

February 12, 2007. **What is phylogenetic signal, and how do you measure it?**

## I. Introduction

The reconstruction of phylogenies requires the existence of characters that exhibit phylogenetic signal, i.e. character states that are shared among related species. The significance of shared homologous traits as evidence of common descent was one of Darwin's great contributions to evolutionary biology, though not what he is remembered for today. We remember Darwin for the theory of natural selection as an explanation for adaptation. Paradoxically, natural selection was in some ways a secondary contribution of the Origin of Species, a theory that Darwin required to explain how phylogenetic signal could be lost given his theory of common descent.

Most modern comparative methods represent attempt to more rigorously test hypotheses of adaptation, or at the very least to reconstruct the timing and frequency of key events in evolutionary history. Starting with the theory of punctuated evolution (1973), paleontologists began to focus much greater attention on the prevalence of evolutionary stasis – the lack of change. Thus the burden shifted to adaptationists to demonstrate the existence of a trait reflected the action of selection, rather than a legacy of past history. Thus, phylogenetic signal (the outcome of stasis combined with cladogenesis) became the 'problem' for the study of adaptation, a problem that comparative methods could potentially solve.

## II. Pattern and Process

Pattern is not process. No matter how many times we hear and repeat this phrase, the temptation to infer the action of specific processes from pattern alone is inevitable. In comparative biology, it has been all too common to equate stasis (pattern) with 'inertia' or 'constraint' (process?) and evolutionary change (pattern) with the action of selection (process). It is essential at the outset to see these dichotomies of pattern and process as orthogonal, setting up a 2x2 table:

<u>Evolutionary process</u>	<u>Pattern of trait evolution</u>	
	Stasis	Change
Adaptive	Stabilizing selection Fluctuating directional selection	Directional or disruptive selection
Non-adaptive	Lack of genetic variation (= constraint?) Antagonistic correlations among traits under selection Swamping by gene flow	Mutation Genetic drift Genetic correlations with traits under selection

In this case, I equate the term 'adaptive' with patterns resulting from the action of selection. It is not a statement about the functional significance of a particular trait, nor is it the same as stating that a particular trait represents an 'adaptation'.

It is critical to remember, as well, that these processes are neither exclusive nor independent of each other. For example, the action of drift and selection in small populations may simultaneously contribute to changes in gene frequency, and it may be difficult or impossible in an individual instance to separate their contributions. Replication in space (populations) or time (across generations) is critical, as selection is the only process we currently know of in evolutionary biology that can cause repeated, heritable change in the same direction in response to environmental conditions. Note also that selection leads to the loss of genetic variation. Thus, strong selection on a trait may lead to fixation of genes responsible for variation, such that future maintenance of the trait is due to the absence of sufficient variation (even if the selective context shifts).

### **III. Phylogenetic signal**

As discussed in the Blomberg and Garland paper, there is a long history of usage of terms such as phylogenetic inertia or constraint, and other more recent terms such as phylogenetic effect and conservatism. To a greater or lesser degree, all of these terms imply the action of some underlying process. The term phylogenetic signal is useful as the implication of pattern alone is clear cut. For the same reason it is a somewhat awkward term; of the available alternatives I prefer phylogenetic conservatism, because I don't think it has a lot of intellectual baggage from the earlier literature and it is fairly agnostic regarding the underlying process.

How can we measure phylogenetic signal? What is desired is a statistical method, utilizing knowledge of phylogenetic relationships, that expresses the degree to which related species are similar to each other. A number of alternatives have been proposed, depending on the specific objectives and the nature of the traits under consideration. It turns out, for reasons that will become clear, that it matters a lot how many states a trait can take on (or for continuous traits, the range of possible states).

### **III. Binary traits: number of steps.**

We will start with simple discrete, binary traits (i.e., traits that take on only two different states). For a binary trait, a simple measure of phylogenetic signal is the number of inferred changes on the phylogeny that are required to explain the extant trait distribution among terminal taxa. The changes can be inferred by parsimony, using standard methods. To get a quick intuition for this, if half of the taxa have state A and half have state B, and these also represent two sister clades on the phylogeny, then only one change is required at the base. All observed similarity among taxa is then 'inherited' from that change (or from the ancestral state, for the other half). In contrast, if the taxa were arranged as a series of sister species pairs, and each pair had one A and one B, then the changes would all be reconstructed very near the tips, and there would be  $N/2$  changes for  $N$  taxa. Clearly, the larger number of changes in this case corresponds to a lower degree of signal.

Let's say we've counted the number of changes – say we get 3. Is that a lot or a little? We might

compare the result to the number of changes for another trait, to test whether some traits exhibit greater signal than others. But we would also like to make an absolute statement for each trait of the significance of the observed pattern. Now things get interesting!

#### IV. Null models for phylogenetic signal

To make a statement of significance, in a statistical sense, we need a null model. We need some way of saying, 'how many changes would we infer if phylogenetic signal were absent?' The problem of null models has generated considerable debate and it helps to focus discussion quite explicitly on our assumptions about the evolutionary process.

Null 1 – Randomized tips: The simplest null model states that the distribution of traits among terminal taxa is independent of their phylogenetic relationships. This is accomplished through randomization procedures, shuffling the traits among taxa (or equivalently, resampling without replacement). Each shuffle provides one instance of what is expected under the null model. A large number of randomizations (999, 9999) provides a distribution of expectations, and this provides the point of comparison for the observed data. If the observed number of changes is less than expected, we can state with a certain probability, the significance of the degree of phylogenetic signal. If the observed number is greater than the random distribution, there is evidence of 'negative signal', a pattern of numerous changes likely distributed near the tips of the tree.

The statistical significance of a pattern, using randomization methods, is calculated by ranking the observed pattern together with the complete array of randomized values. If the test is two-tailed (i.e., you were interested in detecting deviations in either direction from random), the location of the observed value is counted from the nearer end of the list, this number is doubled, and then it is divided by the total number of randomizations. If the test was one-tailed, the location is counted from the appropriate end, and the position is divided by the number of randomizations. If the observed value is tied with some of the randomized values, it is placed towards the center of the distribution so the tied values are all counted. It is conventional to include the observed data as one of the values, along with the randomizations (it is, in a sense, innocent until proven guilty). Hence, 999 randomizations would be done, plus the observed value, to get a total distribution of 1000 values.

Example: A total of 5 changes are inferred for a character. When the character states are randomized on the tree 99 times, and then sorted, the array begins (from the lower end):

3,3,3,4,4,4,4,4,4,5,5,5,5,5,**5**,6,6,6,6,6,6,6,6,6,6,7,

The observed value is in the 15<sup>th</sup> position, out of 100 total. For a 2-tailed test, this indicates  $p = 0.3$ . For a one-tailed test of the hypothesis that observed would be less than expected,  $p = 0.15$ . However, for a one-tailed test that the observed value would be greater than expected,  $p = 0.85$ . Either way, this pattern would not be judged significant at the standard  $\alpha = 0.05$  cutoff level.

As you start working with randomization statistics, the key idea is to identify which aspects of the system you want to preserve, and which aspects will be randomized. For Null 1, we have preserved the topology of the tree, and the number of taxa exhibiting each state of the character.

We have then randomized the position of those states on the tips of the tree.

Null 2 – The second null model we will explore is a bit different, as it holds the number of states constant, but randomizes the topology of the tree. Since it is impossible to randomize the topology and keep the taxa in the 'same' position in any meaningful sense, this also means that we randomize the location of the taxa on the tips. After conducting 99, 999, or however many randomizations are desired, the rest of the test proceeds as above.

We will compare the two null models above in lab, to determine whether they behave similarly or not in detecting significant departures from random patterns. In particular, one of the problems we want to answer is whether the shape of the tree affects the distribution of number of steps under Null model 1. If this is the case, then our ability to detect phylogenetic signal depends on the topology of the phylogeny we are working with. As you work with the two randomization methods, you also want to think about the question of how the null model differs in the two cases. How can you state in words, the null hypothesis under each randomization model?

**V. References** on phylogenetic signal and related terms (and see bibliography of the Blomberg and Garland paper):

Recommended for today's lecture:

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