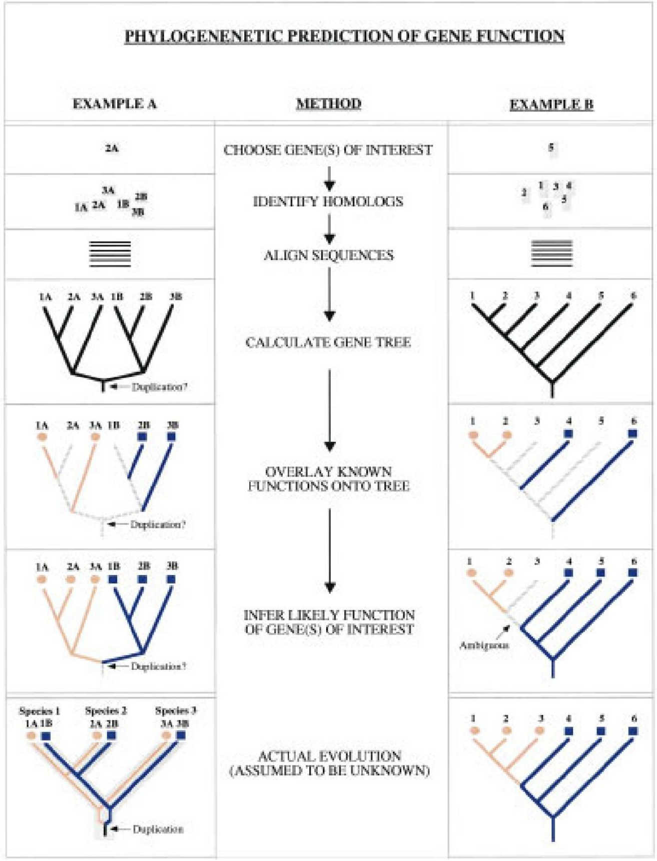
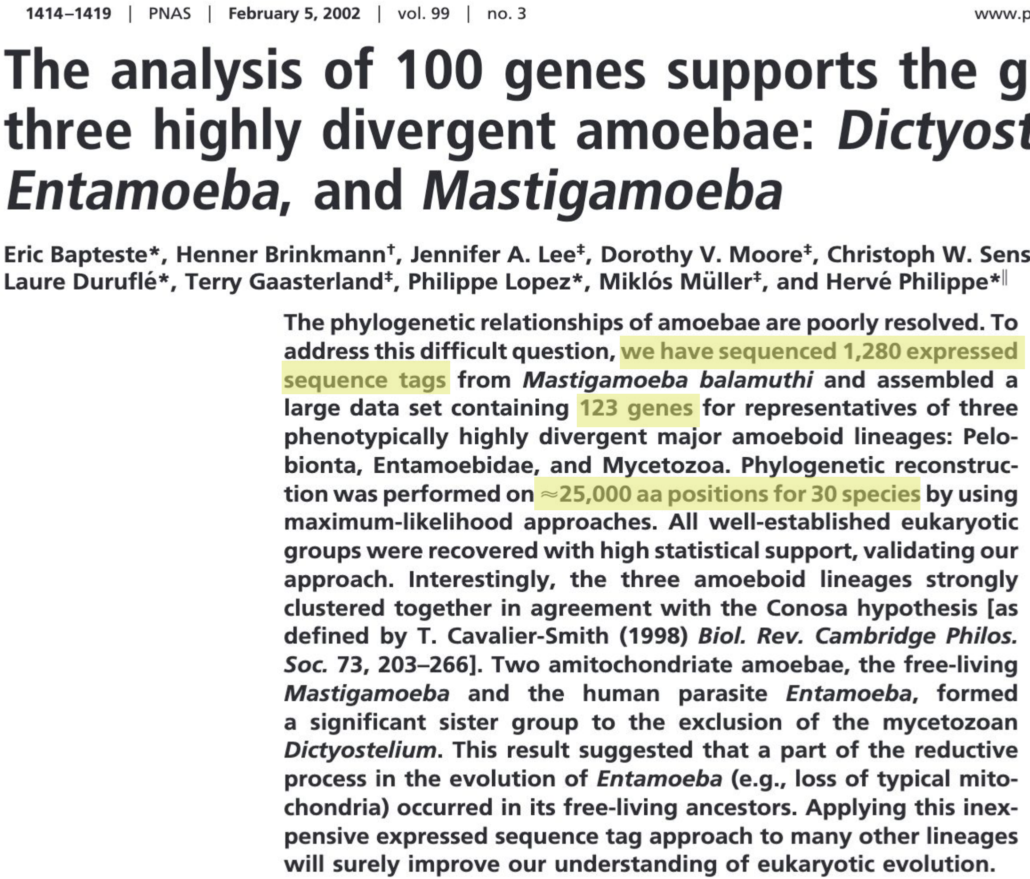

PHYLOGENENETIC PREDICTION OF GENE FUNCTION
EXAMPLE A
METHOD
EXAMPLE B
CHOOSE GENE(S) OF INTEREST
IDENTIFY HOMOLOGS
ALIGN SEQUENCES
CALCULATE GENE TREE
OVERLAY KNOWN FUNCTIONS ONTO TREE
INFER LIKELY FUNCTION OF GENE(S) OF INTEREST
ACTUAL EVOLUTION (ASSUMED TO BE UNKNOWN)
2A
1A 2A 3A 1B 2B 3B
Duplication?
Duplication
Species 1
Species 2
Species 3
1 2 3 4 5 6
Ambiguous

Figure 1 Outline of a phylogenomic methodology. In this method, information about the evolutionary relationships among genes is used to predict the functions of uncharacterized genes (see text for details). Two hypothetical scenarios are presented and the path of trying to infer the function of two uncharacterized genes in each case is traced. (A) A gene family has

The dawn of phylogenomics



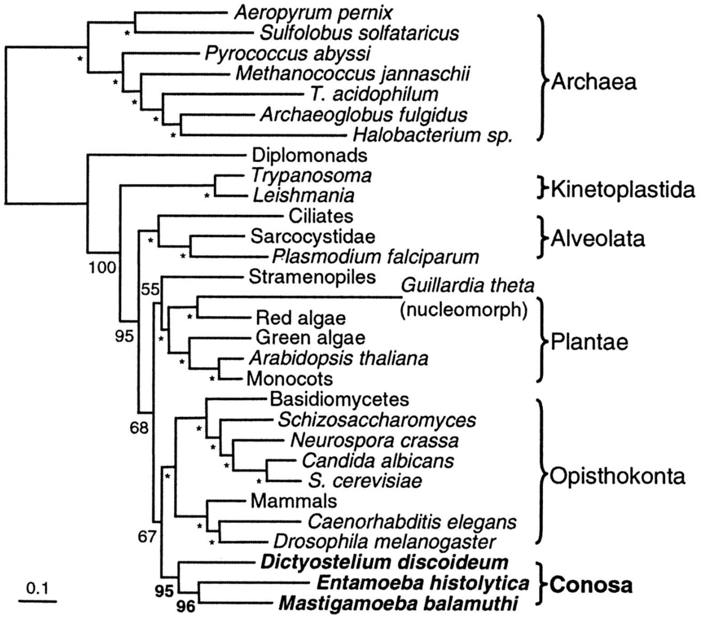
1414–1419 | PNAS | February 5, 2002 | vol. 99 | no. 3 www.pnas.org/cgi/doi/10.1073/pnas.032662799

The analysis of 100 genes supports the grouping of three highly divergent amoebae: *Dictyostelium*, *Entamoeba*, and *Mastigamoeba*

Eric Bapteste[*], Henner Brinkmann[†], Jennifer A. Lee[‡], Dorothy V. Moore[‡], Christoph W. Sensen[§], Paul Gordon[¶], Laure Duruflé[*], Terry Gaasterland[‡], Philippe Lopez[*], Miklós Müller[‡], and Hervé Philippe[*‖]

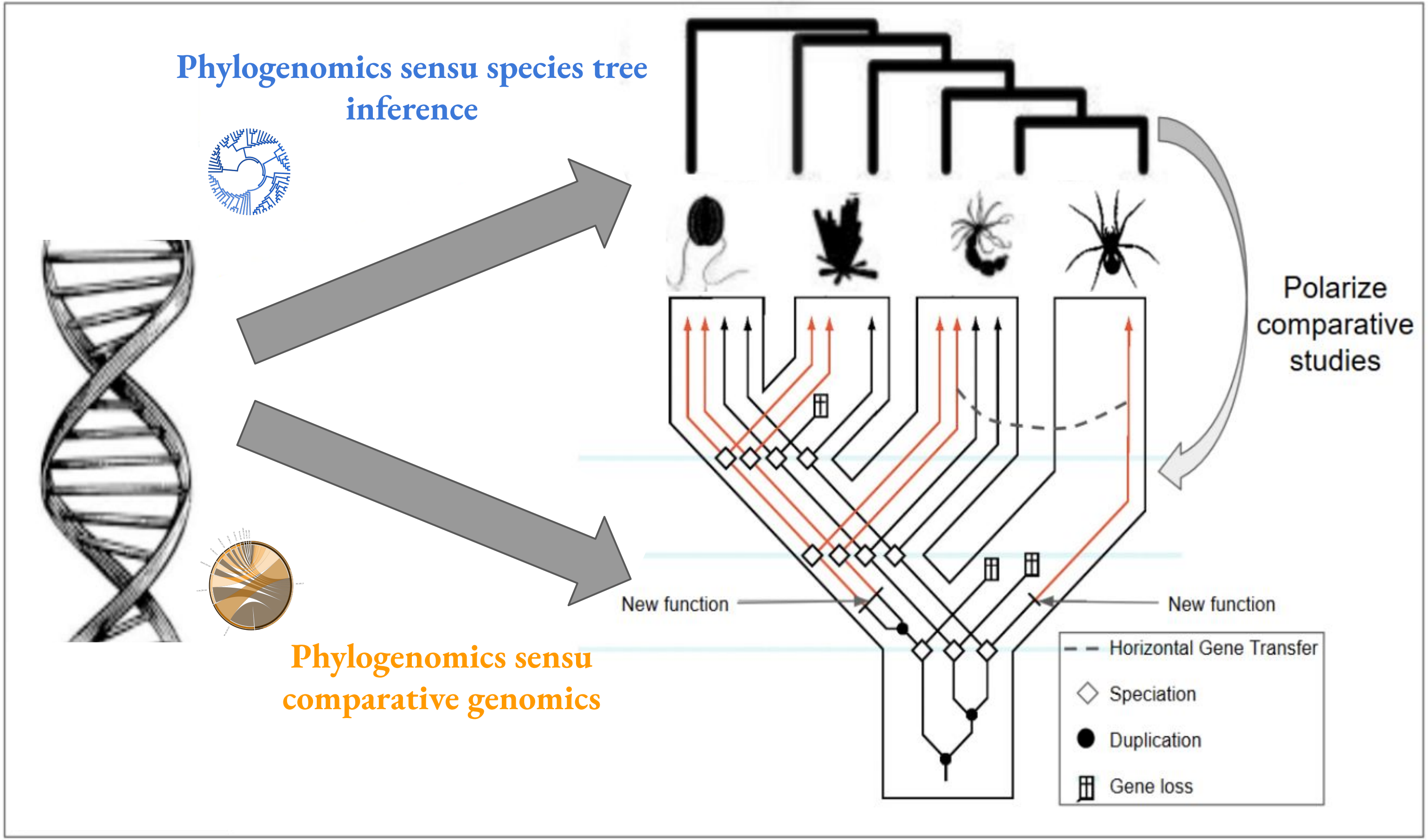
The phylogenetic relationships of amoebae are poorly resolved. To address this difficult question, we have sequenced 1,280 expressed sequence tags from *Mastigamoeba balamuthi* and assembled a large data set containing 123 genes for representatives of three phenotypically highly divergent major amoeboid lineages: Pelobionta, Entamoebidae, and Mycetozoa. Phylogenetic reconstruction was performed on ≈25,000 aa positions for 30 species by using maximum-likelihood approaches. All well-established eukaryotic groups were recovered with high statistical support, validating our approach. Interestingly, the three amoeboid lineages strongly clustered together in agreement with the Conosa hypothesis [as defined by T. Cavalier-Smith (1998) *Biol. Rev. Cambridge Philos. Soc.* 73, 203–266]. Two amitochondriate amoebae, the free-living *Mastigamoeba* and the human parasite *Entamoeba*, formed a significant sister group to the exclusion of the mycetozoan *Dictyostelium*. This result suggested that a part of the reductive process in the evolution of *Entamoeba* (e.g., loss of typical mitochondria) occurred in its free-living ancestors. Applying this inexpensive expressed sequence tag approach to many other lineages will surely improve our understanding of eukaryotic evolution.

Phylogenomics: species tree inference



*ML tree based on 25,032 aa positions. * indicates a constrained node. We used the JTT model, without taking into account among-sites rate variation. The branch lengths have been computed on the concatenated sequences. BVs were obtained by bootstrapping the 123 genes.*

The dawn of phylogenomics

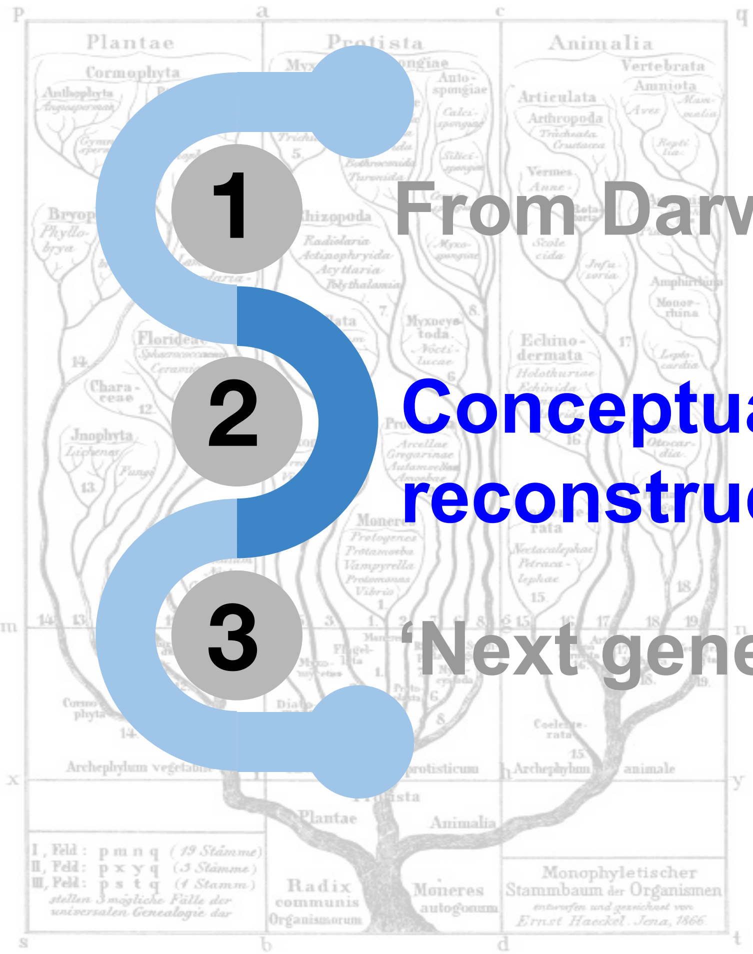


Phylogenomics sensu species tree inference

Phylogenomics sensu comparative genomics

Polarize comparative studies

Content of the lecture

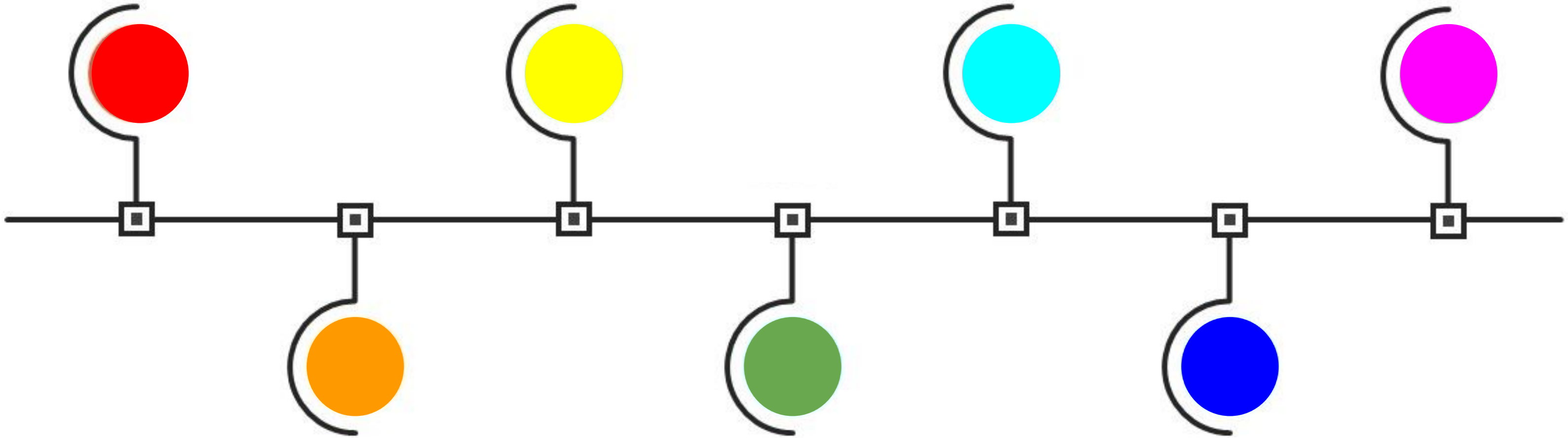


1 From Darwin to phylogenomics

2 **Conceptual framework for phylogenomic reconstruction**

3 ‘Next generation’ phylogenomics

Tutorials and hands-on sessions available at
https://evomics.org/2024-workshop-on-phylogenomics-cesky-krumlov/



01 DATA

02 ORTHOLOGY INFERENCE

03 ALIGNMENT & TRIMMING

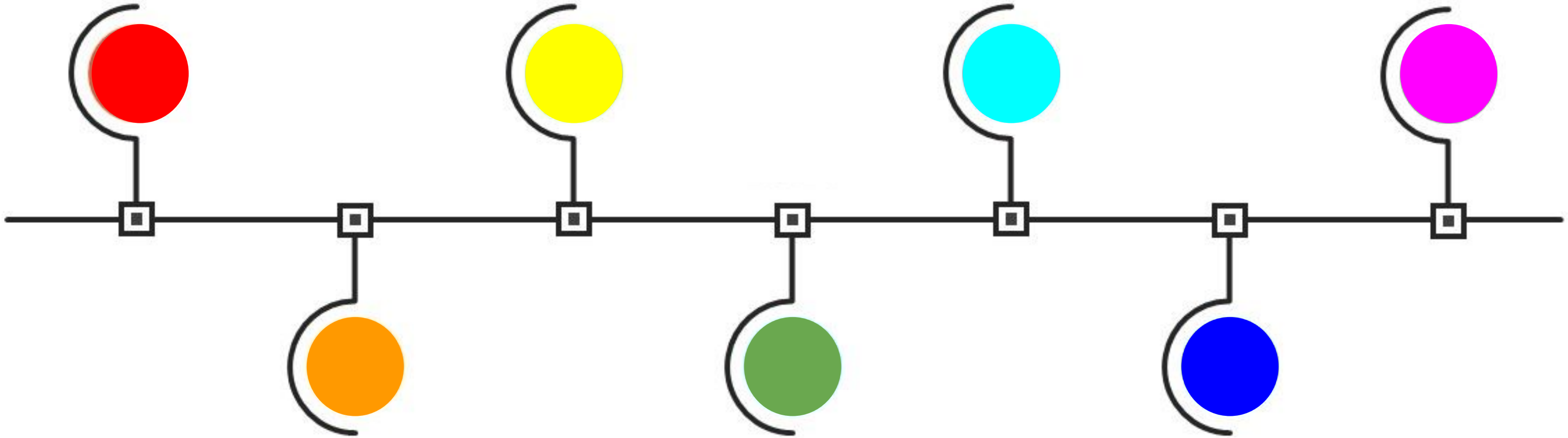
04 PHYLOGENOMIC SUBSAMPLING

05 SUPERMATRIX VS INDIVIDUAL GENES

06 MODEL SELECTION & PHYLOGENETIC INFERENCE

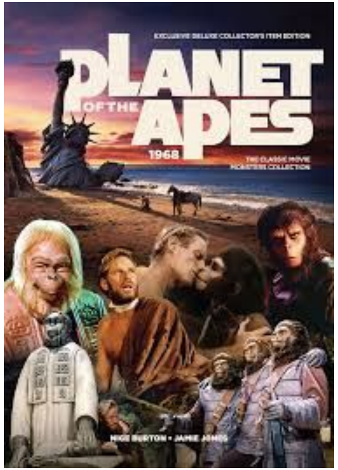
07 TESTING THE ROBUSTNESS OF YOUR TREE

01 DATA



01 DATA

Incomplete, biased, or improper **taxon sampling** can lead to misleading results in reconstructing evolutionary relationships.


EXCLUSIVE DELUXE COLLECTOR'S ITEM EDITION
PLANET OF THE APES
1968
THE CLASSIC MOVIE MONSTERS COLLECTION

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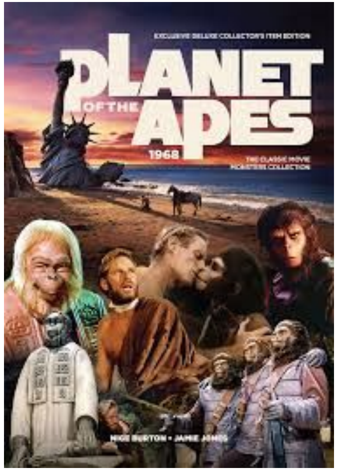
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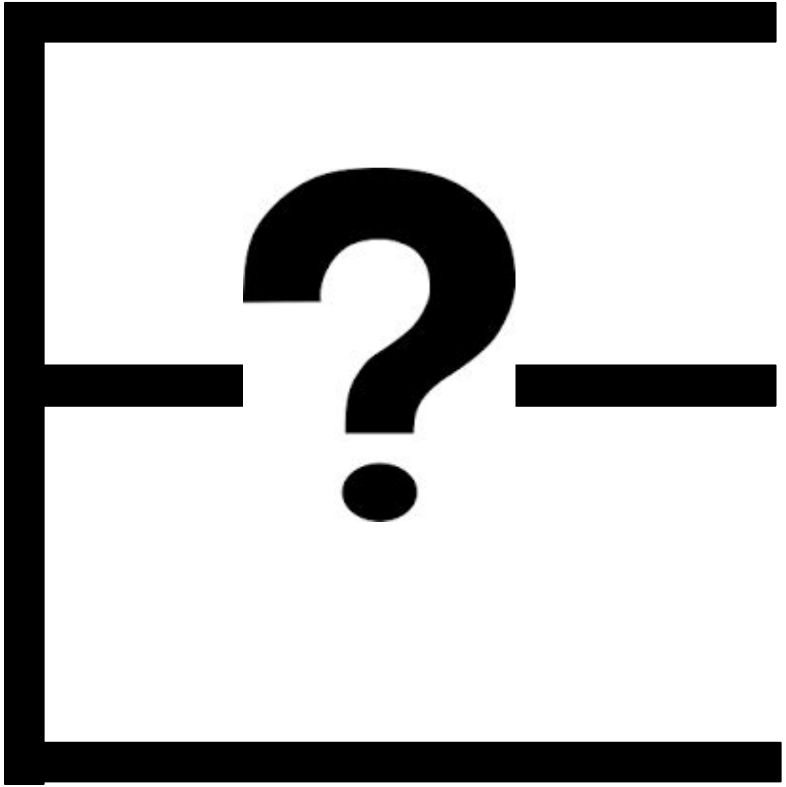
NIGE BURTON • JAMIE JONES



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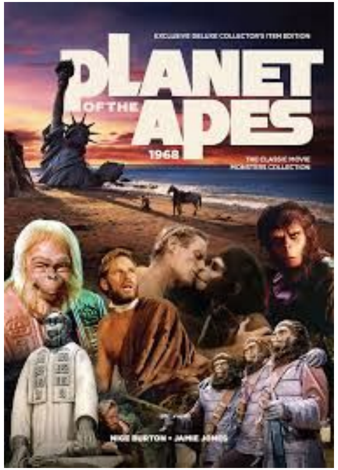

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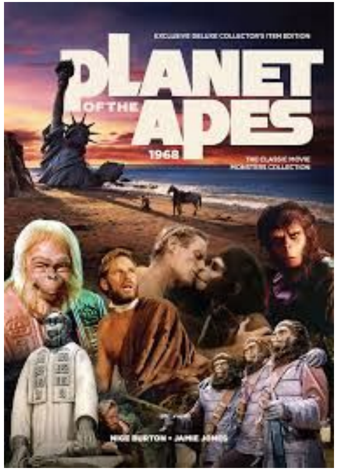
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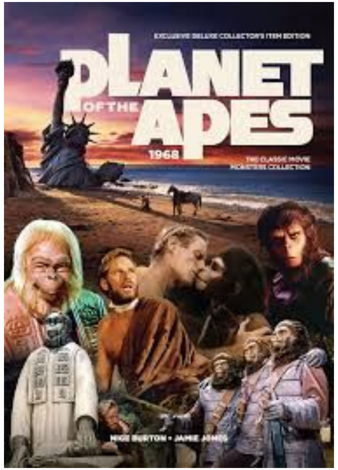

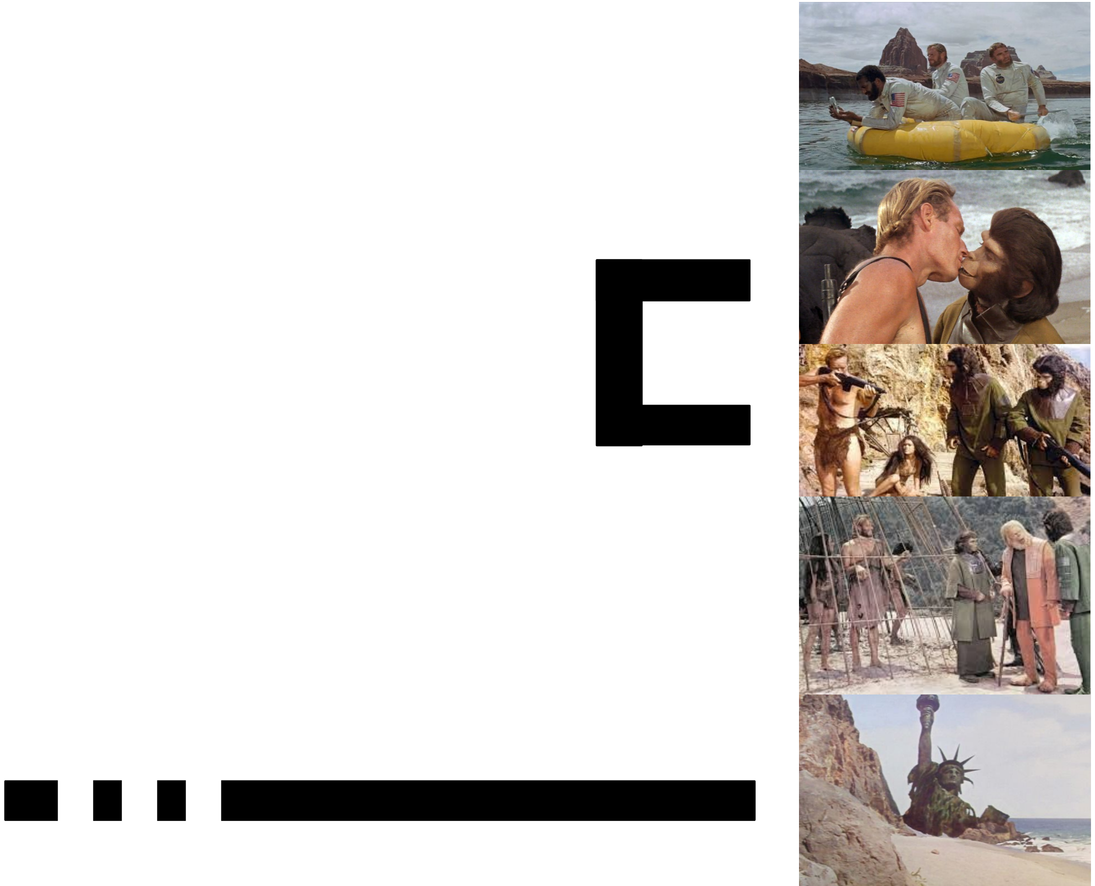

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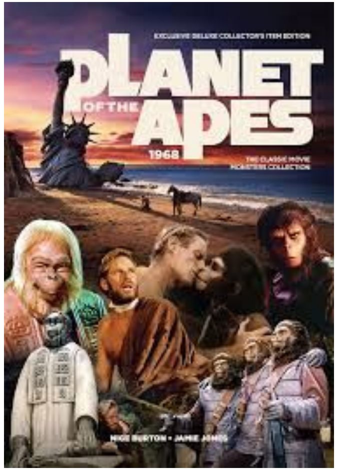
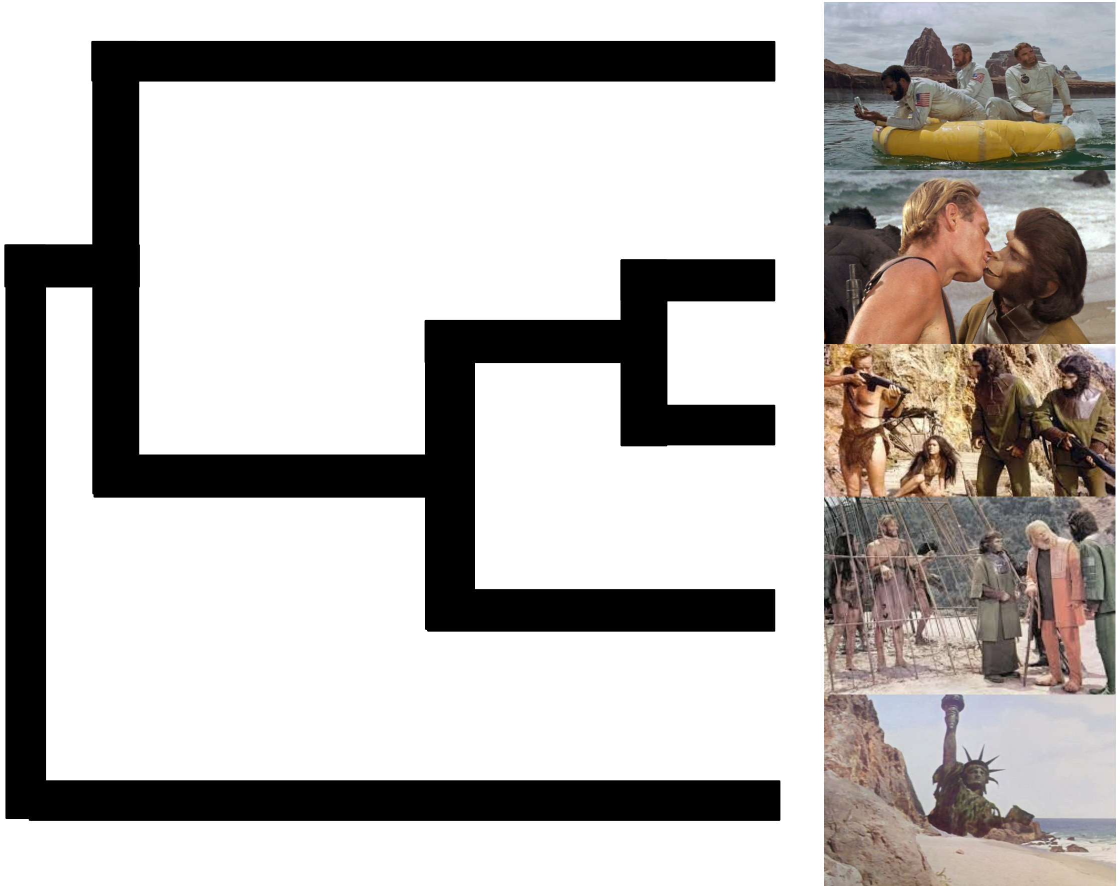


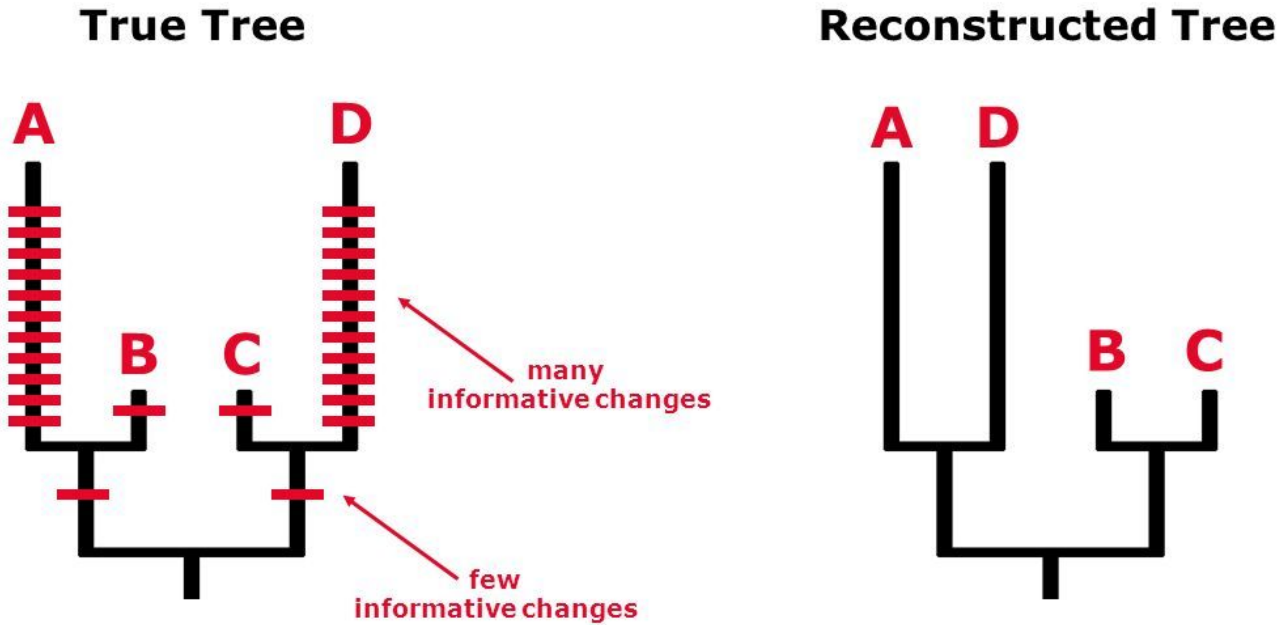


01 DATA

Incomplete, biased, or improper **taxon sampling** can lead to misleading results in reconstructing evolutionary relationships.

Long Branch Attraction

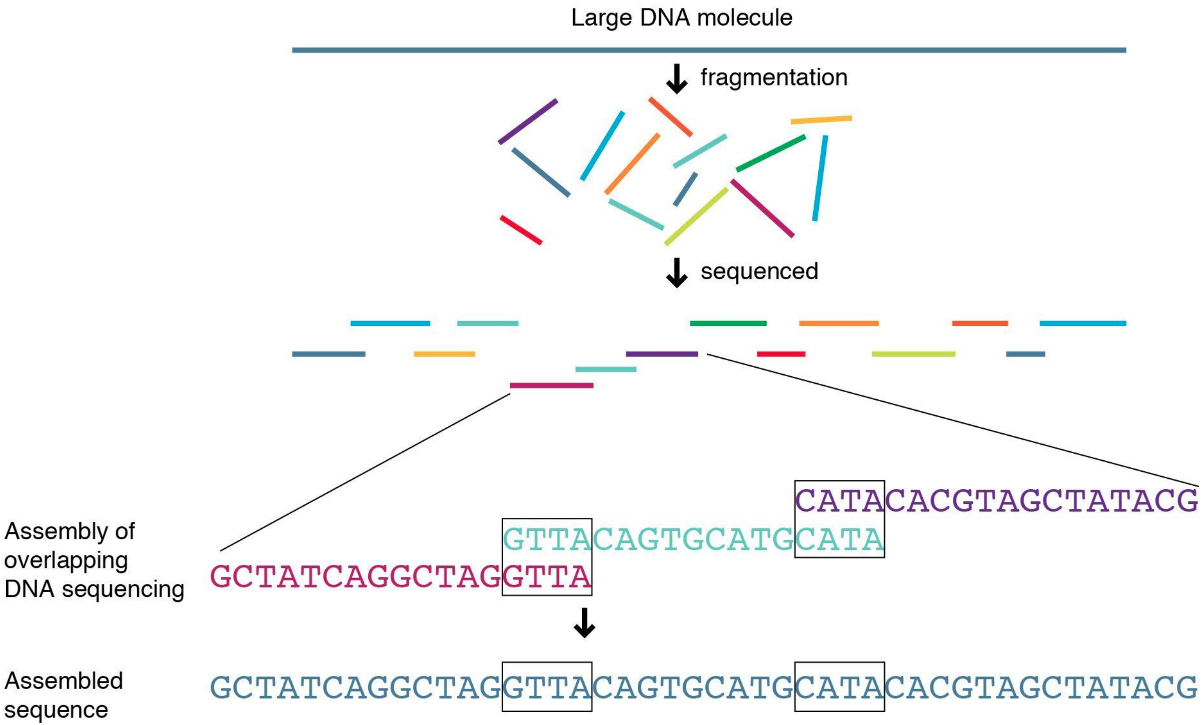
Outgroups / Fast-evolving lineages / Missing data



01 DATA

Source of your data

GENOMES



https://knowgenetics.org/whole-genome-sequencing/

- Assembled and annotated.
- Coding genes are retrieved (longest isoform) -> this is your dataset!

01 DATA

Source of your data

GENOMES

Pros:

- Very large set of genetic markers
- Good identification of full-length genes, less chimeras (if the assembly and annotation are of good quality)
- Good for shallow and deep evolutionary distances
- Ethanol-fixed tissue OK (for draft genomes)

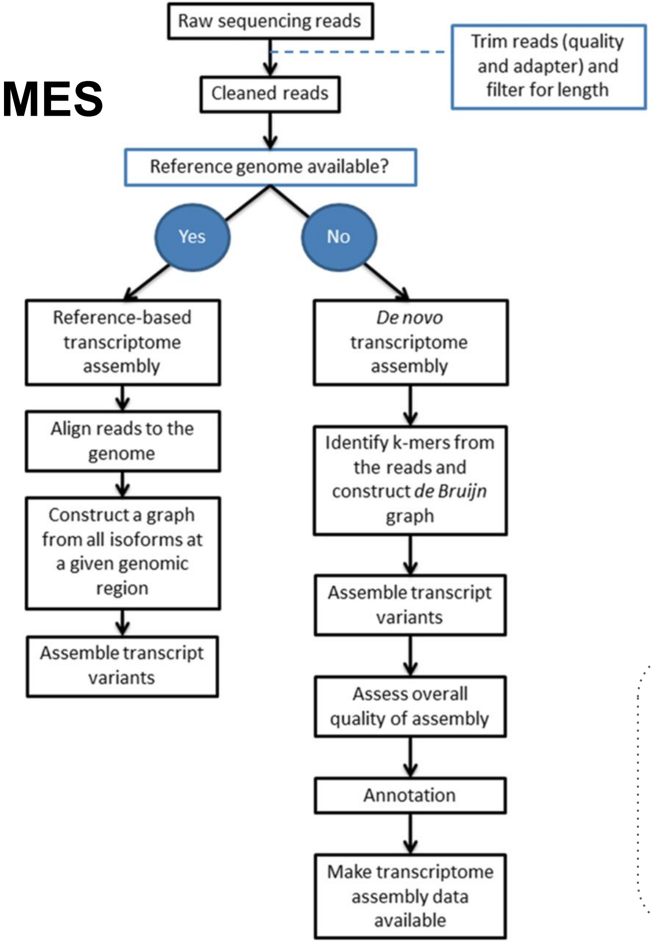
Cons:

- Annotation may vary quite a lot between species (source, software, etc), may not be comparable.
- Expensive (money and computing time)
- More difficult to have a high number of species
- Fresh tissue needed (for chromosome-level genomes)

01 DATA

Source of your data

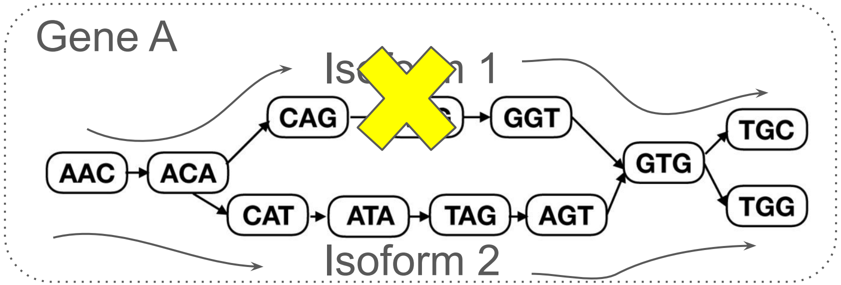
TRANSCRIPTOMES



Raw sequencing reads
Trim reads (quality and adapter) and filter for length
Cleaned reads
Reference genome available?
Yes
No
Reference-based transcriptome assembly
Align reads to the genome
Construct a graph from all isoforms at a given genomic region
Assemble transcript variants
De novo transcriptome assembly
Identify k-mers from the reads and construct *de Bruijn* graph
Assemble transcript variants
Assess overall quality of assembly
Annotation
Make transcriptome assembly data available

- Assembled de novo
- Coding genes are retrieved (after inferring ORFs; longest isoform) -> this is your dataset!

De Bruijn Graph



Moreton et al. 2016

01 DATA

Source of your data

TRANSCRIPTOMES

Pros:

- Very large set of genetic markers
- Much cheaper than sequencing genomes -> easier to have a high number of species
- Not dependent upon a reference genome
- Good for shallow and deep evolutionary distances

Cons:

- Incomplete identification of full-length genes and single-copy transcripts.
- Potential misassembly of transcripts (especially when duplicates are present)
- Missing data as a product of the transcriptome representing a snapshot of expression (but this could also affect genome annotation)
- Fresh tissue needed

01 DATA

Source of your data

ULTRACONSERVED ELEMENTS (UCEs)

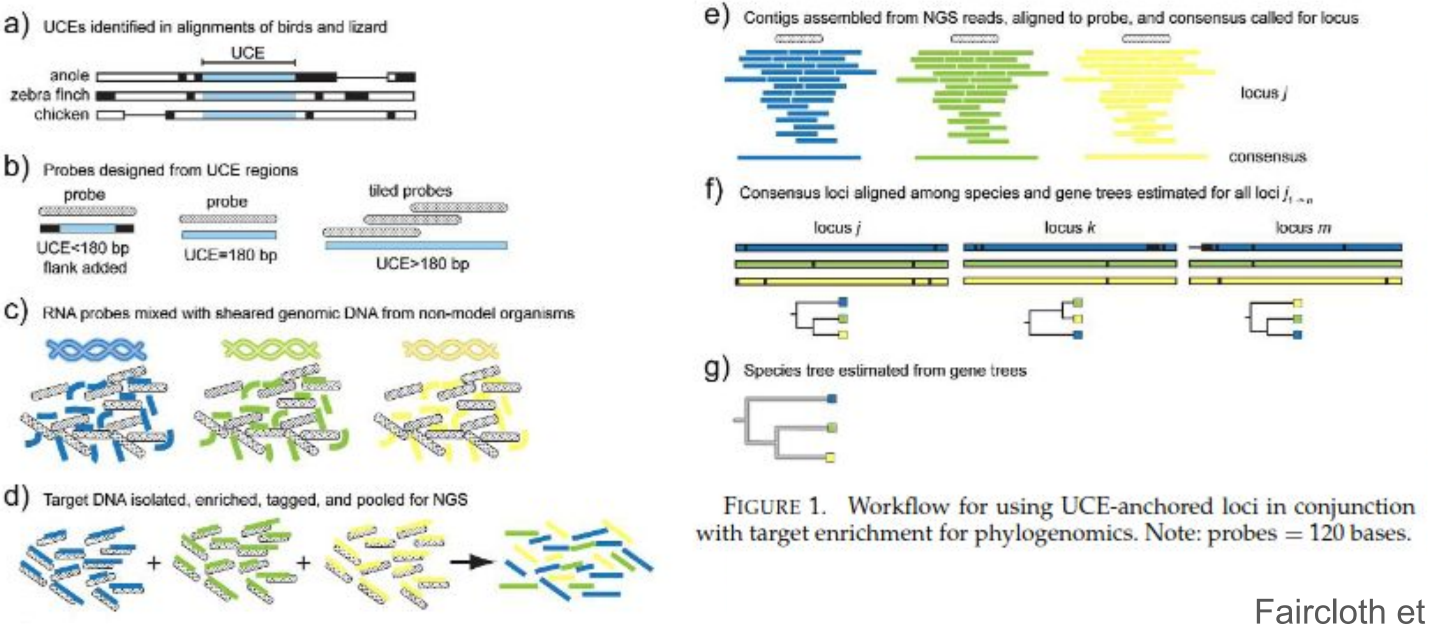

a) UCEs identified in alignments of birds and lizard
UCE
anole
zebra finch
chicken
b) Probes designed from UCE regions
probe
UCE<180 bp
flank added
probe
UCE=180 bp
tiled probes
UCE>180 bp
c) RNA probes mixed with sheared genomic DNA from non-model organisms
d) Target DNA isolated, enriched, tagged, and pooled for NGS
+
+
e) Contigs assembled from NGS reads, aligned to probe, and consensus called for locus
locus j
consensus
f) Consensus loci aligned among species and gene trees estimated for all loci $j_{1 \to n}$
locus j
locus k
locus m
g) Species tree estimated from gene trees

FIGURE 1. Workflow for using UCE-anchored loci in conjunction with target enrichment for phylogenomics. Note: probes = 120 bases.

Faircloth et al. 2012

The UCEs are designed a priori -> after hybridization, sequencing, assembly and mapping, this is your data!

01 DATA

Source of your data

ULTRACONSERVED ELEMENTS (UCEs)

Pros:

- Medium-large set of genetic markers
- Much cheaper than sequencing genomes -> easier to have a high number of species
- Not dependent upon a reference genome
- Tissues fixed in EtOH or museum specimens are OK

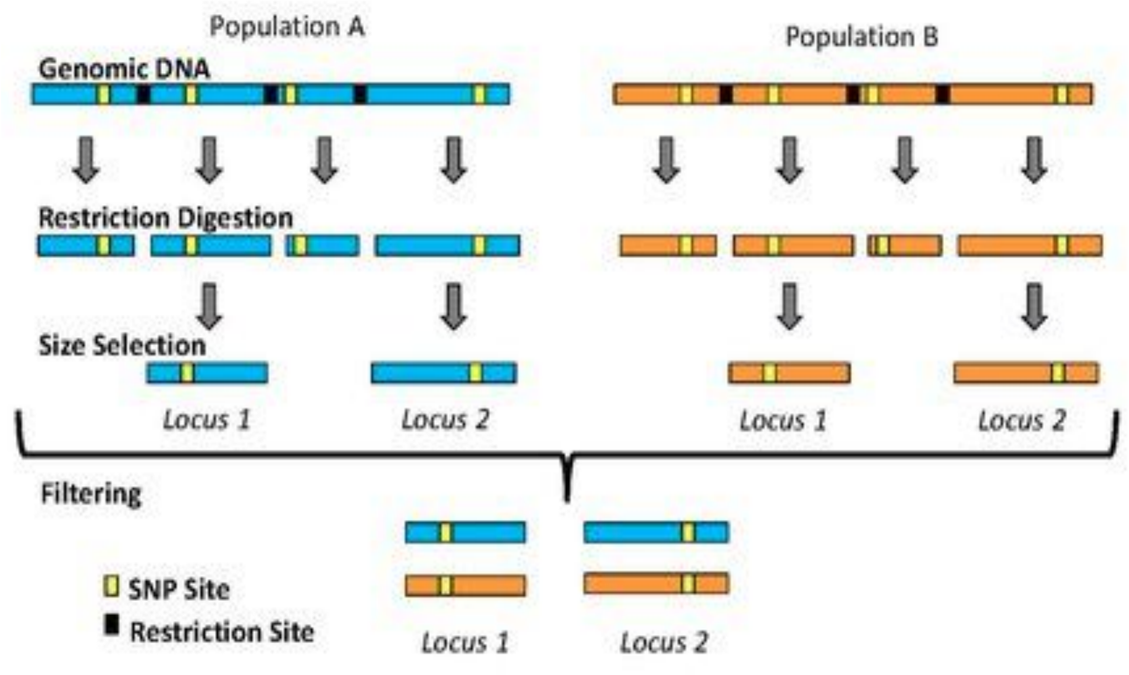
Cons:

- Limited availability of markes outside the designed ones.
- Potential misassembly (if probes are designed with a limited amount of species)
- Retrieval success dependent on DNA quality
- Usefulness of markers known a posteriori
- No proper orthology inference

01 DATA

Source of your data

REDUCED REPRESENTATION (RADseq, GBS)



After digestion, sequencing and mapping, this is your data!

01 DATA

Source of your data

REDUCED REPRESENTATION (RADseq, GBS)

Pros:

- The cheapest of the methods
- Not dependent upon a reference genome
- Samples fixed in ethanol OK
- Markers distributed evenly across the genome

Cons:

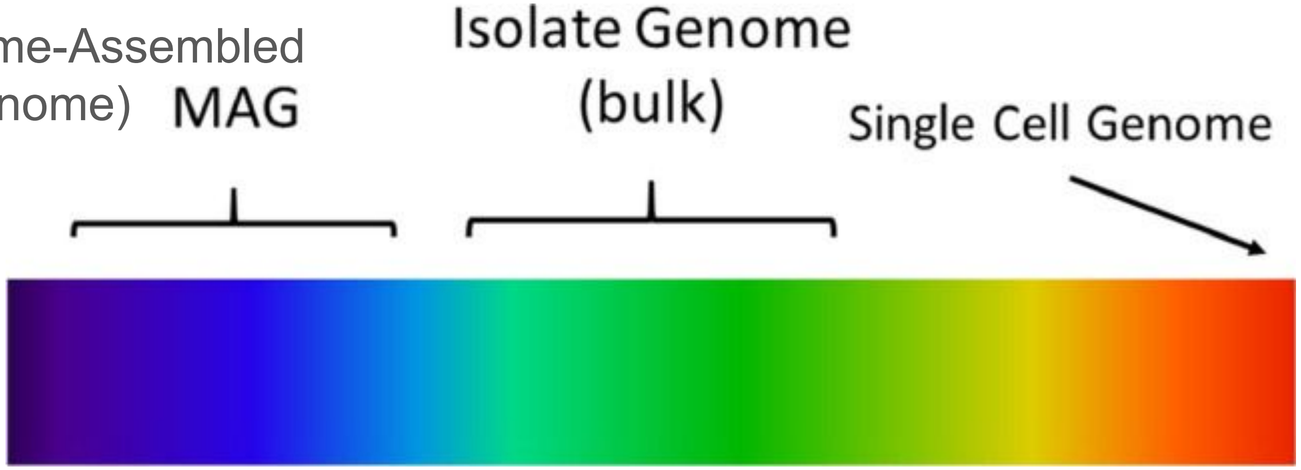
- No full genes, only SNPs
- Only for population genomics or phylogeny including closely-related species
- Missing data as a product of the transcriptome representing a snapshot of expression (but this could also affect genome annotation)
- No proper orthology inference

01 DATA

Source of your data

METAGENOMICS/METATRANSCRIPTOMICS

(Metagenome-Assembled Genome)



One cell, multiple organisms

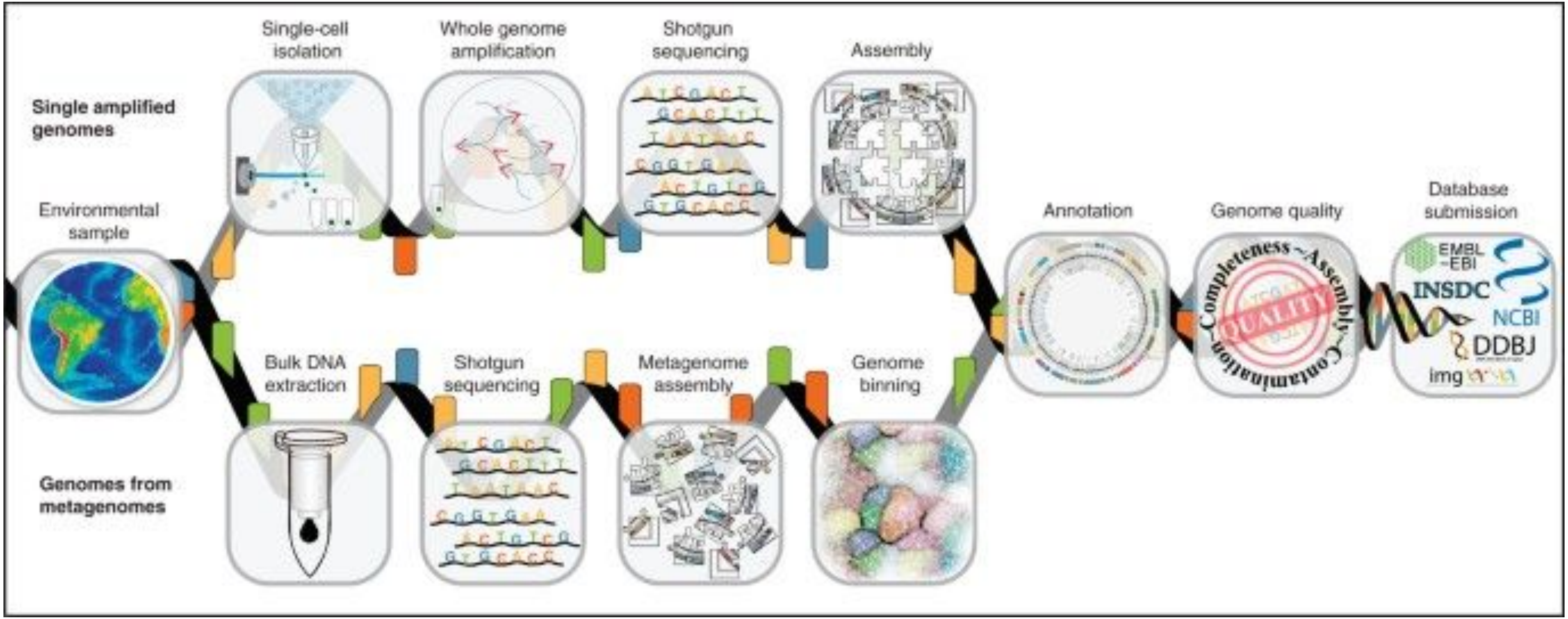
One individual, multiple cells

One cell, one organism

01 DATA

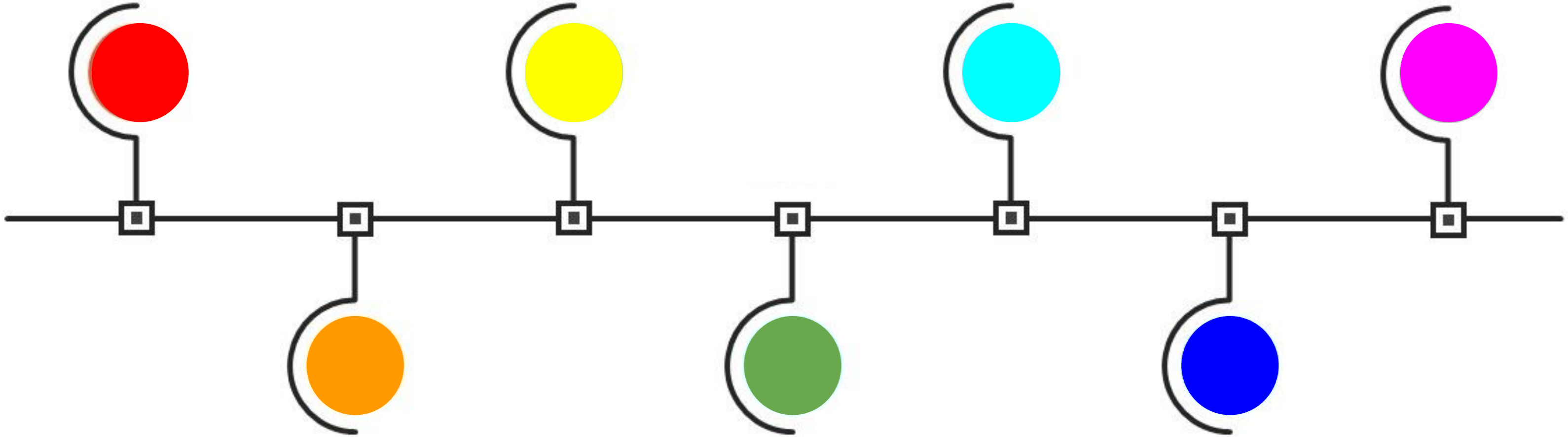
Source of your data

METAGENOMICS - single cell vs MAGs



Bowers et al. 2017

01 DATA



02 ORTHOLOGY INFERENCE

02 ORTHOLOGY INFERENCE

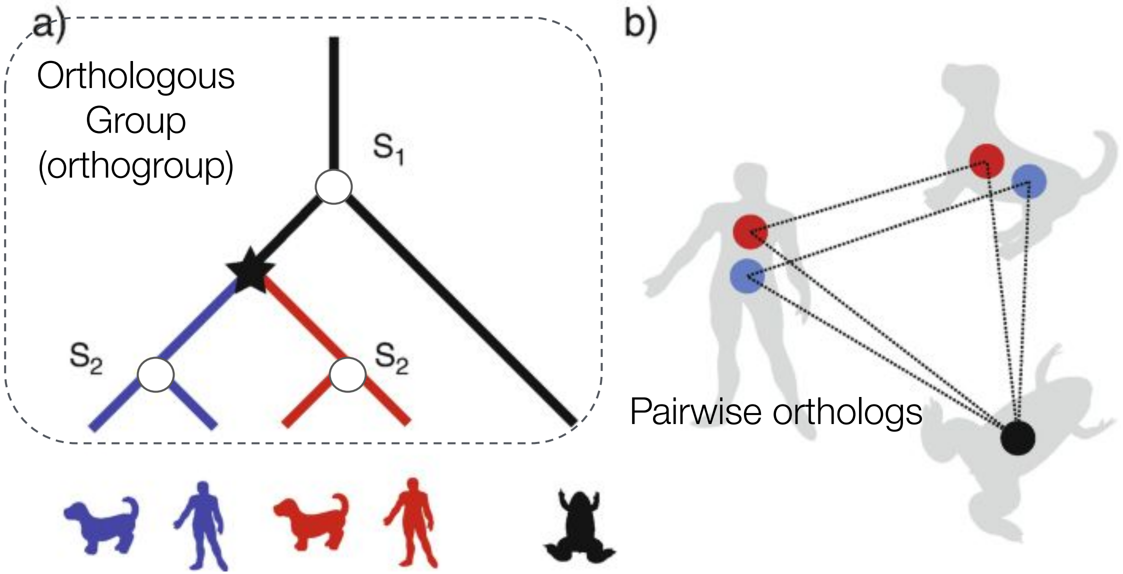
Definitions

- Two genes are **orthologs** if their MRCA is a ***speciation***: ○

- Two genes are **paralogs** if their MRCA is a ***duplication***: ☆

Orthology relationships are inferred *pairwise*

When we have multiple species, we should consider the concept of *orthogroup*



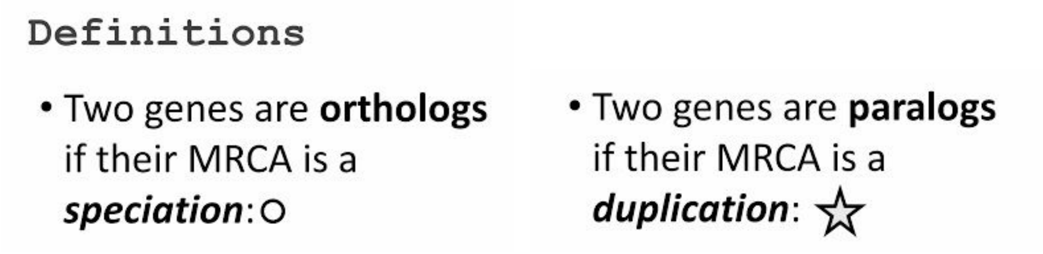
Orthology inference is essential for phylogenomics, as you want to consider only genes that arouse through speciation events

Altenhoff, Glover & Dessimoz 2019

02 ORTHOLOGY INFERENCE

Software:

- OrthoFinder
- OMA
- TOGA (synteny; vertebrates)



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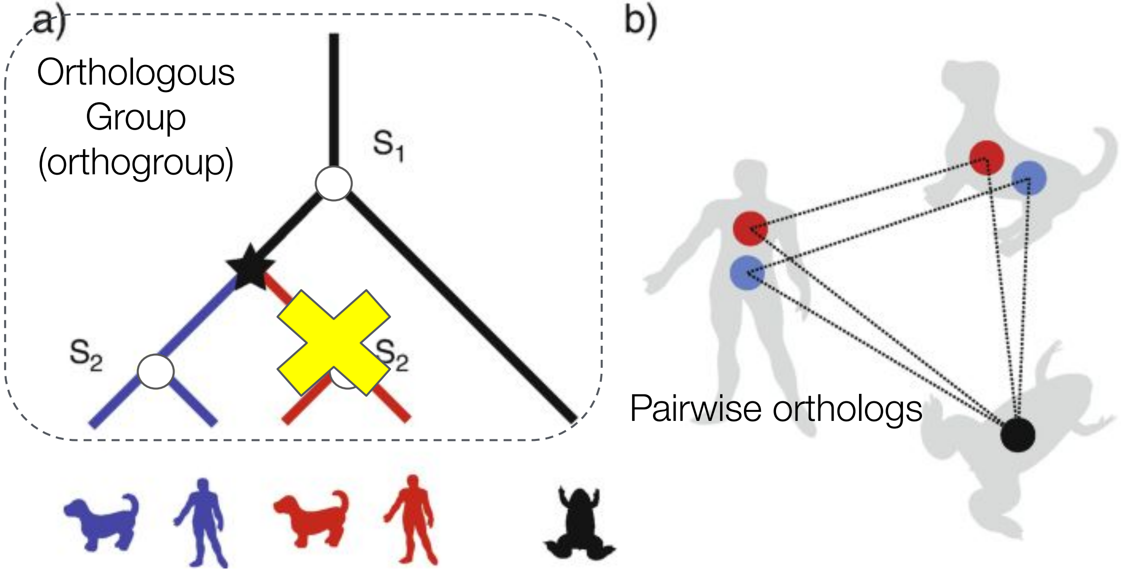
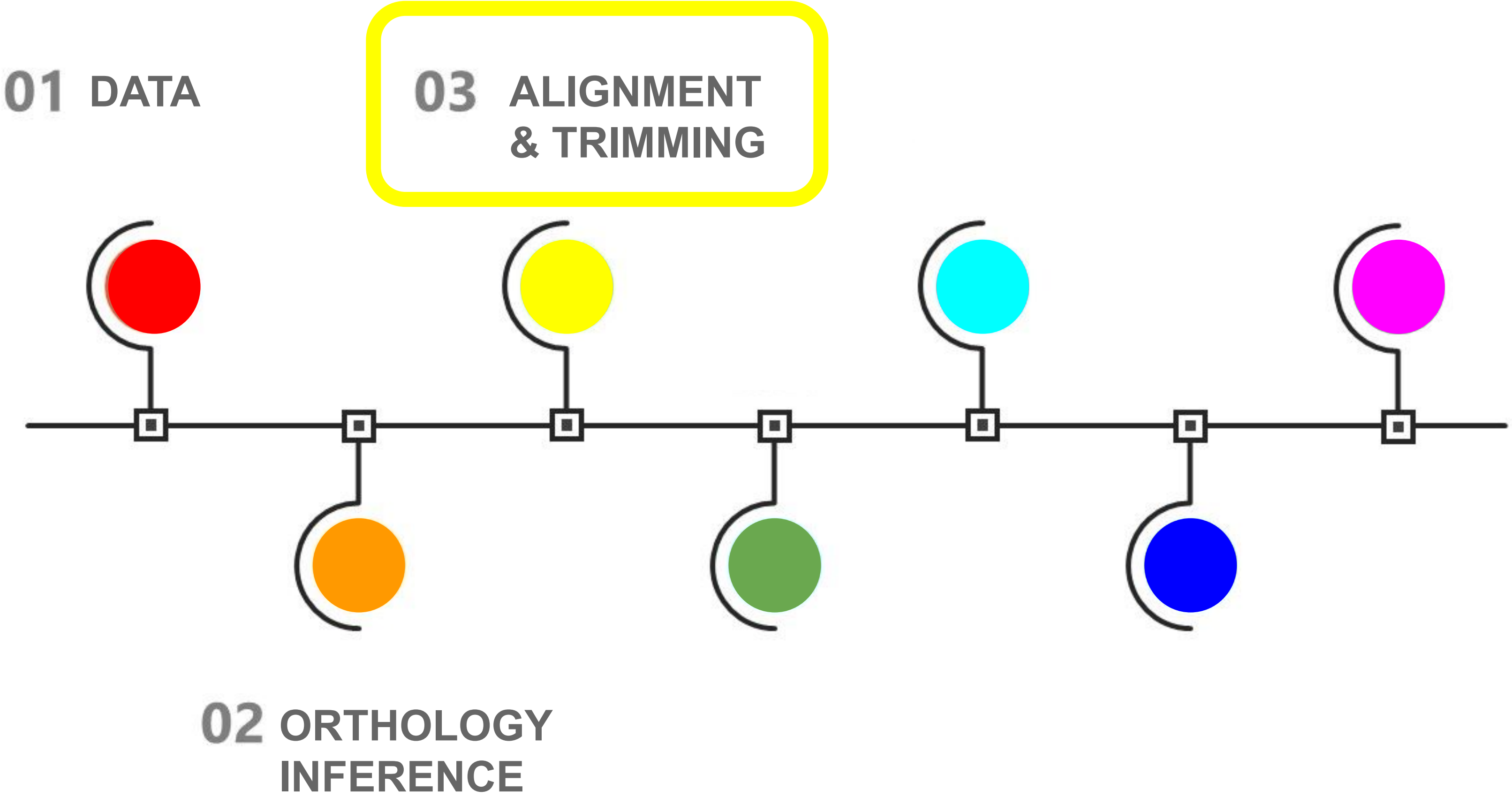


For phylogenomic inference, we want either:

- Single-copy orthogroups (ie, one gene per species)
- Trimmed orthogroups (ie, removing genes from duplication events)

Altenhoff, Glover & Dessimoz 2019



01 DATA

02 ORTHOLOGY INFERENCE

03 ALIGNMENT & TRIMMING

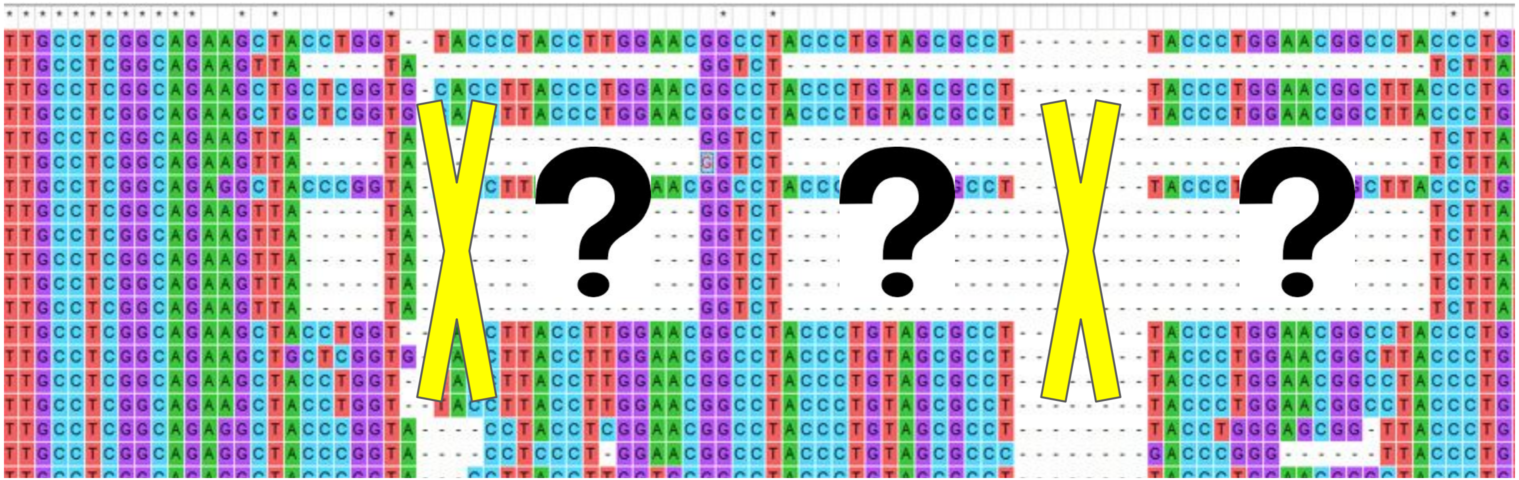
03 ALIGNMENT AND TRIMMING

Software:

- Muscle5, MAFFT
- PhyKIT, trimAL

The goal of the alignment procedure should be to identify the events associated with the homologies, so that the aligned sequences accurately reflect those events.

If the sequences are poorly aligned, you may want to consider trimming the poorly aligned areas.



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Article | Open access | Published: 15 November 2022

Muscle5: High-accuracy alignment ensembles enable unbiased assessments of sequence homology and phylogeny

Robert C. Edgar

Nature Communications **13**, Article number: 6968 (2022) | Cite this article

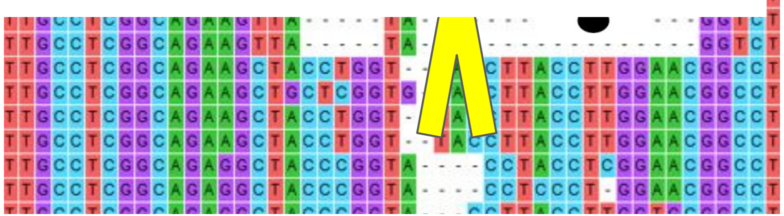
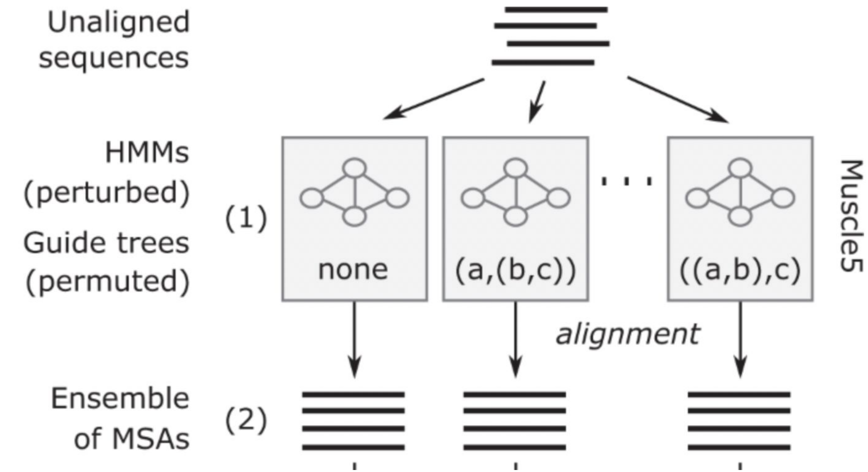
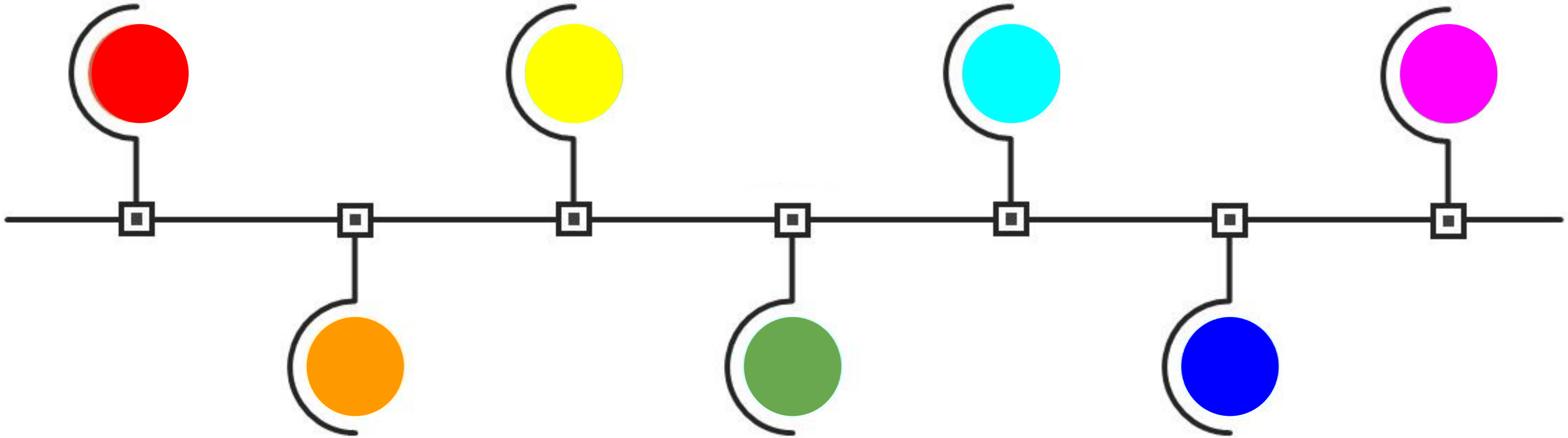


Fig. 1: Typical ensemble workflow for alignment and phylogeny assessment.





01 DATA

02 ORTHOLOGY INFERENCE

03 ALIGNMENT & TRIMMING

04 PHYLOGENOMIC SUBSAMPLING

04 PHYLOGENOMIC SUBSAMPLING

What? Sets of loci are selected from large genome-scale data sets and used for phylogenetic inference.

Why? To avoid an accumulation of nonphylogenetic signals as a product of heterogeneities in evolutionary processes, reduce computing time and improve model fit.

This step can be used to *explore phylogenetic conflicts*, *test specific hypotheses* of relationships, measure the impact of *different sources of bias*, and allow for a *better modeling* of evolutionary processes.

How? By checking the properties of genes or sites and selecting the ones that minimize bias.

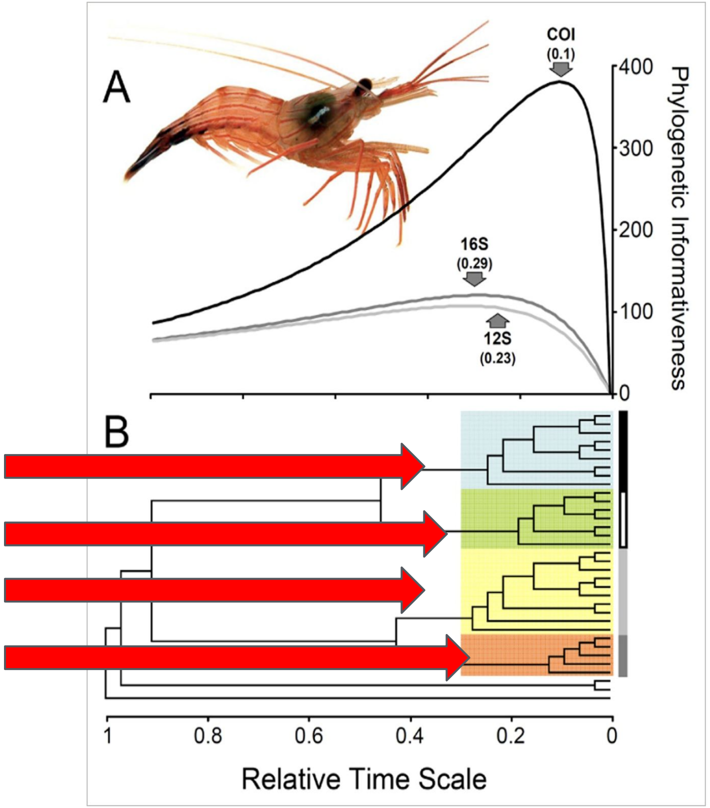
04 PHYLOGENOMIC SUBSAMPLING

Which properties?

Information content

-> length of alignment
-> missing data
-> level of occupancy

Phylogenetic signal



Good information to infer these nodes

Baeza & Fuentes 2013

04 PHYLOGENOMIC SUBSAMPLING

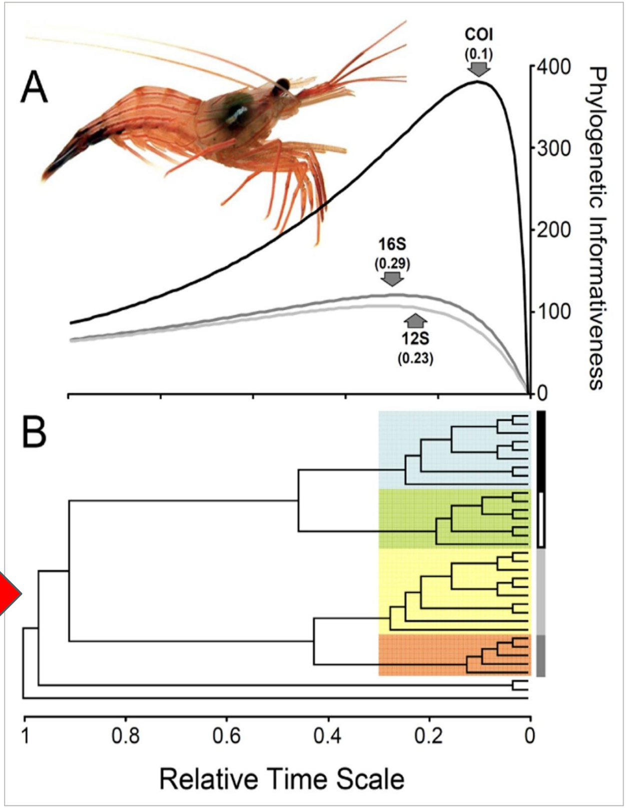
Which properties?

Information content

-> length of alignment
-> missing data
-> level of occupancy

Phylogenetic signal

-> average support
-> Robinson-Foulds distance



Not enough information to infer these nodes

Baeza & Fuentes 2013

04 PHYLOGENOMIC SUBSAMPLING

Which properties?

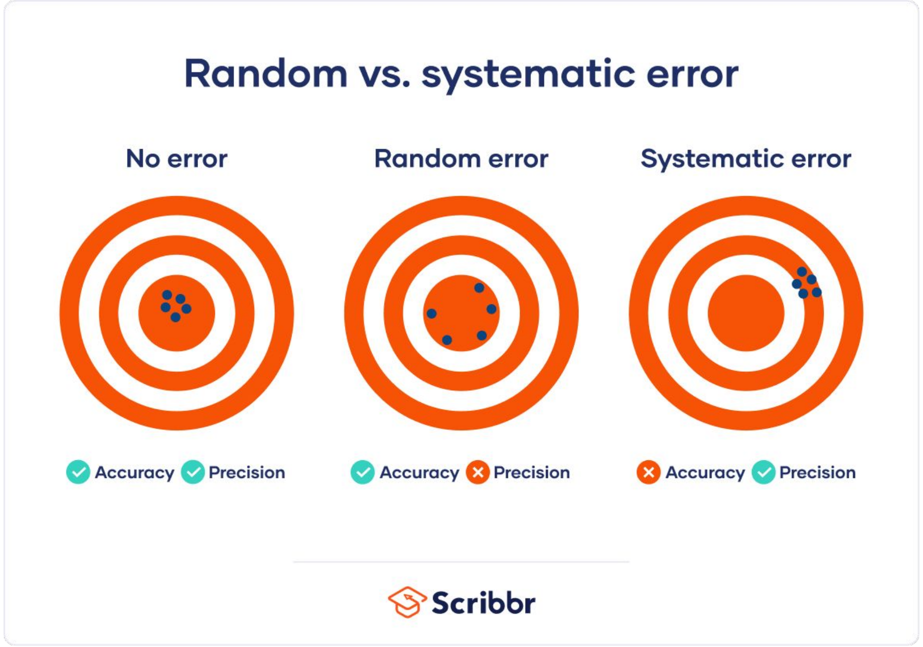
Information content

-> length of alignment
-> missing data
-> level of occupancy

Phylogenetic signal

-> average support
-> Robinson-Foulds distance

Systematic error: when a calculated value deviates from the true value in a consistent way.



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Phylogenetic signal

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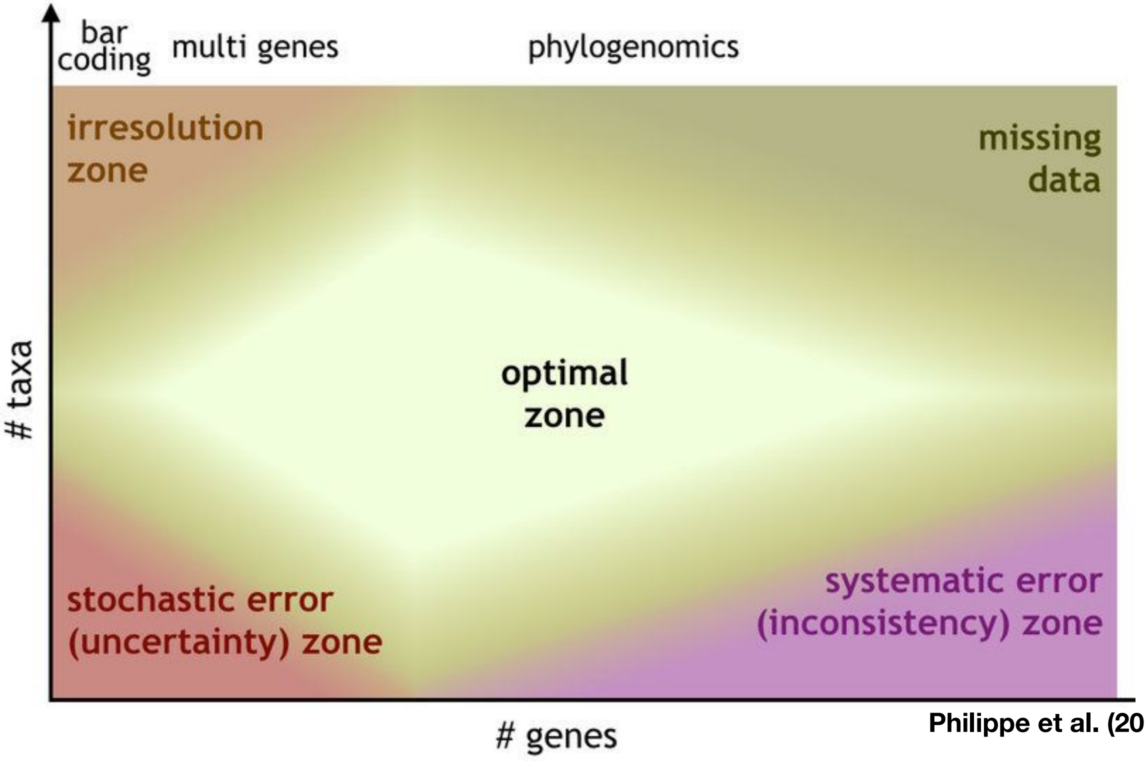
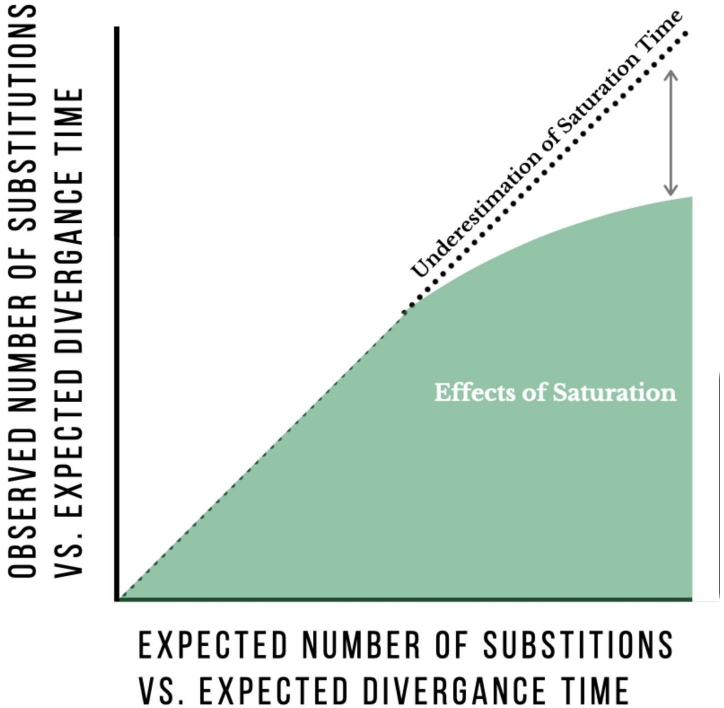
Systematic error:



Philippe et al. (2017)

04 PHYLOGENOMIC SUBSAMPLING

Which properties?



Systematic error

-> root-to-tip distance (ie, the degree of deviation from a strict clock-like behavior)

-> average pair-wise patristic distance between terminals (indicative of susceptibility to long-branch attraction)

-> level of saturation

04 PHYLOGENOMIC SUBSAMPLING

Which properties?

	Gene 1			
	Site 1	Site 2	Site 3…Site n	
Species A	Leu	Met	Lys	Hys
Species B	Leu	Leu	Asn	Pro
Species C	Leu	Met	Lys	Pro
Species D	Leu	Ile	Leu	Leu

Systematic error

-> root-to-tip distance (ie, the degree of deviation from a strict clock-like behavior)

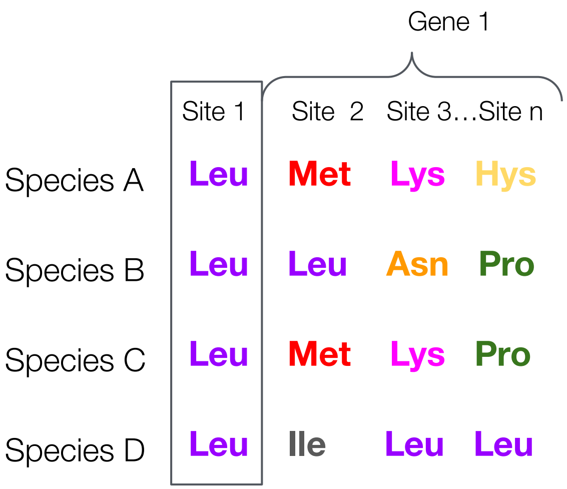
-> average pair-wise patristic distance between terminals (indicative of susceptibility to long-branch attraction)

-> level of saturation

-> compositional heterogeneity

04 PHYLOGENOMIC SUBSAMPLING

Which properties?



Gene 1

	Site 1	Site 2	Site 3…Site n	
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-> level of saturation
-> compositional heterogeneity

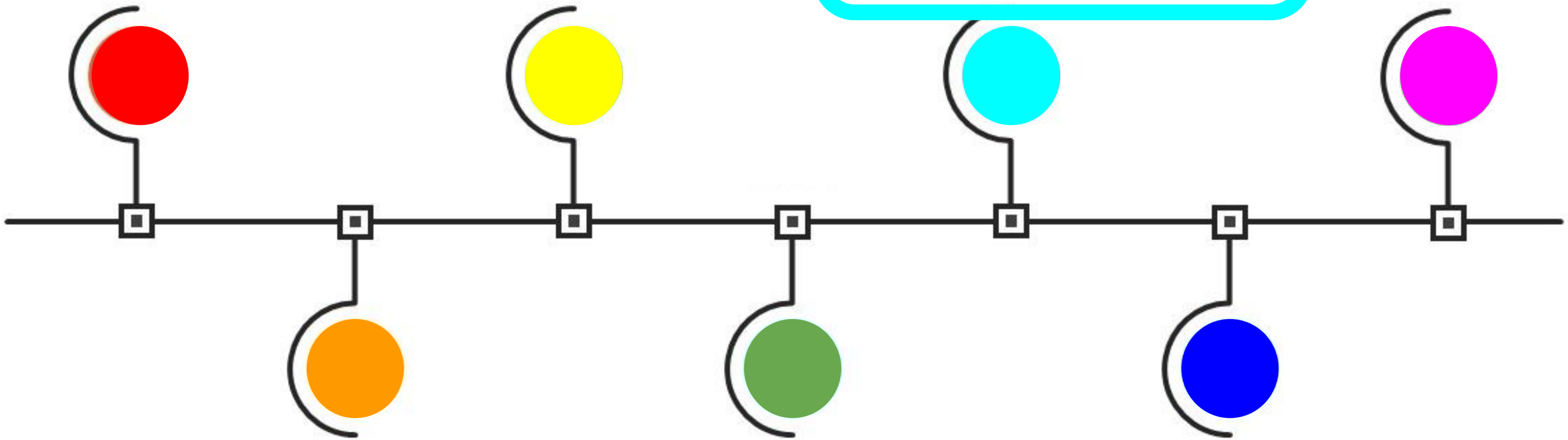
Software:

- PhyKIT
- genesortR

01 DATA

03 ALIGNMENT & TRIMMING

05 SUPERMATRIX VS INDIVIDUAL GENES



02 ORTHOLOGY INFERENCE

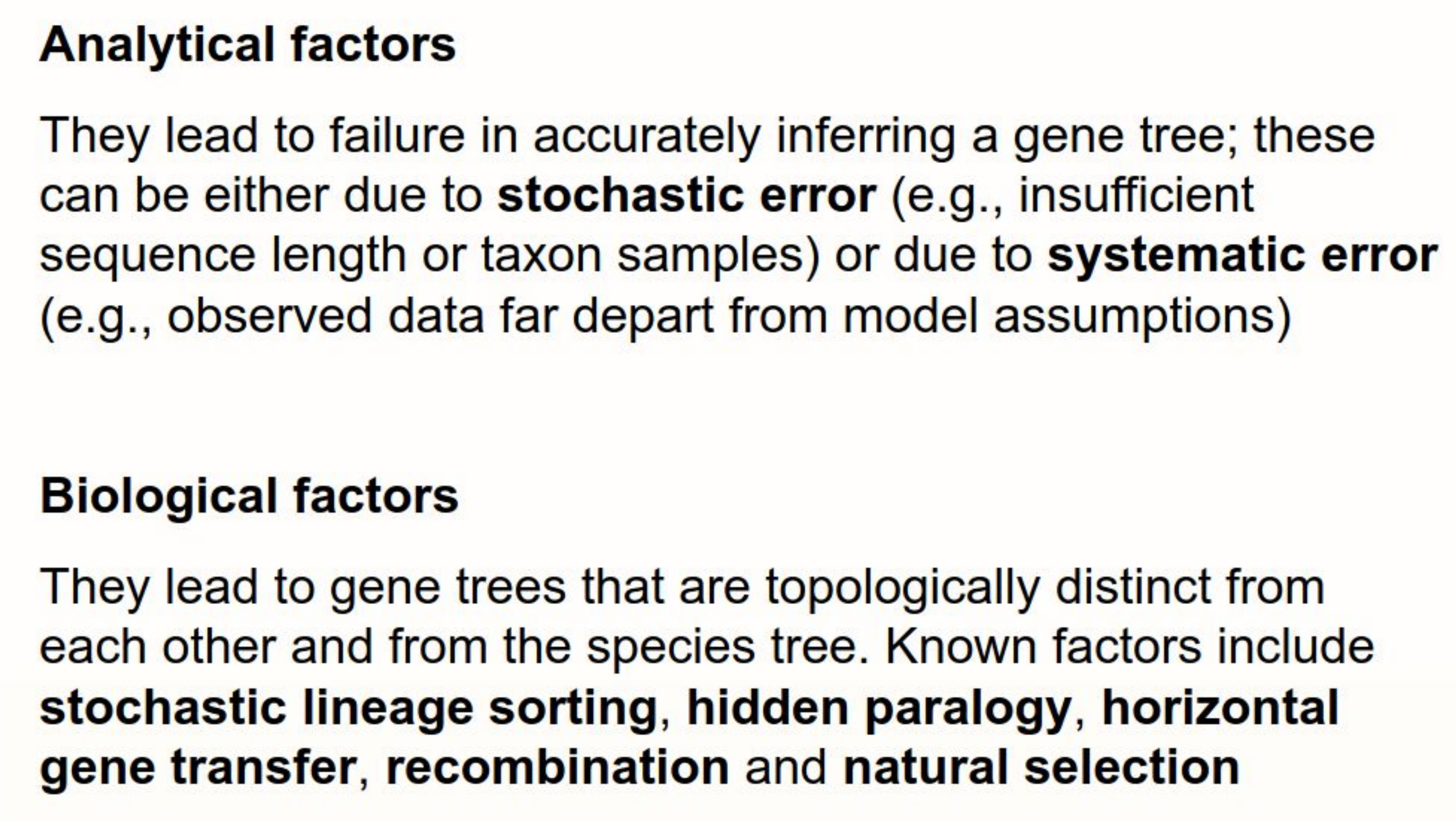
04 PHYLOGENOMIC SUBSAMPLING

05 SUPERMATRIX VS INDIV. GENE TREES

~~Gene tree ≈ Species phylogeny~~

Gene tree ≠ Species phylogeny

05 SUPERMATRIX VS INDIV. GENE TREES



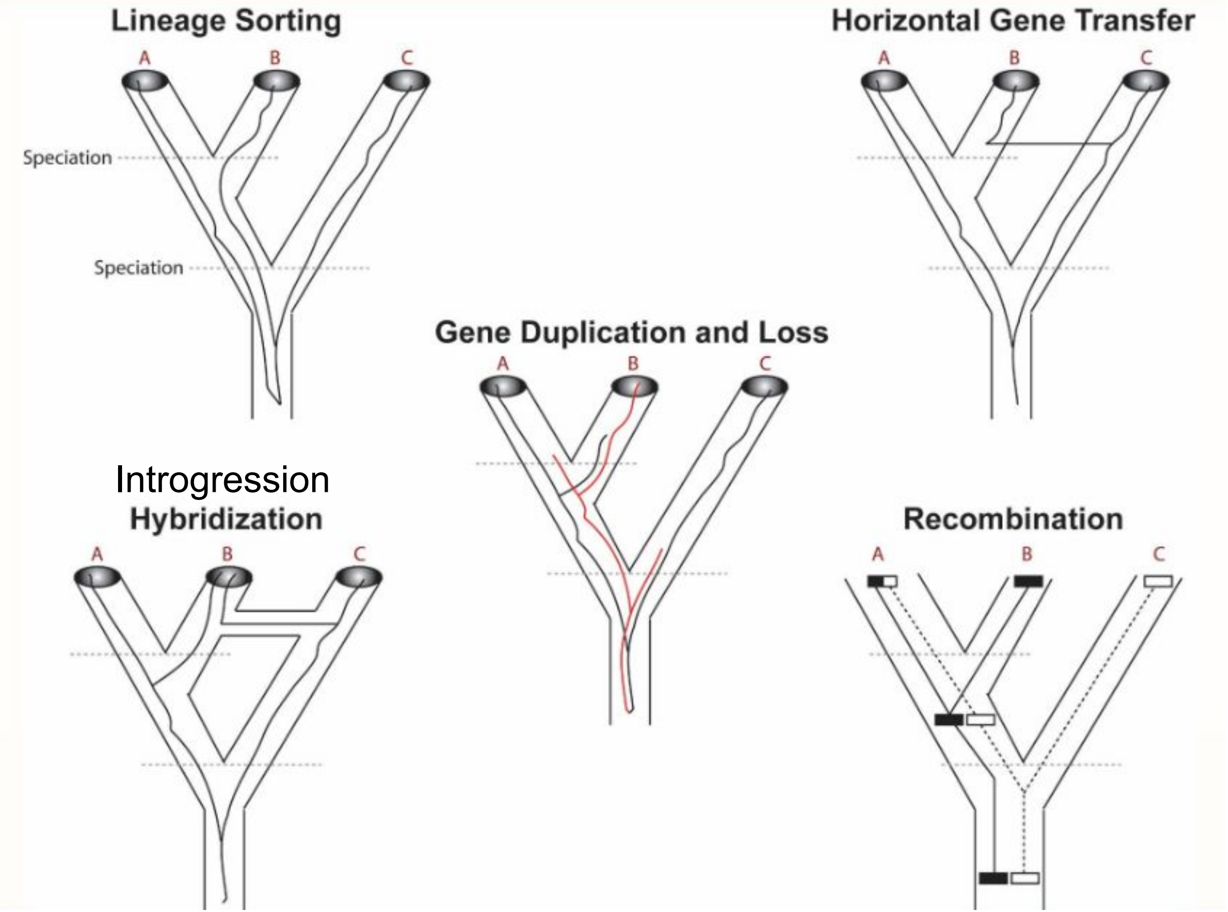
Analytical factors

They lead to failure in accurately inferring a gene tree; these can be either due to **stochastic error** (e.g., insufficient sequence length or taxon samples) or due to **systematic error** (e.g., observed data far depart from model assumptions)

Biological factors

They lead to gene trees that are topologically distinct from each other and from the species tree. Known factors include **stochastic lineage sorting**, **hidden paralogy**, **horizontal gene transfer**, **recombination** and **natural selection**

05 SUPERMATRIX VS INDIV. GENE TREES



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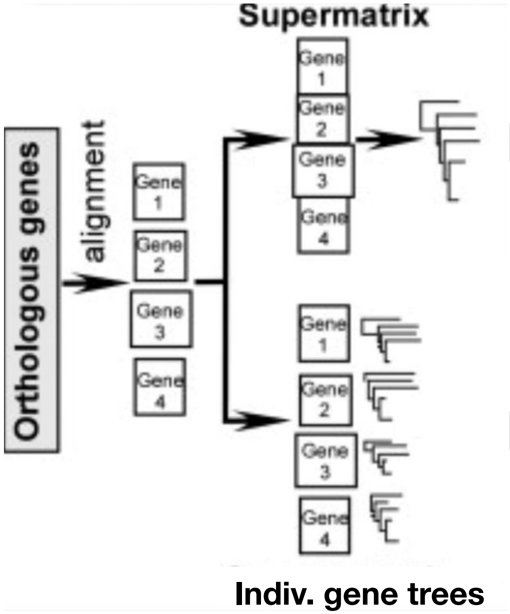


Phylogenetic analysis
(one tree)

Software:

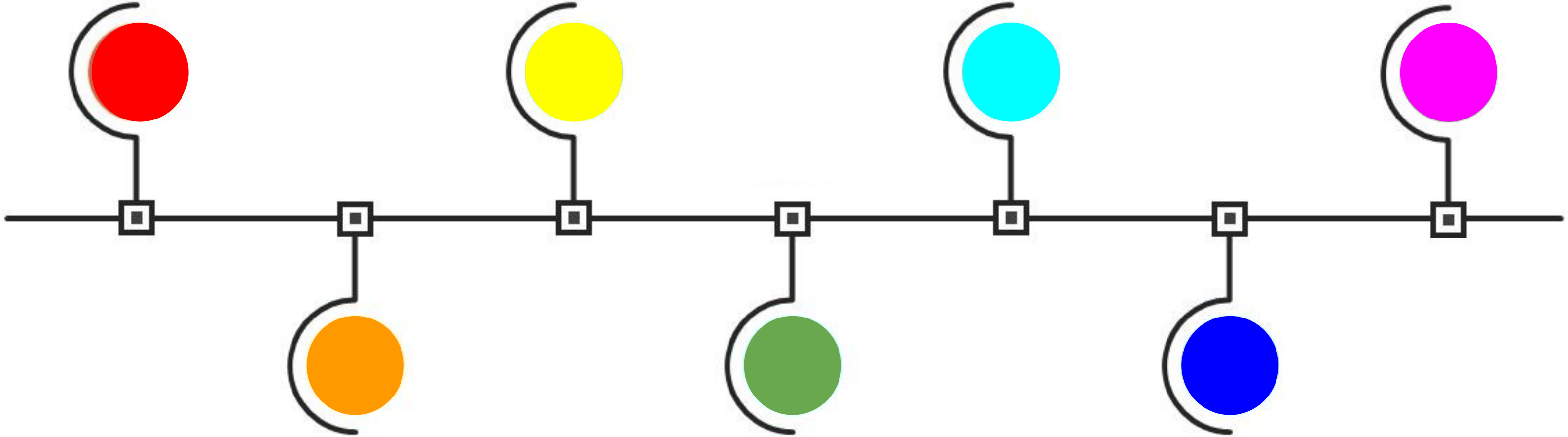
- ASTRAL
- TREE-QMC/TOB-QMC
- StarBeast3

Phylogenetic analysis
(multiple trees)

Estimation of a species tree given a set of gene trees

Multispecies coalescent

Fernández, Hormiga & Giribet (2014)



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06 MODEL SELECTION & PHYLOGENETIC INFERENCE

06 MODEL SELECTION & PHYLOGENETIC INFERENCE

DATA + MODEL OF EVOLUTION

+ METHOD

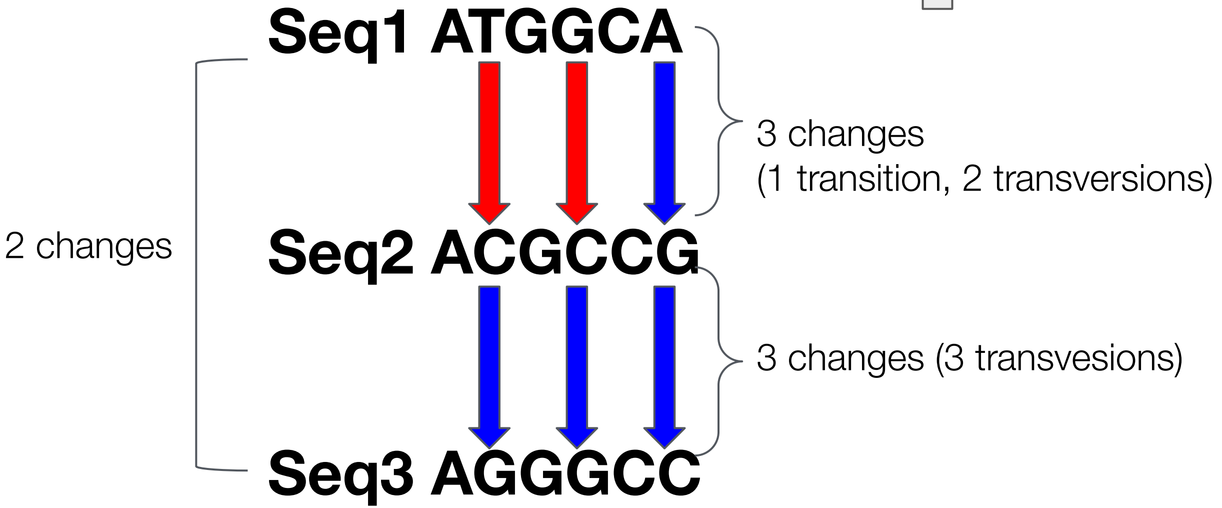
+ A WAY TO ASSESS HOW GOOD YOUR HYPOTHESIS IS

06 MODEL SELECTION & PHYLOGENETIC INFERENCE

DATA + MODEL OF EVOLUTION (= substitution model)

A model that describes changes in sequences over evolutionary time and transforms the number of changes in an evolutionary distance

Observed number of changes + **Equation** = **Evolutionary distance**



Seq1 ATGGCA

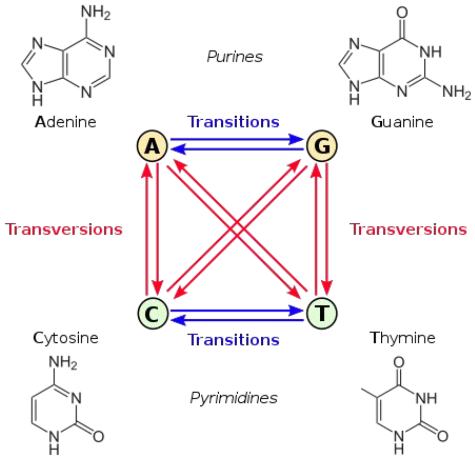
3 changes
(1 transition, 2 transversions)

2 changes

Seq2 ACGCCG

3 changes (3 transvesions)

Seq3 AGGGCC



06 MODEL SELECTION & PHYLOGENETIC INFERENCE

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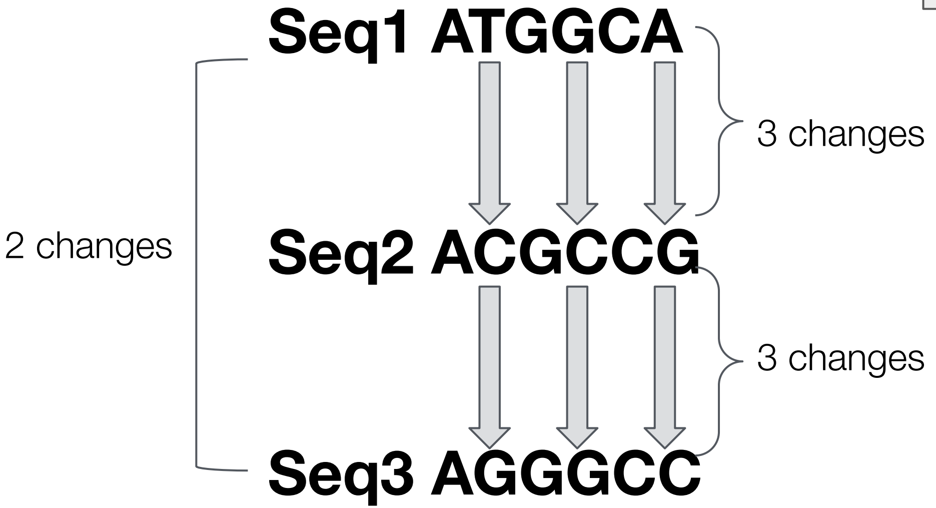

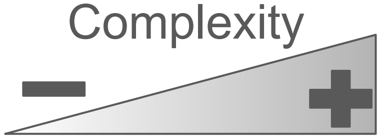

Jukes & Cantor
Kimura 2P
Felsenstein 81
GTR…

nucleotides

PAM
BLOSUM
JTT
LG…

amino acids

06 MODEL SELECTION & PHYLOGENETIC INFERENCE

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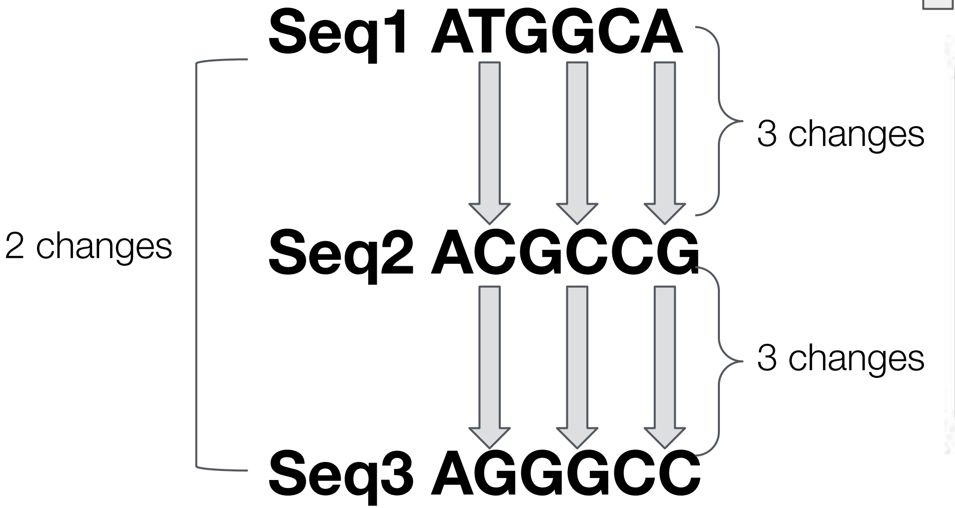


Seq1 ATGGCA

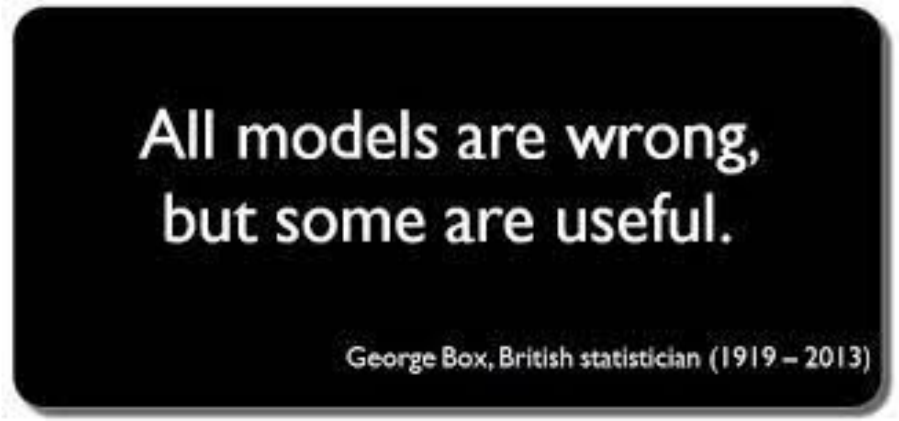
3 changes

Seq2 ACGCCG

3 changes

Seq3 AGGGCC

2 changes



All models are wrong, but some are useful.

George Box, British statistician (1919 – 2013)

06 MODEL SELECTION & PHYLOGENETIC INFERENCE

DATA + **MODEL OF EVOLUTION** (= substitution model)

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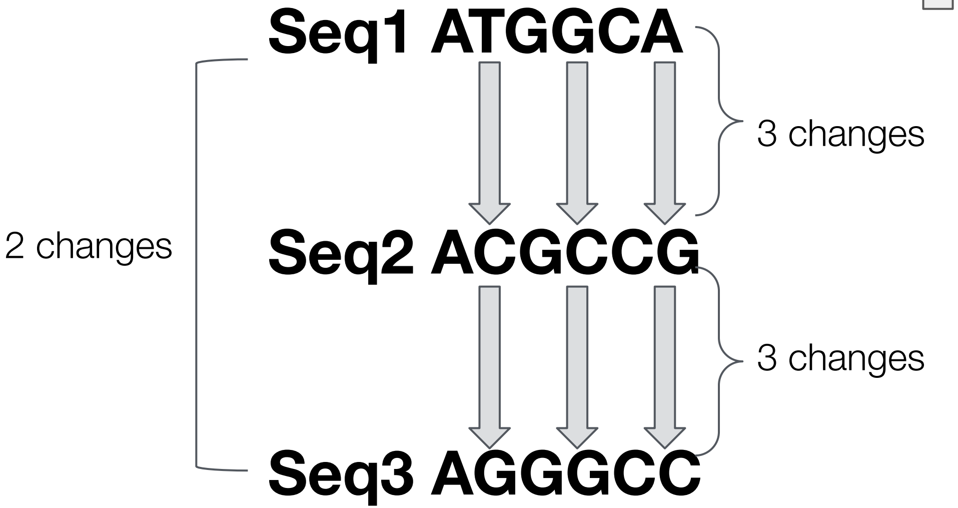


Seq1 ATGGCA

Seq2 ACGCCG

Seq3 AGGGCC

3 changes

3 changes

2 changes

Software:

- ModelFinder (IQ-TREE3)
- ModelTest

06 MODEL SELECTION & PHYLOGENETIC INFERENCE

DATA + MODEL OF EVOLUTION

+ METHOD

Two main methods:

Maximum Likelihood (ML) and **Bayesian Inference (BI)**

Software:

RevBayes
BEAST2
ExaBayes

IQ-TREE3
RAxML-ng
ExaML

Basic question in BI:

'What is the probability that this model (M) is correct, given the data (D) that we have observed?'

Basic question in ML:

'What is the probability of seeing the observed data (D) given that a certain model (M) is true?'

BI seeks P(M|D), while ML maximizes P(D|M)

06 MODEL SELECTION & PHYLOGENETIC INFERENCE

DATA + MODEL OF EVOLUTION

+ METHOD

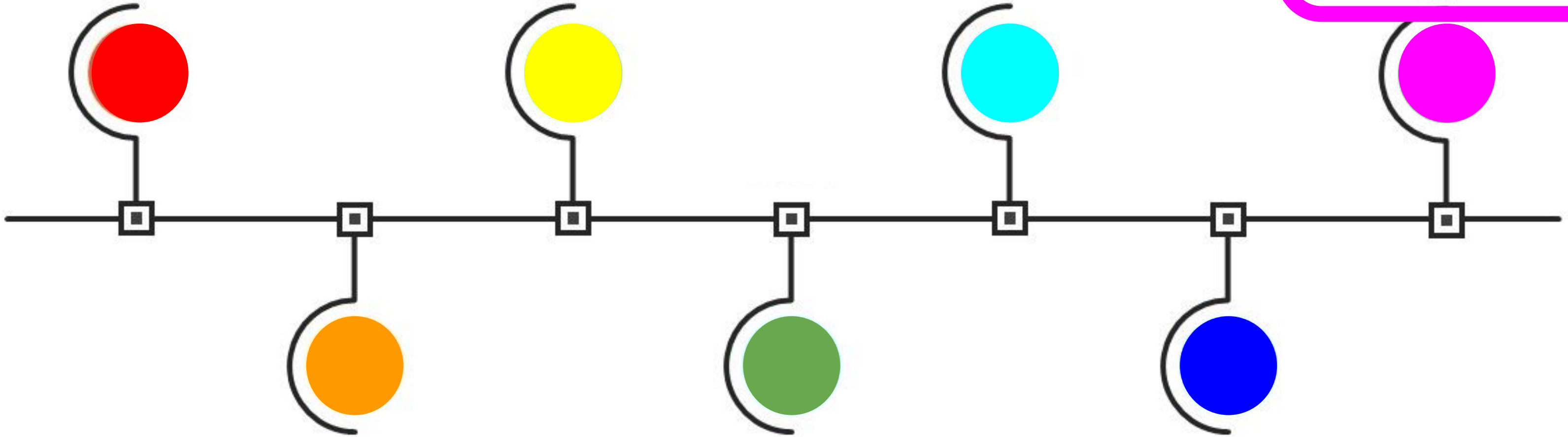
+ A WAY TO ASSESS HOW GOOD YOUR HYPOTHESIS IS

Traditional metrics:

- ML: standard nonparametric bootstrap (100 reps), approximate likelihood ratio test (1,000 reps), ultrafast bootstrap (1,000 reps)(between 1 and 100)
- BI: posterior probability (between 0 and 1)

Novel metrics:

- concordance factor: for every branch of a reference tree, the percentage of “decisive” gene trees containing that branch.
- internode certainty/tree certainty: a measure of the support for a given internode by considering its frequency in a given set of trees jointly with that of the most prevalent conflicting internode in the same set of trees.
- Felsenstein’s bootstrap proportion (FBP)
- Transfer bootstrap expectation (TBE)



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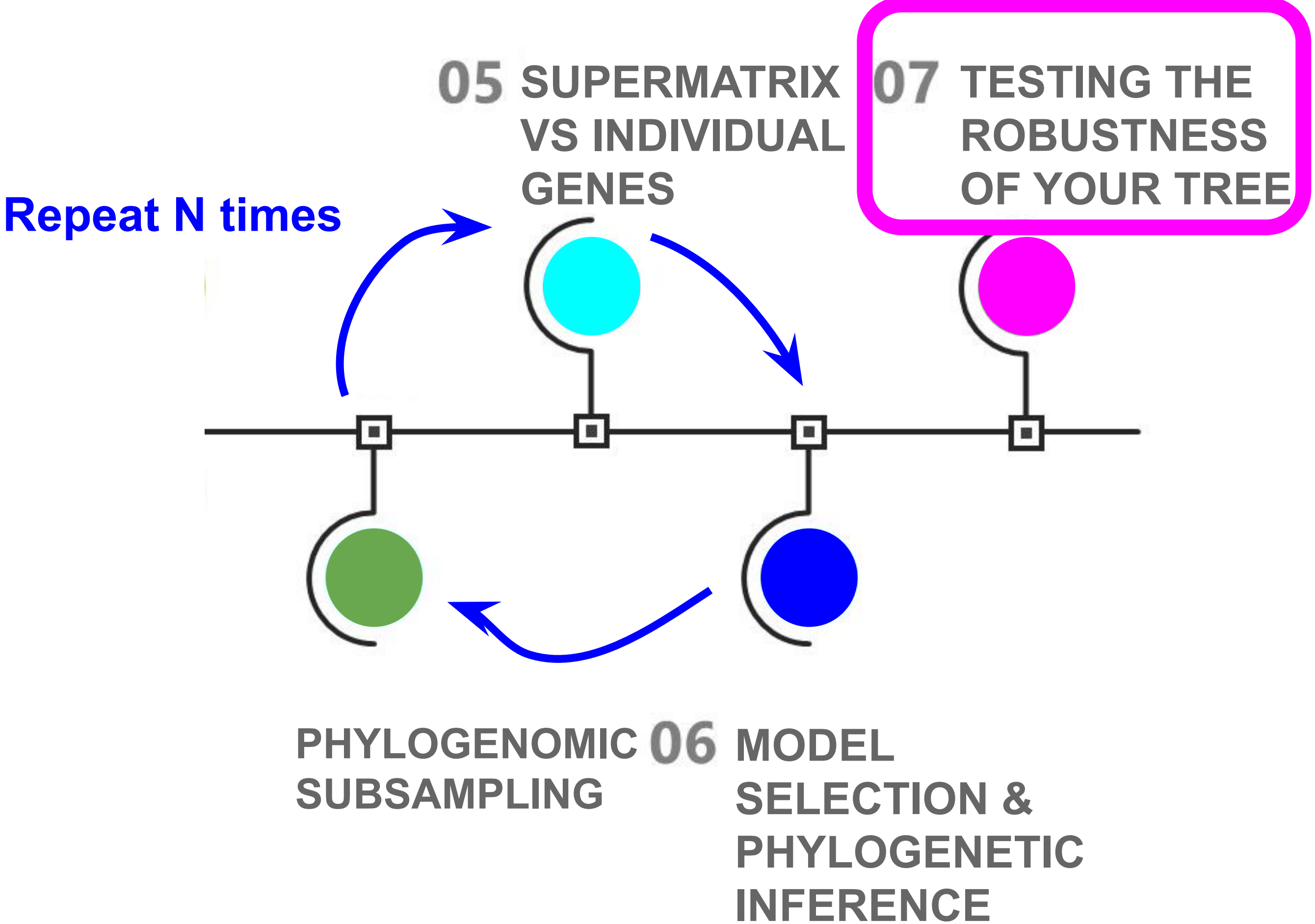
07 TESTING THE ROBUSTNESS OF YOUR TREE

07 TESTING THE ROBUSTNESS OF YOUR TREE



Repeat N times

05 SUPERMATRIX VS INDIVIDUAL GENES

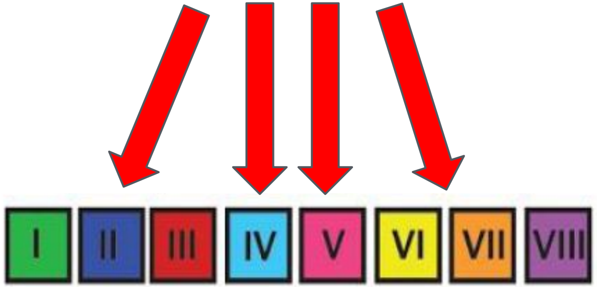
07 TESTING THE ROBUSTNESS OF YOUR TREE

PHYLOGENOMIC SUBSAMPLING

06 MODEL SELECTION & PHYLOGENETIC INFERENCE

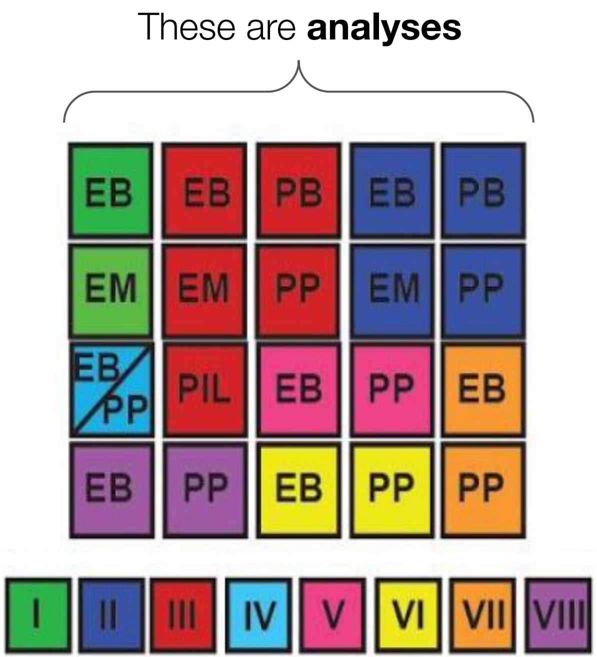
07 TESTING THE ROBUSTNESS OF YOUR TREE

These are **matrices/subsets** of individual gene trees



Fernández, Edgecombe & Giribet (2016) Syst Biol

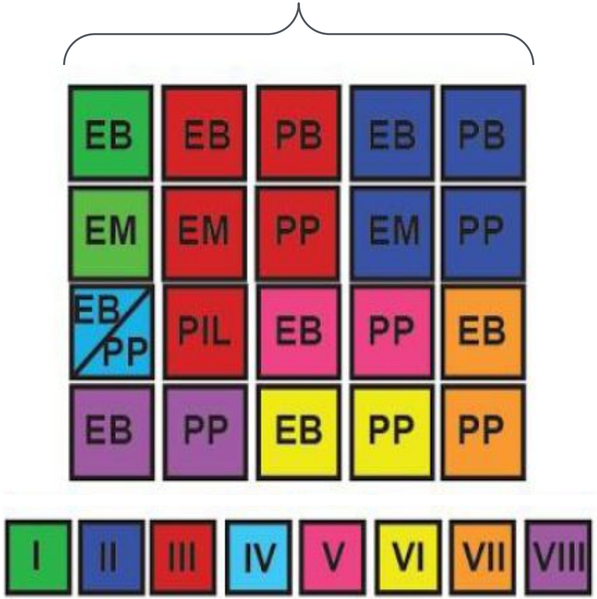
07 TESTING THE ROBUSTNESS OF YOUR TREE



Fernández, Edgecombe & Giribet (2016) Syst Biol

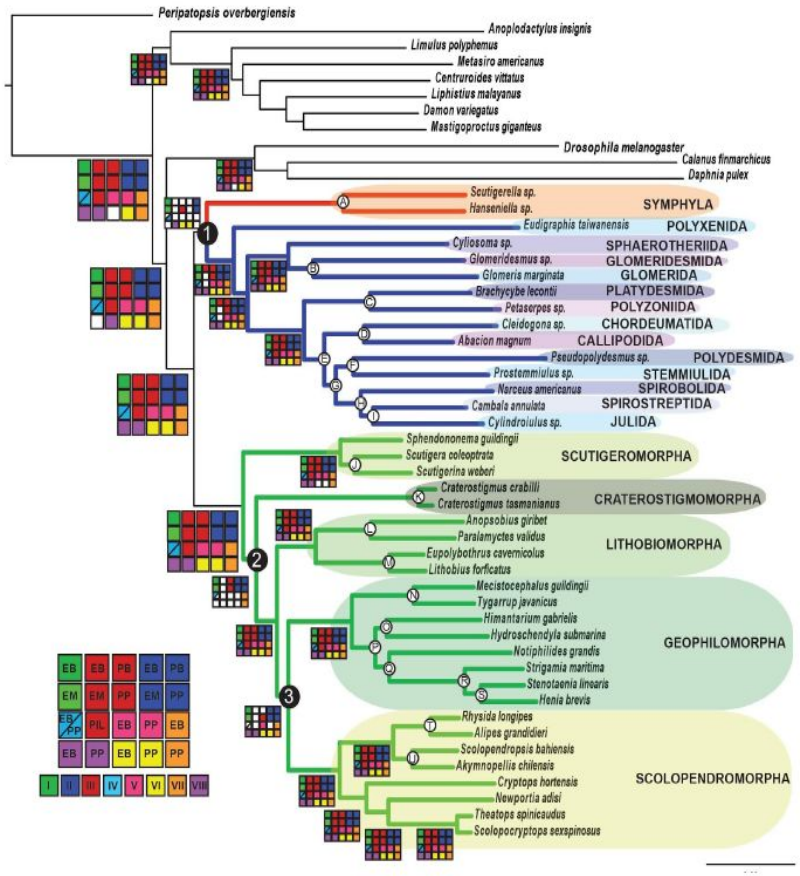
07 TESTING THE ROBUSTNESS OF YOUR TREE

These are **analyses**



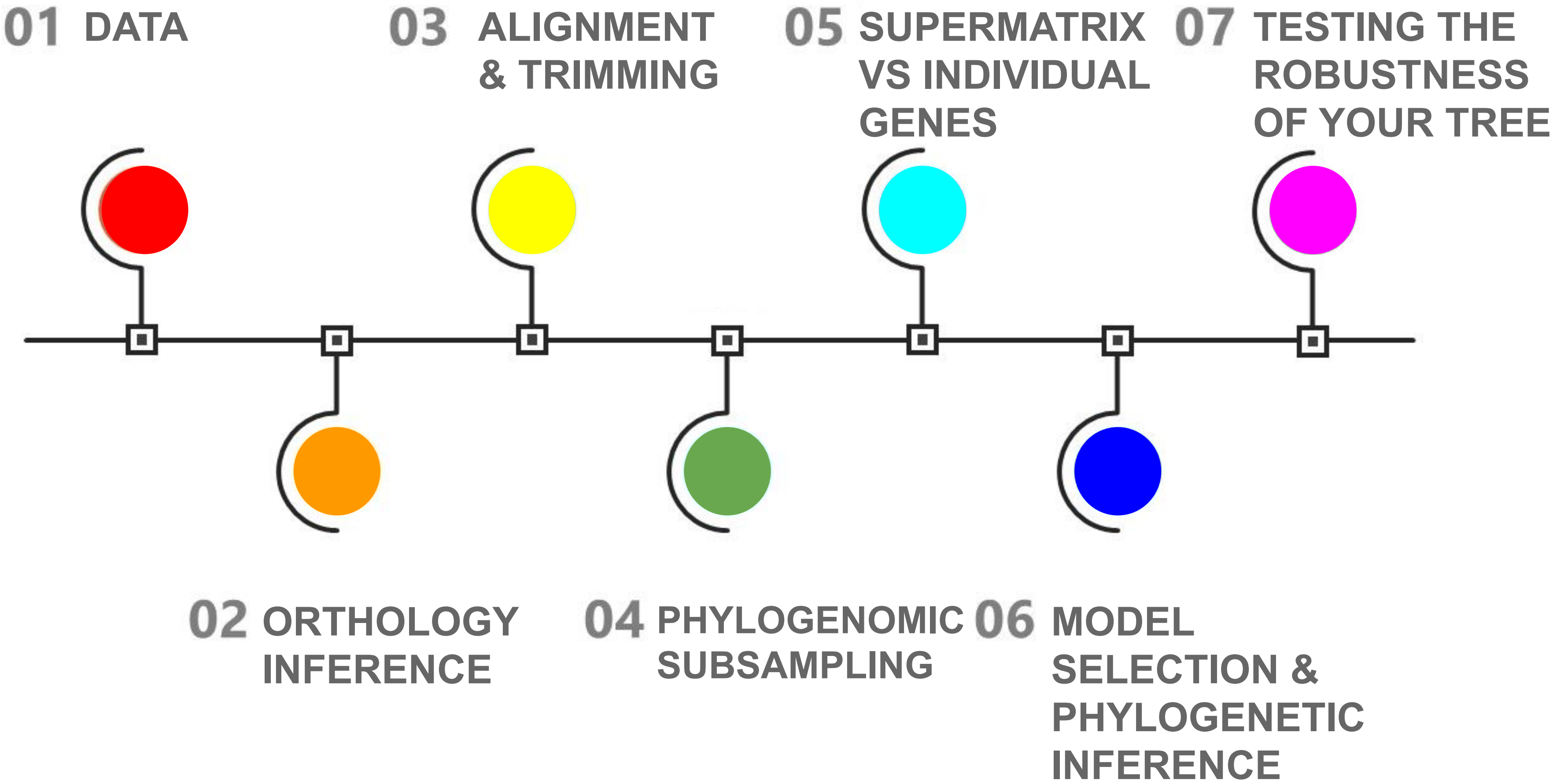
EB	EB	PB	EB	PB
EM	EM	PP	EM	PP
EB/PP	PIL	EB	PP	EB
EB	PP	EB	PP	PP

I	II	III	IV	V	VI	VII	VIII



Fernández, Edgecombe & Giribet (2016) Syst Biol

AND YOU, HOW IS **YOUR** PROJECT?



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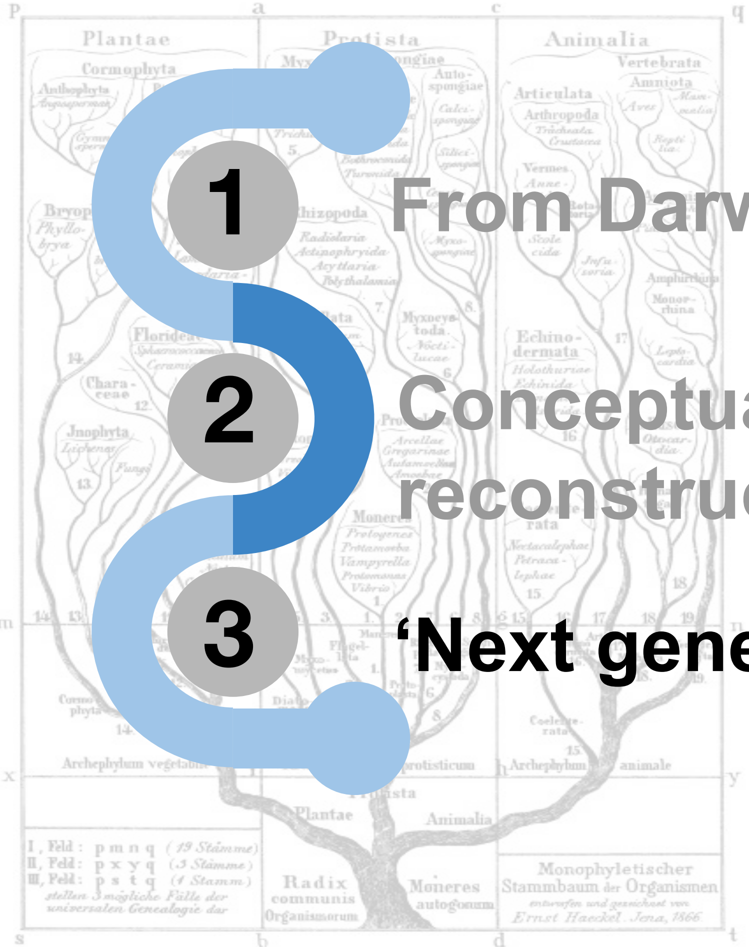
07 TESTING THE ROBUSTNESS OF YOUR TREE

Today’s menu


Plantae
Protista
Animalia
Radix communis Organismorum
Moneres autogonum
Monophyletischer Stammbaum der Organismen
entworfen und gezeichnet von Ernst Haeckel. Jena, 1866

1 From Darwin to phylogenomics

2 Conceptual framework for phylogenomic reconstruction

3 **‘Next generation’ phylogenomics**

“Here be dragons”. This phrase refers to the practice of medieval map makers of drawing dragons and sea serpents in the uncharted areas at the edge of the map.

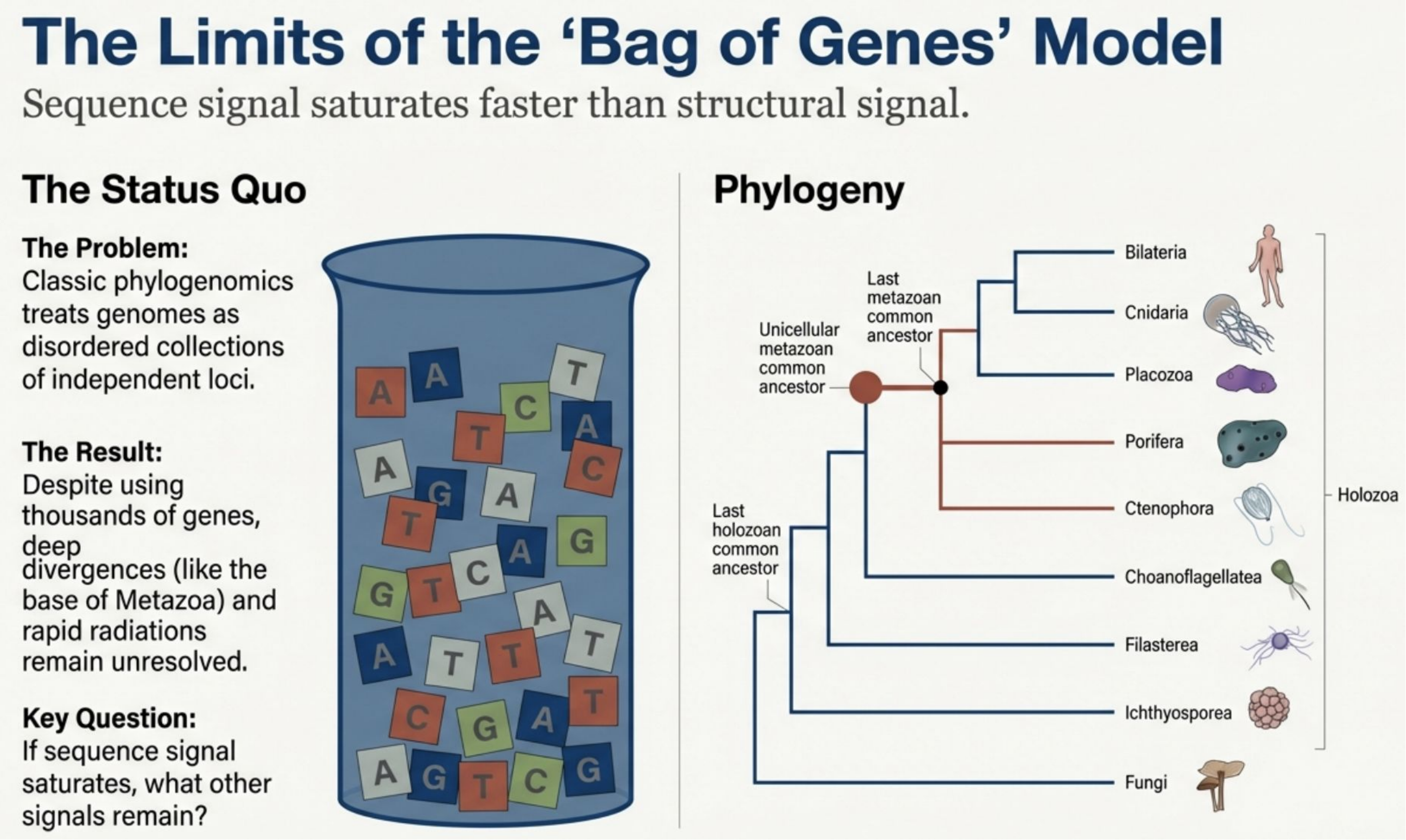
WARNING

THIS PLAY AREA IS USED AT YOUR OWN RISK

‘Next generation’ phylogenomics: Why rethink phylogenomics?

Thousands of loci ≠ resolved trees

- Deep divergences, rapid radiations, short internodes
- Sequence signal saturates faster than we like
- Genomes contain **more information than alignments**



The Limits of the ‘Bag of Genes’ Model

Sequence signal saturates faster than structural signal.

The Status Quo

The Problem:
Classic phylogenomics treats genomes as disordered collections of independent loci.

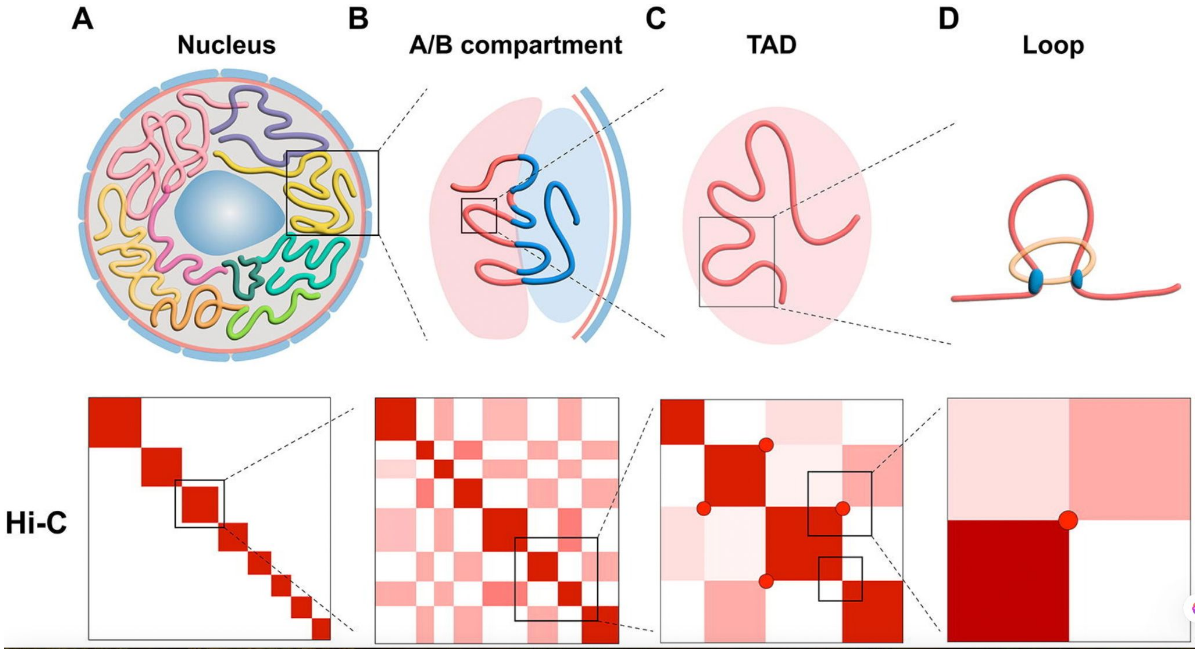
The Result:
Despite using thousands of genes, deep divergences (like the base of Metazoa) and rapid radiations remain unresolved.

Key Question:
If sequence signal saturates, what other signals remain?

Phylogeny

Two new sources of phylogenetic signal

- **Genome architecture**
  - Gene order, chromosomes, 3D folding (chromosome-level genomes galore!)



Yang & Ma 2022

EARTH BIOGENOME PROJECT
sequencing life for the future of life

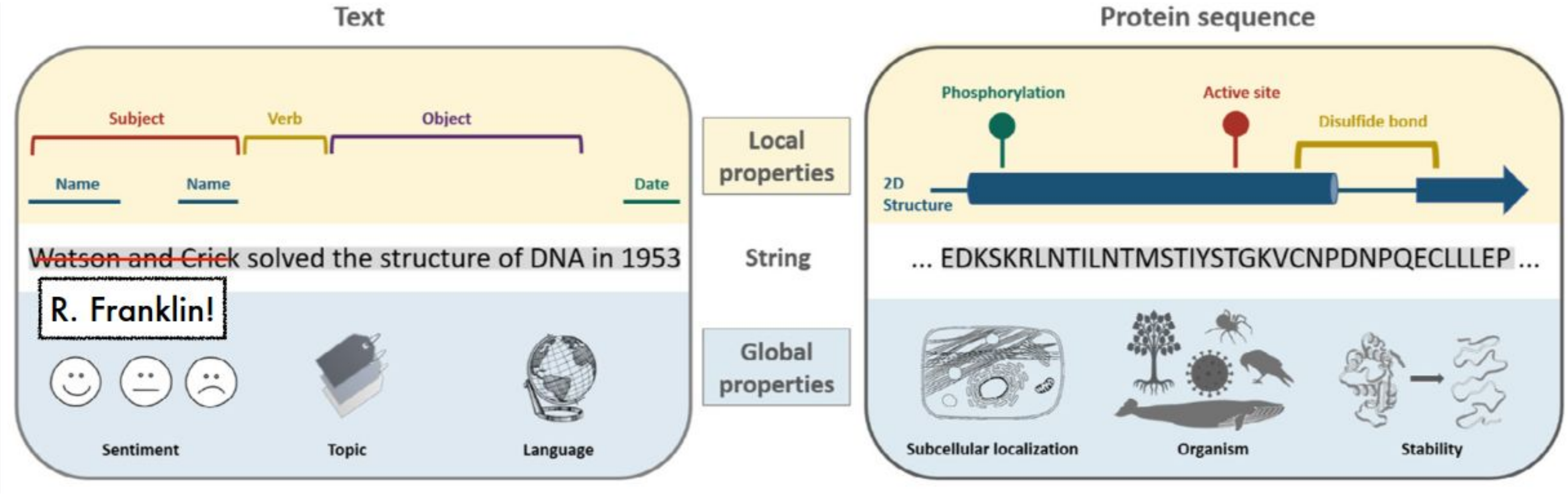
eRGA
European Reference Genome Atlas

Darwin TREE of LIFE

atlasea

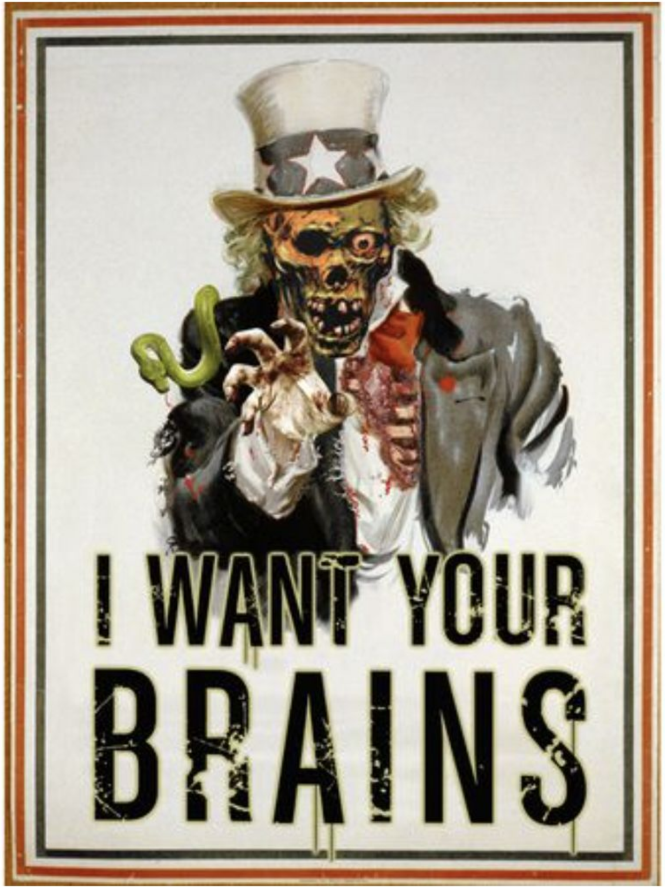
Two new sources of phylogenetic signal

- **Genome architecture**
  - Gene order, chromosomes, 3D folding (chromosome-level genomes galore!)
- **AI-based methods applied to phylogenomics/comparative genomics**
  - Encoding sequences as *'something else'*, based on AI learning



Ofer et al, 2021. 10.1016/j.csbj.2021.03.022. eCollection 2021.

WARNING (AGAIN!!): THIS IS ALL EXPLORATORY



- Uncharted territory, emerging concepts that still need to be properly defined and tested.
  - still exploring: we need your brains!!

- Fields expanding exponentially, great potential, great investment (i.e. chromosome-level genomes, AI in China*)
  - we need to build literacy and critical thinking

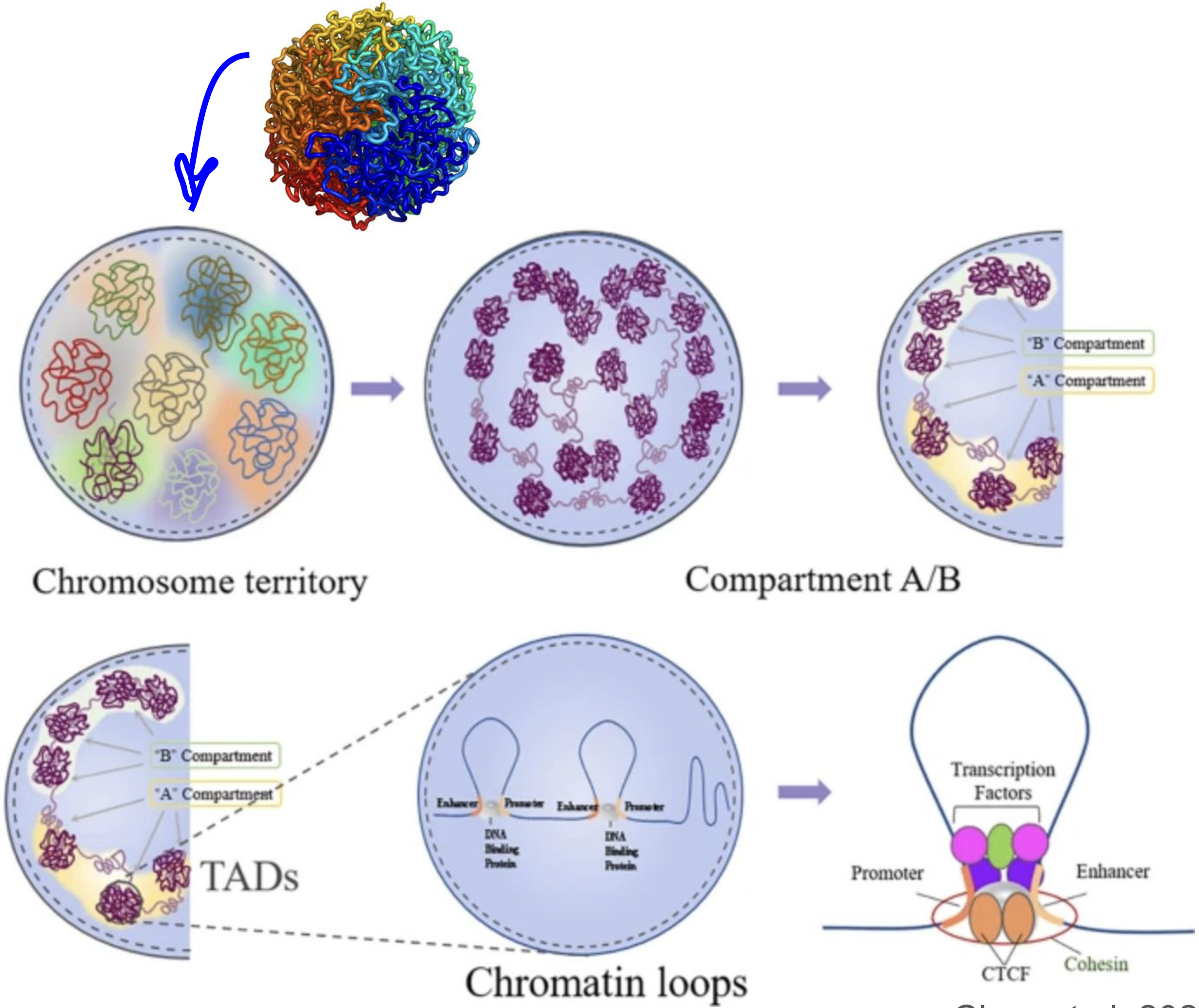
- Results may be GREAT… or may be bullshit

(*China investment in AI surpasses <u>by far</u> that in Europe & USA)

PART I — Genome architecture–aware phylogenomics

Genomes are not bags of genes

- Genes have **order, orientation, neighbors**
- Chromosomes evolve via fusions, fissions, inversions
- Structure persists when sequence similarity is gone: ***SYNTENY***

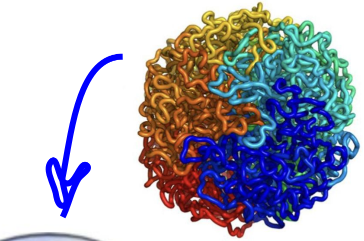


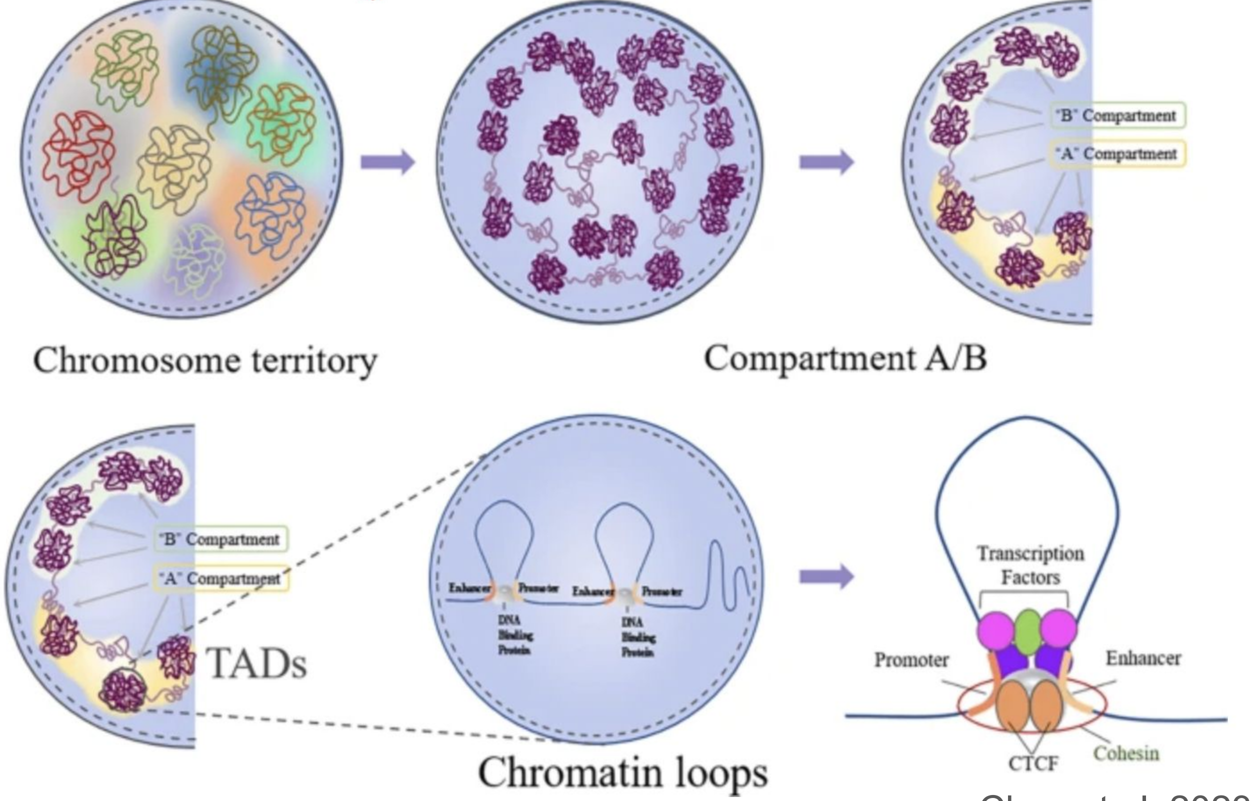
Chen et al. 2023

PART I — Genome architecture–aware phylogenomics

Genomes are not bags of genes

- Genes have **order, orientation, neighbors**
- Chromosomes evolve via fusions, fissions, inversions
- Structure persists when sequence similarity is gone: ***SYNTENY*** (... or does it??)





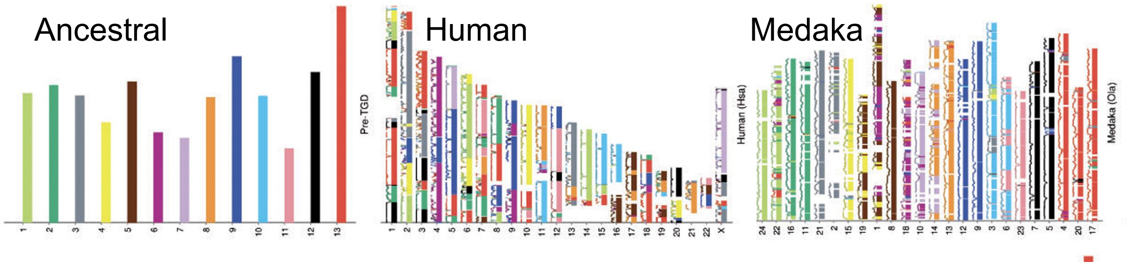
Chen et al. 2023

PART I — Genome architecture–aware phylogenomics

Genomes as documents of evolutionary history: a probabilistic macrosynteny model for the reconstruction of ancestral genomes

Yoichiro Nakatani* and Aoife McLysaght* (2017)

Ancestral Linkage Groups (ALGs): conserved blocks of genes that remained together on ancestral chromosomes over vast evolutionary periods



Macrosynteny survives deep time

- Ancestral linkage groups conserved across animals
- Detected even after >500 My of divergence
- Provides signal when alignments fail

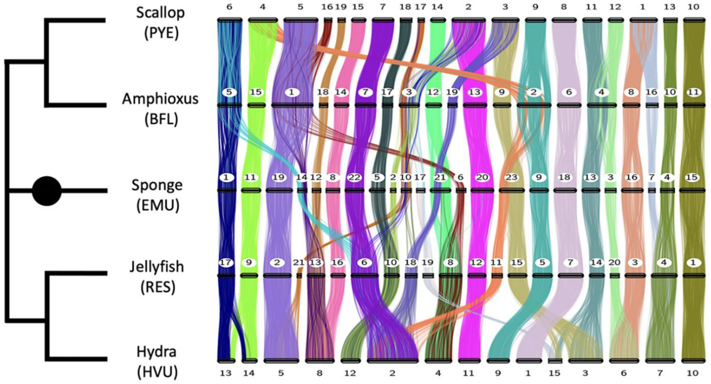
Examples

- Amphioxus as proxy for ancestral chordate genome
- Bilaterian chromosomal blocks conserved across phyla

Deeply conserved synteny and the evolution of metazoan chromosomes (2022)

OLEG SIMAKOV, JESSEN BREDESON, KODIAK BERKOFF, FERDINAND MARLETAZ, THERESE MITROS, DARRIN T. SCHULTZ, BRENDAN L. O'CONNELL, PAUL DEAR, DANIEL E. MARTINEZ, [...], AND DANIEL S. ROKHSAR

SCIENCE ADVANCES • 2 Feb 2022 • Vol 8, Issue 5 • DOI: 10.1126/sciadv.abi



PART I — Genome architecture–aware phylogenomics

Article | Open access | Published: 17 May 2023

Ancient gene linkages support ctenophores as sister to other animals

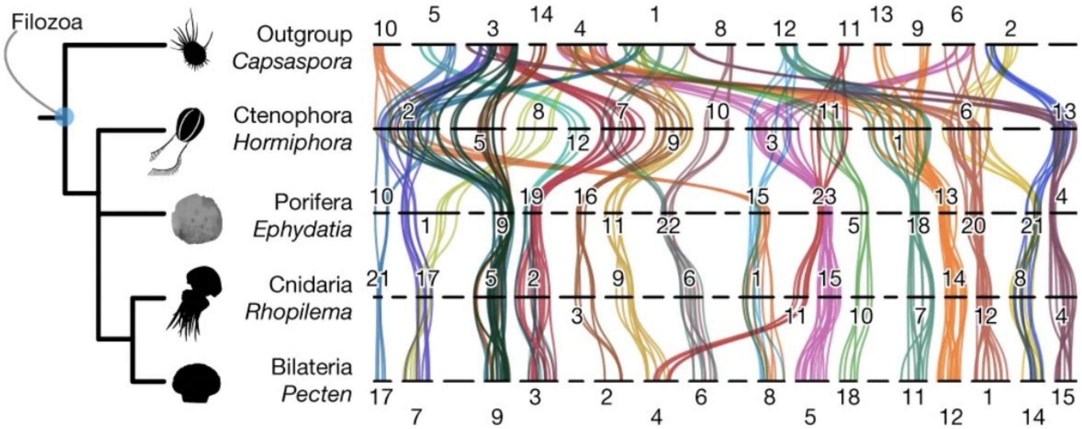
Darrin T. Schultz, Steven H. D. Haddock, Jessen V. Bredeson, Richard E. Green, Oleg Simakov & Daniel S. Rokhsar

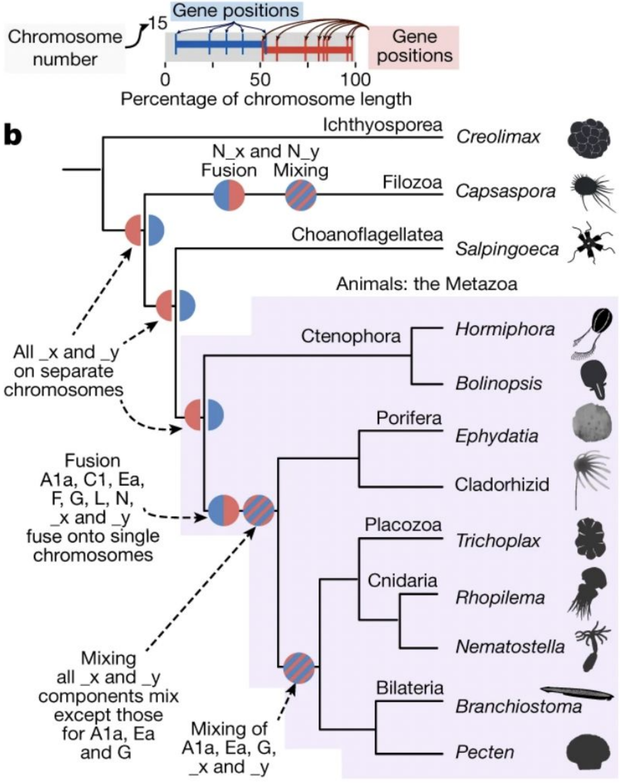
Synteny as a rare genomic change

- Rearrangements = discrete evolutionary events
- Shared fusions/fissions → low homoplasy
- Conceptually similar to indels or retroposons

Key idea

- Fewer characters, but more reliable

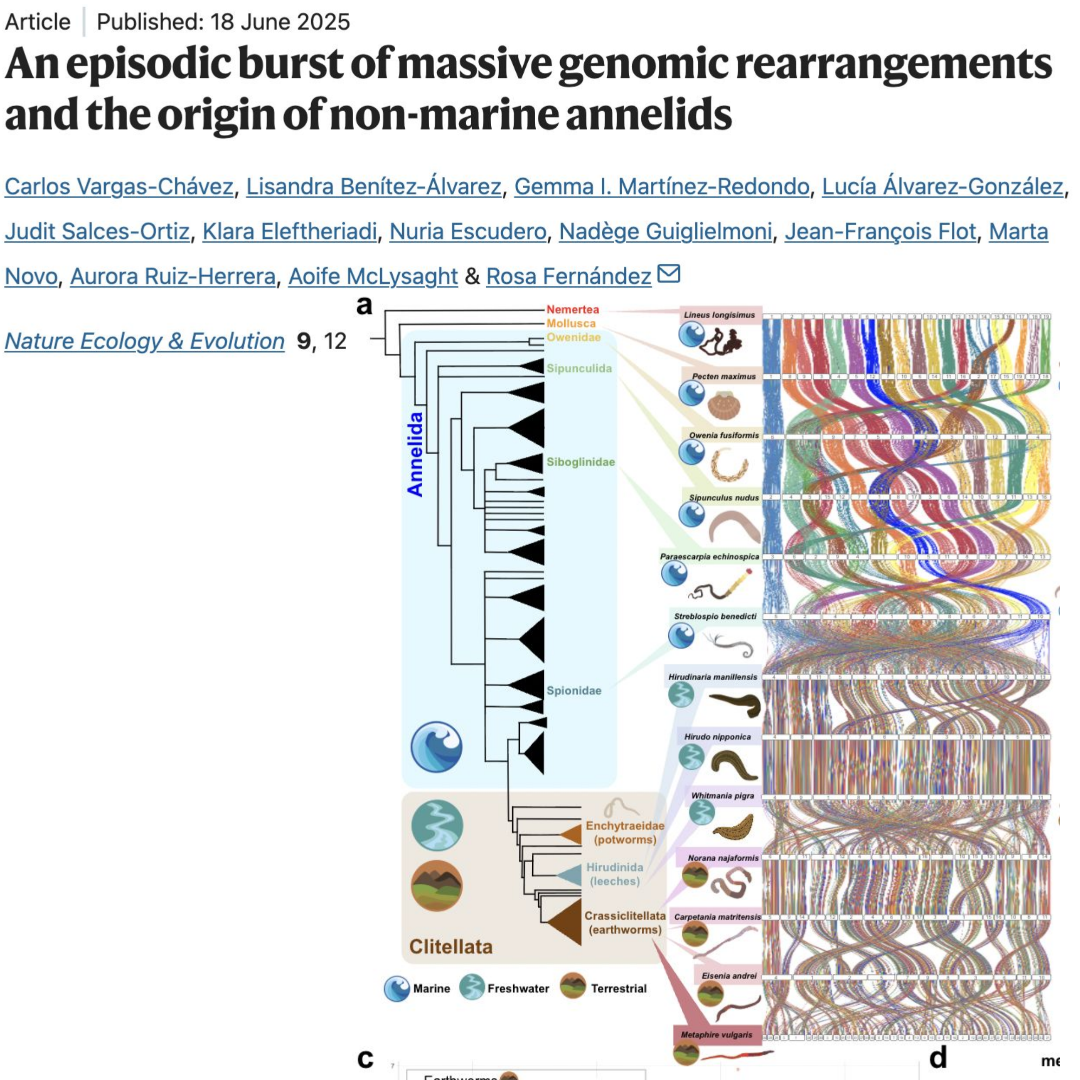




PART I — Genome architecture–aware phylogenomics

Synteny as a rare genomic change

- *REALLY??*



Article | Published: 18 June 2025

An episodic burst of massive genomic rearrangements and the origin of non-marine annelids

Carlos Vargas-Chávez, Lisandra Benítez-Álvarez, Gemma I. Martínez-Redondo, Lucía Álvarez-González, Judit Salces-Ortiz, Klara Eleftheriadi, Nuria Escudero, Nadège Guiglielmoni, Jean-François Flot, Marta Novo, Aurora Ruiz-Herrera, Aoife McLysaght & Rosa Fernández ✉

Nature Ecology & Evolution **9**, 12

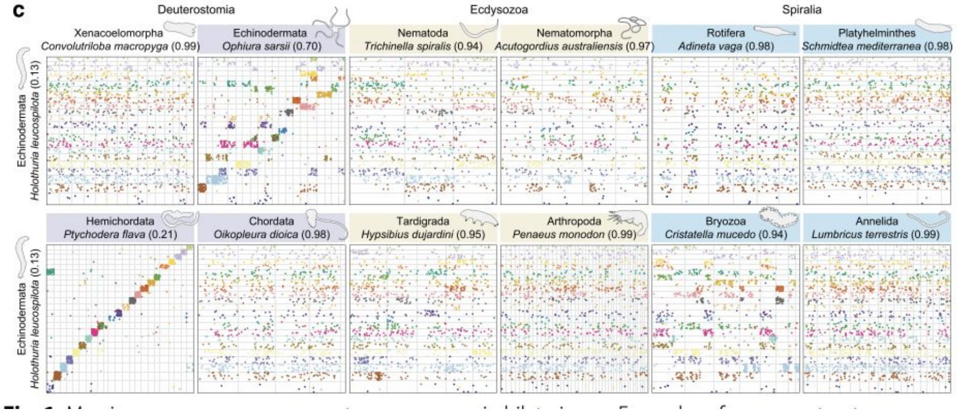
PART I — Genome architecture–aware phylogenomics

Synteny as a rare genomic change

- ***REALLY??***

Conservation of bilaterian genome structure is the exception, not the rule

Thomas D. Lewin[1*], Isabel Jiah-Yih Liao[1] and Yi-Jyun Luo[1*]



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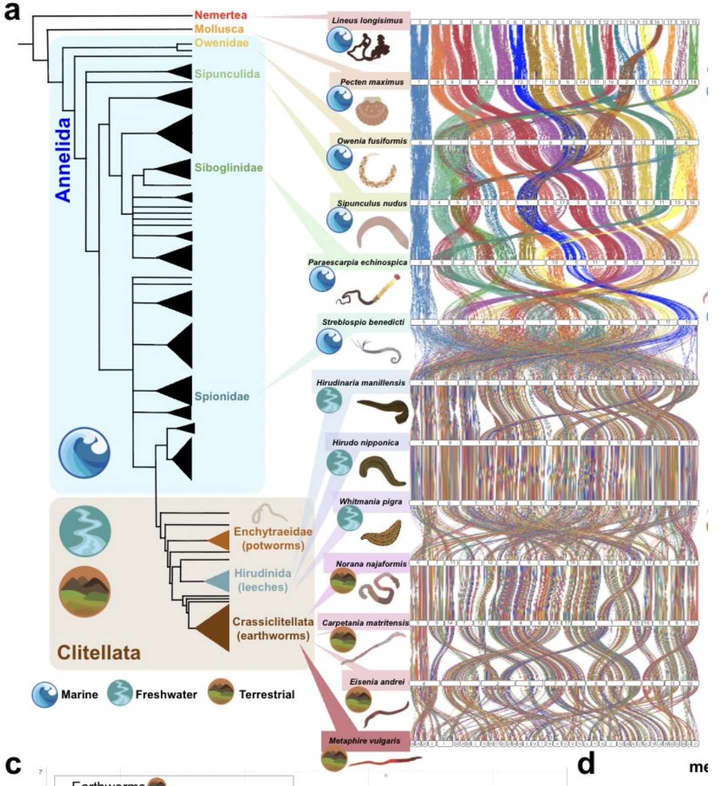
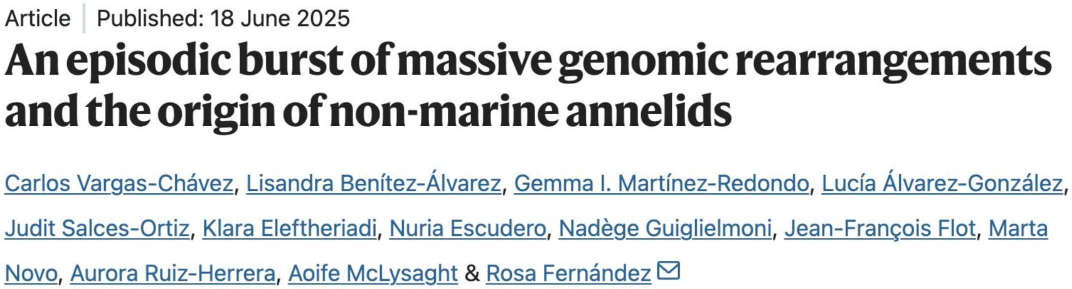
Carlos Vargas-Chávez, Lisandra Benítez-Álvarez, Gemma I. Martínez-Redondo, Lucía Álvarez-González, Judit Salces-Ortiz, Klara Eleftheriadi, Nuria Escudero, Nadège Guiglielmoni, Jean-François Flot, Marta Novo, Aurora Ruiz-Herrera, Aoife McLysaght & Rosa Fernández ✉

Nature Ecology & Evolution **9**, 12



PART I — Genome architecture–aware phylogenomics



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Nature Ecology & Evolution **9**, 12

Synteny as a rare genomic change

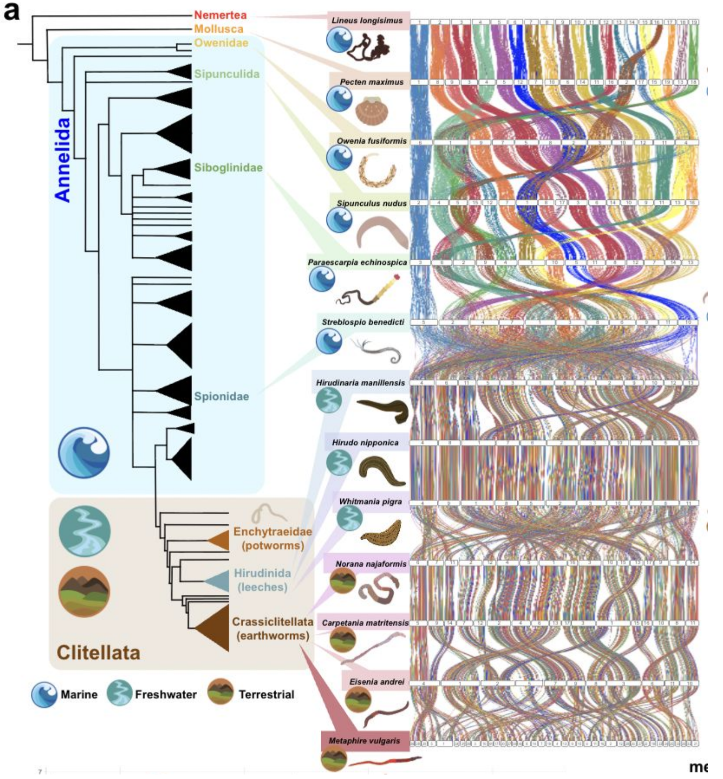
- ***REALLY??***

Rearrangement rate heterogeneity is high

- Some lineages: highly stable genomes
- Others: massive reshuffling (even within phylum/genus!!)
- Rate heterogeneity is lineage-specific: we need *models & new tools* (e.g. to infer ALGs with more precision, simulations of SV scenarios, etc)

Implications

- Architecture works best when reshuffling is not extreme
- If extreme, be creative :-) *(feel free to reach out for tips!)*
- Not all 3D signal is phylogenetically useful



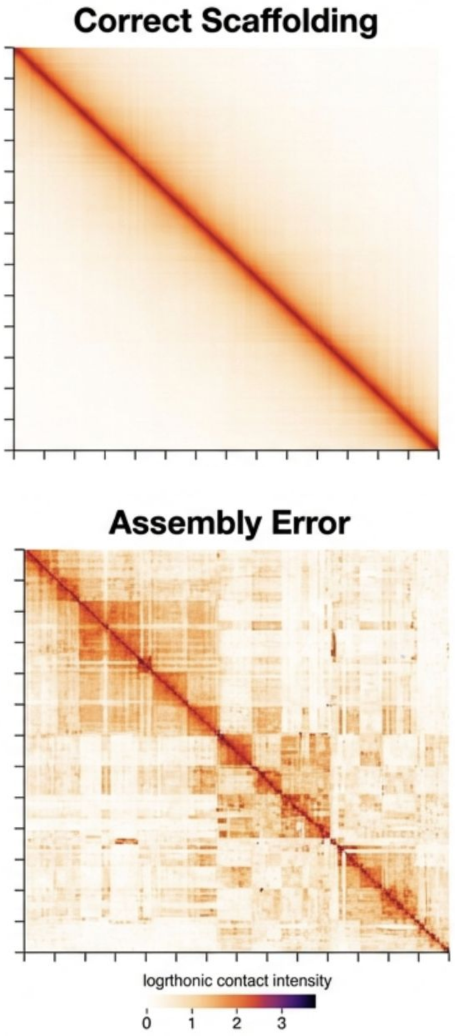
PART I — Genome architecture–aware phylogenomics

When genome architecture can mislead

- Assembly errors mimic rearrangements
- TE-driven convergence of breakpoints
- Paralogy confounds synteny blocks

Rules

- Chromosome-level assemblies are mandatory (good quality!!)
- Hi-C data needs to be comparable (same kits/enzymes) & of enough depth



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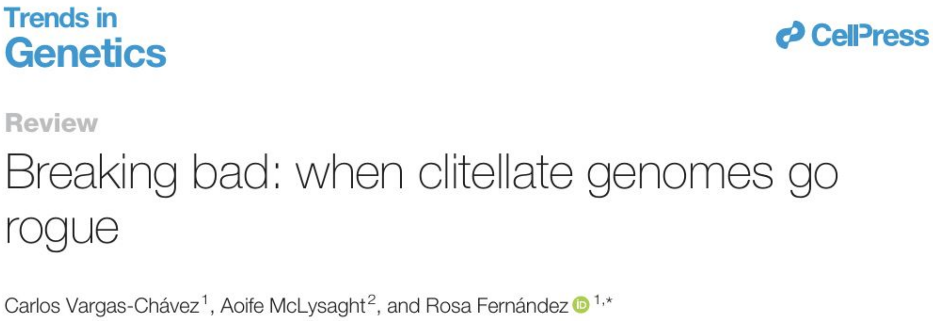


Trends in Genetics

CellPress

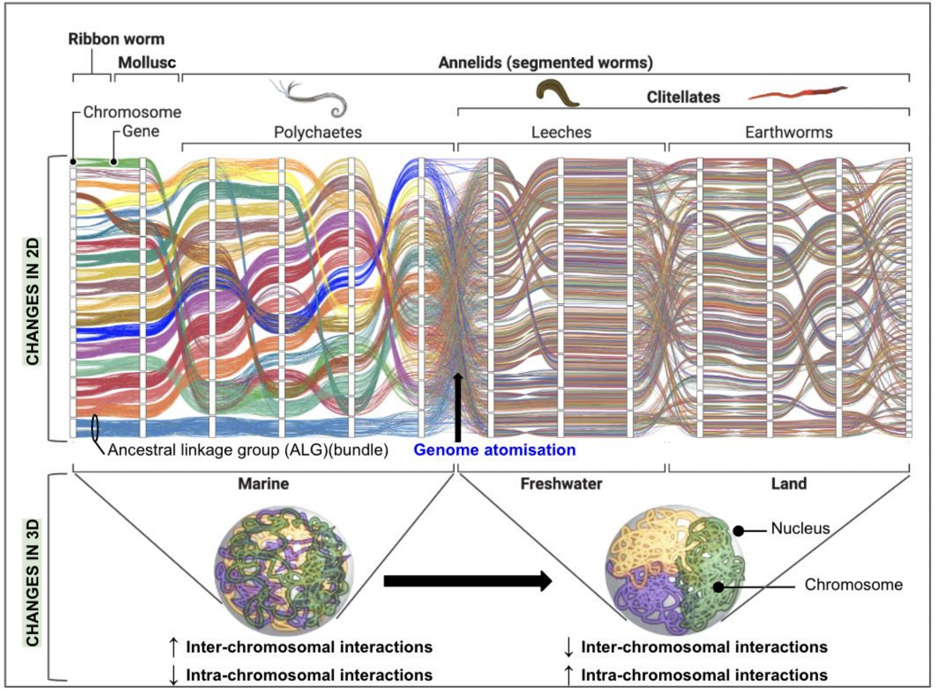
Review

Breaking bad: when clitellate genomes go rogue

Carlos Vargas-Chávez[1], Aoife McLysaght[2], and Rosa Fernández[1,*]

Can 3D data inform phylogeny?

- Comparative contact decay curves
- Compartment similarity metrics
- Architecture-aware distance measures: ‘3D linkage groups’?


Ribbon worm
Mollusc
Annelids (segmented worms)
Clitellates
Chromosome
Gene
Polychaetes
Leeches
Earthworms
CHANGES IN 2D
Ancestral linkage group (ALG)(bundle)
Genome atomisation
Marine
Freshwater
Land
CHANGES IN 3D
Nucleus
Chromosome
↑ Inter-chromosomal interactions
↓ Intra-chromosomal interactions
↓ Inter-chromosomal interactions
↑ Intra-chromosomal interactions

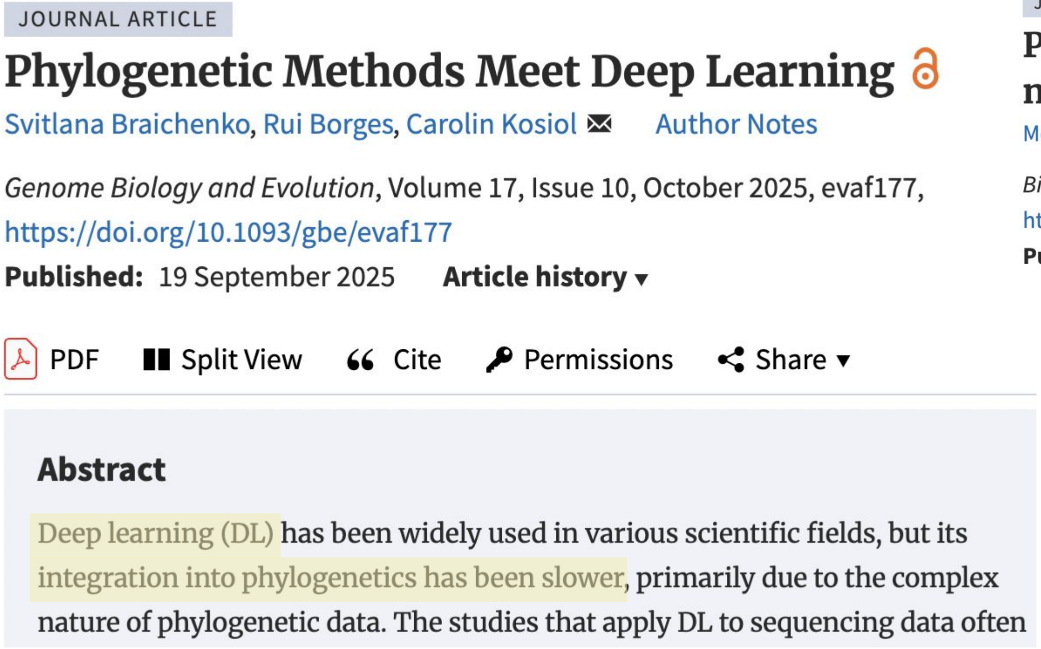
Are we there yet?

- Promising, exploratory, not standardized yet. A lot of fun work to do here!!

PART II — AI-assisted phylogenomics

Two main ‘lines’ of development of methods

- Complex pattern recognition via Machine learning & Deep learning



JOURNAL ARTICLE

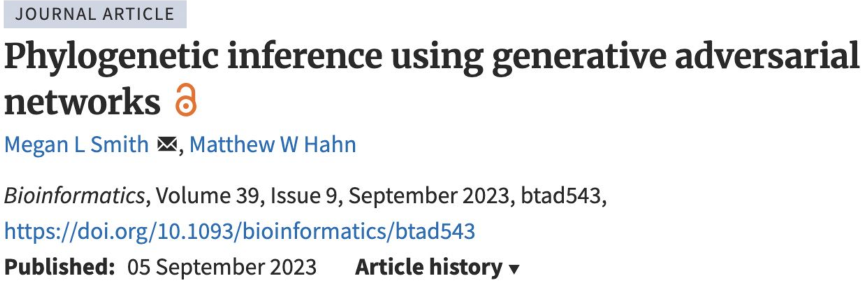
Phylogenetic Methods Meet Deep Learning

Svitlana Braichenko, Rui Borges, Carolin Kosiol ✉ Author Notes

Genome Biology and Evolution, Volume 17, Issue 10, October 2025, evaf177,
https://doi.org/10.1093/gbe/evaf177
Published: 19 September 2025 **Article history** ▾

Abstract

Deep learning (DL) has been widely used in various scientific fields, but its integration into phylogenetics has been slower, primarily due to the complex nature of phylogenetic data. The studies that apply DL to sequencing data often



JOURNAL ARTICLE

Phylogenetic inference using generative adversarial networks

Megan L Smith ✉, Matthew W Hahn

Bioinformatics, Volume 39, Issue 9, September 2023, btad543,
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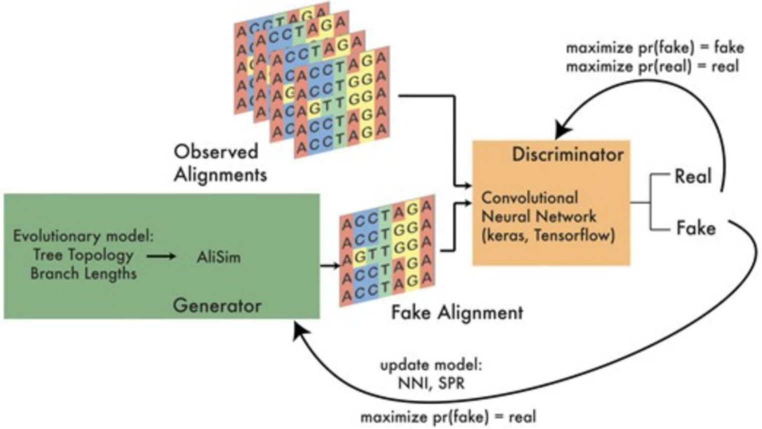
Published: 05 September 2023 **Article history** ▾



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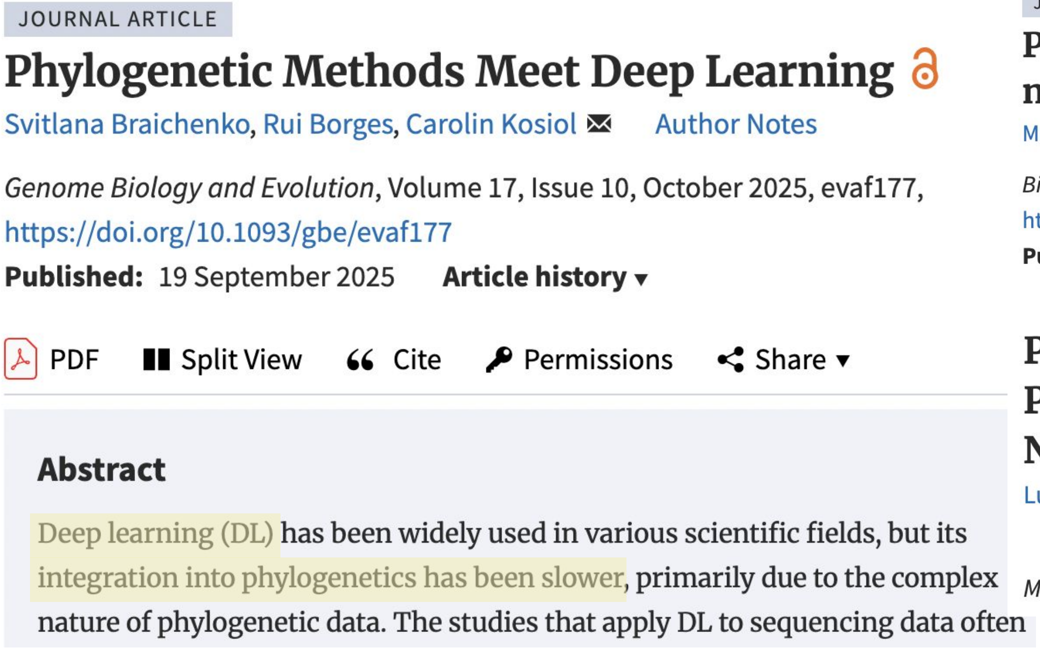

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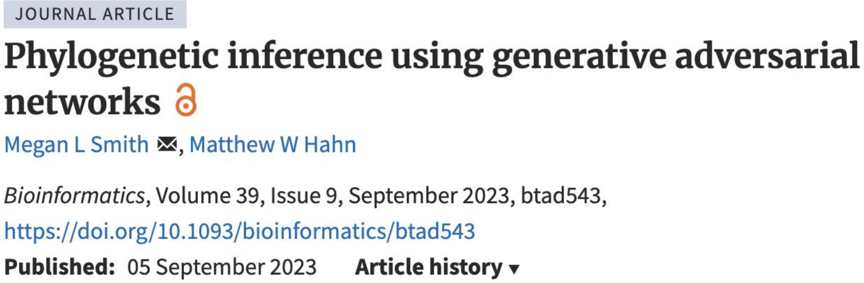

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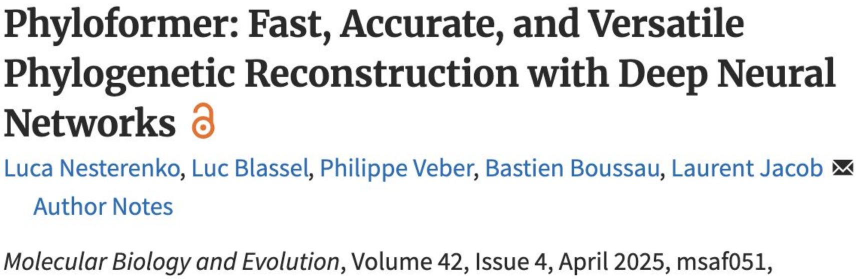

Phyloformer: Fast, Accurate, and Versatile Phylogenetic Reconstruction with Deep Neural Networks

Luca Nesterenko, Luc Blassel, Philippe Veber, Bastien Boussau, Laurent Jacob ✉ Author Notes

Molecular Biology and Evolution, Volume 42, Issue 4, April 2025, msaf051,

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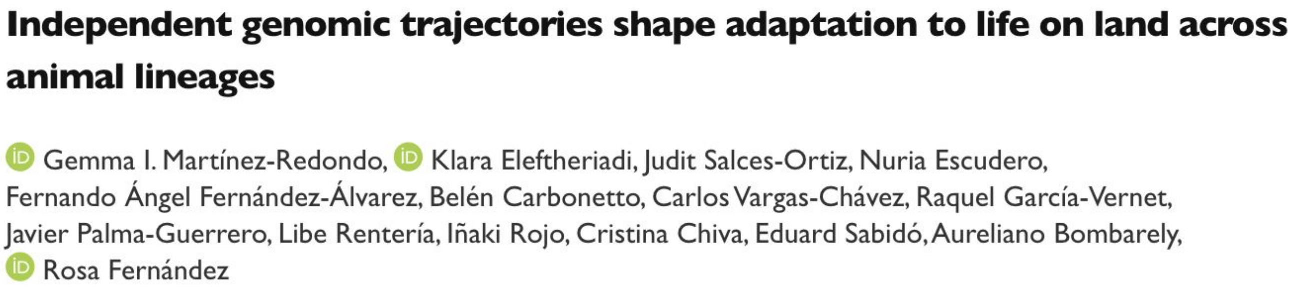
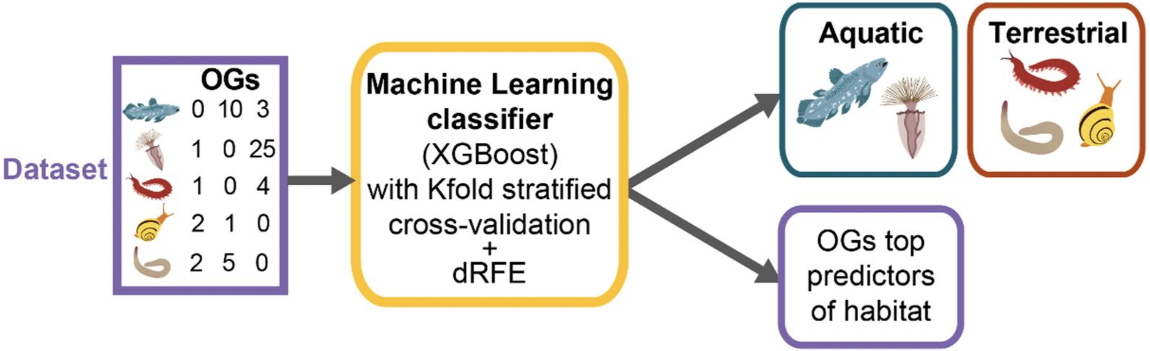
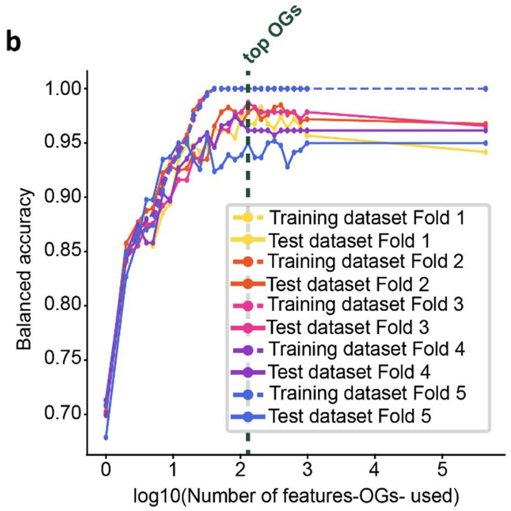


Independent genomic trajectories shape adaptation to life on land across animal lineages

Gemma I. Martínez-Redondo, Klara Eleftheriadi, Judit Salces-Ortiz, Nuria Escudero, Fernando Ángel Fernández-Álvarez, Belén Carbonetto, Carlos Vargas-Chávez, Raquel García-Vernet, Javier Palma-Guerrero, Libe Rentería, Iñaki Rojo, Cristina Chiva, Eduard Sabidó, Aureliano Bombarely, Rosa Fernández

Ca. 1,000 animal genomes, 24M genes, 520k orthogroups (OGs)


Dataset
OGs
0 10 3
1 0 25
1 0 4
2 1 0
2 5 0
Machine Learning classifier (XGBoost) with Kfold stratified cross-validation + dRFE
Aquatic
Terrestrial
OGs top predictors of habitat



130 OGs are relevant for terrestrial animals (none shared across phyla)

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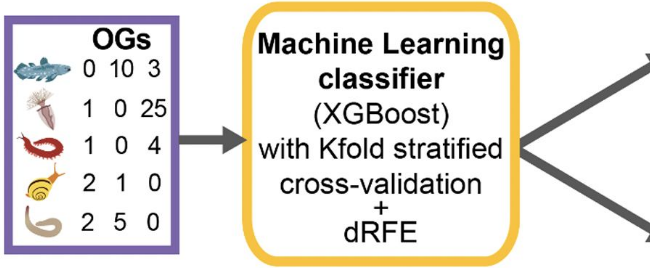
Rosa Fernández

Ca. 1,000 animal genomes, 24M genes, 5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Machine Learning classifier
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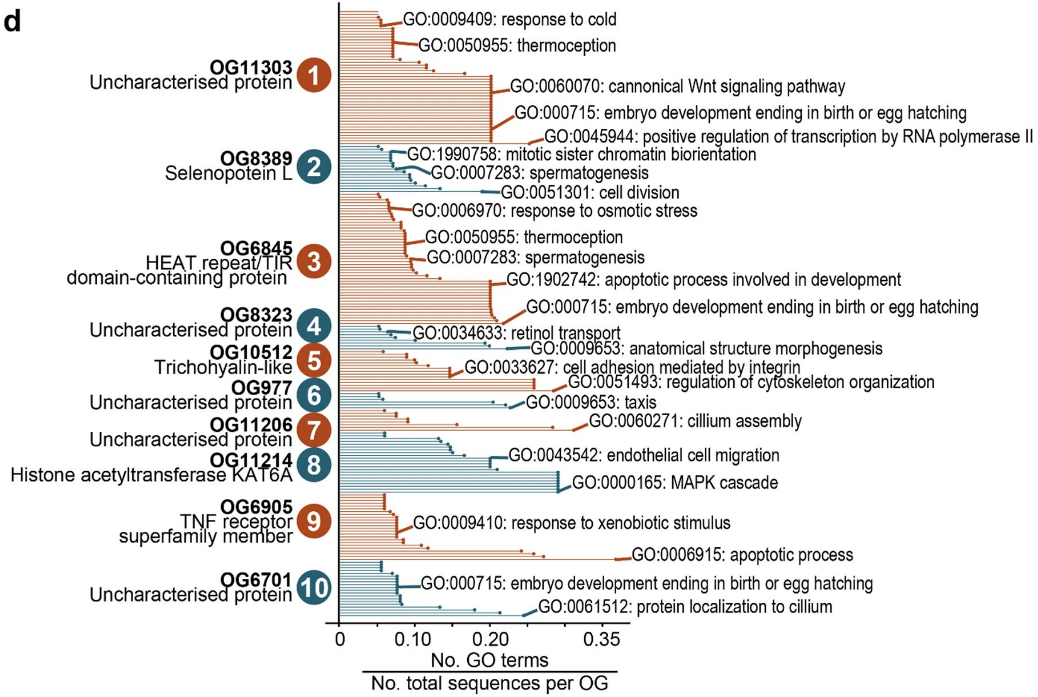
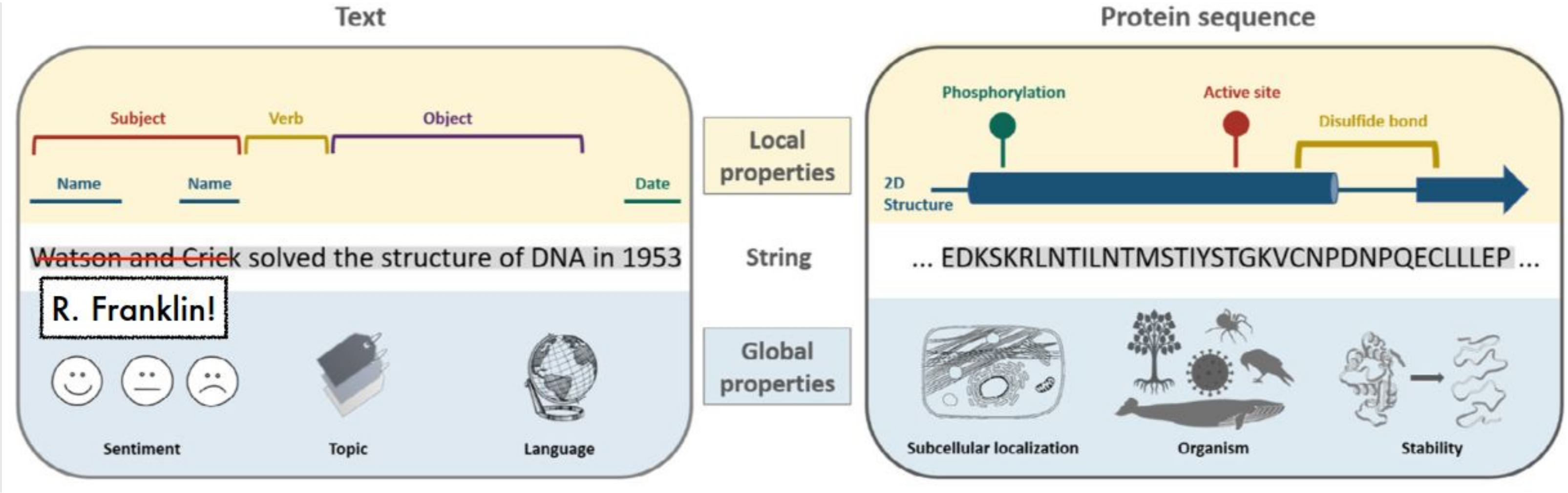


PART II — AI-assisted phylogenomics

Two main 'lines' of development of methods

- Genome/Protein Language Models to recode sequences and 'learn' the *grammar* of genomes

Encoding proteins as numerical vectors ('*embeddings*')



Ofer et al, 2021. 10.1016/j.csbj.2021.03.022. eCollection 2021.

From: www.miro.medium.com

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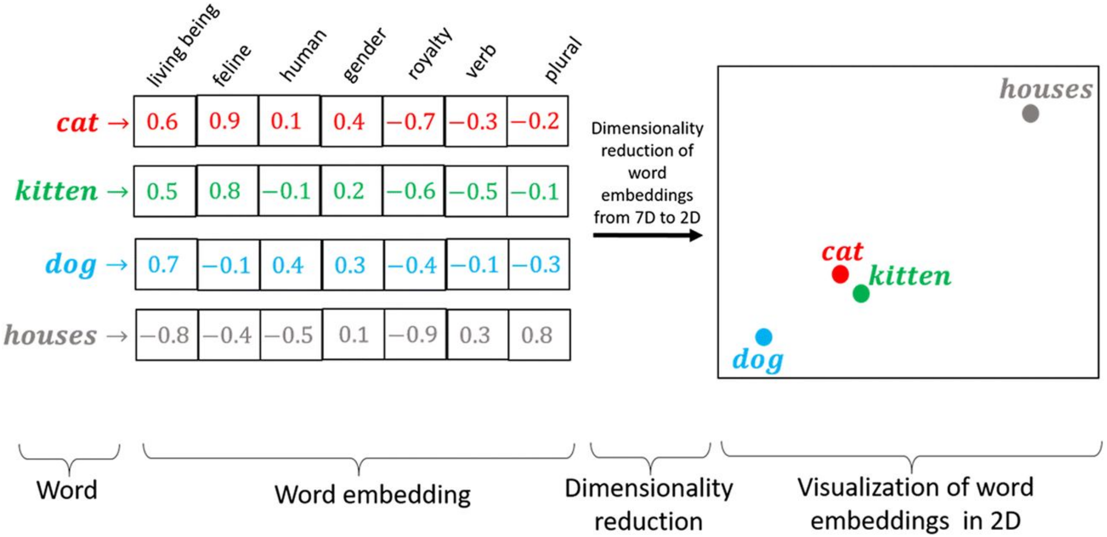
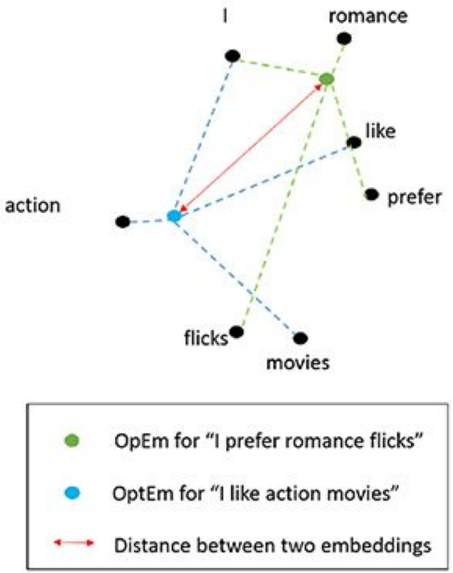


	living being	feline	human	gender	royalty	verb	plural
cat →	0.6	0.9	0.1	0.4	−0.7	−0.3	−0.2
kitten →	0.5	0.8	−0.1	0.2	−0.6	−0.5	−0.1
dog →	0.7	−0.1	0.4	0.3	−0.4	−0.1	−0.3
houses →	−0.8	−0.4	−0.5	0.1	−0.9	0.3	0.8

Dimensionality reduction of word embeddings from 7D to 2D
houses
cat
kitten
dog
Word
Word embedding
Dimensionality reduction
Visualization of word embeddings in 2D



I
romance
like
prefer
action
flicks
movies
● OpEm for "I prefer romance flicks"
● OptEm for "I like action movies"
↔ Distance between two embeddings

From: www.miro.medium.com

Gupta et al. 2020

PART II — AI-assisted phylogenomics

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Protein language models in a nutshell

- Trained on millions of protein sequences
- Learn grammar of evolution implicitly
- No alignments, no trees during training

UniRef50 (ca. 60M non-redundant proteins)

Transformer-based models work best (i.e. **ProtT5**, Ankh3)

Key insight

- Evolutionary constraints are learnable
- More informative than just the sequence
- Less bias due to indels

PART II — AI-assisted phylogenomics

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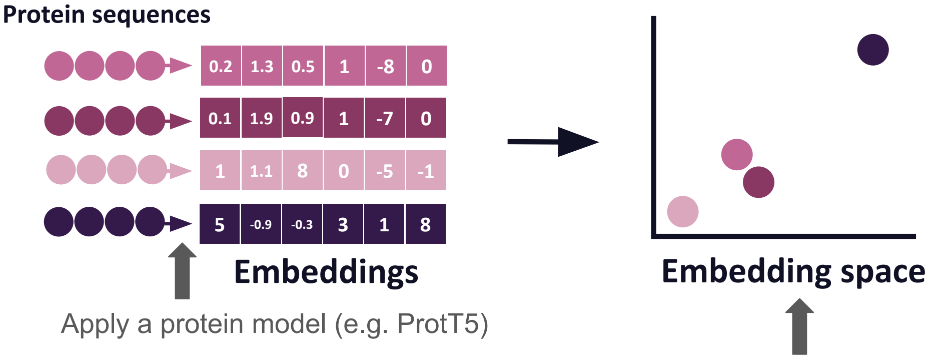
From sequences to embeddings

- Each protein → vector in high-dimensional space

- Similar function/evolution → nearby vectors



Protein sequences

0.2	1.3	0.5	1	-8	0
0.1	1.9	0.9	1	-7	0
1	1.1	8	0	-5	-1
5	-0.9	-0.3	3	1	8

Embeddings

Apply a protein model (e.g. ProtT5)

Embedding space

We can now ask questions!!

PART II — AI-assisted phylogenomics

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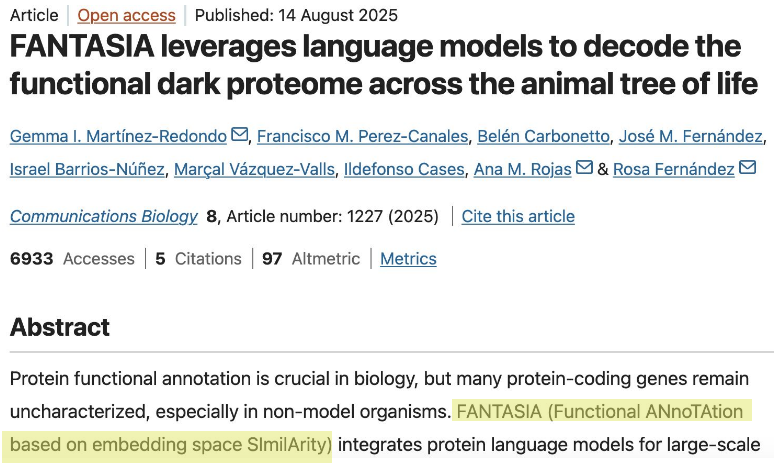


Article | Open access | Published: 14 August 2025

FANTASIA leverages language models to decode the functional dark proteome across the animal tree of life

Gemma I. Martínez-Redondo, Francisco M. Perez-Canales, Belén Carbonetto, José M. Fernández, Israel Barrios-Núñez, Marçal Vázquez-Valls, Ildefonso Cases, Ana M. Rojas & Rosa Fernández

Communications Biology **8**, Article number: 1227 (2025) | Cite this article

6933 Accesses | **5** Citations | **97** Altmetric | Metrics

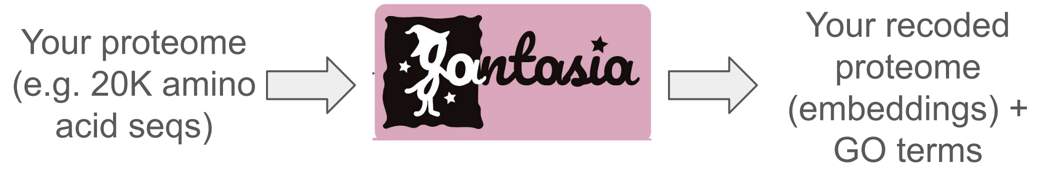
Abstract

Protein functional annotation is crucial in biology, but many protein-coding genes remain uncharacterized, especially in non-model organisms. FANTASIA (Functional ANnoTAtion based on embedding space SImilArity) integrates protein language models for large-scale

JOURNAL ARTICLE EDITOR'S CHOICE

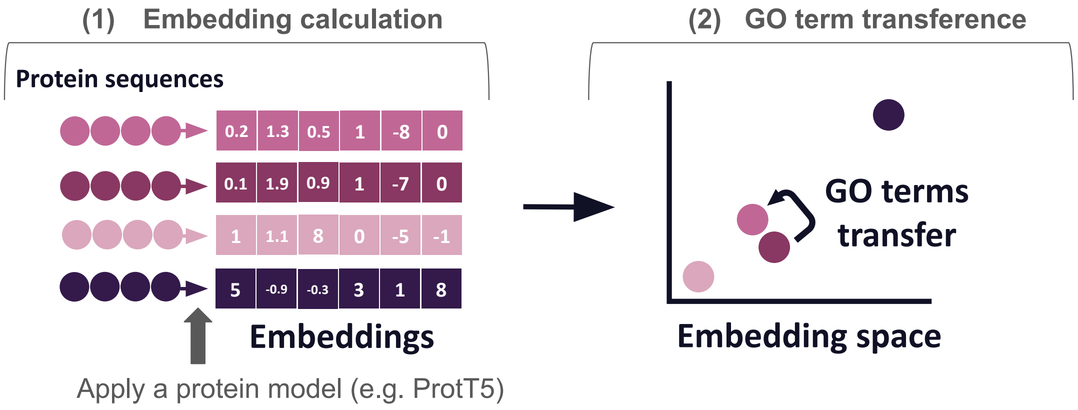
Decoding functional proteome information in model organisms using protein language models

Israel Barrios-Núñez, Gemma I Martínez-Redondo, Patricia Medina-Burgos, Ildefonso Cases, Rosa Fernández, Ana M Rojas Author Notes



Your proteome (e.g. 20K amino acid seqs)

Your recoded proteome (embeddings) + GO terms



(1) Embedding calculation

(2) GO term transference

Protein sequences

0.2	1.3	0.5	1	-8	0
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5	-0.9	-0.3	3	1	8

Embeddings

Apply a protein model (e.g. ProtT5)

GO terms transfer

Embedding space

Different language models (ProtT5, SeqVec, ESM2, Ankh3, etc)

Martínez-Redondo et al., 2025

PART II — AI-assisted phylogenomics

Two main ‘lines’ of development of methods

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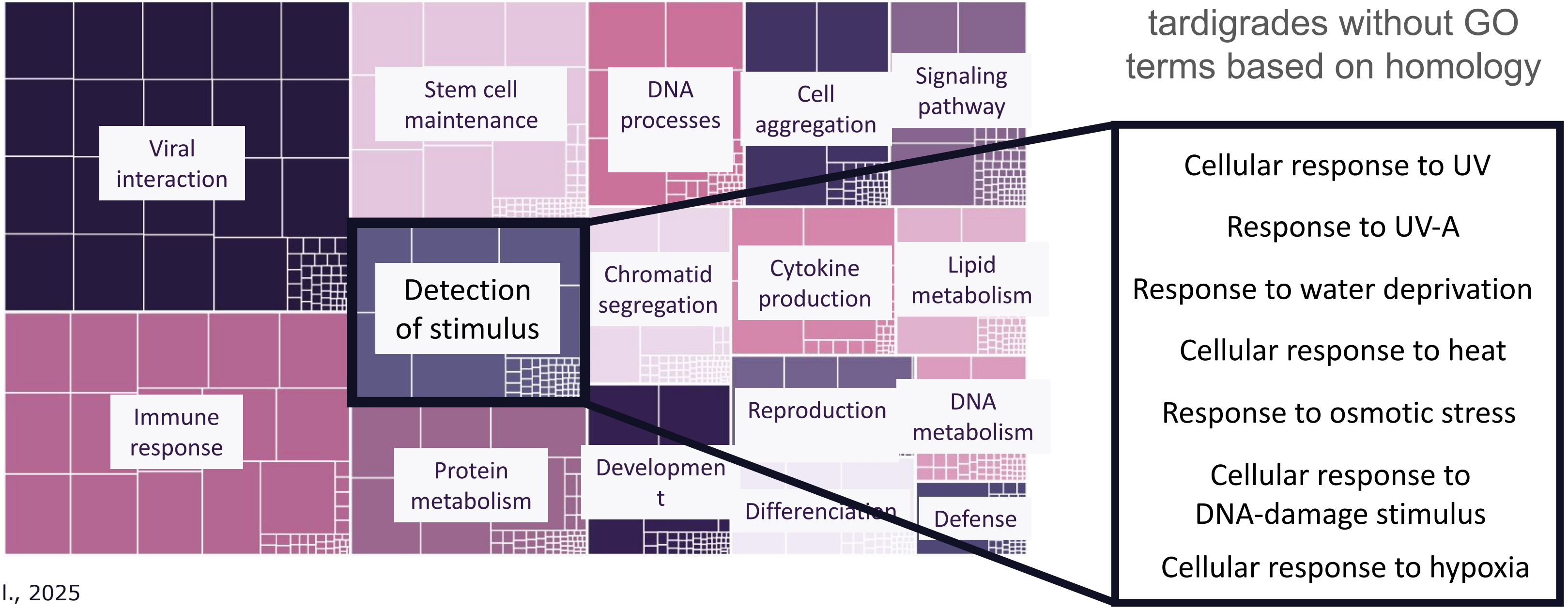
Fantasia

Investigating the ‘dark proteome’ of neglected species/lineages

GO enrichment



Cellular response to UV

Response to UV-A

Response to water deprivation

Cellular response to heat

Response to osmotic stress

Cellular response to DNA-damage stimulus

Cellular response to hypoxia

Ca. 8,000 genes in tardigrades without GO terms based on homology

Martínez-Redondo et al., 2025

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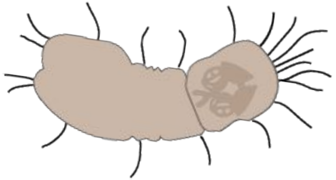
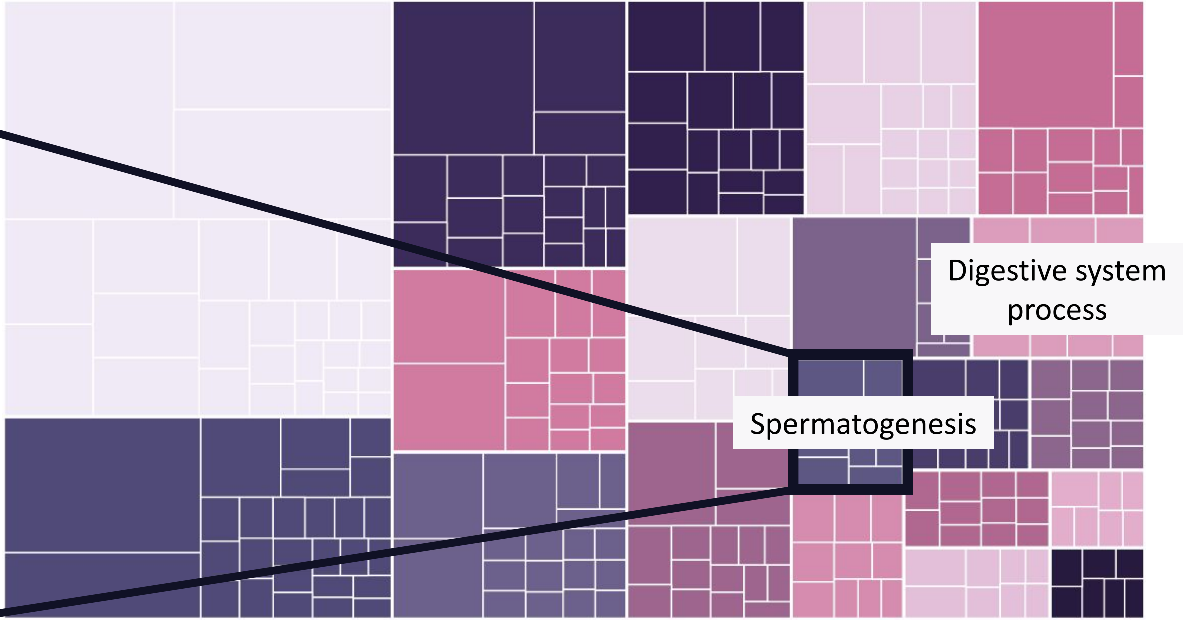


Micrognathozoa



Fusion of sperm to egg plasma membrane involved in singe fertilization

Sperm-egg recognition

Acrosome reaction

Male-female gamete recognition during double fertilization forming a zygote

Spermatogonial cell division

Male germline stem cell symetric division

Martínez-Redondo et al., 2025

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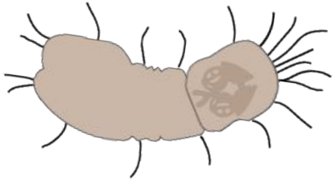

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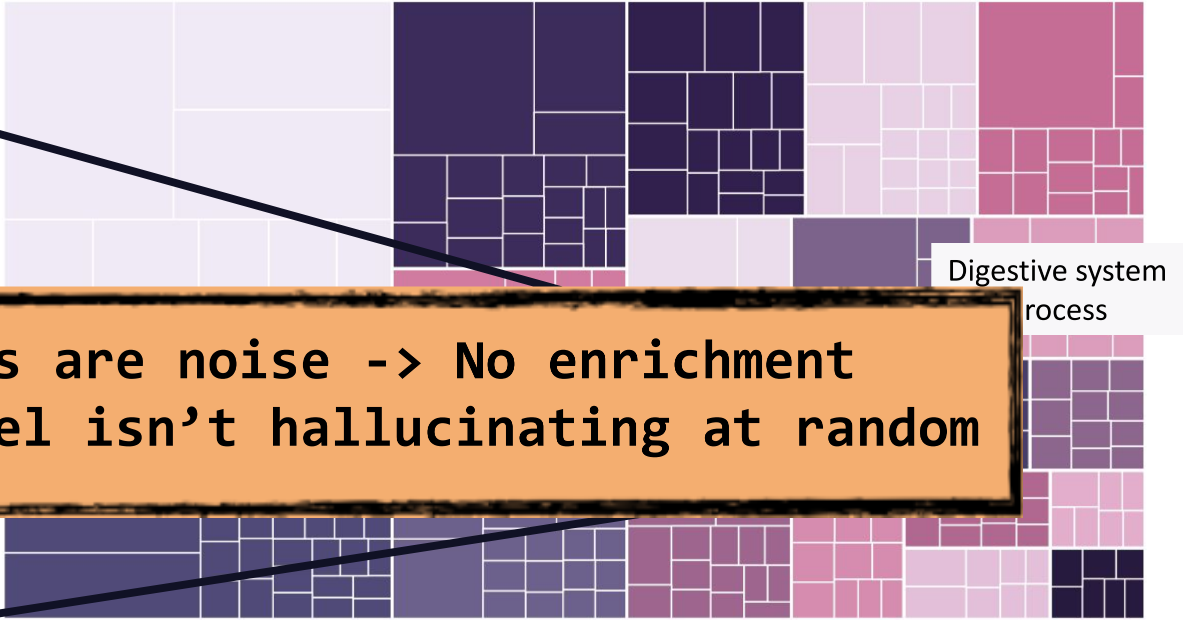

Fusion of sperm to egg plasma membrane involved in singe fertilization

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Male germline stem cell symetric division

If all high scores are noise -> No enrichment
Enrichment -> model isn't hallucinating at random

Martínez-Redondo et al., 2025

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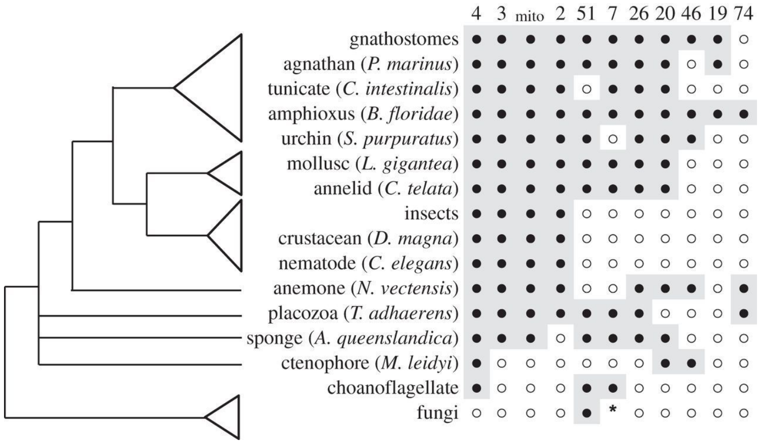
Scaling up comparative genomics (exploration of orthogroups/gene families)

1,000 animal genomes from all phyla, 24 million genes, 520K orthogroups (‘gene families’)

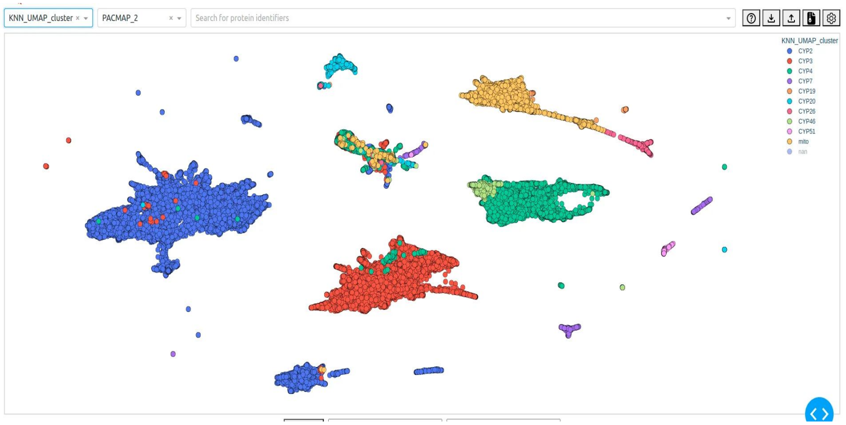
Example 1: Largest orthogroups: **CYTOCHROME P450** (83K genes; **48K** > 300 aa; 11 clans)



	4	3	mito	2	51	7	26	20	46	19	74
gnathostomes	●	●	●	●	●	●	●	●	●	●	○
agnathan (*P. marinus*)	●	●	●	●	●	●	●	●	○	●	○
tunicate (*C. intestinalis*)	●	●	●	●	○	●	●	●	○	○	○
amphioxus (*B. floridae*)	●	●	●	●	●	●	●	●	●	●	●
urchin (*S. purpuratus*)	●	●	●	●	●	○	●	●	●	○	○
mollusc (*L. gigantea*)	●	●	●	●	●	●	●	●	○	○	○
annelid (*C. telata*)	●	●	●	●	●	●	●	●	○	○	○
insects	●	●	●	●	○	○	○	○	○	○	○
crustacean (*D. magna*)	●	●	●	●	○	○	○	○	○	○	○
nematode (*C. elegans*)	●	●	●	●	○	○	○	○	○	○	○
anemone (*N. vectensis*)	●	●	●	●	○	○	●	●	●	○	●
placozoa (*T. adhaerens*)	●	●	●	●	●	●	●	○	○	○	●
sponge (*A. queenslandica*)	●	●	●	○	●	●	●	●	○	○	○
ctenophore (*M. leidyi*)	●	○	○	○	○	○	○	●	●	○	○
choanoflagellate	●	○	○	○	●	●	○	○	○	○	○
fungi	○	○	○	○	●	*	○	○	○	○	○

Nelson et al. (2013)

Embeddings + supervised Machine Learning



Martínez-Redondo et al. (in prep)

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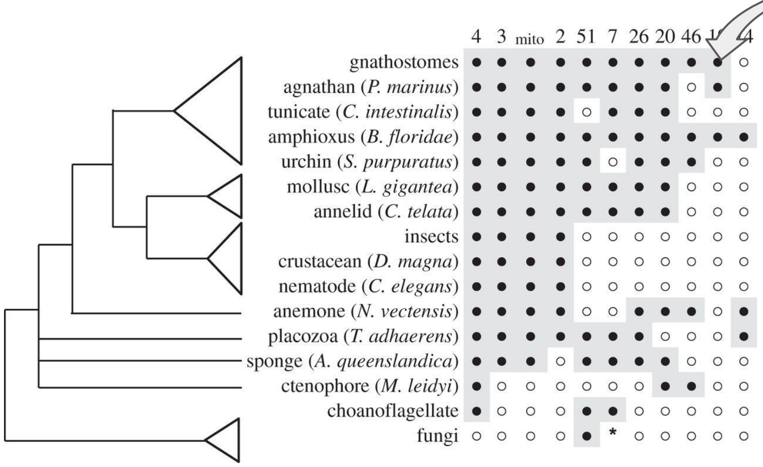

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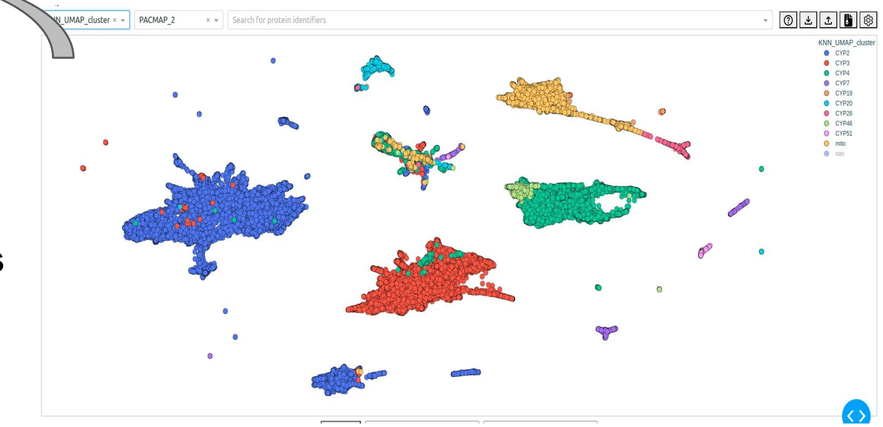
urchin (*S. purpuratus*)	●	●	●	●	●	○	●	●	●	○	○
mollusc (*L. gigantea*)	●	●	●	●	●	●	●	●	○	○	○
annelid (*C. telata*)	●	●	●	●	●	●	●	●	○	○	○
insects	●	●	●	●	○	○	○	○	○	○	○
crustacean (*D. magna*)	●	●	●	●	○	○	○	○	○	○	○
nematode (*C. elegans*)	●	●	●	●	○	○	○	○	○	○	○
anemone (*N. vectensis*)	●	●	●	●	○	○	●	●	●	○	●
placozoa (*T. adhaerens*)	●	●	●	●	●	●	●	○	○	○	●
sponge (*A. queenslandica*)	●	●	●	○	●	●	●	●	○	○	○
ctenophore (*M. leidyi*)	●	○	○	○	○	○	○	●	●	○	○
choanoflagellate	●	○	○	○	●	●	○	○	○	○	○
fungi	○	○	○	○	●	*	○	○	○	○	○

Nelson et al. (2013)

Embeddings

+

supervised Machine Learning



Martínez-Redondo et al. (in prep)

PART II — AI-assisted phylogenomics

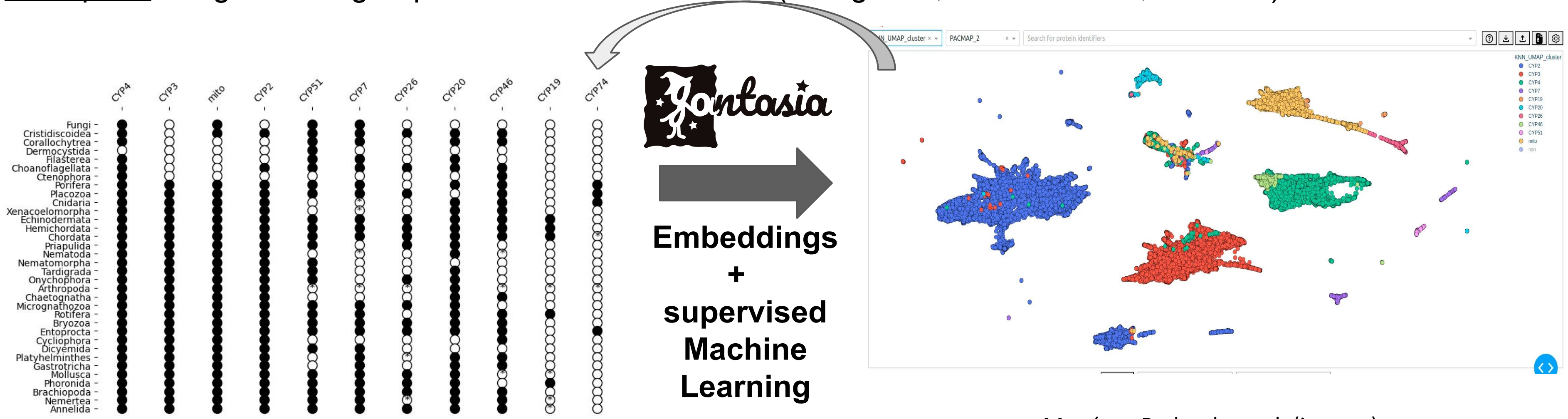
Two main 'lines' of development of methods

- Genome/<u>Protein Language Models</u> to recode sequences and 'learn' the *grammar* of genomes

Scaling up comparative genomics (exploration of orthogroups/gene families)

1,000 animal genomes from all phyla, 24 million genes, 520K orthogroups ('gene families')

<u>Example 1</u>: Largest orthogroups: **CYTOCHROME P450** (83K genes; **48K** > 300 aa; 11 clans)



Embeddings
+
supervised Machine Learning

Martínez-Redondo et al. (in prep)

PART II — AI-assisted phylogenomics

Two main ‘lines’ of development of methods

- Genome/Protein Language Models to recode sequences and ‘learn’ the *grammar* of genomes

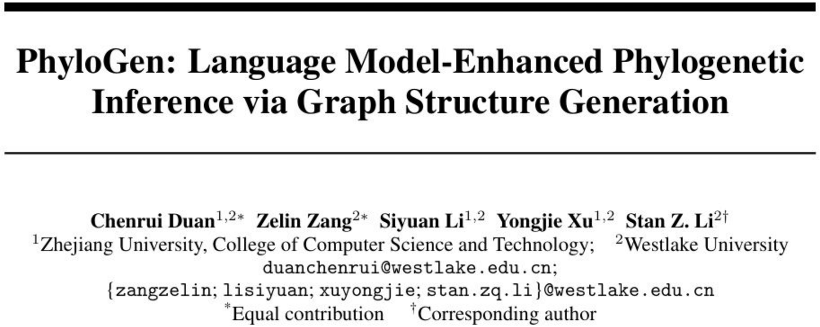
Other potential applications of embeddings

Species trees from embeddings (in progress)

- Aggregate protein embeddings across genomes
- Compute genome–genome distances
- Infer species relationships without MSAs

Caution

- Functional and phylogenetic signals are entangled
- Models also learn dataset biases



PhyloGen: Language Model-Enhanced Phylogenetic Inference via Graph Structure Generation

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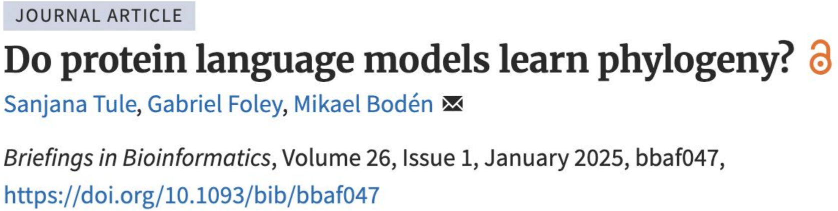
Genome language model (DNABERT2)



JOURNAL ARTICLE

Do protein language models learn phylogeny?

Sanjana Tule, Gabriel Foley, Mikael Bodén ✉

Briefings in Bioinformatics, Volume 26, Issue 1, January 2025, bbaf047, https://doi.org/10.1093/bib/bbaf047

Protein language models
Individual gene trees w/o MSA

PART II — AI-assisted phylogenomics

From gene phylogenies to embedding trees - Conceptual challenges

Embedding-based phylogenomics forces a redefinition of what is being inferred, shifting from explicit models of mutational change to implicit representations of evolutionary constraint. It demands new criteria for interpretation, validation, and trust.

A few (of many) open questions

- **Are embedding distances measures of ancestry, evolutionary constraint, or learned functional similarity? Can these be disentangled?**
- **What replaces explicit models of sequence evolution? What is the implicit evolutionary process acting on embeddings, and can it be formalized and validated?**
- **How should uncertainty and statistical support be defined?**
- **Under which evolutionary regimes (deep time, high divergence, domain reshuffling, convergence) do embeddings provide genuinely new or more reliable signal?**

PART II — AI-assisted phylogenomics

Take-home message

From: https://michellekassorla.substack.com/p/an-ai-playground

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ERGA
European Reference Genome Atlas

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