"PRINCIPLES OF PHYLOGENETICS: ECOLOGY AND EVOLUTION"

Integrative Biology 200   Spring 2016
University of California, Berkeley   D. Ackerly

April 11, 2016 **Lineage diversification**

Reading: Morlon, H. (2014). Phylogenetic approaches for studying diversification. *Ecology Letters*, *17*(4), 508-525.

Optional readings (will be discussed in lecture):
Rabosky, D. L., & Glor, R. E. (2010). Equilibrium speciation dynamics in a model adaptive radiation of island lizards. *Proceedings of the National Academy of Sciences*, *107*(51), 22178-22183.
Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K., & Mooers, A. O. (2012). The global diversity of birds in space and time. *Nature*, *491*(7424), 444-448.

1. Diversity and diversification
     Diversity – measure of number of entities at one point in time, within a defined space
          and/or within a particular clade or functional group
     Diversification –pattern of net change in diversity in a clade through time
          Balance of processes at global scale: speciation and species extinction
     Species may be viewed as fundamentally evolutionary concepts, but paradoxically they
          are usually more easily defined in an ecological context
     Sampling now becomes critical, if we are counting 'species'!
     Change in species from one unit of time to another, in a defined space:
          Demographic equation: $N_{t+1} = N_t + B + I – D – E$
          In evolutionary terms, need to distinguish local vs. global extinction
          Regional diversity studies for some purposes need to separate speciation from
               immigration

2. Current diversity of a clade is a product of age and *average* rate of diversification
     Stem group vs. crown group age (Fig. 1) (Magallón and Sanderson, 2001)
     Rate = relative rate of diversification, e.g. species per species per million years. This is
          what you calculate from the slope of natural log(diversity) vs. time. Same theory as
          calculating population growth rates.

3. Sister taxon and tree topology comparisons avoid need for *absolute* calibration of ages
     Slowkinski and Guyer (1989): A null model based on random splitting will frequently
          lead to imbalanced trees with 1 vs. n-1 taxa. Need to have more than 40 taxa before
          a 1/(n-1) split is 'surprising' (p < 0.05)

4. Most current approaches are based on the distribution in time of branching events, or
     equivalently the pattern of lineages through time (LTT).

     Diversification must always be viewed as the balance of speciation and extinction. But
     extinction is masked from reconstructed phylogenies and there is much debate about

whether extinction can be estimated meaningfully from phylogenies alone (without fossils). See Fig. 3, and Fig. 4 (from Morlon reading)

Starting point is the 'Yule' pure-birth model – constant probability of splitting on every branch and no extinction. The pure birth model results in linear LTT plot on semi-log axis. Alternatively, a constant diversification model may have a fixed speciation and extinction rate. If there is any extinction, the constant diversification model leads to apparent acceleration at tip ('pull of the present') as most recently evolved lineages have not had time for extinction (Fig. 5, Nee 1994). One solution to this problem is to only analyze the tree in linear portion and avoid the most recent part. This problem is exacerbated if you only have partial sampling of the extant taxa in the clade.

Empirically, reconstructed phylogenies tend to be 'stemmy' – decrease in diversification rate with time, despite 'pull of the present'. Does this reflect reality, or sampling and methodological bias?

5. Morlon provides excellent summary of existing models and key empirical results. Perhaps one of the most contested issues in recent years is whether there is evidence of diversity-dependent decline in diversification (ie. Ecological saturation feedback to diversification). See Rabosky and Glor 2010

6. Personally I think one of the most overlooked aspects is the simple question of changes in area with suitable climate for a clade. See Becerra 2005 (Handout Fig. 7)

7. The availability of data and methods for really big and 'complete' trees for large groups at a global scale is opening new windows into studies of diversification. Jetz et al. 2012 bird phylogeny an example. Does not show any signs of slowing down at a global level, and indicates consistent clade-specific differences in diversification rates.
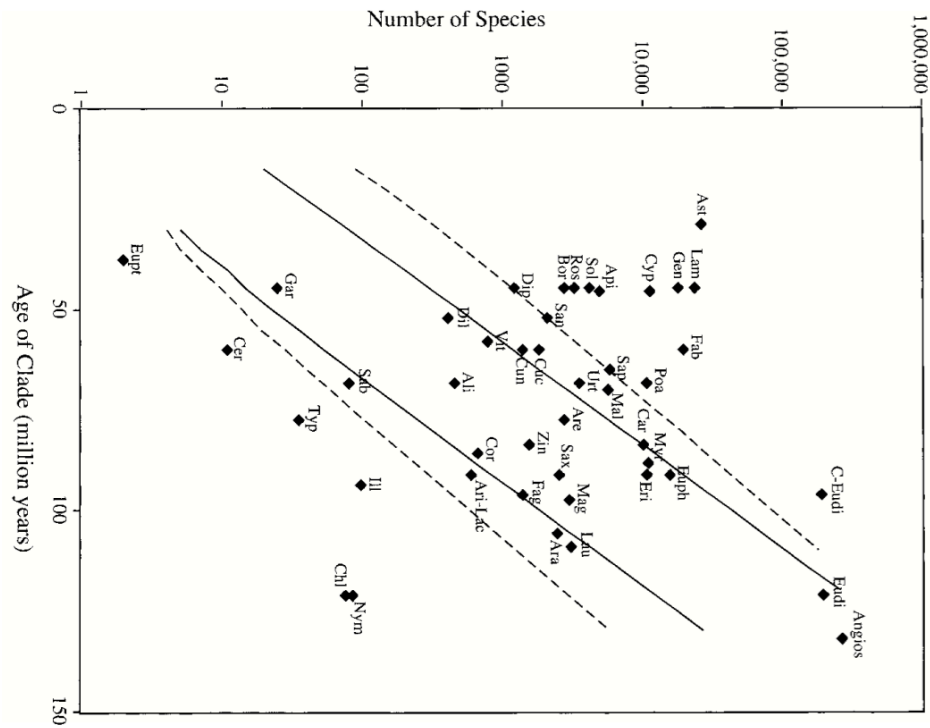
Magallon and Sanderson 2001. Clade age (crown group) vs. diversity. Clades above lines have higher than average rates of diversification, and clades below line lower than average rates.
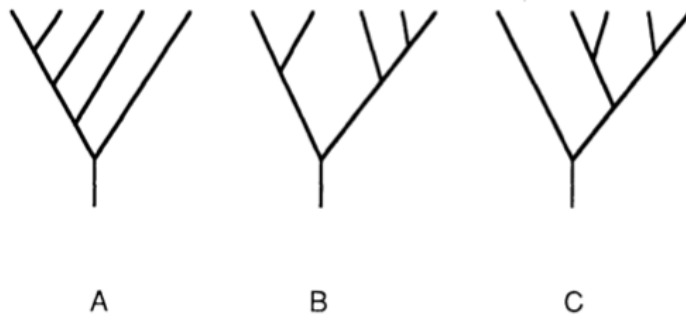
Handout Fig 2:

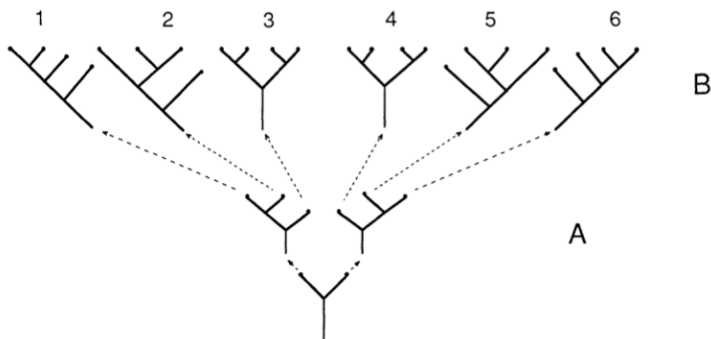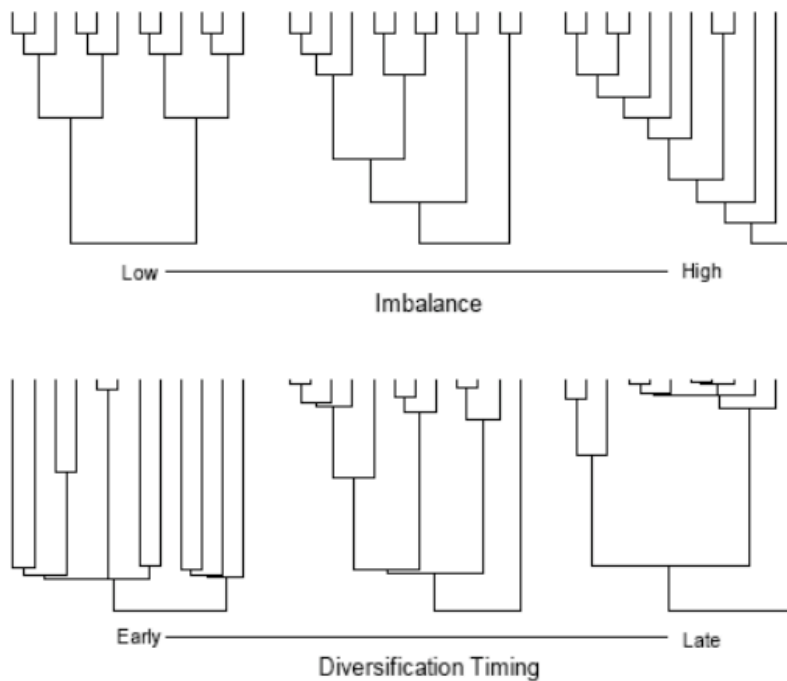
FIG. 1.—The three distinct topological types on five taxa.

FIG. 2.—A diagrammatic representation of the process of lineage-splitting leading to four taxa.

Slowinski and Guyer 1989 Am Nat

Handout Fig. 3

Low ——————————————————— High
Imbalance

Early ——————————————————— Late
Diversification Timing

Handout Fig 4

(a) Expanding **(b)** Equilibrium **(c)** Waxing-waning

1)

Extinction

Speciation

2)

Species richness

Time Time Time

3)

Most recent
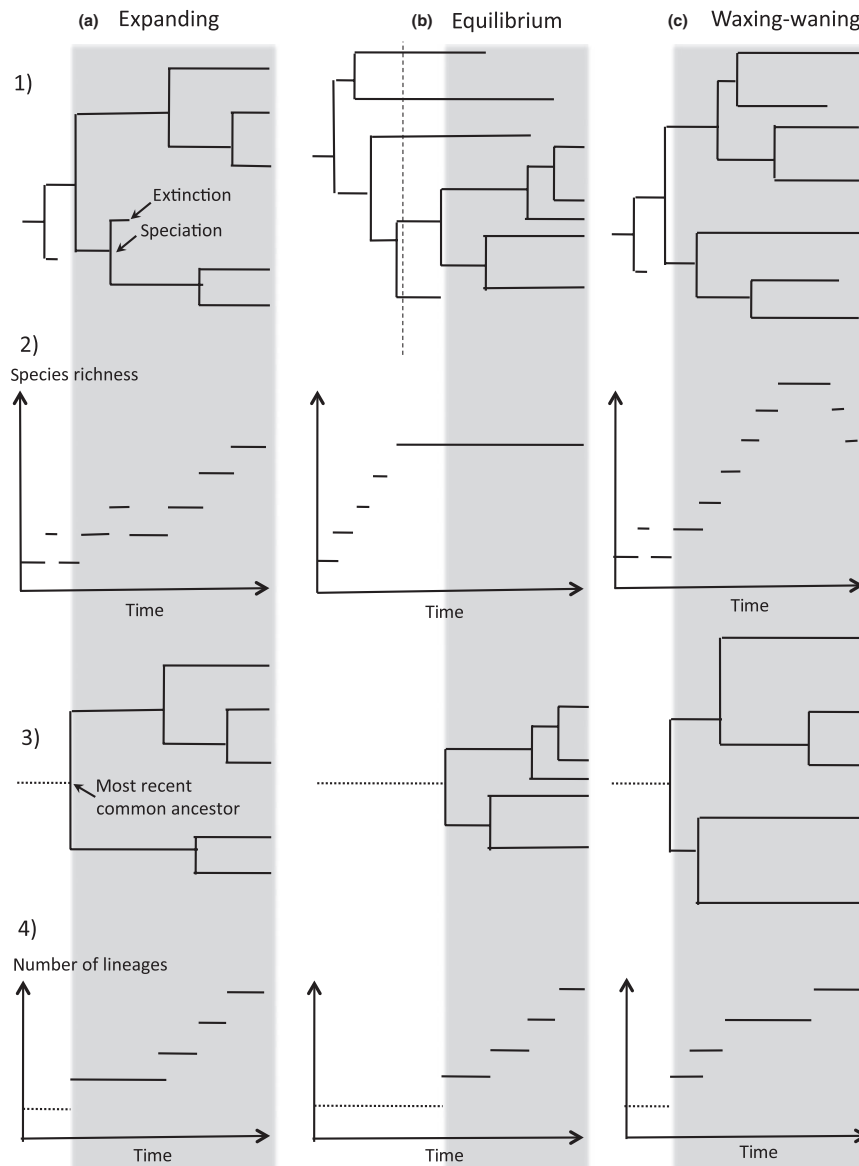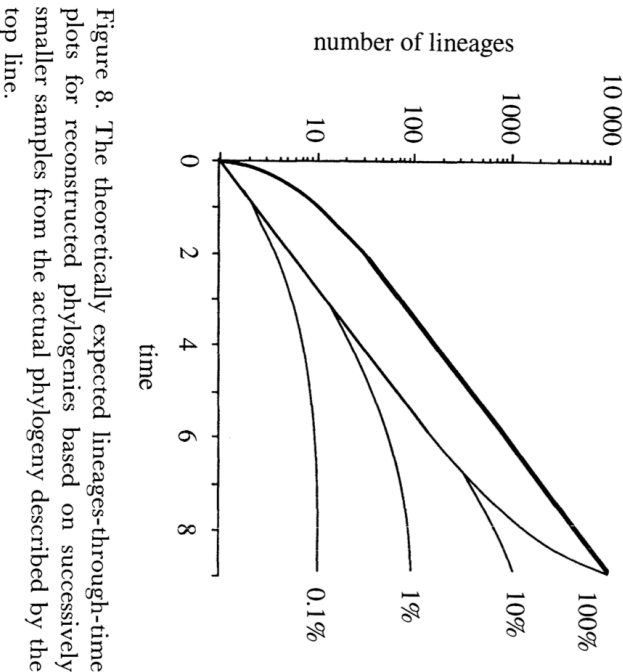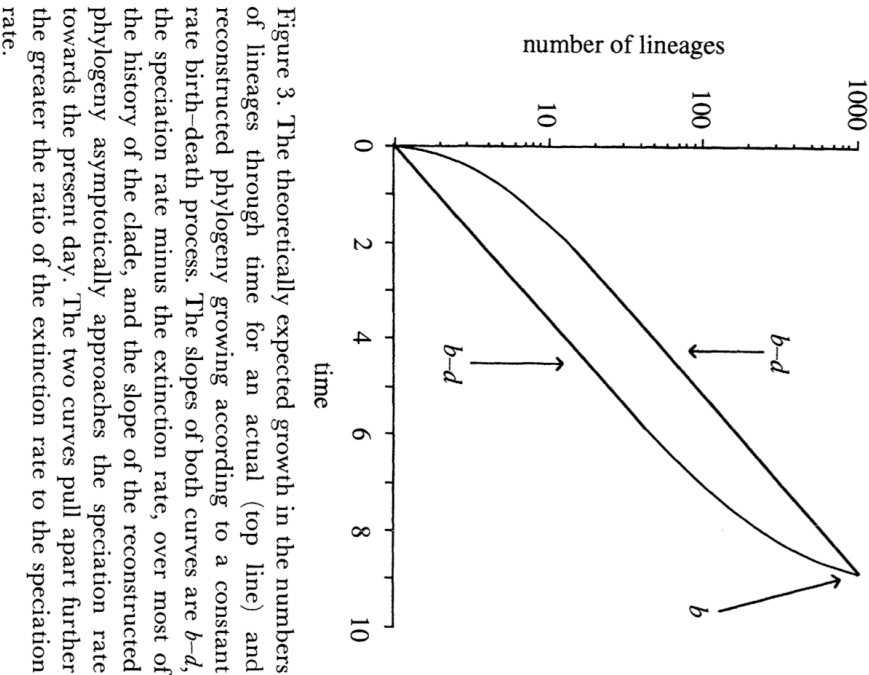common ancestor

4)

Number of lineages

Time Time Time

Figure 1 Analysing diversification with phylogenies. (1) Complete phylogenies representing the birth and death of species, (2) diversity-through-time plot, (3) reconstructed phylogeny and (4) lineage-through-time plot corresponding to scenarios of (a) expanding diversity, meaning that clades' richness increases over time, (b) equilibrium diversity, meaning that clades' richness stay constant over time and (c) waxing–waning diversity dynamics, meaning that clades' richness first increases and then decreases over time. The grey areas correspond to the time period going from the time of the most recent common ancestor in the reconstructed phylogeny to the present. Although the number of lineages in the reconstructed phylogeny always increases from 2 to present-day diversity (4), the corresponding diversity trajectory can be increasing (a), stable (b), or contain periods of diversity decline (c). In (b), starting from the time indicated with the dashed line, each extinction event is immediately followed by a speciation event, resulting in equilibrium dynamics.

Morlon 2014 Ecol Letters

Handout Fig. 5

number of lineages

Figure 3. The theoretically expected growth in the numbers of lineages through time for an actual (top line) and reconstructed phylogeny growing according to a constant rate birth–death process. The slopes of both curves are $b-d$, the speciation rate minus the extinction rate, over most of the history of the clade, and the slope of the reconstructed phylogeny asymptotically approaches the speciation rate towards the present day. The two curves pull apart further the greater the ratio of the extinction rate to the speciation rate.

number of lineages

Figure 8. The theoretically expected lineages-through-time plots for reconstructed phylogenies based on successively smaller samples from the actual phylogeny described by the top line.

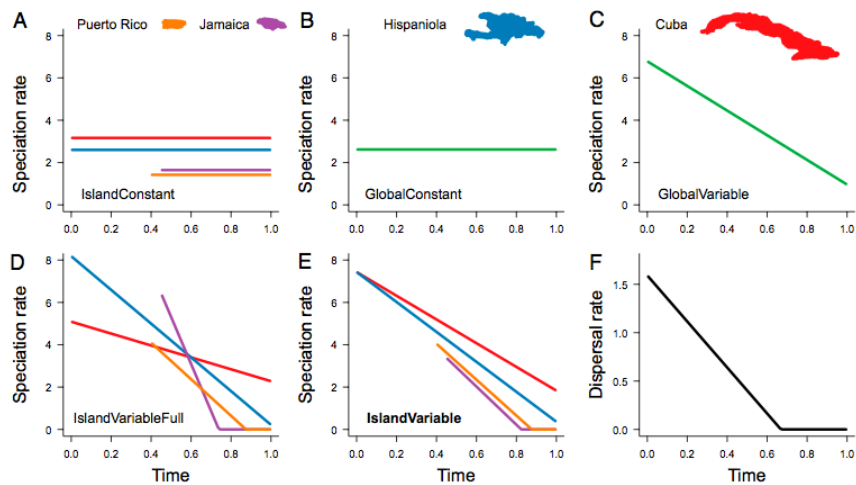**Nee et al. 1994 Phil Trans R Soc**

Handout Fig. 6

Fig. 1. (A–E) Reconstructed speciation-through-time curves under five diversification models fitted to the *Anolis* phylogeny (Fig. 2, Fig. S1). Green curves denote models without state-dependent diversification; other colors describe speciation dynamics on Cuba, Hispaniola, Jamaica, and Puerto Rico. Models are sorted (left to right, top to bottom) from lowest to highest conditional probability (Table 1). Models with island-specific changes in speciation (*D* and *E*) account for $P = 0.995$ of the total probability of the data taken across all models. The maximum-likelihood estimate of the dispersal rate between islands under the IslandVariable model is shown in *F*. Rates are shown in relative time units, as the tree was scaled to basal divergence of 1.0.
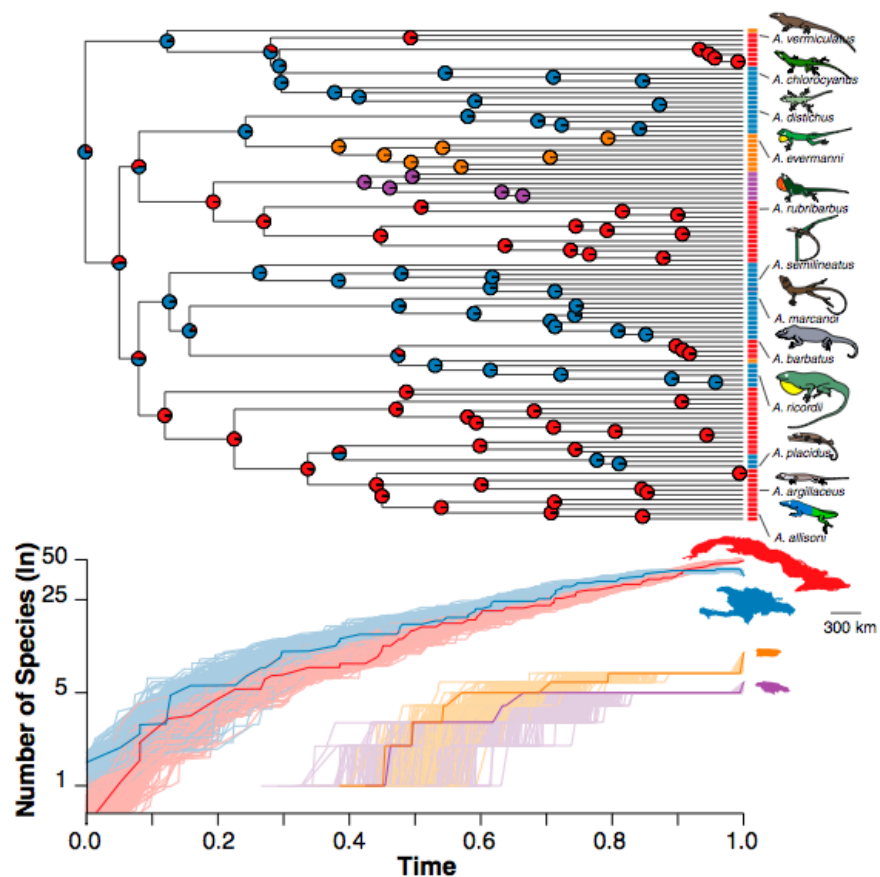

Fig. 2. *Anolis* MCC tree with reconstructed island occupancy probabilities and lineage accumulation curves for Cuba (red), Hispaniola (blue), Jamaica (purple), and Puerto Rico (orange). Occupancy probabilities on internal nodes were estimated under the overall best-fit model (IslandVariable). The MCC tree with all taxon labels is shown in Fig. S1.

Rabosky and Glor 2010
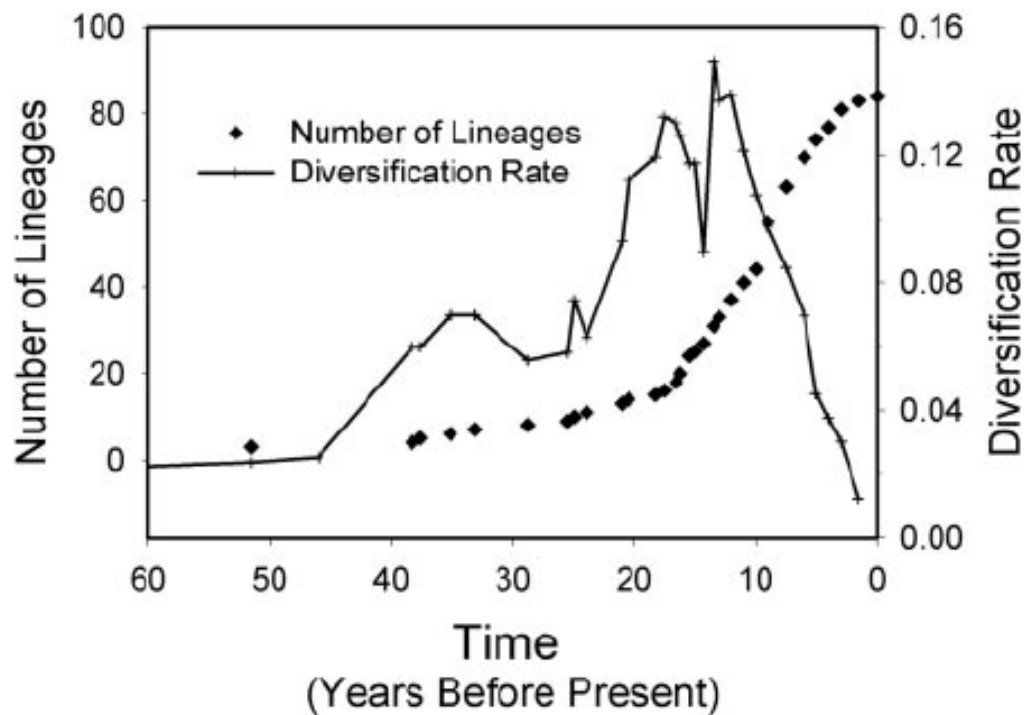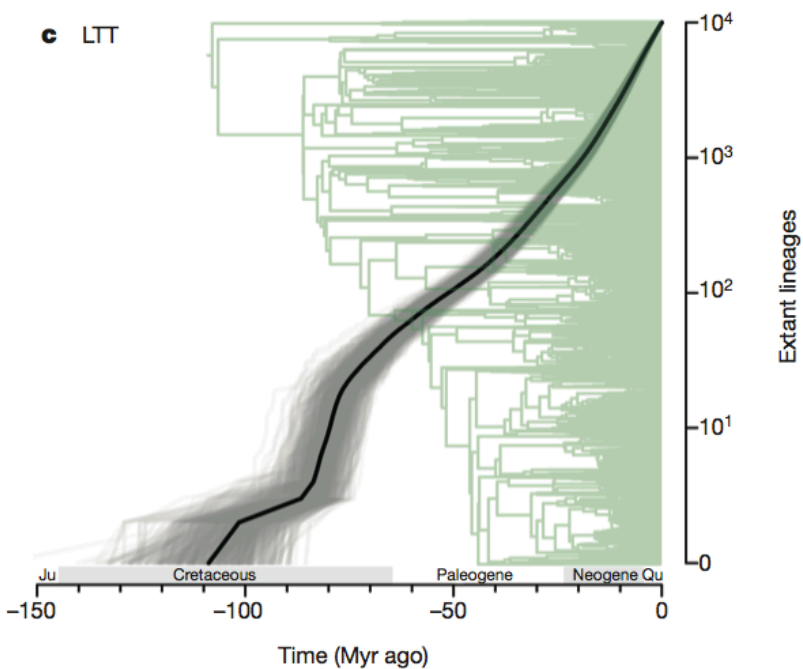
Handout Fig. 7

Fig. 3. Number of *Bursera* lineages through time and 10-MY average diversification rates at different times over the last 60 MY.

Becerra 2005 PNAS

Jetz et al. 2012 Nature

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IB200B 2013 HANDOUT – Great text!
Based on earlier notes by Nat Hallinan

Comparing sister clades within a cladogram: the shape of evolution

I. Summary

The goal today is to introduce a new kind of comparison that often needs to be made: diversity comparisons **among sister clades within a single cladogram** (we'll talk about comparisons **among cladograms** after break). For a long time this subject was the sole purview of paleontologists, but in recent years with the advent of large accurate phylogenies there has been an explosion of research in this field among neontologists as well.

In order to address various questions in both micro- and macro-evolution, we need to address issues such as the symmetry of balance of trees. What is the null expectation? Intuitively, we would expect balanced trees, perhaps, based on some sort of false analogy to coin flips. But, is the this right? How would you generate "random" trees?

What do random trees look like? Slowinski & Guyer (1989) showed a non-intuitive result: the probability of generating a $1 + (n-1)$ tree is $2/(n-1)$, which is equal to the probability of **any** division of species into lineages of unequal size (the probability is $1/(n-1)$ when the species are evenly divided). Thus, even a tree in which one species is the sister taxon to 39 other species is not significantly non-random at the $P = .05$ level ($P > .051$).

What do real trees look like? Often are quite asymmetrical; could this be a methodological bias? Even if real, how do we judge whether it is significantly asymmetric? Furthermore, even if it is significantly asymmetric, how do we associate that with some specific factor postulated to be the cause of that asymmetry? That leads to the topics of "key innovations" and "adaptive radiations" (more next week).

II. "Diversity" or "Diversification"

Diversity is the number of species or of terminal clades (depending on your species concept -- more later on that) in a larger clade. People often use the term *diversification* in this context to refer to the processes that produced the observed diversity. However, I think it is logically better to talk about patterns of diversity (which is what you directly observe in trees) and bring ideas of process in carefully. Just as both birth and death affect the number of organisms, it is better to think of *diversification* as the processes that increase the number of species (terminal clades), and *extinction* as the processes that decrease the number of species (terminal clades). In practice it is very difficult to separate the affects of extinction from the affects of diversification using only extant taxa. N.B., it is common in the literature to talk about *speciation* instead of *diversification*, but this presupposes certain species concepts that are highly controversial (more later in the class).

Several things can lead to a difference in diversity without requiring that there be a cause. For example, we expect older clades to be more diverse. It is also possible for stochastic processes to lead to differences in diversity that we would consider within the range of equivalent outcomes. For example within the monkeys, there are about 50 species of new world

monkeys, 80 species of old world monkeys and 20 species of apes. These are large differences in diversity, but do not require different processes to explain them.

How can we detect processes that cause diversity to vary in a tree? To answer that question we must first explore the types of patterns to be expected, both null models and given certain processes.

III. Tree Shape

Tree shape is a catchall term used to describe the properties of a phylogeny other than the taxa at the tips and the character states either at the tips or reconstructed along the branches and at nodes of a tree. The shape of a phylogeny consists of two properties, the topology and the branch lengths.

Topology is the particular branching pattern for a tree. A *labeled topology* represents the relationships among the taxa at the tips. An *unlabeled topology* has no taxa at the tips and thus consists only of the abstract tree shape. In this way two phylogenies have the same unlabeled topology if the taxa can be rearranged on the tips in such a way that the taxa have the same relationships, but only have the same labeled topology if all the taxa have the same relationships without rearrangement. Today we will mostly concern ourselves with unlabeled topologies; we will only considered labeled topologies at the end, when we include character data.

Imbalance refers to the distribution of taxa among the different clades of a phylogeny. If taxa are evenly distributed among the clades, then the topology is balanced. On the other hand, if some clades have many more taxa than other clades of equivalent rank, then a tree is considered imbalanced.

Excessively balanced topologies could result from several possible causes. Competition among close relatives could create a situation in which there is more competition and thus less diversification in large clades. If there is a period of time after speciation during which a lineage could not speciate again you would get clades that are more balanced than you would expect under equal branching. There are undoubtedly other processes that could increase balance.

Excessively imbalanced topologies are usually assumed to be caused by heritable characters that effect diversification or extinction, such that diversity will show a phylogenetic signal. There is an infinite list of characters that *could* effect diversity. *Key innovations* could be defined as characters that expand the available niche space and thus lead to an increase in diversity. Other types of organismal characters that do not lead to an increase of niche space could also lead to a change in diversity. For example sexual selection has been proposed to lead to increases in speciation. On the other hand broadcast spawners might be expected to show low diversification. Island clades are often more diverse than their closest main land relatives, as they faced little competition upon colonizing the island. One area may also have more available energy or a more heterogeneous environment than another and thus taxa in that area would have higher rates of diversification or lower rates of extinction.

When analyzing or describing macroevolution, the *branch lengths* of a tree are usually

proportional to time; thus these trees are ultrametric. Branch lengths are also of critical importance, when likelihood models are used to analyze a topology. The branch lengths are also interpreted independently of the topology, in order to identify temporal shifts in diversity. *Branching times*, the timings of speciation/splitting events, can be compared on a lineages-through-time plot.
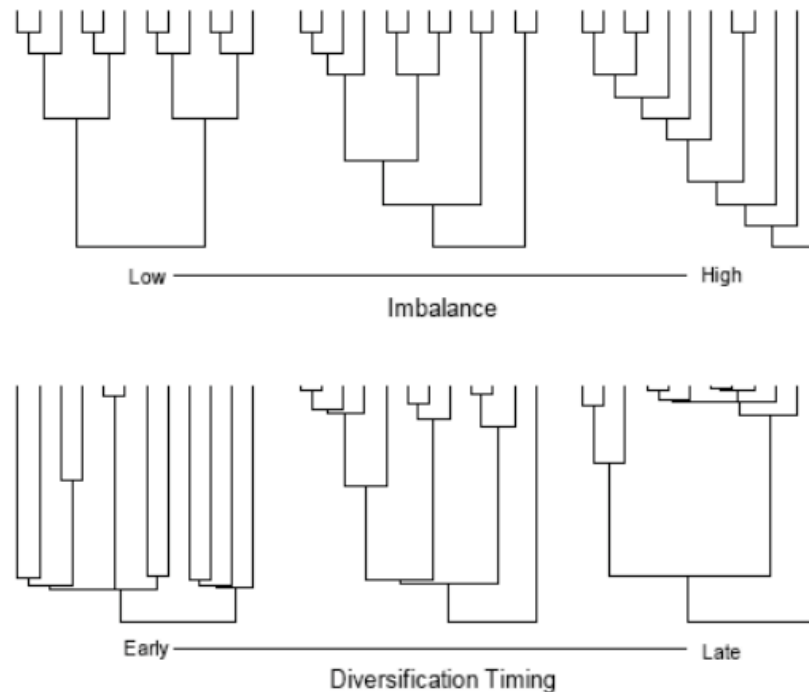
Temporal variation in diversity that effects an entire clade could be attributed to several factors. Adaptive radiations would be expected to lead to high rates of diversification early, and declining diversification through time. On the other hand a mass extinction would clearly show high across the board extinction for a brief period of time. It is difficult to separate the signal of mass extinction from generally high extinction, when examining only extant taxa. Other types of geological events could also affect an entire clade at once, as could a major ecological shift.

IV. Null Models

Before we start asking questions about imbalance and branching times, we must carefully consider what we expect to see if there are no macroevolutionary forces acting. Several different null distributions have been proposed, we will focus only on the simplest and most commonly used.

Equiprobable trees (Maddison and Slatkin 1991) considers each labeled topology to have the same probability. A phylogeny with *n* taxa has $(2n-3)!/2^{n-2}(n-2)!$ possible rooted topologies, and each is equally probable. This distribution produces particularly imbalanced trees.

Random Branching or *Random Joining* (Maddison and Slatkin 1991) is the most commonly used null distribution for these types of studies. It assumes that each branch has an equal chance of splitting, thus it fits our intuition of what a null distribution for diversification

should be.

Although this distribution is more balanced than equiprobable trees it is unexpectedly imbalanced. In the figure above, the middle tree in the top row represents a topology of medium imbalance under the random branching assumption. There is a tendency for people to think of the outcome of random processes as being evenly distributed, but they are only evenly distributed on average. In fact we add a branch to a tip of an already existing tree we would expect it to make any given node less symmetric.

The *Birth-Death Process* assumes that there is a constant rate of speciation/splitting, and a constant rate of duplication. It produces a given topology with the same probability as random branching. However, random branching is only concerned with the probability of a topology, while the birth-death process also allows us to calculate the probability of a set of branch lengths for our topology.

The speciation and extinction rates are usually called l and m, but they are also referred to as *a* and *b*, *b* and *d*, or A and W. The process is often also described using a reparameterization in which the *diversification rate*, *r*, equals *the speciation rate – the extinction rate*, and the *extinction fraction* equals *the extinction rate/the speciation rate*.

You can calculate these parameters in a maximum likelihood framework (or Bayesian for that matter). This would appear to give us an assessment of the role that speciation and extinction had in producing the current diversity in a given clade. Unfortunately, although these models are very good at estimating the standing diversity, they do a notoriously poor job of estimating the role that extinctions and speciations which have left no extant descendants have played in the process.

We use the term birth-death, because this is the same model used to describe population growth with an analogy between diversification as birth and extinction as death. It produces exponential growth in a population if the diversification rate is greater than the extinction rate and exponential decline if the extinction rate is greater. After time *t*, you expect there to be $N_0 e^{rt}$ taxa. It is easy to calculate the probability of getting *N* taxa, after time *t,* or the probability of a particular tree shape.

The Yule process is a modification of the birth-death process, in which the extinction rate is assumed to be zero. This may seem like a terrible assumption. However, the birth-death process is not good at estimating the actual extinction rate, so using a particular extinction rate will rarely have a large effect on the outcome of an analysis. It is often much easier to make calculations, if one assumes that there is no extinction, so test designers often use the Yule process. One should keep in mind when using the Yule process to analyze a tree that you are assuming that the extinction rate is zero. Therefore, even though the estimated parameter is reported as l, the speciation rate, it may be better to think of it as *r*, the diversification rate.

V. Detecting Balanced or Imbalanced Clades

Determining if a clade is imbalanced on a global scale is a fairly straight forward procedure. You calculate the imbalance of a particular clade using one of several measures. Multiple trees of the same size are then simulated from a null distribution (usually equal branching) and the imbalance of the clade in question is compared to this distribution in order to generate a p-value.

The most common measures of imbalance rely only on the topology, and do not consider branch lengths. The two most powerful (Agapow and Purvis 2002) measures of imbalance are Colless's *I* and the mean path length from root to tip. Both these indices increase as imbalance increases.

Colless's I is the sum of the absolute values of the difference between the number of taxa in every pair of sister clades.

The mean path length is the sum of the number of nodes below each tip.

There is some indication that phylogenies may in general be much more imbalanced than one would expect under random branching (Blum and Francois 2006). This implies that macroevolutionary forces are rampant. It is common practice to check for imbalance in a clade before trying to explain why some clades are more diverse than others. It is also possible that different types of clades are more or less imbalanced, or that balance varies between different levels of a tree.

VI. Detecting Unexpectedly Large or Small Clades

Sister Clades. The classic way to identify an excessively large or small clade is to compare two sister clades. If one is much bigger than the other, then there presumably must be some reason. This method has two major problems. The first is that every possible division of taxa between two sister clades is equally likely (Slowinski and Guyer 1989). Thus, having 50 taxa in one clade and 50 in its sister is just as likely as having 99 taxa in one clade and 1 in its sister. Therefore the one tailed p-value for a diversity comparison between sister clades is $n_s/(n_s+n_l-1)$.

The second problem with this type of analysis has a potentially even more devastating affect on power. When a researcher is comparing the diversity of two sister clades, they have almost always picked it as an example from a much larger phylogeny in which this pair of sisters is particularly different in diversity. Therefore a test of sister clade diversity actually is a two tailed test, and is only one of many potential tests. The number of other possible tests is equal to the number of other internal nodes in the tree, which means that in any tree you should almost always expect to see one pair of sister taxa with the maximum possible difference, as there are so many places to conceivably observe such a difference.

Global Comparisons. One potential resolution to the problem is to test every node in a phylogeny. One can then make the Bonferroni correction for multiple tests to estimate p-values. This approach is more honest, but it still does not resolve the problems with power.

Several likelihood tests involving the birth-death process have been shown to have much greater power in detecting oversized clades. One approach is to estimate l and m for the entire clade and then use those values to calculate the probability of each clade being large as it is (Magallon and Sanderson.,2001). Clades found to be outside some reasonable confidence interval are assumed to be significantly large or small. Several other tests revolve around using the likelihood ratio test to determine if there are different rates between sister clades (McConway and Sims 2004) or pairs of sister clades and their nearest outgroup (Moore et al.. 2004). P-values for these statistics can be calculated by simulation.

VII. Detecting correlations between traits and diversification

This is what all these tests are aiming towards. If we are interested in the affects of macroevolutionary forces, we want to know what characters are influencing that affect. If one character state has a large effect on diversification or extinction it can bias the distribution of lineages across the tree. A strong macroevolutionary affect can lead to the appearance that a trait is highly selected for on a microevolutionary scale, when it is actually not (Maddison 2006). In fact the proportion of taxa with a given character state is a consequence of a balance between microevolutionary and macroevolutionary forces. In the last couple of decades many tests have been designed to test for just such a correlation. We will discuss several, at least in outline.

Pseudoreplication is as big a problem for calculating these types of correlations as it is for any other correlation calculated on a phylogeny. However, test designers in this field tend to ignore it. They seem to think that since they are using a phylogeny in their test, they have already dealt with this problem. That is not the case. If diversity is dependent on a character and that character is dependent on the history of the taxa, then diversity may appear to be dependent on another character as a consequence of their shared history alone. In other words imbalanced phylogenies could indicate spurious correlations between characters and diversity, if the null distribution is not carefully chosen.

Sister clade comparisons represent the simplest test that can be done. They inherently deal with the pseudoreplication problem. If you can identify pairs of sister clades for which every member of one clade has the same character state for a discrete character and every member of the other clade has another state or the two clades have non overlapping ranges for a continuous character, then a straight forward sign test can be done (Maddison 2006). The fraction of clade pairs with differences in the same direction can be compared to a binomial distribution. However, the power of your test is severely limited by the number of such clade pairs that you can identify.

If you can only identify sister clades in which some portion of taxa have the same character state or they have overlapping ranges for a continuous character, then your path is not so clear. These tests are nonetheless commonly done. Two sister clades can be compared; the clade with a higher percentage of taxa that have a particular character state can be compared to the difference in clade size. For a continuous character a phylogenetically independent contrasts for a pair of sister clades can be generated and this can then be compared to the difference in size between clades. Both these tests are semi-phylogenetic at best, as they fail to account for the effect that character changes within these clades had on diversity.

Nested comparisons are often done using these same methods throughout the tree. For example Independent Contrasts would be generated for some character for an entire tree, and then plotted against the differences in clade size for that pair of nodes (eg. Isaac et al. 2005 and Seddon et al. 2008). These methods have been shown by simulation to generate appropriate p-values (Isaac et al. 2003). However, they still have the same problem as the sister clade comparisons above, and these problems are compounded by potential pseudoreplication. The differences in size between clades are not equivalent to independent contrasts and have not had historical signal properly filtered out, thus the same change in diversity may be counted multiple times. It is not appropriate to generate independent contrasts for diversity data (although it is done), as the values are not equivalent in age and independent contrasts do not account for effects on diversification below the tips.

Large Clades. Another group of methods rely on comparing larger clades to smaller clades. In one type of test a slice is made through the tree at some point in time, so that all the clades are of the same age. The size of these "equivalent clades" is then compared to the distribution of some character (Nee et al. 1992). In another test one attempts to find a correlation between the extremely large clades identified in the previous section, and some character (Magallon and Sanderson. 2001). Neither of these tests deals with the pseudoreplication problem at all.

Full likelihood models. Three tests have been developed that calculate the likelihood of a character and diversity patterns together throughout a tree. Two of the tests are for discrete characters and one is for continuous characters. These types of tests have a potential to resolve the problems found in the other tests.

Both tests for discrete characters rely on calculating the probability of the tree and a binary character if the rates of speciation and extinction depend on the state of the character. The likelihood of that model is then compared to one in which the speciation and extinction rates are constant. One test relies on comparing maximum likelihoods using the likelihood ratio test (Maddison et al. 2007); the other uses a Bayesian MCMC to "paint" reconstructed character states onto the tree (Ree 2005).

The test for correlation between a continuous character and diversity is far more restricted (Paradis 2005). It assumes no extinction, and a particular reconstruction of one or more continuous characters on the tree. It then tests for a linear relationship between those characters and $log(l/1\text{-}l)$. This is compared to a regular Yule process by a LRT.

All of these tests use an incorrect model for their null distribution, in which the tree is a random draw from a birth death distribution. This does not account for the fact that the tree in question may in fact be imbalanced as a consequence of diversity being dependent on characters that they did not consider. This problem can easily be resolved by comparing their test statistics to a null distribution of characters randomly evolved on the same tree.

VIII. Issues

"Taxonomic data"- Unresolved Terminals. It is often the case that one wants to draw conclusions about diversification processes without a fully resolved tree. Almost all of these methods can work at least theoretically with "taxonomic data". That is to say the terminals of the tree can have numbers of species instead of a resolved phylogeny. Many of these tests are set

up to work with this type of data, and most of the others could potentially be. Of course you must assume that your terminals are in fact monophyletic, that species numbers are comparable in different groups, etc. This is not recommended except in emergencies -- much better to use phylogenies!

Fossils. Throughout this lecture we have been forced to talk about diversity, instead of separating it into the two distinct processes of speciation (diversification) and extinction, because our methods have no power to distinguish the two. Fossils have the potential to provide a great deal of information on patterns of extinction. In fact there is already a large literature on this subject; unfortunately most of it is not phylogenetic (Jablonski 2008). One could potentially add fossils to phylogenies, and then modify any of these methods to generate an equivalent test, but with much more power to separate extinction from speciation. However, there is a problem with sampling...

Sampling. Pretty much all of these methods assume that you have a complete sample of species (terminal clades). This is obviously an unrealistic assumption. A few do have modifications to deal with incomplete sampling, but they are mostly *ad hoc*. The question is, how much of a problem does it cause? It depends on whether the noise is evenly distributed, or sampling is biased. There have not been many studies done on this subject, but my inclination is to say that the problem is not too big, as long as your sampling is random. You should be aware that diversity estimates are probably a little low, but as long as they're low across the board that's OK.

You can get into a problem if your sampling is biased, in particular if it is biased in favor of a clade that you are trying to show is more diverse. For example I would expect all the following groups to appear relatively more diverse than their sister: clades of large organisms, heavily studied clades, clades with many representatives in heavily studied areas, terrestrial clades, clades with showy taxa with easy to distinguish species. Thus if you're trying to show that small animals are more diverse than large ones, you're OK. On the other hand, if you're trying to show that more strongly sexually selected taxa are more diverse, you may have a problem.

Species definitions can also have a large affect on your conclusions. If you're asking questions about numbers of species, then what you think species are can have a big affect. This may be a bigger problem if you are including species defined by different groups of researchers.

If you reject species as an independently existing level of organization for analysis, then this problem ends up reducing to the same problem as differential survival in microevolution but being played out on a much larger scale than just within populations.

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