Molecular phylogenetics

Molecular Phylogenetics

- Lectures in the mornings: Niklas Wahlberg and Jadranka Rota
- Practicals in the afternoon: joined by Hamid Ghanavi and Leidys Murillo-Ramos

- https://github.com/niklas-w/Molecular-systematics-course

Introduction to the course

- Aims of course
- Overview – why molecular phylogenetics?
- Some basic concepts e.g. phylogeny, monophyly, homology & analogy
- Exploring patterns in sequence data

Aims of the course:

- To introduce the theory of phylogenetic inference from molecular data
- To provide an introduction to some of the most used methods (and computer programs)

The questions

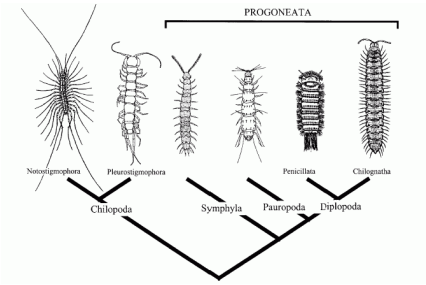
- What is a phylogeny?
- Why are we interested in phylogenies?
- Why should we use molecular data (sequences) to infer phylogenies?

The very basic facts

- What we see today in nature is the outcome of what has happened in the past
- Ecology and evolution are inseparable
- "Species" or "genes" are not individual entities without any connections to other species or genes
  - phylogeny

What is a phylogeny?

- A phylogeny is the historical genealogy of a group of species



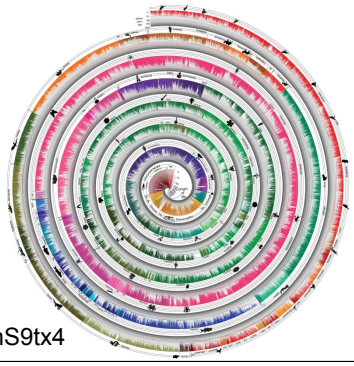
A phylogeny is an inference

- Envisioned as a dichotomously branching tree

- A phylogeny cannot be observed
- A phylogenetic hypothesis can be inferred from observed data

What we are after

- Phylogenies – the Tree of Life
- With phylogenies we are attempting to get a good working framework for Life
- Getting to the root of how evolution has worked



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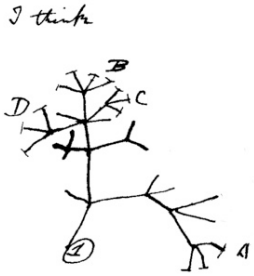
- ”Nothing makes sense in biology except in the light of evolution”

  – Dobzhansky 1973

- ”Nothing in evolution makes sense except in the light of phylogeny”

  – Savage 1997

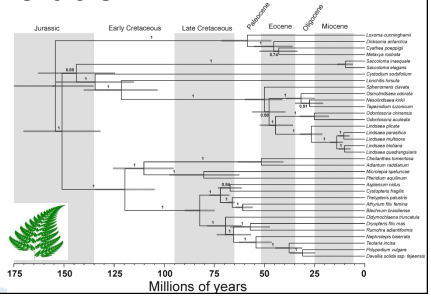


The rise of systematics

- Within the last 25 years the number of phylogenetic studies has skyrocketed
- Largely due to the advent of easy DNA sequencing methods
- Is helping us understand biodiversity and evolutionary processes better

Systematics is…

- The study of the kinds and diversity of life
- The study of character evolution
- The study of historical biogeography
- The study of the temporal framework of evolution
- The study of molecular evolution



Why *molecular* systematics?

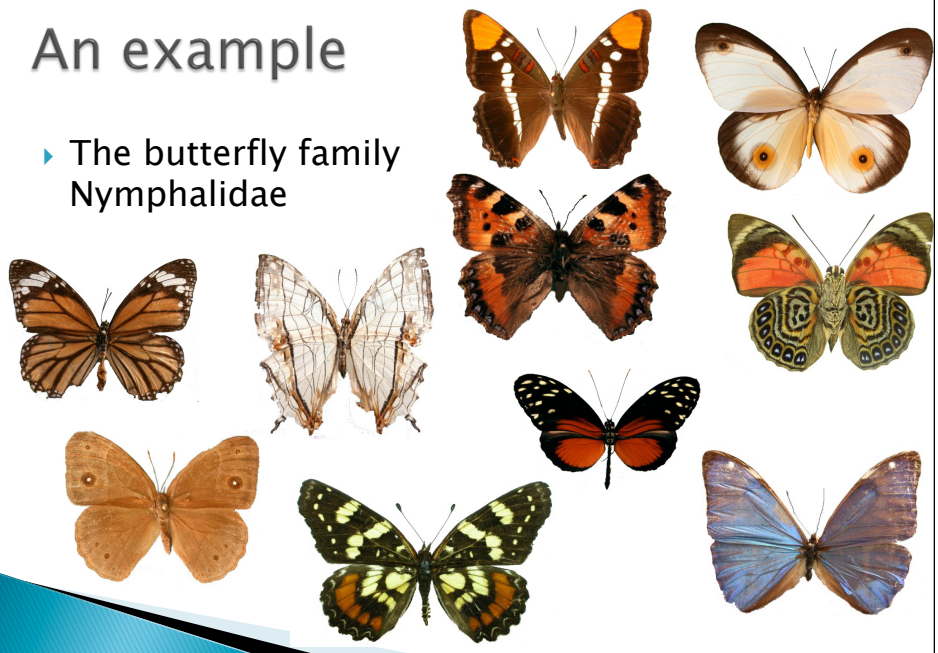
- Ease of data generation for large numbers of taxa
- Ease of generating a large number of independent data sets for given taxa
- Molecular characters behind the morphological characters we see

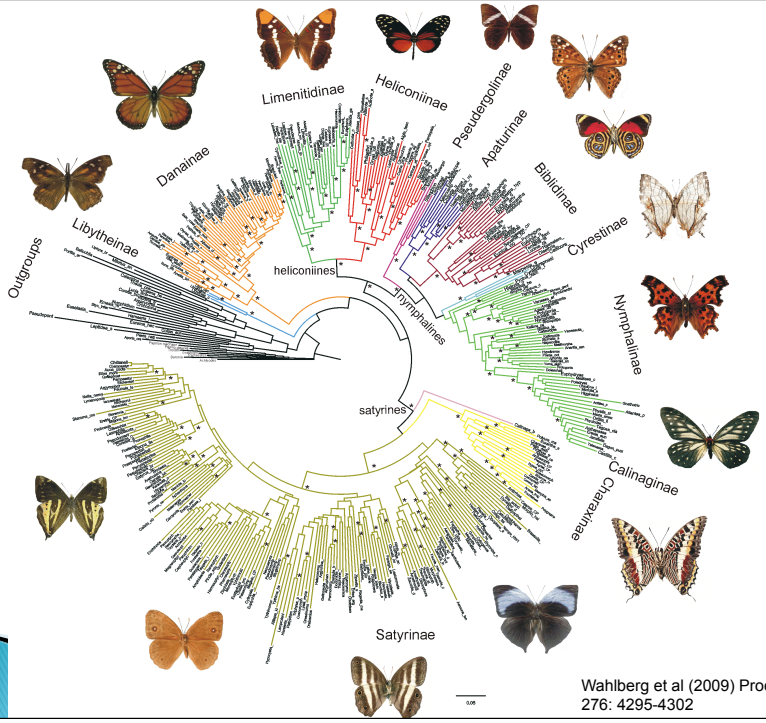
Molecular systematics as a part of molecular evolution

- Biochemistry — basic low-level processes (e.g., nucleotide substitution, amino acid interactions)
- Molecular genetics — fundamental genetic processes (e.g., DNA replication, recombination)
- Population genetics — micro-evolutionary processes
- Systematics — macro-evolutionary processes

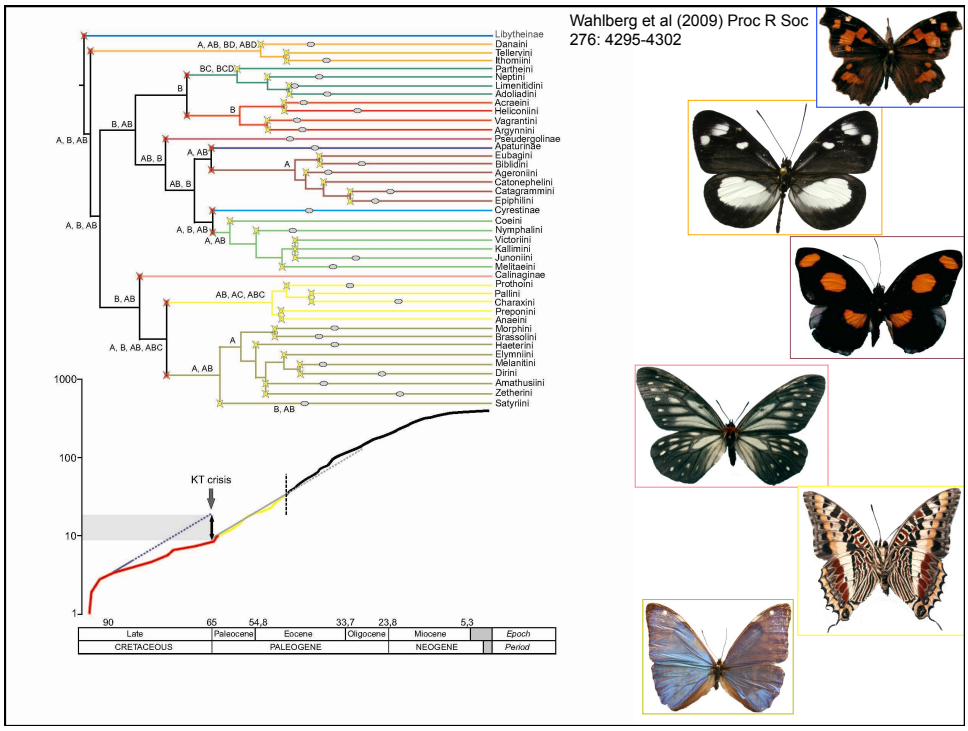
An example

- The butterfly family Nymphalidae





Wahlberg et al (2009) Proc R Soc 276: 4295-4302



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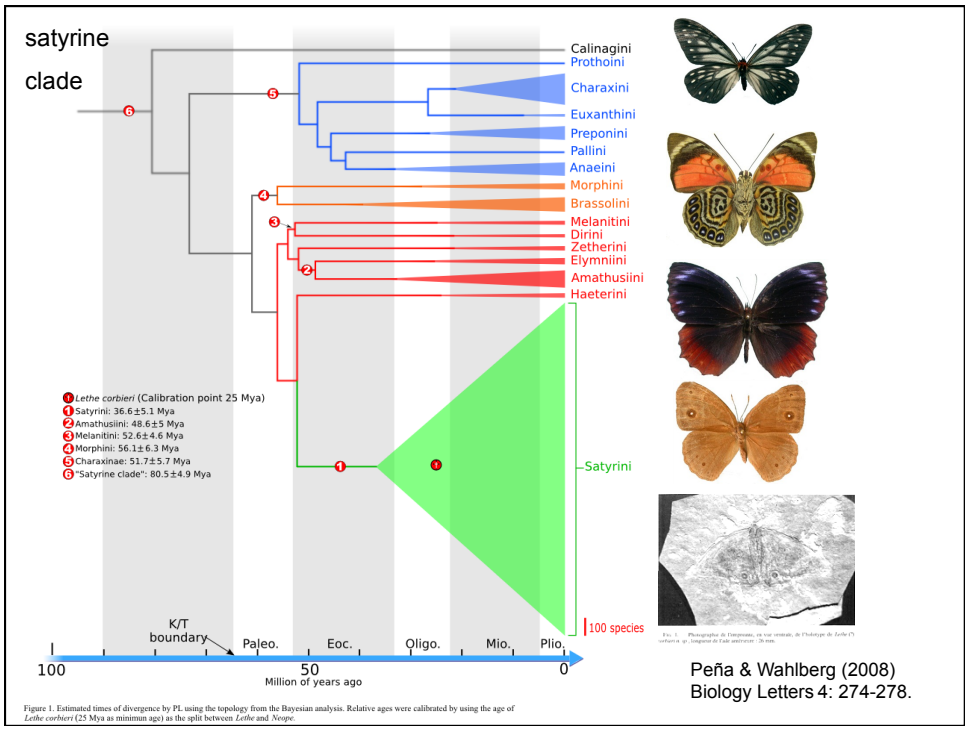
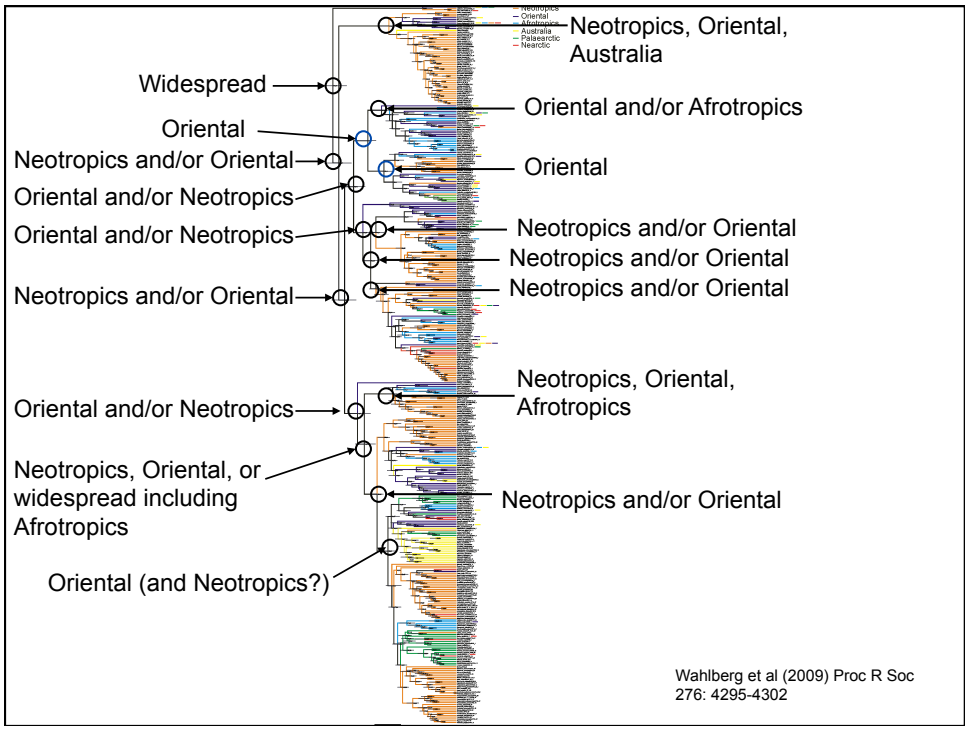


Figure 1. Estimated times of divergence by PL using the topology from the Bayesian analysis. Relative ages were calibrated by using the age of *Lethe corbieri* (25 Mya as minimum age) as the split between *Lethe* and *Neope*.

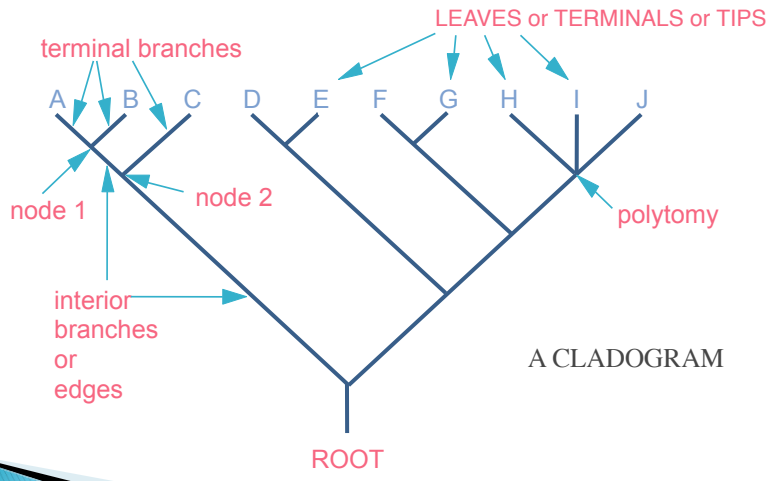
Peña & Wahlberg (2008) Biology Letters 4: 274-278.



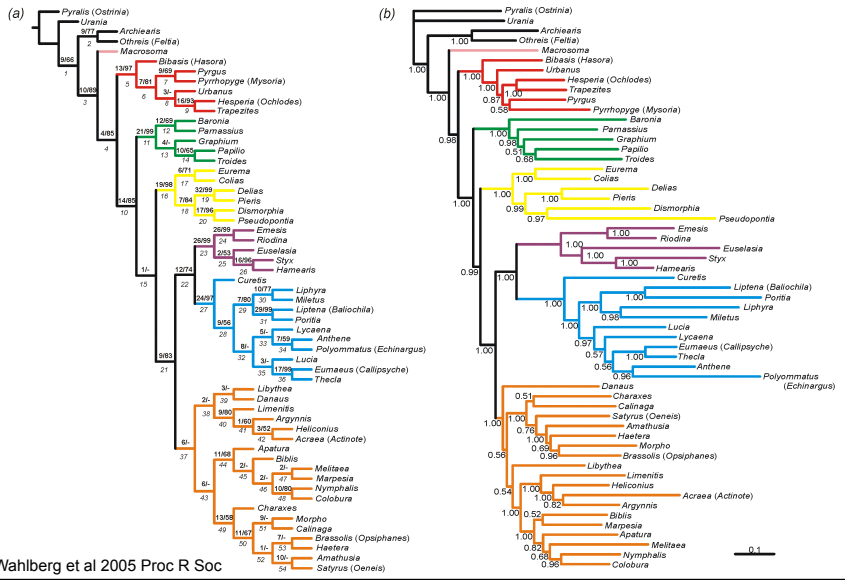
Some basic concepts

- Cladogram – a tree diagram which depicts a hypothesised evolutionary history
- Phylogram – a tree which indicates by branch length the degree of change believed to have occurred along each lineage
- Chronogram – a tree in which branch lengths are directly in proportion to time

Phylogenetic Trees

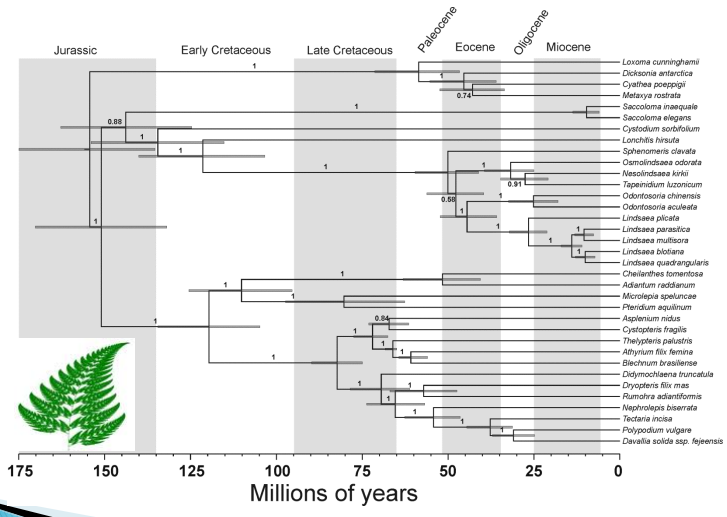


Cladograms and phylograms



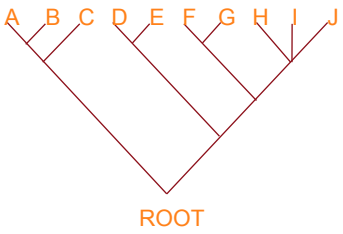
Wahlberg et al 2005 Proc R Soc

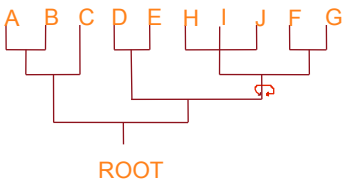
Chronogram

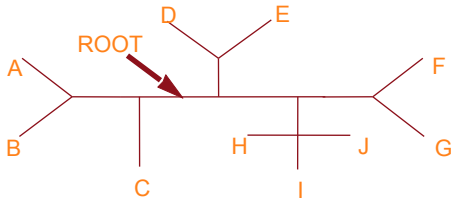


Lehtonen et al. 2012: Bot J Linn Soc 170:489.

Trees – Rooted and Unrooted





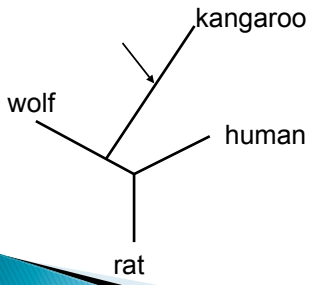


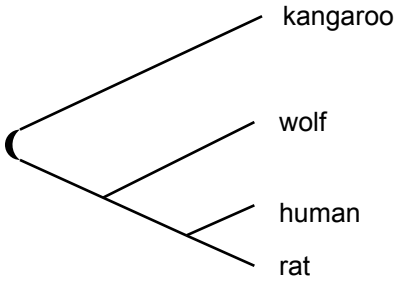
Rooting a tree

- Rooting a tree using outgroups
  - Place the root on the branch leading to the outgroup taxon
  - Use outgroup taxa in the analysis (rarely done)
- Other ways of rooting a tree
  - Assume a molecular clock
  - Midpoint rooting (root on the longest branch)

Outgroup rooting of unrooted trees

- Outgroup – related sequence that definitely diverged earlier (paleontological evidence)
- Not too distantly related (tree method becomes unreliable)

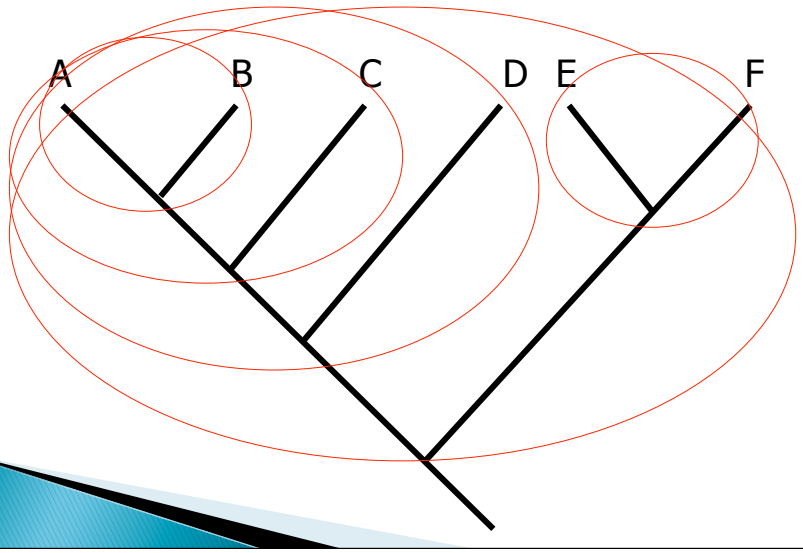




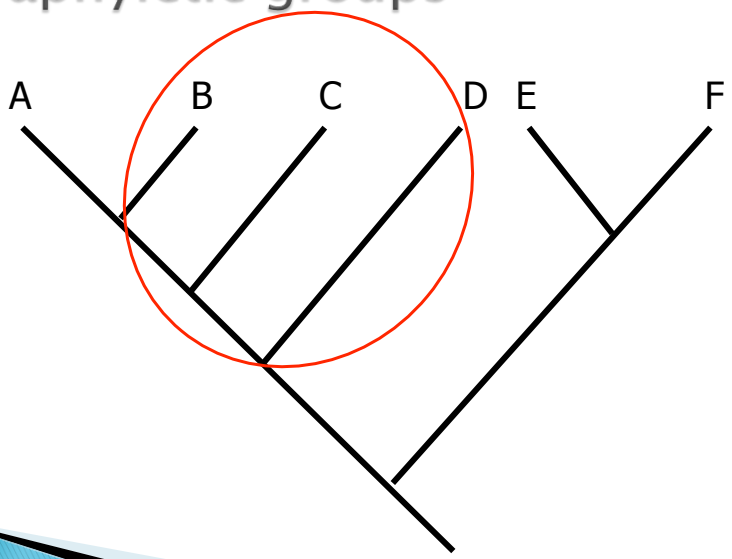
Phylogenetic systematics

- Uses tree diagrams to portray relationships based upon recency of common ancestry
- Monophyletic groups (clades) – contain species which are more closely related to each other than to any outside of the group

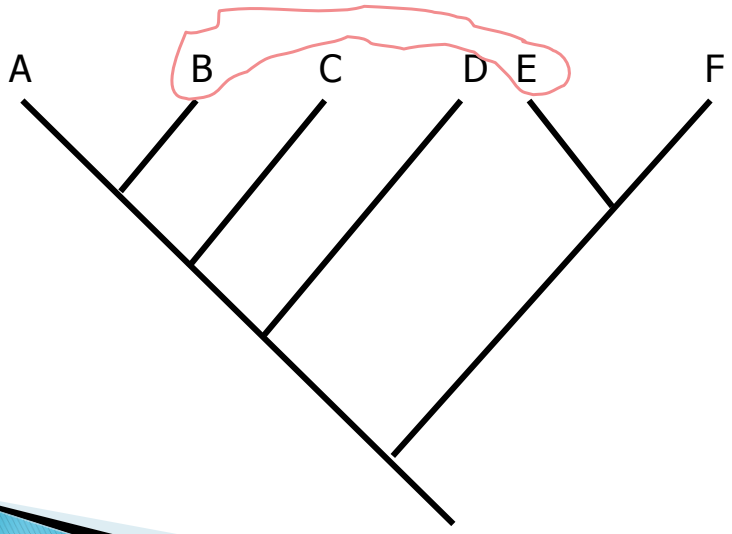
Monophyletic groups



Paraphyletic groups



Polyphyletic groups

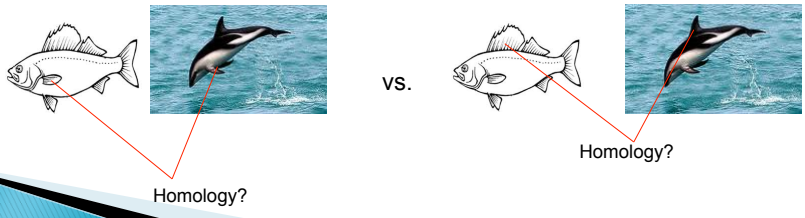


Some premises underlying phylogenetic inferences

- Sees homology as evidence of common ancestry
- Phylogenetic inferences are premised on the inheritance of ancestral characters, and on the existence of an evolutionary history defined by changes in these characters
- A tree-like model of evolution
  - paralogy and lateral transfer?

Homology

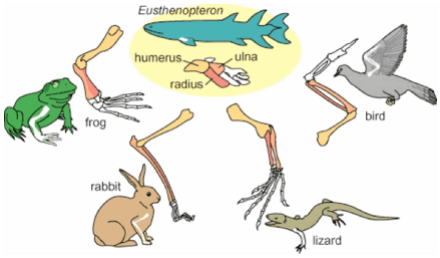
- The most fundamental concept in inferring phylogeny is homology
- We need to be sure the characters we are studying are homologous, ie "the same" character in different organisms
- Otherwise our analyses will be misled



Owen's definition of homology

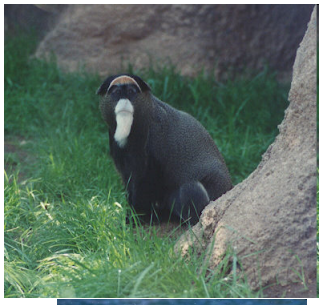
Homologue: the same organ under every variety of form and function (true or essential correspondence)

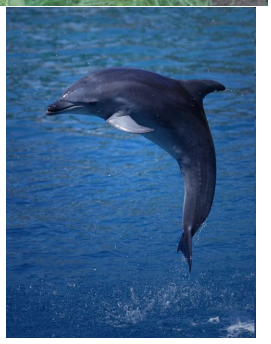
Richard Owen 1843



Homologies can be:

- Apomorphic
  - Shared derived
- Plesiomorphic
  - Shared ancestral





Character evolution

- Heritable changes (in morphology, gene sequences, etc.) produce different character states
- Similarities and differences in character states provide the basis for inferring phylogeny (i.e. provide evidence of relationships)
- The utility of this evidence depends on how often the evolutionary changes that produce the different character states occur independently

Unique and unreversed characters

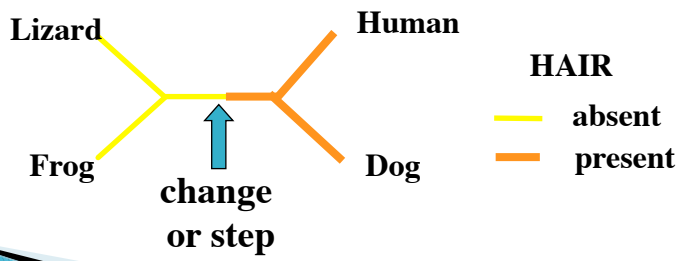
- Given a heritable evolutionary change that is unique and unreversed (e.g. the origin of hair) in an ancestral species, the presence of the novel character state in any taxa must be due to inheritance from the ancestor
- Similarly, absence in any taxa must be because the taxa are not descendants of that ancestor

Unique and unreversed characters

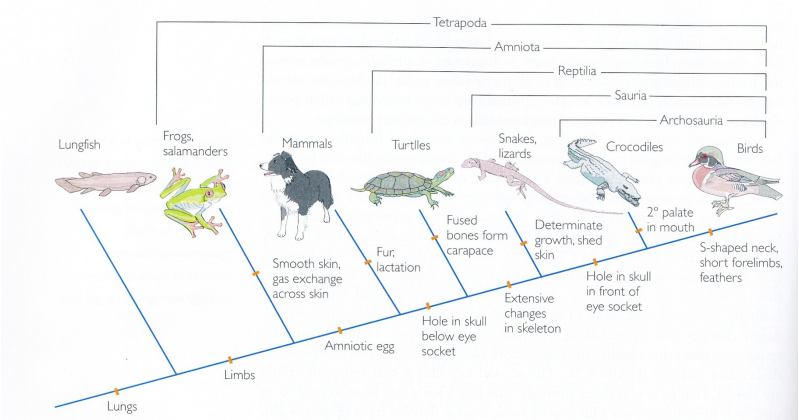
- The novelty is a *homology* acting as badge or marker for the descendants of the ancestor
- The taxa with the novelty are a clade (e.g. Mammalia)

Unique and unreversed characters

- Because hair evolved only once and is unreversed (not subsequently lost) it is *homologous* and provides unambiguous evidence for of relationships



In the ideal world



The converse of homology

- Analogy: superficial or misleading similarity
  - Homoplasy

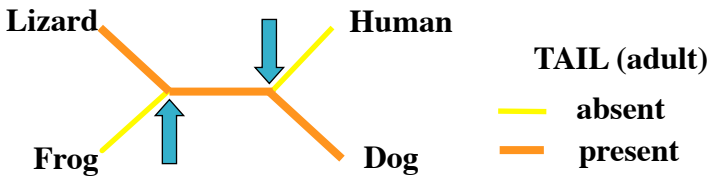
Falcon	Bat	Pteradactyl	Butterfly
↑	↑	↑	↑
Ancestral bird	Ancestral mammal	Ancestral reptile	Ancestral hexapod

Homoplasy – Independent evolution

- Homoplasy is similarity that is not homologous (not due to common ancestry)
- It is the result of independent evolution (convergence, parallelism, reversal)
- Homoplasy can provide misleading evidence of phylogenetic relationships (if mistakenly interpreted as homology)

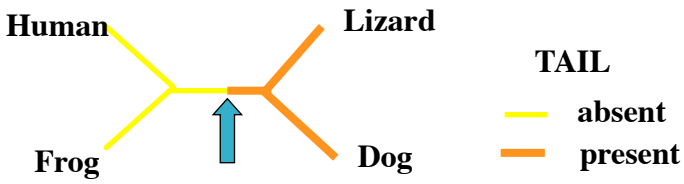
Homoplasy – independent evolution

- Loss of tails evolved independently in humans and frogs - there are two changes on the true tree



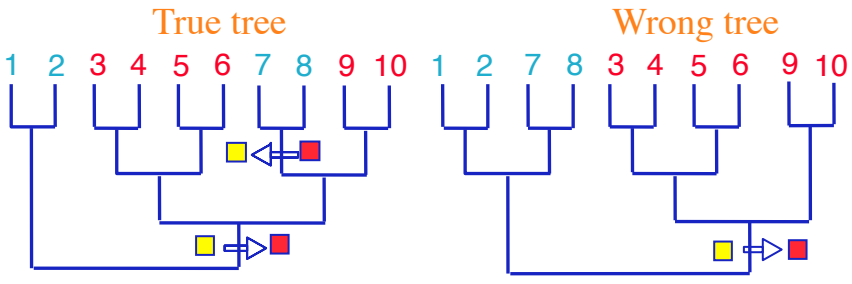
Homoplasy – misleading evidence of phylogeny

- If misinterpreted as homology, the absence of tails would be evidence for a wrong tree: grouping humans with frogs and lizards with dogs



Homoplasy – reversal

- Reversals are evolutionary changes back to an ancestral condition
- As with any homoplasy, reversals can provide misleading evidence of relationships



Homoplasy – a fundamental problem of phylogenetic inference

- If there were no homoplastic similarities inferring phylogeny would be easy – all the pieces of the jig-saw would fit together neatly
- Distinguishing the misleading evidence of homoplasy from the reliable evidence of homology is a fundamental problem of phylogenetic inference

Homoplasy in molecular data

- Incongruence and therefore homoplasy is common in molecular sequence data
  - There are a limited number of alternative character states (e.g. Only A, G, C, T and "gap" in DNA)
  - Rates of evolution are sometimes high

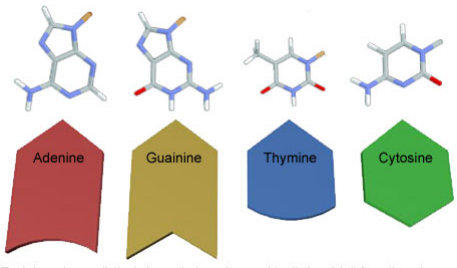
Homoplasy in molecular data

- Character states are chemically identical
  - homology and homoplasy are equally similar
  - cannot be distinguished by detailed study of similarity and differences

Purines

Pyrimidines

Figure B-3: The Four Nitrogenous Bases



Each base has a distinct shape that can be used to distinguish it form the others. 3D representations of the four bases are shown, with the corresponding chemical structures drawn above.

Phylogenetic analysis is an attempt to infer the past

- Inferring a phylogeny is an attempt to produce a best estimate of an evolutionary history based upon incomplete information
- Our direct information about the past is limited
  - Fossil record very incomplete
  - Access to contemporary species and molecules

Phylogenetic analysis requires careful thought

Phylogenetic analysis is frequently treated as a black box into which data are fed (often gathered at considerable cost) and out of which "The Tree" springs

– (Swofford et al 1996, Molecular Systematics)

Why DNA is the Ultimate Source of Information

- Higher levels lack information
  - For example, one can infer protein sequence from DNA sequence data, but not complete DNA sequence from protein sequence data
- Lower levels provide no additional useful information
  - For example, sub-atomic structure does not provide information about historical relationships

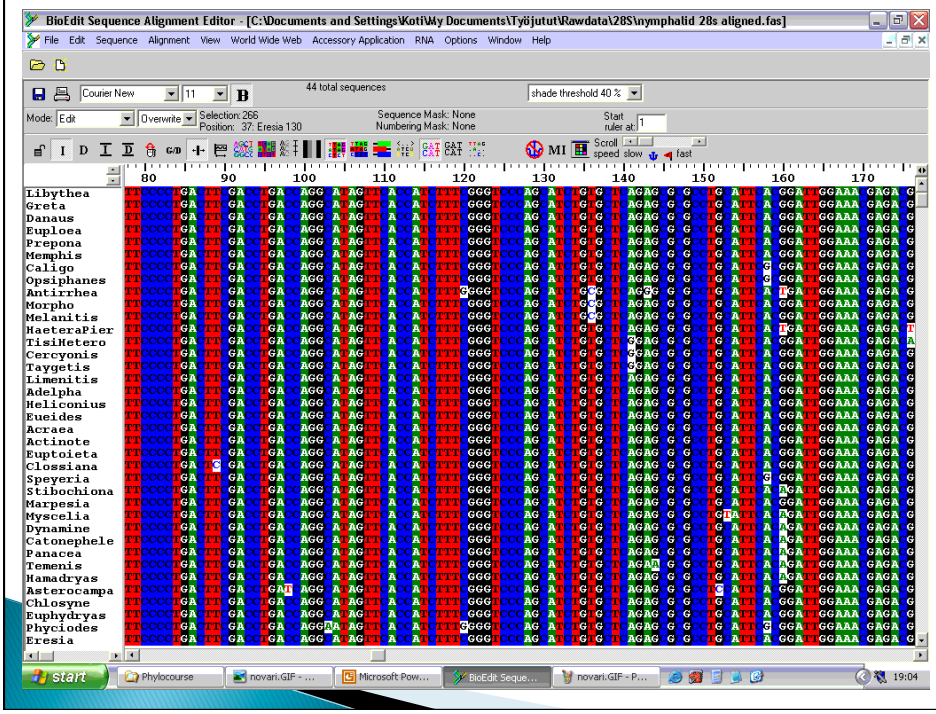
What is "Molecular" in Molecular Systematics?

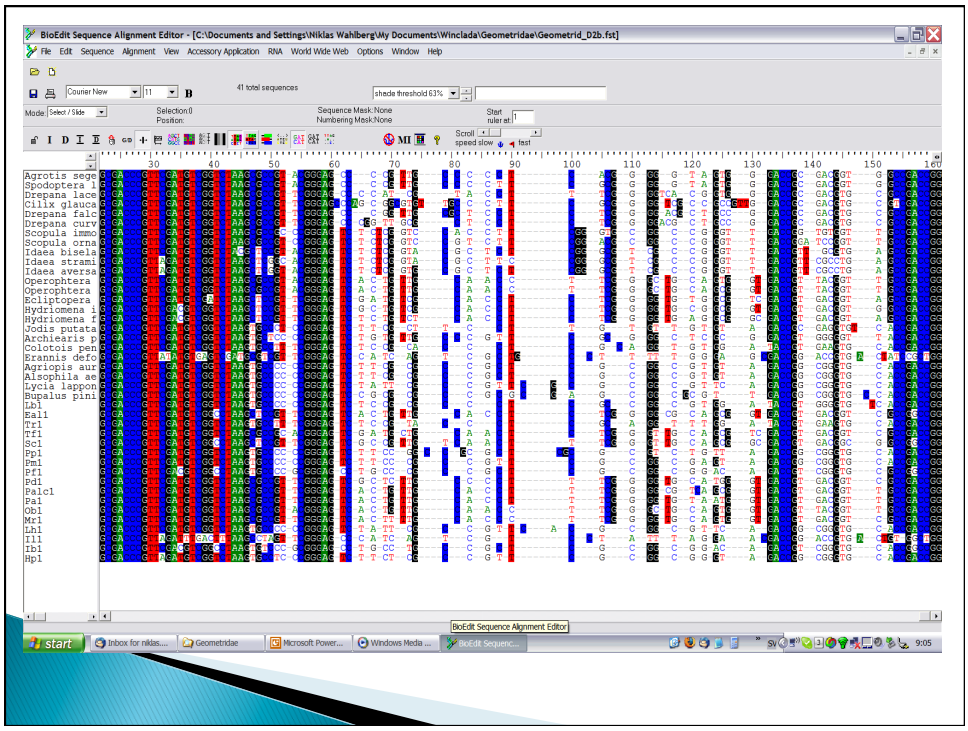
- Carbohydrates — No
- Lipids — No
- Secondary metabolites — No
- Proteins (amino acids) — Yes
- Nucleic Acids (DNA and RNA) — Yes

Proteins and DNA are sometimes described as "informational macromolecules"

What sequences should we use?

- Choice of sequence – appropriate for question (fast or slow evolving – close or distant relationships).
- Many sequences are a mosaic of different rates
  - rRNA different structural regions evolve at different rates
  - Proteins – synonymous (silent) rate (codon position 3) is often faster than nonsynonymous (positions 1 & 2 – changes aa) rate of change
  - Transitions occur more readily than transversions

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Adelpha107 1
Aglais63 3
Agraulis 24
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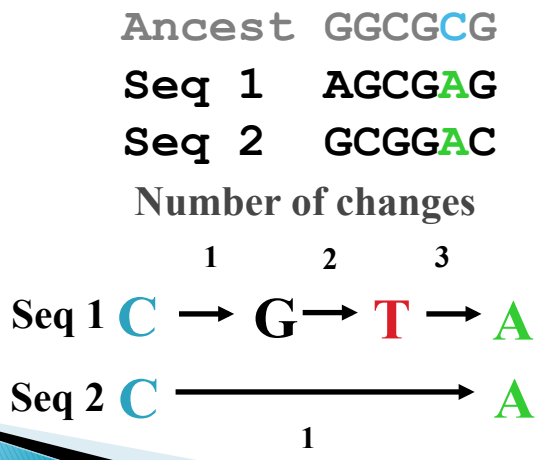
Exploring patterns in sequence data:

- Do the sequences contain phylogenetic signal for the relationships of interest? (too conserved or too variable)
- Are sequences saturated for change at the level of relationship to be investigated?
- Do sequences manifest biased base compositions (e.g thermophilic convergence) or biased codon usage patterns which may obscure phylogenetic signal?

Saturation in sequence data:

- Saturation is due to multiple changes at the same site subsequent to lineage splitting
- Models of evolution attempt to infer the missing information through correcting for "multiple hits"
- Most data will contain some fast evolving sites which are potentially saturated (e.g. in proteins often position 3)
- In severe cases the data becomes essentially random and all information about relationships can be lost

Multiple changes at a single site – hidden changes



Data

- For long, the field of systematics was restricted by the amount of data
- 10 years ago, datasets comprising 3–5 genes were the norm
- 5 years ago the genomic revolution swung into full effect
- We are now faced with an abundance of potential data, but where can we get it?

The Era of Phylogenomics

- Genomes can now be sequenced relatively easily
- Whole genomes contain a lot of information that is irrelevant for systematics, especially at deep levels
- The field of systematics is still trying to figure out how best to utilize genomic level data

Phylogenomic quandries

- What parts of the genome should be used?
- How can we get at those parts in the most efficient way?
- Where can we access specimens for our chosen methods?

Phylogenomic data

- Transcriptomes
  - All genes that are being expressed in a certain tissue at a certain time
- Ultra-Conserved Elements
  - Probes to pull out UCEs and flanking regions
- Anchored Hybrid Enrichment
  - Probes for e.g. exons of protein coding sequences
- Whole Genome Sequencing