

Adaptation and the comparative method

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Over the past two decades, it has become widely accepted that phylogenies need to be incorporated into statistical analyses of interspecific data. However, recent debate has focused on whether it is appropriate to apply phylogenetic comparative methods (PCMs) to the study of adaptation. Although some of the criticisms are serious, it is premature to stop applying PCMs altogether. New statistical methods designed explicitly for the comparative study of adaptation overcome these criticisms and offer fresh insights into the evolution of phenotypes.

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Evolutionary biologists have used the phylogenetic comparative method (PCM) to study adaptation (Box 1) for a long time. The development of modern statistical tools for analysing comparative data has generated much excitement. However, recently the use of modern PCMs has come under criticism from authors who argue that PCMs might be inadequate for the task of studying adaptation^{7–11} and that we should focus instead on mechanistic or empirical approaches. Rather than throw the baby out with the bathwater, other researchers^{12–17} have begun to develop PCMs specifically for use in the study of adaptation. Here, I discuss a few of the more serious statistical criticisms, and present highlights of new PCMs that address these criticisms directly and lead us to new directions in the study of adaptation.

Inaccurate estimates of ancestral states?

The traditional cladistic approach to studying adaptation is to reconstruct the phenotypes of putative ancestors and to determine whether evolutionary changes in the phenotype have been associated with major shifts in the environment (Box 1). The main criticism of using statistical PCMs with this approach is that ancestral estimates are probably inaccurate^{11,14}, especially given the unrealistic models of evolution assumed by most available statistical methods. In particular, whenever the rate of evolution is sufficiently high or the ancestor sufficiently old, it might be best to accept our limitations and not even try to estimate ancestral states from comparative data.

This discussion has been reinvigorated by the development of two new PCMs^{14,18,19} for estimating ancestral

states. However, in spite of gloomy theoretical predictions, computer simulation tests^{20,21} show that estimates from these methods can be reasonably accurate. For example, in a simple case, correlations between true values of ancestral phenotypes and those estimated using the new PCMs were over 90% for six out of eight ancestors estimated²¹. Of course, it is difficult to know whether the ancestor in which we are particularly interested is one of the six that were estimated correctly or whether our situation is comparable to the simple conditions used in computer simulation studies. Still, the problem might not be nearly as bad as anticipated.

The two new PCMs are also promising because they include ways of estimating confidence intervals for each ancestral state, thus offering a direct way of determining how accurate our estimates truly are^{14,18,19}. Unfortunately, measures of uncertainty (i.e. standard error) appear to be far less accurate than estimates of the ancestral states themselves, especially when selection has been working to erase history²¹. We might also be able to use diagnostic tools (e.g. spatial autocorrelation statistics) to determine whether the comparative data contain enough phylogenetic signal for accurate estimation of ancestral states¹¹. Existing diagnostic tools are not powerful²² and might cause us to reject the use of PCMs more often than necessary. Thus, further statistical development of uncertainty measures (e.g. randomization tests) and of diagnostic tools are needed. Although we need to be cautious about the details of underlying evolutionary models and measures of uncertainty, it is too soon to dismiss the use of statistical PCMs for studying adaptation based solely on estimation accuracy.

Evolutionary maintenance versus origins

Another worry of the historical approach discussed previously is that it focuses on evolutionary change and can ignore important adaptations that have simply been maintained for long periods. The hope is to identify specific periods when phenotypes changed in response to natural selection. However, because evolutionary changes can be exceedingly rare, the focus on the few resulting changes often makes the sample size too low for statistical analyses to be meaningful. For example, consider the evolution of display frequency in *Liolaemus* lizards (Fig. 1). Although Chilean *Liolaemus* produce more visual head-bob displays than Argentinian *Liolaemus*, it seems that the difference is the result of a single change that happened at the initial divergence of the two clades. Following one possible argument²³, a single evolutionary change gives us only a single data point for our comparative analyses, and we are left with no hope of finding an association between evolutionary change in display frequency and environmental factors. Adding data from more species does nothing to increase our statistical power and we would be tempted to conclude that a statistical PCM cannot be used to understand this potential adaptation any further.

However, this approach does not take into account that traits remaining stable for long periods might also be the products of natural selection^{3,13}. Normally, we expect species to become more different from one another over time simply because of stochastic events occurring along each of the independent evolutionary trajectories. Small changes in the environment are likely to occur at each generation, such that each taxon is living in a slightly different selective regime. Random genetic drift might also cause traits to arise or be lost. An observation that display frequency has been retained at a high level, in spite of long periods of evolutionary diversification, might thus suggest that the trait has been actively retained as an adaptation.

One of the most exciting new comparative methods uses this type of evolutionary stasis as a starting place for studies of adaptation (Box 2). Hansen's¹³ model envisions organisms as groups of traits evolving in response to stabilizing selection imposed by a variety of potentially conflicting environmental factors. His PCM uses comparative data and information on how long each taxon has been subjected to a particular environmental state to estimate the relative impact of the specified environmental factor on phenotypic evolution. Variations

on this basic model can also be used to explore the possibility of character displacement¹⁵, adaptive radiation and many other selective phenomena. The approach offers an interesting complement to empirical and mechanistic approaches for determining exactly why certain phenotypes might be advantageous and whether these phenotypes are retained owing to a lack of genetic variation, developmental constraints or other factors.

Correlation versus causation

Some researchers have taken a simpler approach to studying adaptation, using PCMs to search for relationships between phenotypes and environments in clades exhibiting large numbers of evolutionary changes (Box 1). Although this might seem to be a reasonable approach at first, it suffers from several extensions of the well known correlation versus causation problem.

First, relationships between traits and environments in extant taxa do not address the question of evolutionary origin. As pointed out by several authors⁷, a current relationship between traits and environments does not necessarily mean that there was a relationship between traits and environments when the adaptation arose long ago. This is a problem in most of evolutionary biology and is not specific to the comparative method. We have little direct evidence of what happened millions of years ago and, therefore, must infer what happened from any available evidence.

Second, correlated changes in traits and environments do not necessarily imply that environmental change is triggering phenotypic changes^{6,10,24–27}. Relationships among traits or between traits and environments can be the result of forces other than adaptation. For example, such relationships might occur because the trait is evolutionarily linked to a second trait (the true adaptation) that is being selected on by the environment. The observed correlation might also be the result of a reversed causal relationship; for example, we would expect to find a correlation between trait and environment when organisms directly impact their environments.

The problem of correlation versus causation is a serious one and one that is difficult to address directly using PCMs. One suggestion is to look for repeated, but independent, examples of support for the same adaptive hypotheses. Repeated tests of this sort might serve as weak evidence for causation. Another possibility is to measure many variables and apply comparative methods that allow all to be analysed simultaneously (e.g. use multiple regression and

Box 1. General approaches to the study of adaptation

Although the definition of adaptation has been under debate for a long time, most researchers agree that adaptations are phenotypes exhibiting higher evolutionary fitness than other phenotypes in the same environment^{1–3}. In addition, phylogenetic researchers have argued that the phenotype must have been shaped by natural selection over evolutionary time to serve its current role^{4–6}. This has been termed the historical approach.

In general, there are two phylogenetic approaches to the study of adaptation. Traditionally, researchers have focused on specific traits, understanding the mechanistic basis of the trait and understanding exactly how that trait might function to increase fitness. The ancestral states of the trait can then be reconstructed on a phylogeny to determine which phenotypes are new forms derived in response to natural selection^{5–8}. This approach depends on thorough understanding of single traits and small numbers of evolutionary changes.

Other scientists have searched for statistical relationships between phenotypes and environments, while taking phylogenetic information into account². This approach is purely statistical, and relies on having large numbers of taxa and evolutionary changes in both the phenotype and the environment. Researchers often gather data from the literature on traits that are only poorly understood, with the hope of finding strong general patterns that will generate hypotheses for future studies.

multivariate statistics instead of simple correlations). Far more promising is the use of experimental manipulations in conjunction with comparative studies^{28–30}. For example, we might measure the genetic correlation between traits in existing species or consider the impacts of environmental change on these traits. We can use direct tests of the causal connection between traits and environments

in extant taxa to make inferences about the relationships in ancestral lineages. Again, the solution is not to give up on PCMs altogether, but rather to use them cautiously and in conjunction with other approaches.

Null models

Most PCMs initially involve determining how much trait variation can be explained

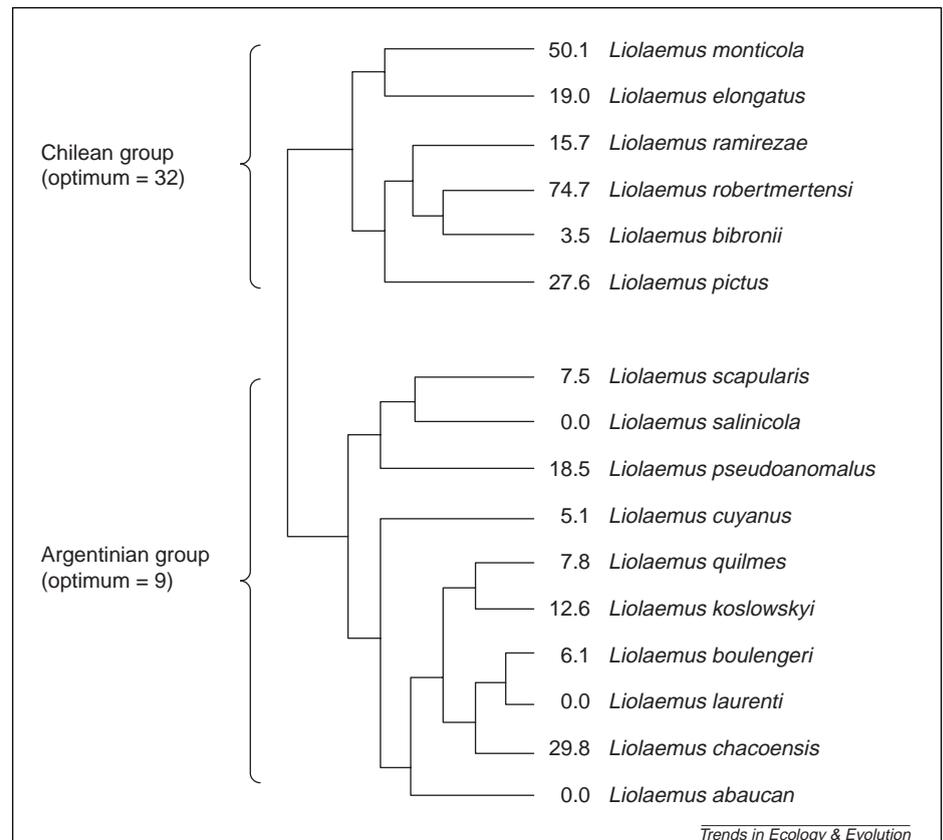


Fig. 1. An example of display frequencies and phenotypic optima estimated using Hansen's 1997 approach¹³ for 16 species of the South American *Liolaemus* lizards in two hypothetical environments. Although there are major differences in display frequencies between Chilean and Argentinian groups, these seem to be the result of a single evolutionary change early in the diversification of the Chilean group. Some cladistic approaches would consider this to be a single data point and would be unable to statistically determine whether display frequency is an adaptation. However, using Hansen's method (Box 2) we can use these data to answer several questions involving adaptation.

Box 2. A new perspective on adaptation

Hansen¹³ developed an exciting new mathematical approach to explore questions regarding adaptation. He envisions phenotypes evolving primarily in response to stabilizing selection, with traits tracking the environment as they change through evolutionary time. However, the environment is composed of several factors, most of which have only a small impact on the actual phenotype expressed. Hansen's method involves a statistical regression of measured taxon phenotypes on the amount of time each taxon evolved in a particular environmental state. The slope and overall fit of this regression provide estimates of the relative importance of a specific environmental factor in the evolution of a particular phenotype.

For example, we might imagine an increase in display frequency as a result of the radiation of *Liolaemus* across the Andes and into a novel Chilean environment, perhaps with more dense vegetation making a single display easy to miss (Fig. 1). We can then use Hansen's model to determine the relative historical importance of this Chilean vegetation on display frequency against a background of other environmental factors that might be similar for the two clades.

Using Hansen's model, we estimate a large difference between display frequencies in the two environments (23.0, S.E. = 8.99; Fig. 1) – this is strong evidence that display frequency increased with a single environmental shift and has been maintained at a high level as long as the environment remained constant. Under historical definitions (Box 1), our results are consistent with the trait being an adaptation to this particular environment.

by the stochastic evolution of traits along a phylogeny. For example, Cheverud *et al.*'s³⁰ spatial autocorrelational approach estimates the trait variation explained by

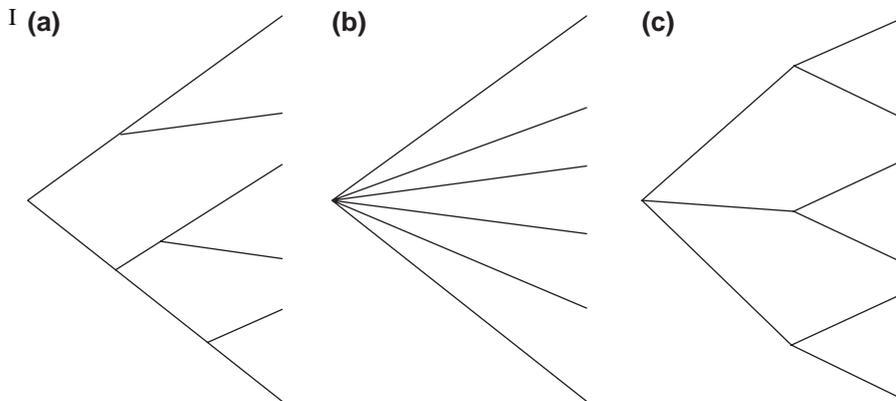
phylogeny, arguing that whatever is left over in the error term can be explained by other forces (e.g. adaptation). Baum and Donoghue¹⁶ suggest that likelihood

ratio tests can be used in a similar way, by comparing the fit of different models of phenotypic evolution to the data. For example, if a model describing phenotypic evolution under natural selection fits the data significantly better than a model of evolution under random genetic drift, we could conclude that the trait is an adaptation. Statistical likelihood can be estimated from most population-genetic models describing trait evolution (e.g. Pagel's³¹ model for categorical traits or the models reviewed in Hansen and Martins³²), thus offering a large number of possibilities.

As Westoby *et al.*⁹, and Orzack and Sober¹⁷ point out, it might not always be reasonable to consider phylogenetic explanations as the null hypothesis. In some cases, we might prefer to increase our statistical power by considering ecological or adaptive explanations first. Although the spatial autocorrelational method does not allow for this possibility, Baum and Donoghue's¹⁶ approach can easily be adapted to this scenario. The basic idea is also similar to that taken by Hansen^{13,15} (Box 2), in which it is assumed that the trait is evolving via natural selection. Although the approach does not allow us to distinguish between true adaptations and correlated selection, it does allow us to answer further questions (e.g. what is the optimum phenotype in a particular environment) and offers new areas for future research.

Box 3. Assumptions and the comparative method

Various authors have suggested that when phylogenetic information is unreliable we should not apply a phylogenetic method because of the unreasonable assumptions that are required. Unfortunately, not applying a phylogenetic method also requires making several assumptions. In fact, the assumptions underlying Felsenstein's contrasts²³ are not much more restrictive than those required by pairwise comparisons or refusal to apply phylogenetic methods, and they are based on firmer evolutionary ground. Below is an example (Fig. 1) of the assumptions that might be made during the application of each method.



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Felsenstein's method (Fig. 1a)

- Phylogeny is completely known and accurate. All ancestors lead to exactly two descendants.
- Traits evolve along the phylogeny via something similar to Brownian motion (BM). BM has been used in population genetics to describe several sorts of evolution^{24,32}; for example, it can be used to describe the effects of directional selection when the direction of selection has shifted stochastically through evolutionary time.

Nonphylogenetic analysis (Fig. 1b)

- Phylogeny is completely known and accurate. Specifically, the phylogeny looks like a star, with all taxa radiating from a single point. (Alternatively, we might assume that evolution is so rapid that taxa are not expected to show any similarities owing to shared phylogenetic history.)
- Each taxon has had the same opportunity for evolutionary change and the mean change in phenotype along each branch of the phylogeny is zero. (BM would lead to this sort of pattern.)

Pairwise comparison methods (Fig. 1c)

- Phylogeny is known and accurate, to the extent that taxon pairs are known not to overlap on the phylogeny.
- The two taxa within each pair have had the opportunity to undergo the same amount of evolutionary change and that amount of change is the same for all pairs. This would be true under BM if all taxon pairs also diverged for the same amount of time.
- Evolution after each speciation event is independent of evolution before that point. (This would also be true under BM.)

Invalid assumptions

Other authors have questioned whether comparative methods are appropriate for studying adaptation because of the restrictiveness of their assumptions^{9,10}. For example, Felsenstein's²⁴ independent contrasts method was designed to deal only with phenotypic traits (not environments) and requires an assumption of Brownian motion-type evolution (usually described as random genetic drift; Box 3). Use of this method to study adaptation probably violates its assumptions and we know that the method can perform poorly when given data that violate its assumptions^{10,33,34}.

However, the Brownian motion assumption, underlying Felsenstein's²⁴ method of independent contrasts (and many other PCMs), is a powerful assumption that covers a lot of evolutionary ground. For example, Brownian motion can also be used to model directional selection with a constant push towards a single optimum throughout the clade or when the direction shifts frequently and at random throughout the clade^{25,33}. When phylogenetic information is only somewhat inaccurate or assumptions are only mildly violated, Felsenstein's

method usually does at least as well as not doing anything at all^{22,35}.

The alternative situation, not taking phylogenetic information into account, can lead to worse problems. All comparative analyses make some assumptions (Box 3). Choosing not to apply a PCM, not to use branch length information, or not to apply a mathematically complex method (e.g. applying pairwise comparisons instead) usually requires making more, not fewer, assumptions. Several computer simulation studies^{22,35} have shown that not taking phylogenies into account can lead to inaccurate results. This is a statistical problem of pseudoreplication similar to that occurring when the same individual is measured repeatedly. Whenever such dependence exists it needs to be taken into account, whether the study has anything to do with evolution or with phylogenetics.

The problem is that we are usually not sure how much dependence there is and how seriously our particular case violates the assumptions of existing methods. Existing methods are also still somewhat inflexible about the types of phylogenetic dependence they can consider. For example, we still do not have a method designed specifically for Price's¹⁰ model of adaptation. Fortunately, the general method described by Martins and Hansen¹⁸ can be used to design statistical analyses to address virtually any comparative question and situation, including that described by Price¹⁰. Although doing this requires some theoretical skills, the approach is becoming more and more accessible each year. For example, Butler *et al.*³⁵ showed how the basic tools can be implemented in a standard statistical package. Other authors are developing new tools for particular questions regarding adaptation^{12,13,15,16} and randomization tests can also provide a powerful way to develop phylogenetic methods for a particular situation¹⁵.

Conclusions

Recently, a handful of methods have been published allowing adaptation and natural selection to be included explicitly in phylogenetic comparative analyses. These methods offer a new perspective on the study of adaptation and directly address the criticisms outlined here. Most of the methods are still in a developmental stage, thus requiring further theoretical exploration and computer implementation tools. However, the number of recent manuscripts on the topic suggests that such development is well under way.

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