

3. Is a particular feature adaptive or not?

Comparative Methods: the ghost of history

- “Comparative methods extract information from comparisons of two or more species in large-scale patterns **not accessible** to experimentation.” (Steve Stearns) →
- It is a method of parceling out variation to:
 1. **history** and inheritance from ancestors, vs...
 2. **adaptation** to current environments.
- There are two types (classes) of “comparative methods.”
 1. **Phylogenetic trait analysis** (we’ll come back to this).
 2. **Comparative trend analysis.**
 - This is used to compare changes in 2 or more traits across species or higher groups to reveal **correlated changes**.
 - These trends can be interpreted either as...
 1. revealing **intrinsic constraints** associated with certain body plans or phylogenetic histories, or...
 2. documenting **patterns of adaptation** to **extrinsic** selection forces (typically, **ecology**).

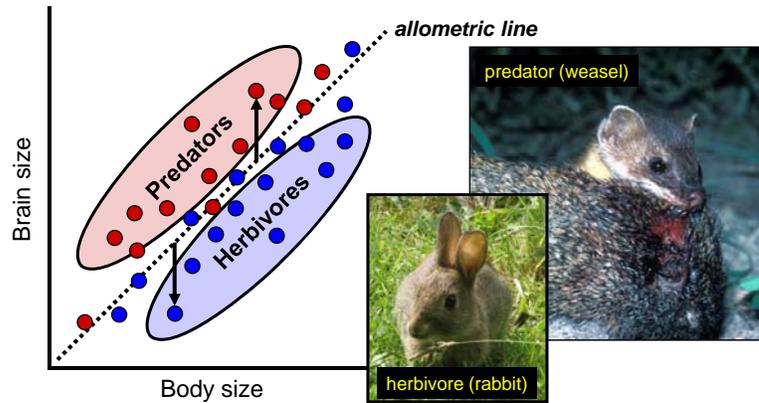


Comparative trend analysis

- Comparative trend analysis is as old as biology.
- It’s a search for associations between traits and ecological (or other) variables, as ways of testing hypotheses of adaptation.
 - **Example:** Is there an association (correlation) between long, nectar-producing tubes in flowers, and the incidence of pollination by hummingbirds?
 - The inference is that tubes evolved as an **adaptation** making hummingbird pollination more effective and, perhaps, exclusive.
- **We use comparative methods because it’s impossible** (usually) **to experimentally re-create the conditions of the past.**

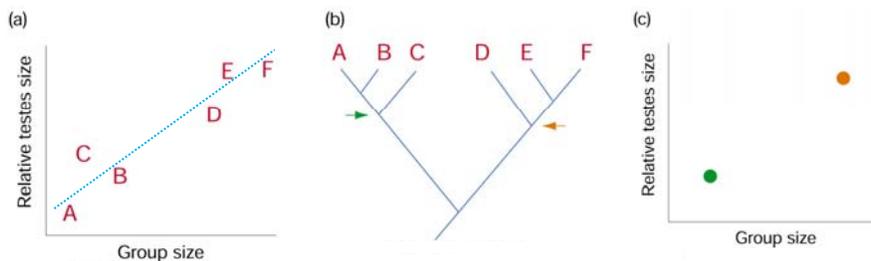


A classic example: **Comparing brains** in predators and herbivores



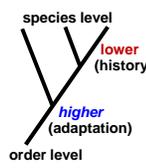
- This all started with work done by **Jerison** in 1973 in mammals.
- The diagonal line is the null hypothesis: allometry between brain size and body size.
- Deviations (arrows) above or below the line – [the residuals of the regression](#) -- contain the evolutionarily interesting phenomena.

A **simple scatterplot** may provide only **weak evidence** that two traits evolve in tandem



- Here, testis size has been adjusted for body size.
- And “group size” serves as a surrogate for male-male competition.
- What looks like six independent data points are actually just two, based on phylogenetic information.
- **One approach:** restrict your comparison to a **single clade** of closely related species, to factor out the phylogenetic effect.
- **More versatile approach:** **separate** the phylogenetic signal from the adaptational signal.

**Separating the phylogenetic from the adaptational signal:
“Culling the variance” (see Harvey & Pagel 1991)**

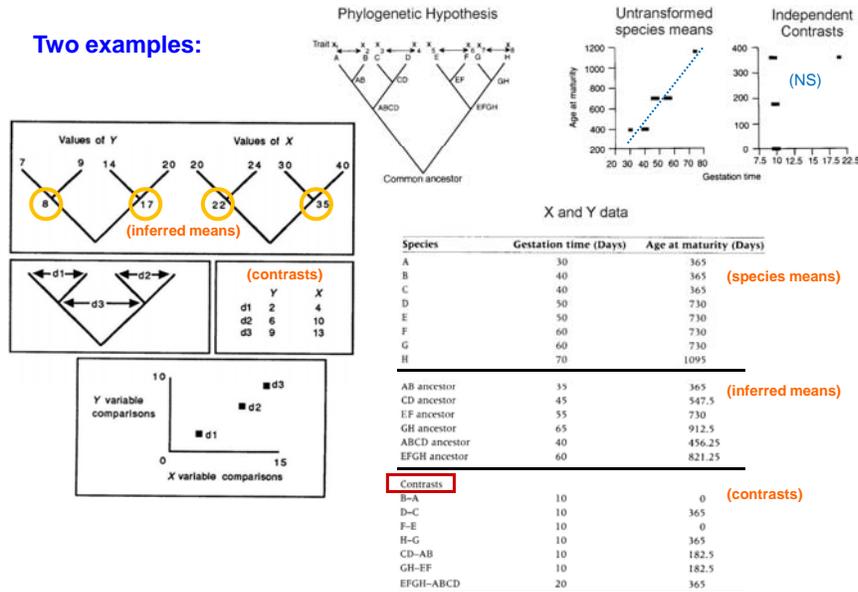
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- I. **“Take out the phylogeny”** (history)
- A. Stearns’ *phylogenetic-subtraction* method (1983) – This routine statistically removes from the data the lineage-specific variation associated with the *higher* nodes, and analyzes the remaining variation.
 - e.g., subtract from each *species* the “mean value” for its *order*.
 - B. Cheverud et al.’s *phylogenetic autocorrelation* method (1985) – Conceptually similar to Stearns’.

- II. **“Take out the comparative data”** (ecology)
- A. Lynch’s *maximum likelihood* approach (1991) – This uses the phylogenetic component to *test* the comparative relationship, the latter being the “phylogenetically uninformative *residual deviation*.”

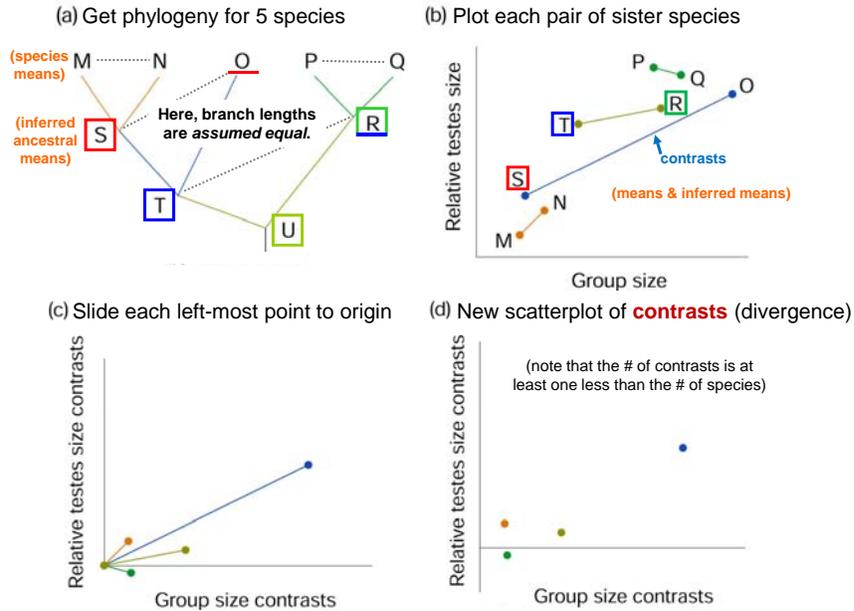
- III. **“Independent comparisons”** (Uses all the variation.)
- A. Felsenstein’s *phylogenetically independent contrasts* (1985) – Recognizes that what is phylogenetic inheritance at one (*lower*) level may constitute part of an adaptive difference at a *higher* level.

Independent Comparisons: Assuming equal branch lengths and a Brownian motion model of evolution (Harvey & Pagel 1991)

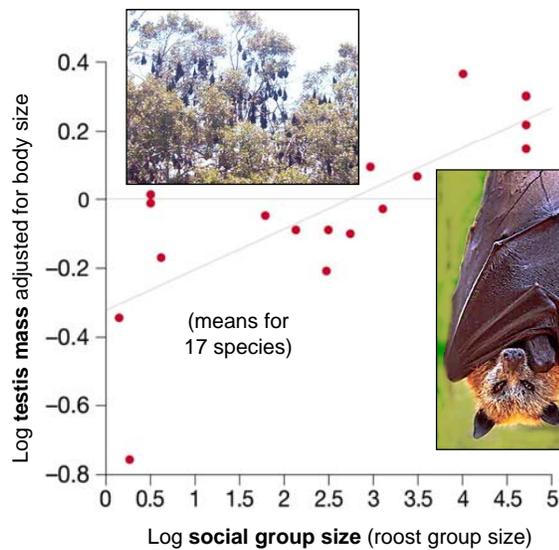
Two examples:



Independent comparisons: a graphical example (Freeman & Herron 2007)



Scatterplot of variation in testis size among fruit bats & flying foxes ("s.o. Megachiroptera") (Hosken 1998)

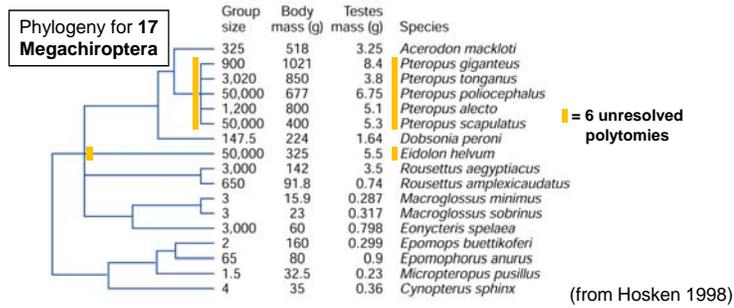


Hypothesis: larger testes are an adaptation for sperm competition.

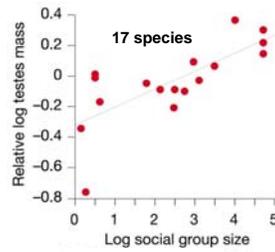
Females in larger groups would have more opportunities for multiple matings.

Males living in those larger groups would thus experience greater sperm competition.

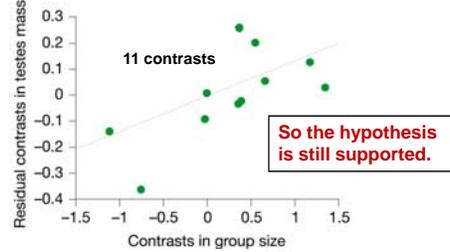
Looks to be a good correlation.



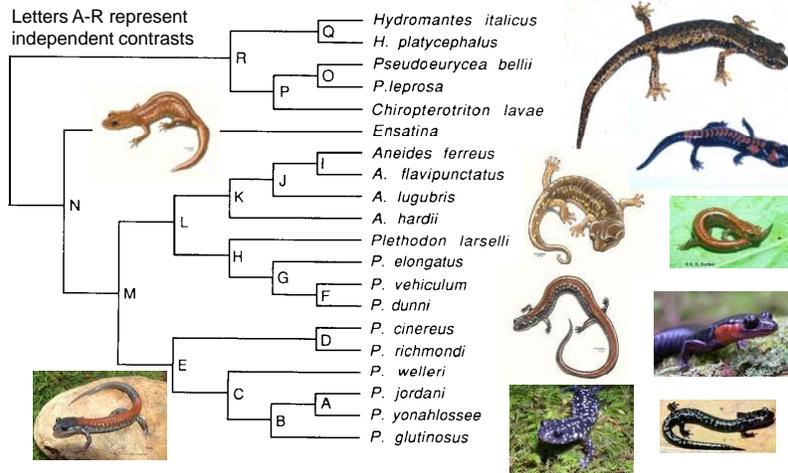
Plot of testis size vs. social group size



Plot of phylogenetically independent contrasts



Independent contrasts: Limb regeneration rate vs. genome size in 20 plethodontid salamanders (Sessions & Larson 1987)

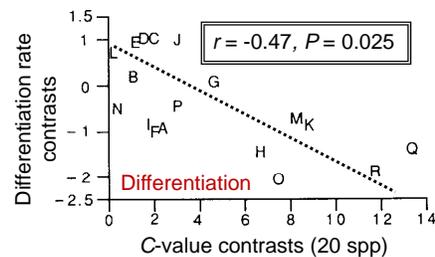
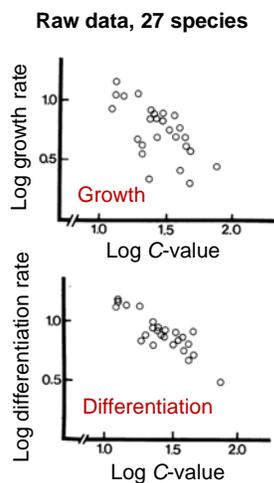


This was a test for an association between developmental (regeneration) rate and genome size (C), to see if the latter constrained the former.

Details of the study (Sessions & Larson 1987)

- “Junk DNA” hypothesis predicts DNA will accumulate in the genome until the costs of transcribing it impose too great a cost on the organism.
- Specific **prediction**: Genome size (**C-value**) should vary *inversely* with the development rate during limb regeneration in a species (larger C = slower development).
- Tested this in 20 (of 27) species of Plethodontidae
 - Phylogenetic nodes (20 spp) were reconstructed using parsimony and ancestral means were inferred at each node.
 - Difference scores (independent contrasts) were calculated for each of the 18 pairwise comparisons for three variables:
 - **C-value** (size of genome, measured as weight in picograms)
 - Rate of limb *differentiation* } (components of **development**)
 - Rate of limb *growth* }
 - Estimates of **branch lengths** (in units of time) were taken from the molecular data (for standardized contrasts).

Results (Sessions & Larson 1987)



- Both growth rate and differentiation rate are *negatively* correlated with C-value for the 27 species tested.
- But **only differentiation rate** retains any significant association when contrasts are plotted.
- i.e., large positive differences for C-value within clades tended to go with large negative differences in limb differentiation rates within clades.

Comparative methods without PICs: Breeding systems in plants

- Alternatives are **dioecious** (separate sexes) and **monoecious** (“perfect” flower, hermaphroditic).
- Givnish (1980, 1982) and Givnish & Bawa (1980) hypothesized an association between breeding system and **seed type** in **gymnosperms** (pines, yews, ginkgoes).
 - dry, wind-dispersed seeds were associated with monoecy (95%).
 - fleshy, animal-dispersed “fruits” were associated with dioecy (90%).

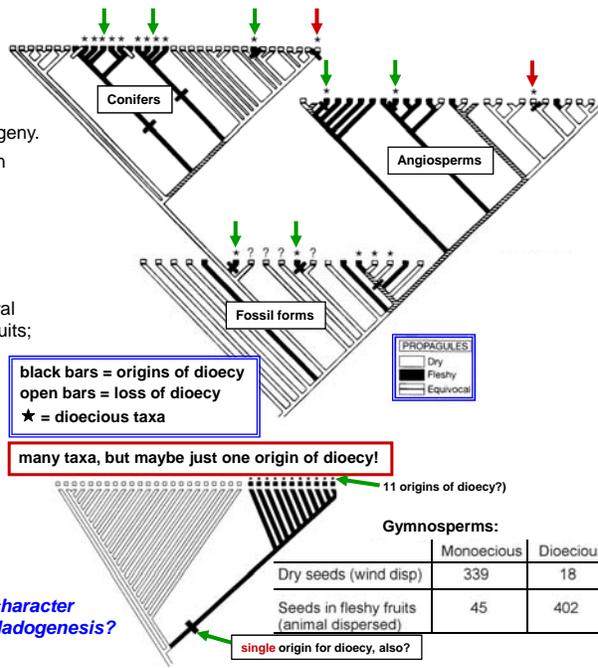


Seed type vs. breeding system in gymnosperms

- **Explanation** for the observed associations:
 1. A mutation arises that puts a fleshy coat on the seed.
 2. That greater investment in female function achieves higher fitness:
 - a. Birds are attracted to the new food source.
 - b. Birds inadvertently disperse the seeds.
 - c. The seeds are then more likely to germinate and grow.
 3. So dioecy evolved **after** the fleshy fruits, to maximize efficiency through division of labor.
- **Problem** with this is the same as before:
 1. Dioecy could have arisen just **once**, not many separate times due to the same selective pressures.
 2. So we would have just one data point – the others represent a form of *pseudoreplication* (Kroodsma 1989).
- **Solution**: One has to know the *phylogenetic relationships* of the constituent gymnosperms – **then map characters of interest onto the tree**.
- **Donoghue et al.** (1989) performed the necessary procedures:

Donoghue et al. 1989:

- Bigger data set that includes angiosperms.
- Mapped traits on phylogeny.
- Dioecy arose **7 times** in clades that already had fleshy fruits...
- and only **twice** in those clades with dry seeds ($p = 0.02$).
- But this assumes several fossil taxa had fleshy fruits; if not, then $p = 0.13$.



- Note that dioecy evolved just a few times, compared to the enormous number of dioecious species with animal-dispersed fruits.
- **What is it about this character suite that promotes cladogenesis?**

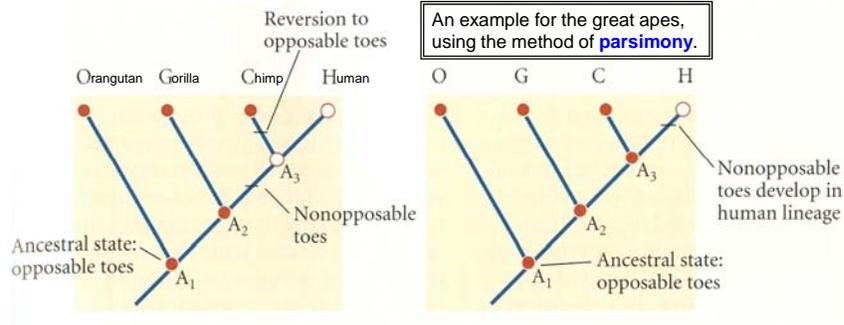
Later work on correlates of dioecy: Vamosi et al. 2003

Using a large-scale molecular phylogeny of the **angiosperms**, the authors tested hypotheses of **character correlation** across the entire clade. A simple Bonferroni correction was used to assure phylogenetic independence.

	Dioecy	Fleshy fruit	Woody habit	Tropical distribution	Inconspicuous flowers	Inconspicuous inflorescences	Many-flowered inflorescences	Abiotic pollination
Dioecy		correlates	+	+	+	+	NS	+
Fleshy fruit	NS		+	+	+	+	NS	NS
Woody habit	NS	causal pathway		NS	NS	NS	NS	NS
Tropical distribution	D,Te ⇒ D,Tr	Dr,W ⇒ F,W	n/a		NS	NS	NS	NS
Inconspicuous flowers	NS	Dr,W ⇒ Dr,H	n/a	n/a		NS	+	+
Inconspicuous inflorescences	ND,I ⇒ D,I	Dr,W ⇒ Dr,H	n/a	n/a	n/a	Not examined	+	+
Many-flowered inflorescences	NS	I,Dr ⇒ I,F	n/a	n/a	n/a	NS	NS	NS
Abiotic pollination	NS	NS	n/a	n/a	NS	NS	NS	-

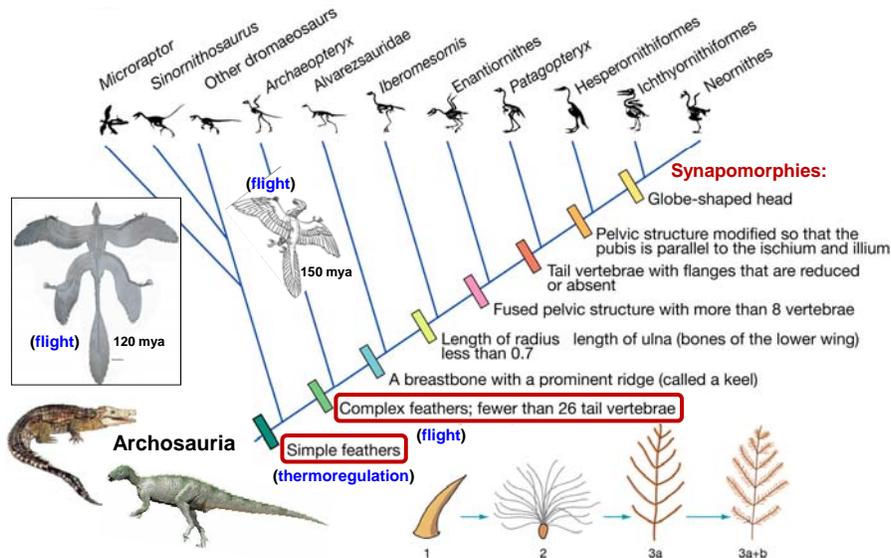
- Maximum likelihood was used to analyze the presence of correlations and the sequence of acquisition of traits (= evolutionary pathway).
- Certainly dioecy is strongly *correlated* with fleshy fruits in angiosperms, but it is also significantly correlated with other characteristics – e.g., woody habit, tropical distribution, inconspicuous flowers or inflorescences, and abiotic (wind!) pollination.
- Results *do not support the hypothesis* that dioecy is more likely to evolve in lineages possessing any of those six traits.

4. What is the pattern of character evolution in a group?



- The goal is to reconstruct, through inference, the history of evolutionary change in interesting characteristics – once again, exploring **adaptation**.
- This is done by “**mapping**” character states on the phylogeny and inferring the state in each common ancestor (the “**ancestral state**”).
- The requirements are:
 1. a known *phylogenetic tree*, based on an independent character set.
 2. an *explicit method*, such as parsimony, minimum evolution, maximum likelihood, or Bayesian inference.

Character evolution: dinosaurs & birds (Chiappe 1995, Xu et al. 2001)

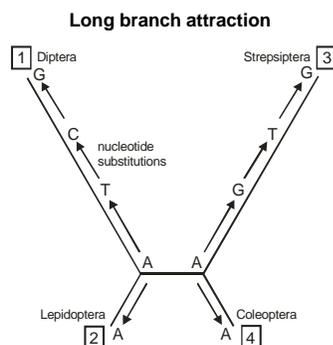
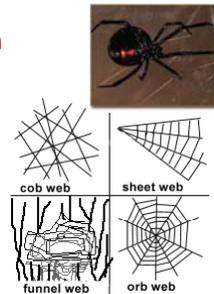


Needed: Explicit methods of character analysis

- A. The nature of the *phylogenetic tree*:
- Its accuracy is the basis for **all** subsequent inferences and conclusions, so you need to have confidence in it.
 - It should(?) be based on characters that do not include the character you are interested in, otherwise your procedure will be circular.
- B. The *method of analysis*: MP, ME, ML, or Bayesian inference?
- **Maximum parsimony** (MP) is a commonly used method for reconstructing ancestral character states.
 - The underlying logic is familiar: the mapping that entails the fewest changes (gains or losses) in a character across a tree is the best one.
 - *Example*: David Maddison's *Macclade*.
 - But there are potential problems, due to...
 - very **rapid evolution** in parts of the tree, and...
 - any violation of the assumption that **the probability of gains and losses are equal** across the tree.

Parsimony & rapid evolution

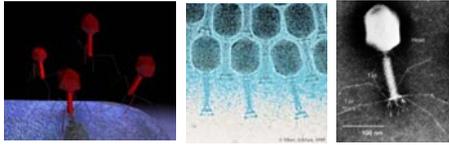
- The method of testing hypotheses of adaptation using ancestral character states under parsimony can be traced to Coddington's landmark paper (1988) on the evolution of spider webs (but see Bond et al. 2014).
- He argued that many adaptationist hypotheses become meaningless without information about the **order** and **timing** of character state changes.



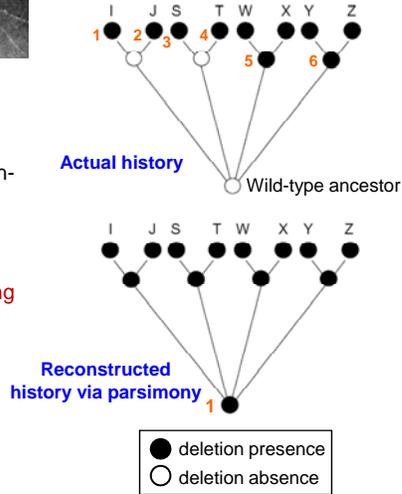
It is parsimony's attempt to **minimize the number of evolutionary events** that causes problems.

1. When **rates** of evolution are rapid, long branch attraction can lead to incorrect conclusions (Felsenstein in 1973) due to **undetected changes**.
2. When **gains** and **losses** are not **equally likely**, parsimony will also reconstruct an incorrect mapping, because by default it weights character states equally.

An **example** of a parsimony problem (Cunningham *et al.* 1997)

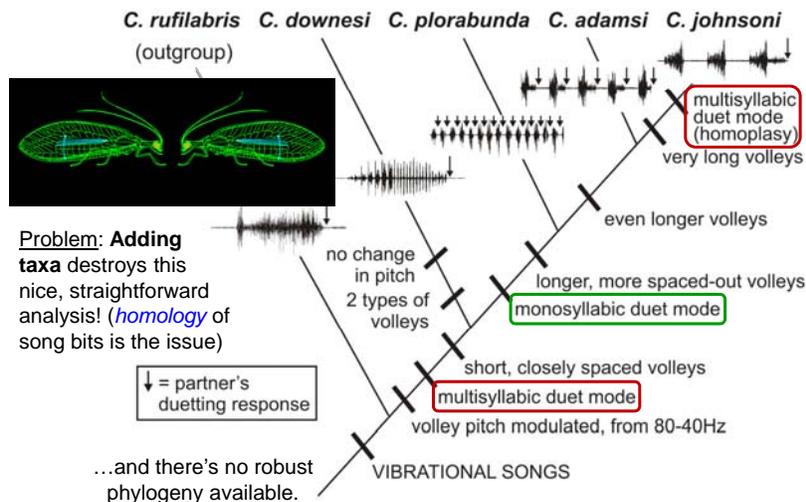


Four lineages of T7 bacteriophages



- This shows the effect of rapid evolution on MP reconstruction *when gains are much more likely than losses*.
- An identical **deletion** removing several non-essential genes appeared **6 times** independently (6 “gains”).
- Being large, the deletions are irreversible, **making the probability of “loss” (re-acquiring the gene) much higher than that of gain**.
- Parsimony incorrectly reconstructs the deletion at all ancestral nodes.
- Parsimony was unable to distinguish accurately between homology and convergence (homoplasy).

Parsimony-based reconstruction of song evolution in North American green lacewings (mtDNA phylogeny)

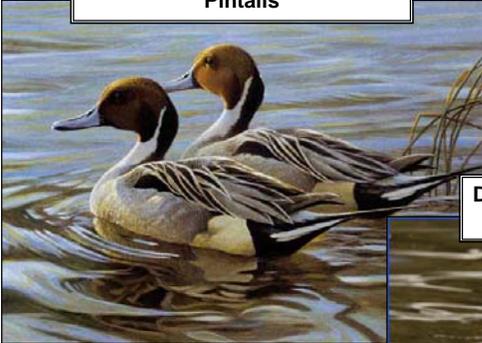


Correcting the parsimony problem (Cunningham *et al.* 1998)

- Under parsimony, it is nevertheless possible to compensate for unequal gains vs. losses by applying a **range of weights** to the character transformations.
- A formal solution is to use "**sensitivity analysis**" (an *iterative* technique).
 - First you set up a "step matrix" of **costs** associated with each type of character-state change (i.e. gain or loss).
 - Then, you set up and apply a **series of step matrices** to the data, to reconstruct ancestral states over a **range** of transformation probabilities.
 - At one extreme, you would use a matrix favoring **repeated loss** of a character state.
 - At the other extreme would be a matrix favoring **parallel gains**.
- Sensitivity analyses make a lot of intuitive sense, because for most characters we would never expect the rate of gains and losses to be exactly equal (see Ree & Donoghue 1998).
- But this method **cannot be standardized**: one mapping may hold up while another fails, but perhaps only because the treatments were different.

An example from dabbling ducks (Omland 1997)

Monochromatic plumage color:
Pintails



Sexual dimorphism in plumage color has long been thought to be **ancestral** in dabbling ducks (Ernst Mayr, 1940's).

Monochromatism was therefore assumed to have arisen many times independently.

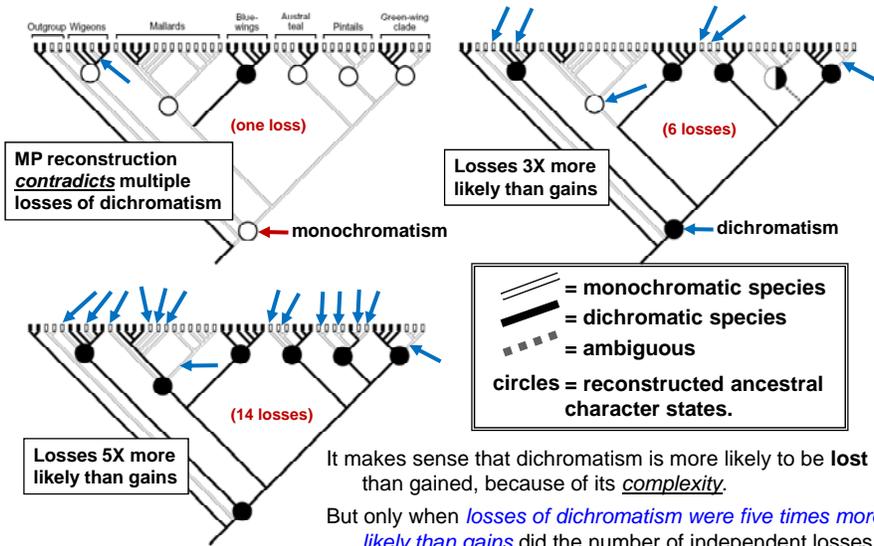
Dichromatic plumage color:
some Mallards



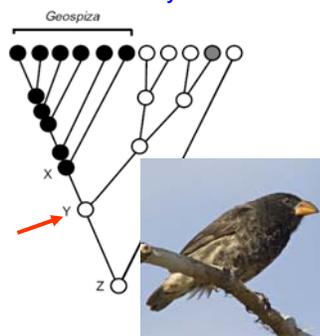
Omland (1997) tested this hypothesis using **sensitivity analysis**.

For parsimony under *equally weighted* and *unordered* gains and losses, the inferred ancestral state for *Anas* was **monochromatism**.

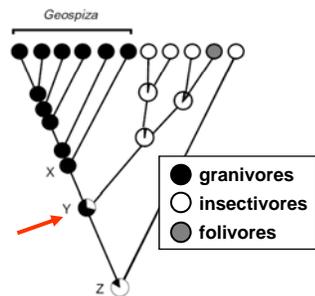
Sensitivity analysis of character-state change in dabbling ducks



Parsimony



Maximum likelihood

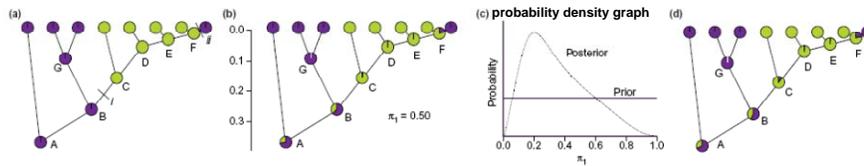


Maximum-likelihood reconstructions (Schluter et al. 1997)

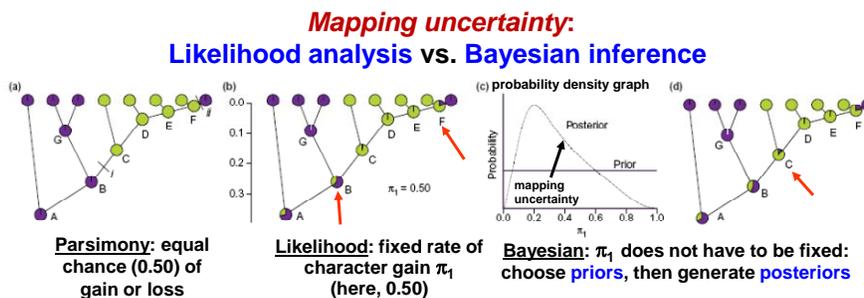
- This method uses explicit models of evolution to estimate the probabilities of all possible character state reconstructions at every node on the tree.
- The reconstructions are additionally controlled by:
 - A. the **distribution** of character states in the terminal taxa;
 - B. the **rate** of evolution of the character; and
 - C. the **length** of the internodal branches.
- **Example** – Schluter’s work (1997) on feeding behavior in **Darwin’s finches**:
 - A. Parsimony finds no support for granivory outside the *Geospiza* clade.
 - B. But ML reconstructs the ancestor just basal to *Geospiza* as **granivorous**, w/ 70% confidence.
 - C. Due to **short branch** from Y to X; it’s unlikely that a character evolving so **slowly** would have changed over that short a time period.

Tools: SIMMAP, MRBAYES, & MESQUITE

Making the reconstruction of evolutionary history more credible (Ronquist 2004)

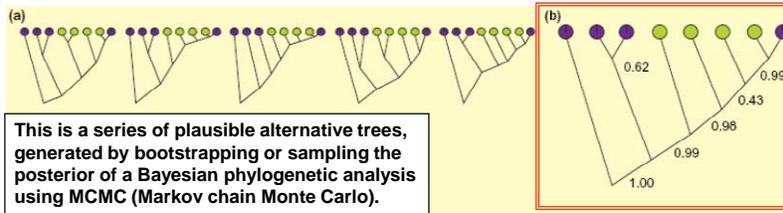


- As stated earlier, inferring ancestral character states and character changes using parsimony is satisfying.
- **But** there are **two major sources of error** under **ANY** approach.
 1. There is usually a range of *alternative reconstructions* on the same tree that are almost as likely: **mapping uncertainty**.
 2. The tree itself is almost never known without error. Given a range of plausible trees, it is possible that the evolutionary history of a trait could differ depending on the tree: **phylogenetic uncertainty**.
- **Sensitivity analysis** can determine the sensitivity of the results to slight changes in the analytical conditions, for both mapping uncertainty and phylogenetic uncertainty. But it's a somewhat *unrepeatable* method.



- **Likelihoods** are calculated from an evolutionary *probability* model:
 - **Markov model** for discrete and non-continuous characters.
 - **Brownian motion model** for quantitative characters.
- The relative or conditional probability of each ancestral state can be calculated, given the observed states at the tips (**existing data**).
 - But the value π_1 (relative rate of character gain) has to be a fixed value, even though its value is not known with certainty and is typically an ML estimate!
- **Bayesian** inference does not lock π_1 to a fixed value. Instead, π_1 is allowed to vary over the whole range of possible values. The analysis calculates its resulting or **posterior probability**, based on **prior probabilities** plus **existing data**.

Phylogenetic uncertainty:
* **Bootstrapping**



This is a series of plausible alternative trees, generated by bootstrapping or sampling the posterior of a Bayesian phylogenetic analysis using MCMC (Markov chain Monte Carlo).

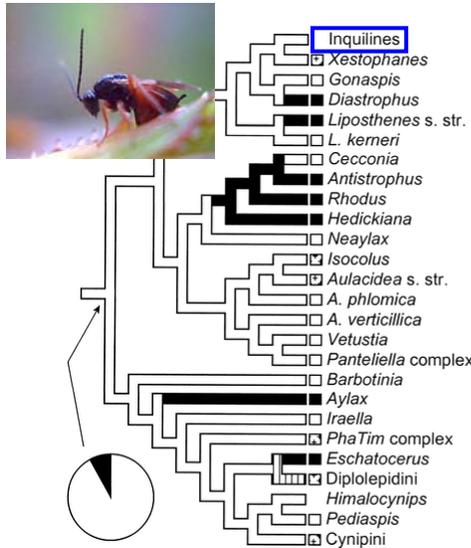
The **phylogenetic uncertainty** is summarized in the tree shown in (b).

How does phylogenetic uncertainty affect mapping?

- Bootstrapping can be used to address phylogenetic uncertainty, but **not mapping uncertainty per se**.
- But bootstrapping can provide insights into mapping uncertainty indirectly.
- The method is to map characters onto each of the bootstrap estimates of phylogeny and use the distribution of these mappings to show uncertainty.
- Example: Evolution of gall formation in gall wasps (Ronquist & Liljebad 2001)

* Drawing randomly with replacement from a set of data points

Estimating phylogenetic uncertainty with bootstrapping:
Gall types in gall wasps (Cynipidae) (Ronquist & Liljebad 2001)

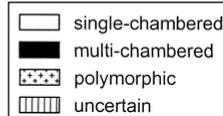


Traditional view:

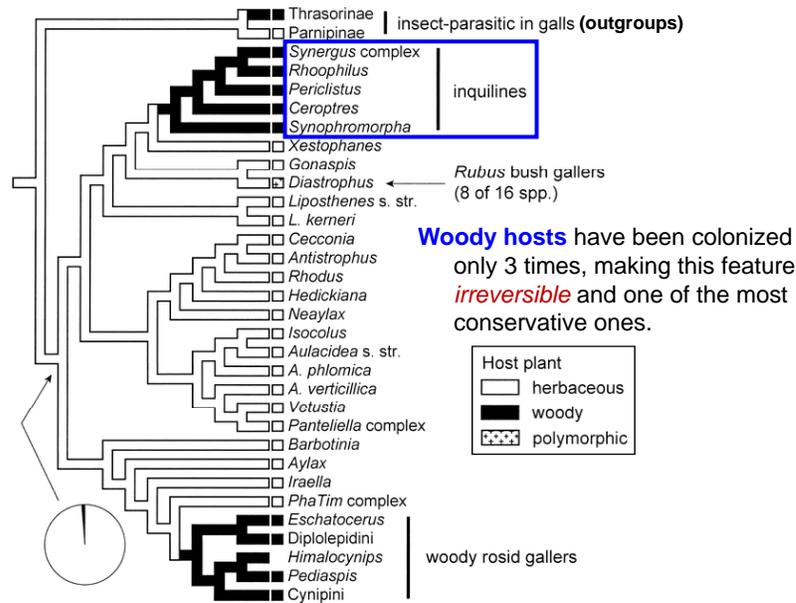
- Earliest = Asteraceae stem feeders; no galls
- Multi-chambered galls evolved first, then single-chambered.

New view:

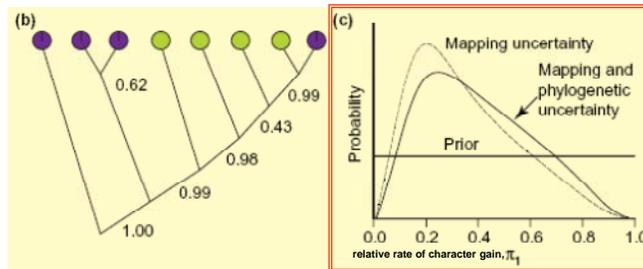
- Earliest = single chambered galls on Papaveraceae (poppy)
- Stem feeders (no galls) are derived, as are multi-chambered galls.



Host associations of gall wasps

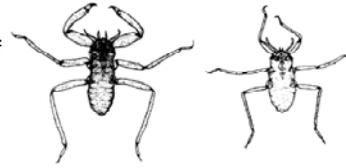


Phylogenetic and mapping uncertainty: Bayesian inference

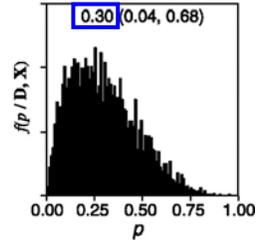


- Bayesian inference can account for **both** phylogenetic uncertainty **and** mapping uncertainty, in one model.
- The method is to expand the probability model (described earlier) to include **topology**, **branch lengths**, and **other** parameters necessary to infer phylogeny.
- The posterior probability distribution is complex, but can be sampled using stochastic simulation in, say, MCMC techniques.
- Note that because Bayesian posterior probabilities are always based on **ALL** the available data, one usually includes the character to be mapped in the analysis (circularity be damned).

The sum of the **posterior probabilities** of phylogenetic trees having different numbers of reconstructed gains and losses of the **horned soldier caste** character in 34 taxa of Aphidae (Huelsenbeck et al. 2000)



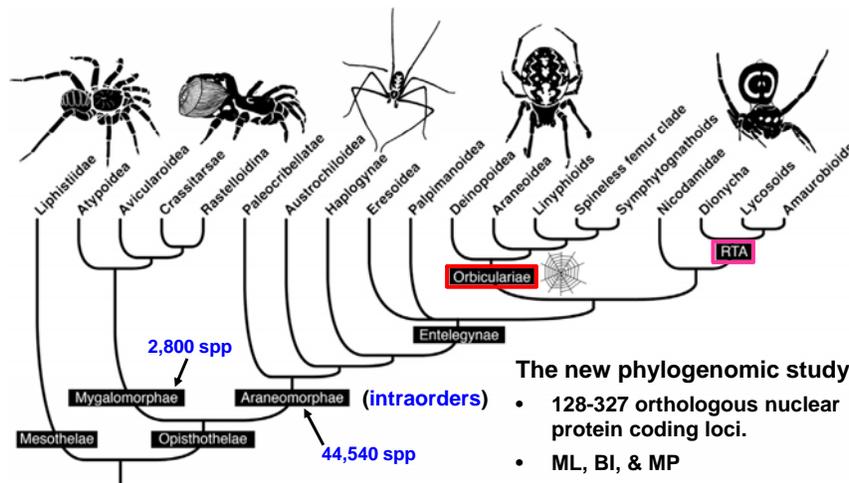
Number of Gains	Number of Losses										
	0	1	2	3	4	5	6	7	8	9	10
0											
1		0.01	0.18	0.58							
2		0.03	0.18								
3		0.01									
4	0.01				0.01	0.01	0.01				
5		0.01	0.02	0.02	0.02	0.01					
6		0.03	0.04	0.05	0.02						
7		0.02	0.07	0.08	0.04						
8		0.07	0.10	0.06							
9		0.12	0.08								
10	0.10										



Note that there's a high degree of uncertainty in such reconstructions, such that the strongest solution under MP has a sum of only **0.18**.
(The light shading shows the result when all trees are equally likely – e.g., no phylogenetic signal)

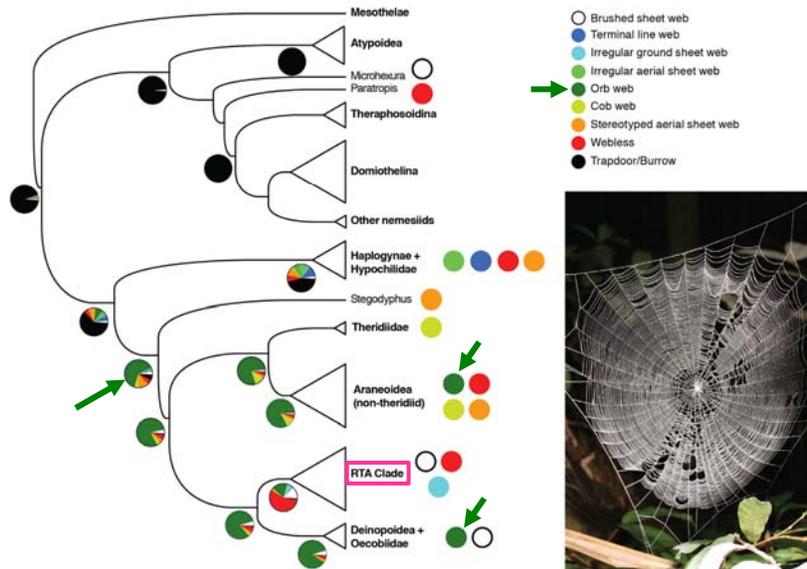
Using a single Bayesian inference model, the result suggests that **losses are 3x more likely than gains**, but the 95% credibility range is quite large...

A recent example: The orb-weaving habit in spiders
(Bond et al. 2014). Current view shown below.



- The new phylogenomic study:
- 128-327 orthologous nuclear protein coding loci.
 - ML, BI, & MP
 - Mesquite for ancestral state reconstructions (likelihood)

...but instead, orb-weaving may be ancient and not monophyletic



The orb web either evolved **much earlier** than previously hypothesized and is ancestral for a majority of spiders *or else it has multiple independent origins*, as hypothesized by precladistic authors.