

An integrative view of phylogenetic comparative methods: connections to population genetics, community ecology, and paleobiology

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Recent innovations in phylogenetic comparative methods (PCMs) have spurred a renaissance of research into the causes and consequences of large-scale patterns of biodiversity. In this paper, we review these advances. We also highlight the potential of comparative methods to integrate across fields and focus on three examples where such integration might be particularly valuable: quantitative genetics, community ecology, and paleobiology. We argue that PCMs will continue to be a key set of tools in evolutionary biology, shedding new light on how evolutionary processes have shaped patterns of biodiversity through deep time.

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Introduction

Phylogenetic trees are important tools for classifying organisms and studying evolutionary patterns.¹ Rapidly decreasing costs of sequencing coupled with increasingly sophisticated tree-building methods and software (e.g., MrBayes,² BEAST,³ RAxML⁴) have meant that robust phylogenetic trees with reliable branch lengths are now available for many groups, with more to come. Along with this increase in trees, the number and variety of phylogenetic comparative methods (PCMs) have exploded.⁵ Novel PCMs have been developed to study a wide range of phenomena. For example, new methods allow scientists to explore the dynamics of diversification in evolving clades, testing for changes in speciation and/or extinction rates through time and across groups.^{6–10} Other new methods focus on testing hypotheses about the tempo and mode of evolution of morphological and ecological traits.^{11–15} These innovations have spurred a renaissance of research into the causes and consequences of large-scale patterns of biodiversity.

This is undoubtedly an exciting time for PCMs. In this essay, we outline how recent advances in PCMs can help us to address long-standing questions in evolutionary biology across various temporal and

spatial scales. We discuss how further developments in PCMs, which have long been a key tool for interdisciplinary research, might contribute to other fields focusing on quantitative genetics, ecology, and paleobiology. But, as we argue throughout this essay, this is not a one-way street; the further development of PCMs will crucially depend on incorporating models, concepts, and ideas from seemingly disparate areas of research. PCMs will continue to be a key set of tools in evolutionary biology, shedding new light on how evolutionary processes have shaped patterns of biodiversity through deep time.

The current state of PCMs

PCMs are a set of statistical approaches that can be used to analyze phylogenetic trees and, often, associated data on species' traits. The first PCMs were designed as statistical tools for accounting for shared ancestry while testing for correlations between pairs of traits.^{16–18} Essentially, the tree was treated as a statistical nuisance—something that needed to be considered lest it mislead tests of adaptation. More recently, researchers have viewed phylogenies as historical frameworks and used PCMs to model evolutionary processes along their branches, such as models of speciation and

extinction;^{19,20} trait evolution;^{11,13,21–23} and the influence of traits on diversification.^{24,25} In this section, we briefly review the early development of PCMs and give examples of their most common applications.

Methods for analyzing trait evolution

Testing for evolutionary correlations among characters. One of the first PCMs was Felsenstein's phylogenetic independent contrasts.¹⁶ Independent contrasts are most commonly used to test for correlated evolution between two characters. By *evolutionary correlation*, we mean that we can predict the magnitude and direction of change in one character given knowledge of evolutionary changes in another—which differs from standard correlation, in which the goal is to predict the value of one trait given the value of another. For example, Garland *et al.*²⁶ used independent contrasts to test for an evolutionary correlation between body mass and home range area across mammal species. Independent contrasts can also be used to estimate the rate of trait evolution and compare rates among clades²⁷ as the average squared independent contrast is exactly the restricted maximum likelihood (REML) estimate of the evolutionary rate parameter of a Brownian motion (BM) process.²⁸

An approach that is closely related to independent contrasts but more flexible is phylogenetic generalized least squares (PGLS).¹⁷ PGLS uses linear models to fit statistical models to comparative data accounting for the phylogeny (specifically, so that the covariance structure of the residuals reflects the structure of the phylogeny). Using PGLS, one can test hypotheses about character correlation (as above), but PGLS also allows one to construct more complex and multifactor models of correlations among characters. For example, one could use PGLS to explain variation in one response variable, say body size Y , using any number of explanatory predictor variables X_1, X_2, \dots, X_n , which may be either continuous or discrete. Although PGLS can seem quite distinct from independent contrasts, the two methods are actually statistically equivalent to one another when one assumes that traits evolve according to a BM model.^{28,29} However, PGLS has the additional flexibility that one can also assume other (non-Brownian) models of evolution, like Ornstein–Uhlenbeck (OU),²¹ early burst,^{30,31} or Pagel's tree transformations.^{32,33} These issues are

discussed in detail in Refs. 34 and 35, and we will not delve into them further here. There are also a number of methods for testing for evolutionary correlations without an explicit model, such as phylogenetic eigenvector regression (PVR)³⁶ and partial Mantel tests;³⁷ however, recent studies have demonstrated that model-based methods generally have superior statistical properties to their nonparametric counterparts.^{38,39}

An alternate method for testing for evolutionary correlations between traits that is rapidly becoming very popular is the phylogenetic mixed model.^{33,40–42} The phylogenetic mixed model is adapted from the “animal” model from quantitative genetics,^{43,44} wherein observations of individual trait values are linked through a pedigree. Lynch⁴⁰ demonstrated that an analogous model could be used for comparative data, with the phylogeny taking the place of a pedigree. Hadfield and Nakagawa⁴² extended Lynch's idea, making the case that this connection to quantitative genetics has very broad implications. Sophisticated statistical methods for fitting complex models have been well developed in quantitative genetics,^{45,46} and these can be applied to phylogenetic comparative data. By making use of these innovations, researchers can test for correlations between traits with a wide variety of distributions (standard PGLS requires that the response variable be normally distributed) and include intraspecific sampling. Bayesian Markov chain Monte Carlo (MCMC) methods, similarly adopted from the quantitative genetics literature,⁴⁵ can be used to efficiently estimate parameters, even for complex models and these are currently implemented in flexible software for fitting mixed-effects models (MCMCg1mm).⁴⁷ We think the phylogenetic mixed model is very widely applicable and anticipate that its use will only continue to grow. Future research is needed to fully understand the statistical connections between this and other approaches that were developed explicitly in the context of PCMs to solve similar problems.^{48–52}

Using models to understand the tempo and mode of trait evolution. While testing for correlated evolution between characters on a phylogeny requires a model, the model itself is often not of interest.³⁴ A conceptually distinct approach to the comparative analysis of traits focuses on using the models in order to make inferences regarding the

evolutionary processes that may have generated the data we observe^{53,54}—in essence, the tree is treated as a source of information rather than as being a nuisance parameter.³⁸ These models are still being developed, but already allow us to fit a wide range of models to phylogenetic comparative data. One can focus on estimating parameters that are of interest to a wide range of biologists, or on comparing the fit of alternative models. For example, perhaps a biologist is interested in average rates of trait change through time, perhaps to compare the rate to what is known from studies of short-term evolution. A simple way to estimate that rate is to use maximum likelihood or Bayesian methods to fit a model of BM evolution to characters on trees, estimating the rate parameter σ^2 .⁵⁵ For example, this approach has been used to compare rates of body size evolution across a range of animal groups.³¹ Similar approaches are also possible for characters with discrete states.^{22,50,56}

Model-fitting approaches show tremendous promise due to their potential flexibility. Many key approaches involve comparing the fit of simple models—like BM—to various alternatives. For example, many macroevolutionary hypotheses of interest to organismal biologists hypothesize that rates of evolution might vary across a phylogenetic tree. To address this question, O'Meara *et al.*¹¹ developed an approach in which researchers could assign branches on the phylogeny to “evolutionary regimes” and ask whether the regimes had significantly different BM rate parameters (see Ref. 12 for a related approach). An early example of an application of this method can be seen in Collier *et al.*,⁵⁷ who showed elevated rates of evolution in the morphology of the feeding apparatus in the sunfishes (*Lepomis*) compared to rates in their sister clade, the black basses (*Micropterus*). A more flexible approach, developed by Eastman *et al.*, uses reversible-jump MCMC (RJMCMC)⁵⁸ to fit multitrate BM models to trees (AUTEUR).¹³ (Similar machinery was developed by Revell *et al.*¹⁴ and Venditti *et al.*⁵⁹) The RJMCMC approach of Eastman *et al.* can be used to explore the data without requiring particular *a priori* hypotheses about how rates vary across the tree, or it can be constrained to test specific hypotheses⁶⁰ as to where rate shifts occur in a matter similar to that of O'Meara *et al.*¹¹ An alternate statistical approach for investigating heterogeneity in rates of evolution across the tree is to use approximate Bayesian computation (ABC).^{61,62}

This is useful for situations where a closed-form likelihood is difficult to derive or does not exist. An example of this is fitting evolutionary trait models for continuous characters to unresolved clades (“MECCA”).⁶³ (Kutsukake and Innan⁶⁴ used a related technique to estimate parameters for a more complex suite of models.)

One can also consider models that depart from the unconstrained random walk of BM. One common alternative is the OU model for trait evolution. This model can be qualitatively described as “Brownian motion on a spring”; characters evolve with a random walk component but also tend to be drawn toward some medial value (often denoted θ) by a restraining force (captured by the parameter α). This model can be equated to Lande's model of stabilizing selection on a constant adaptive landscape,⁶⁵ although model parameters estimated on phylogenetic trees are almost never compatible with this interpretation of OU (see below; Ref. 31). As discussed above, a number of methods have been developed to model heterogeneity in the rate of BM evolution across the phylogeny; the same is true of OU models. The approaches of Butler and King,⁶⁶ Beaulieu *et al.*,¹⁵ and Ingram and Mahler⁶⁷ allow the parameters of the OU model to vary among clades in a phylogenetic tree. (See Ref. 68 for a conceptually related method for investigating evolutionary correlations between traits.)

We focus here on BM and OU processes due to their explicit connections to population genetic models (see below). However, a number of other models of trait evolution have been developed for comparative data. These include the accelerating/decelerating change model (ACDC),³⁰ also known as the early burst model³¹ when referring to decelerating rates of evolution. The early burst model has often been interpreted by researchers^{31,69} as being consistent with predictions from classical theory on adaptive radiations,^{70,71} though this connection is mostly heuristic and is not rooted in any formal mathematical theory. Other models that have been considered include cladogenetic evolution models, in which traits evolve during speciation events;^{54,72} phyletic trend models,^{49,73–75} and a “saltational evolution” model based on a Lévy process.⁷⁶ O'Meara⁵ provides an excellent overview of the mathematical connections between these various models, and we refer interested readers to his paper for technical details.

Methods for analyzing lineage diversification

Using tree topology to investigate patterns of diversification. Phylogenetic trees contain information regarding the historical patterns of diversity dynamics, and characterizing the patterns of diversification has long been a major focus of evolutionary biologists.⁷⁷ The earliest approaches for comparing diversification rates on trees relied on measurements of tree balance. These metrics compare the number of species in sister clades—that is, pairs of clades that descend from a single common ancestor. Any differences in diversity between sister clades can then be compared to the expectation under a null model such as birth–death, a simple model where each lineage has a constant probability of speciating or going extinct. Dramatic differences in species number between these sister clades are a sign of different diversification rates in these clades.⁷⁸ One limitation of this approach is that birth–death models create nodes that are quite unbalanced in diversity, so that significant results require dramatic differences in diversity.⁷⁹ Researchers gain power by combining data from many independent pairs of sister clades. For example, studies combining pairs of sister clades have found associations between the diversity of angiosperms (flowering plants) and a variety of traits (e.g., mating system,⁸⁰ floral symmetry⁸¹). One can also summarize the pattern of balance across entire trees. There are a number of metrics to do so, each with its own advantages;^{82,83} modern versions of tree balance tests use ML (Symmetree: Refs. 84 and 85) or Bayesian⁸⁶ approaches. Taken as a whole, studies on tree balance have shown that phylogenetic trees are generally more imbalanced than one would expect from birth–death models.⁸⁷ This general result—which seems to apply broadly to many taxa and tree depths—suggests that rates of speciation and extinction vary across clades in the tree of life, a finding consistent with the plethora of studies showing this in the fossil record.^{88,89}

Model-based approaches to investigate rates of diversification. One can also directly fit birth–death models to phylogenetic trees. Although birth–death models have long been applied to various types of evolutionary problems,^{90,91} most current approaches stem from an influential paper by Nee *et al.*¹⁹ who derived likelihood equations for phylogenetic trees of extant taxa generated under a birth–death process. More recently, the approach of Nee

*et al.*¹⁹ has been extended to allow for various modifications. For example, Alfaro *et al.*⁶ use MEDUSA, which makes use of a stepwise Akaike information criterion (AIC) algorithm, to identify vertebrate clades that have unusual rates of speciation and/or extinction compared to their relatives. Similarly, multiple researchers have developed statistical models to investigate how patterns of diversification rates change through time.^{7,8,10,92,93} It is also worth noting that the time-dependent models mentioned in the preceding sentence were also independently implemented in the R package *Diversitree*²⁵ using the approach originally derived by Maddison *et al.*²⁴ Extinction estimates from phylogenies of present-day species can be unreliable, with some researchers suggesting that one should never try to estimate extinction from living species.⁹⁴ Although one might view extinction estimates with some skepticism, extinction certainly affects the shape of phylogenetic trees, and it seems a mistake to leave it out of diversification models.^{95,96}

Methods for analyzing the influence of characters on diversification. Researchers in paleobiology and macroevolution have long been intrigued by the question of how traits may influence speciation or extinction rates.^{97–100} In an extremely innovative paper, Maddison *et al.*²⁴ derived a model (the binary state speciation–extinction (BiSSE) model) that simultaneously accounts for trait evolution, diversification, and the influence of the former on the latter. The original BiSSE model²⁴ was developed to test for a relationship between the state of a two-state discrete character and rates of speciation or extinction. For example, Goldberg *et al.*¹⁰¹ used the BiSSE model to investigate the effect of mating system (self-incompatible versus self-compatible lineages) on diversification within the plant family Solanaceae. They found that self-incompatible lineages diversified at significantly higher rates than their self-compatible relatives, maintaining self-incompatibility in the clade despite a tendency for transitions away from self-incompatibility to occur at a higher rate than transitions to self-incompatibility. Such a result suggests a role for species selection in the evolution of biodiversity, something that has long been of immense interest to many evolutionary biologists.^{102,103} However, the importance of these xxSSE class of models goes beyond investigating

questions of species selection. Studying trait evolution on its own without considering the possibility that variation in the trait of interest may be correlated with variation in diversification rates may lead researchers to make spurious inferences regarding the rates of evolution, as eloquently demonstrated by Maddison¹⁰⁴ and Goldberg and Iqic.¹⁰⁵

Extensions of BiSSE now allow one to test for a relationship between speciation and/or extinction and a quantitative character (QuaSSE),⁴⁹ multistate characters (MuSSE),²⁵ and geographic range (GeoSSE).¹⁰⁶ One can also evaluate whether evolutionary change is concentrated at speciation events or not (ClaSSE and BiSSE-ness; Refs. 107 and 108, respectively). This variety of approach highlights the flexibility of the mathematical approach upon which BiSSE *et al.* are based;²⁴ certainly more extensions of this framework are possible for future work.

Progress and directions toward a more integrative comparative biology

Comparative methods can give insights into questions in a wide range of fields. What all of these approaches have in common is that they are concerned with patterns expected when one extrapolates evolution along a single branch of a tree to a whole clade of related species. That is, comparative methods at their core are methods that relate processes that happen within species to patterns of variation across species. It is this aspect of comparative methods that makes them so well suited to interdisciplinary questions in many different areas of biology, anthropology, and beyond.

We believe that comparative methods will increasingly play a key role in bridging gaps, both across disciplines and over short and long timescales. Below, we highlight three areas where recent work in comparative methods has shown great promise in building these bridges.

Quantitative genetics and PCMs

The often-made distinction between microevolution and macroevolution is partially arbitrary. The fundamental processes of evolution (selection, mutation, drift, and gene flow) that determine the frequencies of alleles in a population also act across geological timescales.¹⁰⁹ Consequently, comparative methods have a long-standing connection with quantitative genetics. Authors commonly connect models used in comparative methods with mod-

els of trait evolution from quantitative genetics. The best known of these are Lande's⁶⁵ models of drift and stabilizing selection. Lande's model assumes constant additive genetic variation and a static adaptive landscape. If this landscape is flat, then the model predicts that species mean phenotypes will evolve according to a BM model, while a landscape with a single peak results in evolution according to an OU model.

However, there remains a serious disconnect between Lande's quantitative genetics models and macroevolutionary analyses. When one fits macroevolutionary models to empirical data, the model parameters that one obtains are almost always incompatible with Lande's models and what we know about genetic variation and population sizes of species in the wild.^{21,31} For example, we know from observations of wild and experimental populations that selection is often strong^{110–113} and that there is often abundant additive genetic variation for selection to act upon.^{114–117} However, at macroevolutionary scales, rates of phenotypic evolution inferred from the fossil record appear to be exceedingly slow;^{118,119} this phenomenon has been referred to as the paradox of stasis.¹²⁰ This paradox implies that the simplest quantitative genetic interpretation of macroevolutionary models (i.e., those based on Lande's models^{53,65}) are probably incorrect.^{31,121} Some authors have proposed that the patterns that we see across long evolutionary time spans reflect the dynamics of changing adaptive landscapes. For example, selection that varies randomly in strength and direction from one generation to the next will also produce BM evolution;⁵³ this result was first discussed by Felsenstein.¹²² A number of mechanisms have been proposed to explain macroevolutionary stasis, such as depletion of additive genetic variance,¹²³ ephemeral divergence (in which phenotypic changes are common but only rarely established for long periods of time),^{116,124} and constraints due to multivariate selection^{125,126} (reviewed in Ref. 120).

Over long timescales, simple quantitative genetics models of evolution are almost certainly wrong. Perhaps because of this, models used in comparative methods are often considered to be heuristic or phenomenological, and specific ties to population genetic models are often not considered. We believe that the answer to this problem is not to abandon quantitative genetics models entirely, but

rather, as we discuss below, to develop models that more accurately capture evolutionary processes on phylogenetic trees.

Important steps in this direction have come from Hansen and collaborators. Hansen's view of the conflict between Lande's models and macroevolutionary data is that long-term patterns of trait evolution reflect the dynamics of adaptive landscapes more than they reflect species adapting to static adaptive peaks.^{21,68} More work is certainly possible in this area.

We believe that comparative methods still have a lot to learn from quantitative genetics—and vice versa. A number of quantitative genetics models have been developed and applied to long-term data, but not applied to comparative data. Two prominent examples include the house-of-cards^{127,128} and continuum-of-alleles¹²⁹ models. These two models, which differ in their assumptions and predictions, both relax the assumptions of constant additive genetic variance within populations and allow genetic variation to evolve, but have not been applied to comparative data. As another example, Estes and Arnold¹³⁰ outline a suite of quantitative models of long-term evolution—including drift, stasis on an adaptive peak, peak climbing, and peak shift models—which could, in principle, be fit to data in a comparative framework.¹³¹ Methods for this have not been fully developed (but see Ref. 121).

Another important direction for future research is in the development of multivariate approaches in comparative methods. A few steps have been taken in this direction,^{131–134} but the majority of comparative studies are univariate—or, if they are multivariate, consider one trait at a time. Most quantitative models generalize relatively easily to multiple traits. In addition, multivariate comparative methods connect to the vast literature about the effects of genetic covariances (and the **G** matrix) on evolution. Multivariate comparative methods could also potentially provide a powerful test for the effects of antagonistic selection, which is thought by some to act as a major constraint on rates of evolution over long timescales,^{125,126} as well as of correlative selection, indirect selection, and of how the genetic axes of divergence⁴⁴ have changed across the phylogeny. As stated above, the phylogenetic mixed model^{40–42} provides a natural conduit between multivariate approaches in quantitative genetics and PCMs, via the use of the animal model.⁴³ However, to date, this

connection has been largely phenomenological—that is, making use of the structural similarity between phylogenies and pedigrees rather than modeling traits with quantitative genetics parameters. It may be possible to use this or related models to explicitly address some of the questions regarding multivariate evolution that we have discussed here.

Community ecology and PCMs

Another area where PCMs have played an increasingly important role is in community ecology.¹³⁵ Studies of local and regional patterns of species composition and diversity often emphasize the interplay between short-term local processes and longer term regional processes.¹³⁶ Various approaches—typically lumped together as the field of phylogenetic community ecology—use information about historical processes gleaned from phylogenies to untangle the interactions between contemporary and historical processes. These studies seek to understand how community composition is shaped by both short-term, local process like competition, along with longer term processes, like species movement and dispersal across a geographic landscape, patterns of trait evolution, and rates of speciation and extinction.

Studies of phylogenetic community ecology use the phylogenetic relationships among species in local communities to draw conclusions about how communities are structured and organized.¹³⁵ Pioneering studies looked for patterns of clustering or overdispersion of species in a local community on a phylogenetic tree and related these to ecological processes, such as habitat filtering and competition. These approaches have more recently been extended to look at changes in phylogenetic structure with spatial scale (e.g., phylogenetic diversity–area relationships¹³⁷) and across metapopulations.¹³⁸

However, and as pointed out by Webb *et al.*¹³⁵ in their important review paper over a decade ago, the interpretation of most patterns of phylogenetic structure depends critically on how traits have evolved (see also Refs. 139–141). In particular, interpretation of patterns of communities on trees is heavily dependent on the tempo and mode of trait evolution—and, in particular, whether close relatives in the phylogenetic tree tend to have similar trait values.¹³⁵ Consequently, many recent studies of phylogenetic community ecology include data on traits that are important to species' survival and

competitive abilities, or (in some cases) direct measures of the function of those traits.^{139,140,142,143} This most often takes the form of measuring the phylogenetic signal of traits, and using that to aid in the interpretation of community phylogenetic patterns (Ref. 140 but see Refs. 144 and 145). However, the phylogenetic signal itself is agnostic with respect to the process of evolution, and a wide range of very different processes can lead to traits with a high or low signal.¹⁴⁶ The consequence of this is that patterns of trait variation and phylogenetic clustering are often complex and difficult to ascribe to any one particular ecological explanation. Increasingly, studies of phylogenetic community ecology have compared patterns of phylogenetic community structure across a range of temporal or spatial scales.^{137,147,148} Such replicated studies can help to uncover the ecological causes of phylogenetic patterns in communities.

Despite this rapid progress in the area of phylogenetic community ecology, many processes of great importance to ecologists—like differences in abundance across species, competition, and ecosystem functioning—are still mostly absent from comparative methods. For example, many studies of community ecology are focused on species interactions, but almost all current comparative models (such as those described above) assume that evolution along each branch of a phylogenetic tree is independent of evolution along every other branch. Such an assumption precludes competition, or any other process of species interactions, from affecting patterns of trait evolution. This limitation has affected the field of phylogenetic community ecology by making it difficult to evaluate the relationship between implemented models and the actual processes of interest. We agree with other recent reviews that believe that the solution to this dilemma awaits development of models that capture the processes of interest to community ecology, such as habitat filtering and competition, but can also be applied to phylogenetic comparative data^{139,140}—a development that is already well underway.¹⁴⁵

We can also imagine a broader application of phylogenetic methods beyond the study of local and regional species diversity. For example, ecologists have focused decades of research on studying species abundance distributions. There are tremendous differences in species' abundances in natural communities: most species are rare, a few are com-

mon, and abundance distributions of most communities follow a characteristic pattern.¹⁴⁹ Species' abundances also change through time, and population size is critical to rates of long-term evolution, affecting both rates of genetic drift and mutation-selection balance.⁴⁴ However, differences in population size are typically not reflected in comparative model-fitting approaches, which effectively assume that all species have equal and constant population sizes.¹⁵⁰

One can imagine comparative methods that consider the evolution of species traits along with changes in their population size through time along the branches of a tree. A starting point for these models is provided by Hubbell's unified neutral theory (UNT) of biodiversity.¹⁴⁹ The UNT is an individual-based neutral model of metacommunities. Under the UNT, all individuals are ecologically equivalent, that is, differences among species do not matter for ecological dynamics. By modeling the processes of birth, death, migration, and speciation, the UNT makes a wide range of predictions about species' abundances, phylogenetic relatedness, and geographic distributions (reviewed in Ref. 151). The UNT is well known and controversial for its assumption of ecological equivalence—and perhaps future models might relax that assumption.^{152,153} But it is also worth noting that the UNT—and any other model that considers the process of community drift along with speciation—can be used to model species' abundances along the branches of phylogenetic trees, and perhaps to evaluate the effects of these changes on comparative patterns of trait evolution.¹⁵⁴

Another example of a subfield of ecology where comparative methods have not often been applied, but might be useful, is in studies of ecoevolutionary dynamics (reviewed in Ref. 155). In particular, we know there are connections between species traits and ecosystem functioning.¹⁵⁶ Since species trait evolution is in the realm of comparative methods, it stands to reason that phylogenetic patterns could reveal something about ecosystem functioning—and, perhaps, how function changes through time.^{157–159} This is an area that is ripe for more work (for an example, see Ref. 160).

In summary, the idea of applying phylogenetic trees to capture historical effects on ecological communities is now well established. Many recent studies include both phylogenetic trees and actual

measurements of species' traits. Methods for analyzing such data are rapidly being developed, although we still lack models that directly incorporate many processes of interest, like competition among species. Finally, there are a number of key ideas from ecology that have not yet been placed in a comparative framework. We believe that there is still great potential for PCMs to provide novel insights into fundamental ecological questions.

Paleobiology and PCMs

There is much to gain from the intersection of PCMs and paleobiology. Paleobiology and PCMs have converged on very similar research themes and questions, despite coming from different intellectual backgrounds (paleontology and molecular phylogenetics, respectively). Both comparative methods and paleobiology have long sought to answer questions such as: How have diversity and disparity (trait differentiation) changed through time?^{23,161} What are the drivers (abiotic and biotic) of these changes?^{10,162} What roles do evolutionary novelties (i.e., key innovations) play in generating patterns of diversity?^{9,70} Are community dynamics stable over long periods of time?^{163,164} How important are higher level macroevolutionary processes (e.g., species selection) and what types of traits are most important for predicting the evolutionary trajectory of a lineage?^{97,102,103} What is the relative importance of cladogenetic versus anagenetic change?^{107,165} All of these questions are currently being investigated using modern PCMs in a wide variety of systems. Macroevolution has gone mainstream, without much of the controversy that surrounded the field even a decade ago (for example, see Ref. 99). Species selection, for example, once heretical to many neontologists, is now commonly addressed using molecular phylogenies (reviewed in Refs. 100 and 103), thanks in part to the advent of novel statistical approaches.²⁴

Rather than viewing paleobiology and PCMs as competing research programs, we believe that phylogenetic data and paleontological data have complementary strengths and weaknesses. The problems with the fossil record have long been noted by evolutionary biologists.¹⁶⁶ First, for many groups, the fossil record is so scanty that it is impossible to make reliable inferences regarding the dynamics of diversity. This is especially true of many of the groups, including iconic adaptive radiations such as Dar-

win's finches, Caribbean anoles, African rift lake Cichlids, and Hawaiian silverswords. Second, even for groups for which there is a reasonably good fossil record, such as Phanerozoic marine invertebrate taxa, different strategies for dealing (or not) with sampling have yielded different conclusions regarding the dynamics of diversity.^{167–170} Third, there is a difficulty in defining a species from the fossil record. While species delimitation is a thorny issue in neontological data sets,^{171–173} it is even more challenging using morphological data sets from extinct lineages. As a result, most broadscale paleobiological studies make use of data at the generic level, or higher, as estimates of species-level diversity are notoriously unreliable and subject to myriad sources of bias.^{174–176} For many questions, such as examining trends in diversity through time, lineages that have endured to the present are valuable data points and these may not be included in fossil data sets.

Using molecular phylogenies to study macroevolution also has its own distinct problems. These studies are necessarily limited to a single time slice in history—viewing evolution from such a perspective (no matter when the time of observation) is likely to present a warped view of history. Whole clades, once historically dominant, may have gone extinct before the present, leaving no trace in molecular data sets.¹⁷⁷ Second, in the absence of direct historical information, it becomes very difficult to say anything concrete regarding the characteristics of any particular ancestor^{178–182} and some macroevolutionary patterns, such as directional trends, are impossible to detect with extant-only data.^{75,180} Furthermore, modern PCMs rely on explicit statistical models to describe evolution through time; these models may not adequately capture the general historical patterns we are interested in.

Fortunately, where fossil data sets are deficient, molecular data sets are valuable and vice versa. Fossils provide direct historical information at multiple time intervals, while molecular phylogenies are less burdened with sampling issues as the paleontological data (though, in practice, most phylogenies of extant lineages are incomplete at the species level and incorporating sampling schemes is also important for making reliable inferences from these data sets).^{183,184} It has become apparent that a truly comprehensive macroevolutionary research program will involve some

combination of PCMs and fossil data. There are three possible paths for this synthesis, which we describe below.

Adding fossils to phylogenies. The first is to combine phylogenetic data from extant lineages with data from extinct lineages—this has been referred to as the *total evidence* approach. Despite the promise of a total evidence approach in some cases,¹⁸⁵ this has, in general, proven to be difficult. For one thing, the definition of what a lineage (e.g., species, or worse, genera) is likely differs between extant and extinct taxa, and this will influence our interpretations of any results.^{173,186} Placing extinct taxa within a backbone molecular phylogeny is fraught with challenges. One approach that has recently been used is to coestimate the topology and the divergence times of both extinct and extant taxa using combinations of molecular data (for extant taxa) and morphological data (for both).^{187,188} Using these combined phylogenies, one could then fit macroevolutionary models to the data. Fitting models of trait evolution to paleotrees is straightforward only if we assume that lineages are sampled randomly with regard to their trait values, an assumption that may very well be violated both in paleontological data sets (due to taphonomic bias) and in neontological comparative data sets (due to differential sampling), though this problem has received very little attention (but see Refs. 42,189 and 190).

However, when investigating diversity dynamics, it is essential to incorporate information on the taxon sampling—as different sampling regimes across lineages will influence estimates of diversification rates.^{183,184,191,192} The influence of sampling also limits the application of any approach that relies on a simple model of diversification (e.g., BiSSE²⁴). As sampling in the present is fundamentally different from sampling in the fossil record, it will be essential to devise probabilistic sampling models in which sampling through time is estimated along with the diversification parameters. Stadler¹⁹³ and Didier *et al.*¹⁹⁴ made some progress on this front, but these models have yet to be applied to empirical data. Furthermore, sampling and extinction can be confounded,^{184,195} making inferences challenging.

Applying comparative methods to paleotrees. Another area of intersect between paleobiology and PCMs is in the application of statistical meth-

ods designed for molecular phylogenies to trees containing only fossil taxa (hereafter paleotrees). We note that while the use of phylogenetic analysis to make evolution inferences is hardly new in paleobiology,¹⁹⁶ fitting statistical models borrowed from the molecular systematics literature has only become prevalent within the past few years.

A number of studies have compared the fit of various evolutionary models to make inferences regarding large-scale patterns of trait evolution.^{197–202} For example, Sallan and Friedman²⁰⁰ fit multiple evolutionary models (BM, OU, and Pagel's δ^{32}) to both cranial and postcranial morphological data from ray-finned fishes. They found support for different rates of evolution between the axes, but also found that in both cases, an OU model was strongly supported over other models in the candidate pool, which they suggested may result from an adaptive peak or internal constraints. As stated above, it is important to keep sampling issues in mind, even when the models do not explicitly consider sampling.

It is important to consider that fitting a statistical model either of trait evolution or lineage diversification to a phylogeny requires a chronogram with robust branch lengths. Until recently this has been a major hurdle to the adoption of PCMs as the vast majority of paleotrees have been constructed using parsimony-based analyses of morphology. Methods for time-calibrating cladograms is an area of active research in paleobiology.^{203–205} These methods will be especially useful for groups for which phylogenetic relationships can be well resolved with morphological characters in conjunction with a reasonably good stratigraphic record to allow for reliable time calibration.¹⁸⁵

Integrating conventional paleobiological and phylogenetic approaches. A third approach that has to date received far less attention is to integrate conventional paleobiological approaches with conventional PCMs on molecular phylogenies— inferences would be based on the joint estimation of parameters from the two approaches. For example, in their study of coral diversity dynamics, Simpson *et al.*²⁰⁶ used a time-binning approach to analyze the fossil record together with a model-fitting approach to analyze the molecular trees. They found that across these two analyses, the inferences were

broadly congruent. It would be possible to formally integrate analyses of the fossil record and molecular phylogenies, by calculating the joint probability distribution of both sets of data and estimating parameters shared across both data sets (e.g., speciation rate—though as we noted above, a speciation rate derived from a molecular phylogeny may have a different interpretation from one derived from a fossil data set). Such a combined approach might be a profitable line of research that could potentially make use of existing methods but not necessarily require that fossil data sets be artificially forced into a phylogenetic framework. An unresolved question relating to the above is whether molecular phylogenies and fossil data sets from the same group are statistically independent observations, and if they are not, how we should deal with the covariance between them. This problem certainly warrants serious attention.

A step in this direction was recently made by Slater *et al.*⁷⁵ Slater and colleagues⁷⁵ used data from fossils as informative priors on the state at internal nodes of a molecular phylogeny. They demonstrated for the case of trait evolution that, including information from fossils, can drastically improve our ability to correctly identify models—this is especially the case for certain models such as a trend toward larger body sizes (Cope's rule).⁷⁵ While we think that this approach certainly has its merits, we envision a slightly different methodological framework going forward. Rather than including the fossil data before, to help constrain analyses using molecular data, the flow of information could go both ways. That is, both data types could be used simultaneously in a single integrative analysis to make inferences regarding the dynamics of diversification and/or trait evolution.

Conclusion

Phylogenetic trees provide a central conceptual framework for understanding the diversity of life. In the past few decades, tree thinking has been adopted across biological disciplines and fundamentally transformed how we as biologists think about and investigate patterns through deep time and the processes that have generated them. This has been greatly aided by the rapid development of statistical techniques for making sense of phylogenetic comparative data and tremendous progress has

been made on this front. However, we argue that we are approaching something of an impasse with current approaches and that making further substantial progress with PCMs will require serious consideration of ideas and mathematical models developed in disparate fields. A more integrative comparative biology will be necessary to address long-standing questions in evolutionary biology and form a more comprehensive understanding of biodiversity across spatial and temporal scales.

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Conflicts of interest

The authors declare no conflicts of interest.

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