Primer

Phylogenetic comparative methods

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Phylogenetic comparative methods (PCMs) enable us to study the history of organismal evolution and diversification. PCMs comprise a collection of statistical methods for inferring *history* from piecemeal information, primarily combining two types of data: first, an estimate of species relatedness, usually based on their genes, and second, contemporary trait values of extant organisms. Some PCMs also incorporate information from geological records, especially fossils, but also other gradual and episodic events in the Earth's history (for example, trait data from fossils or the global oxygen concentration as an independent variable). It is important to note at the outset that PCMs are not concerned with reconstructing the evolutionary *relationships* among species; this has to do with estimating the phylogeny from genetic, fossil and other data, and a separate set of methods for this process makes up the field of phylogenetics. PCMs as a set of methods are distinct from, but are not completely independent of, phylogenetics. PCMs are used to address the questions: how did the characteristics of organisms evolve through time and what factors influenced speciation and extinction?

Evolution at large scales is mostly a branching process, with speciation and extinction occurring through time. In rare instances, these branches may come back together via hybridisation or horizontal gene transfer, but for the most part the branches diverge. Graphically, this branching process is represented as a phylogeny. However, the nature of the evolutionary branching process means that some pairs of species are closely related and others are distantly related, and this violates an important assumption of standard statistical methods — independence
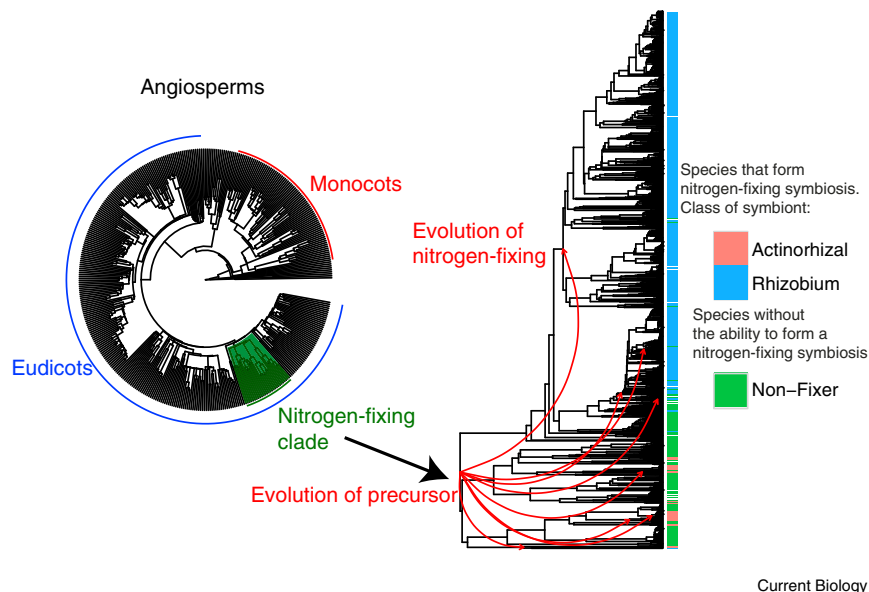

Figure 1. An example use of a PCM for understanding trait evolution.
Werner and colleagues were interested in the evolution of the ability to form nitrogen-fixing symbioses among angiosperms. The symbionts of the individual plant taxa differ, and are traditionally grouped into two types: rhizobial and actinorhizal associations. The ability to form such symbioses is only found is a small set of species, which are located within the 'nitrogen-fixing clade'. The authors reconstructed the evolution of the trait using a PCM that included the possibility of the evolution of an unknown precursor to the actual trait; the red arrows connect the precursor to the places in the phylogeny where PCMs infer the final evolution of the symbioses. The PCM used strongly supports a pathway to nitrogen fixation via a single precursor that evolved only once in the history of angiosperm evolution. They estimated when this precursor evolved (about 100 million years ago) and which extant clades should still have the precursor but not the ability to form a nitrogen-fixing symbiosis. The identity of this precursor is still unknown and is an area of active research. Figures modified and adopted from Werner *et al.* (2014).

among data points. The first viable solution to this problem came in a paper entitled "Phylogenies and Comparative Method", which was published by Joseph Felsenstein in 1985. His method is referred to as Phylogenetically Independent Contrasts, and is essentially a clever reformulation of linear (regression) models, which biologists routinely use, in which species are no longer seen as the data point. Rather each evolutionary branching point or 'divergence' is treated as a replicate in a statistical sense. Unlike species themselves, evolutionary divergences are independent events, and classical statistical models can be applied.

Subsequent to Felsenstein's pioneering work, the field of PCMs has grown and diversified. New methods have been directly and indirectly extended by many researchers and made more flexible by including not only continuous traits but also discrete traits. We are currently witnessing a

rapid increase in the number of PCMs and their applications, not only to a diverse array of evolutionary questions, but also to questions outside of evolutionary biology.

Methods for trait evolution

There are two related goals of trait-evolution PCMs. The first is to model the "tempo and mode of evolution" to use a phrase from paleontologist G.G. Simpson in 1944. This process means representing the change in trait value along each branch of the phylogeny mathematically; or put another way, this type of PCM attempts to reconstruct the way that the traits change through time. Connecting Simpson's terminology to modern methods, *tempo* describes the speed of trait evolution and *mode* describes the manner of evolution (for example, slow and gradual or with big jumps).

Modern models of the tempo and mode of evolution are mathematically intricate, but in many cases there are
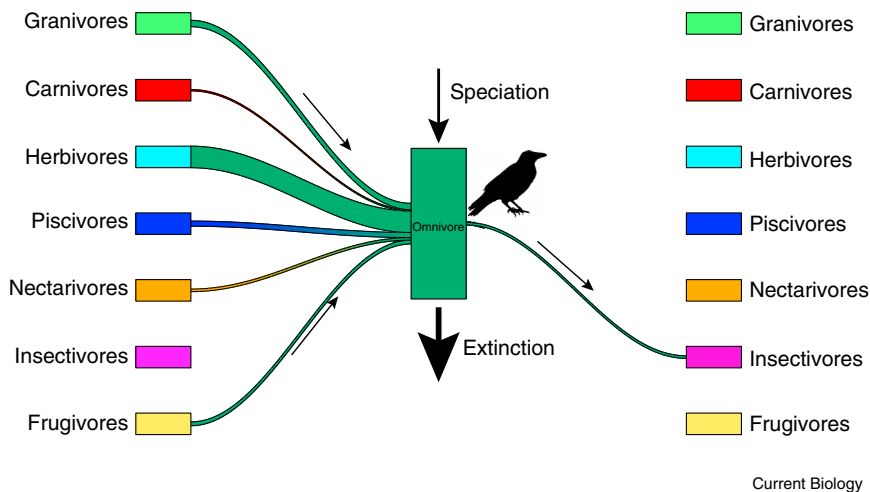
Current Biology

Figure 2. An example of lineage-diversification PCMs.
Lineage-diversification PCMs can simultaneously estimate the rates of transition among different groups, speciation, and extinction. In an analysis of bird-feeding guilds, Burin and colleagues used a PCM that found omnivores to be an 'evolutionary sink' — that is, there are significant transitions into that class from many others, but relatively infrequent transitions out of that class. The rates of transitions are represented by the thickness of lines. Within omnivores, the PCM also reconstructed a relatively high rate of extinction. Simultaneously understanding trait evolution, speciation, and extinction is one of the keys to understanding adaptive radiations across all taxa. Figure modified and adopted from Burin *et al.* (2016).

simple analogies to explain them. For example, the most common model of trait change through time is built on 'random walks' of Brownian motion. In this model, after a speciation event, the descendent species drift away from each other following mathematics that were first worked out by Albert Einstein in 1905. Another model of evolution describes a process where Brownian drift is supplemented by a pull towards one or more evolutionary attractors, (known as the Ornstein–Uhlenbeck process). This pull can be thought of as a 'rubber band' with the strength of the pull getting stronger the further the distance from the attractor. Yet another model represents the likelihood of a hidden 'precursor' that is necessary for the evolution of a particular state (see for example Figure 1). The exciting part about the development of these models is that they represent progress on answering some of Simpson's original questions about the tempo and mode of trait evolution in many different lineages. One of the big unknowns in the field is what aspects of the tempo and mode of trait evolution are common for all taxa on Earth? And what aspects of the tempo and mode are specific to particular groups of organisms?

The second major goal is to examine evolutionary links, both amongst traits and also between traits and the environment. This point is perhaps easiest to explain by example. Take the simple question — across many species, does environmental variable X influence values of trait Y? Ignoring evolution's branching structure, this question may be usefully addressed with linear regression. However, as Felsenstein pointed out, such analysis would ignore the non-independence of the species. Felsenstein's method works well for this particular case. More recently, more general solutions have been found such that phylogenies may now be used in the context of many more types of linear models (for example, generalized linear mixed models and structural equation models). Further, these more complex phylogenetic models can now be used along with other modern statistical techniques for model selection, averaging, validation, and prediction.

Methods to investigate lineage diversification
The simple and compelling question — why are some lineages more speciose than others of similar age? — has driven development of a second

category of PCMs, which we term here 'lineage diversification PCMs'. There is evidence for shifts in speciation and extinction rates in different lineages from both the fossil record and the relative diversity of extant lineages. Differences in diversity among same-aged clades are apparent in many if not all large taxa. For example, there are >300,000 extant angiosperms and <2,000 extant gymnosperms, even though the groups are 'sister clades' and therefore must be exactly the same age. Since both groups descended from a single species after they split, the greater current diversity of angiosperms must be the result of either an uptick in speciation rates or a downtick in extinction, or both, in the time since they split. Shifts like these in 'net diversification rate' (the speciation rate minus the extinction rate) leave a signal in the phylogenetic tree. In the simplest sense, shifts in net diversification create 'unbalanced' trees with lots of tips on some branches and few (or none) on others.

Lineage-diversification PCMs assemble and combine the evidence for these upticks and downticks and try to explain them. In other words, they ask two related questions: first, where and when on the phylogeny were there shifts in diversification rate? And second, why did those shifts occur? Lineage diversification PCMs not only examine patterns of trait diversification but also elucidate how certain characteristics of the organisms can facilitate or impede speciation, extinction, or both. With Figure 2, we illustrate these methods with an example using a large avian data set of diversification rate difference between passerine versus non-passerine taxa. In this case, the authors hypothesized that feeding guild might affect both the transition rates to other guilds, as well as speciation and extinction rates.

Rise of PCMs in different disciplines
In principle, PCMs represent an increasingly flexible class of statistical models used to describe change in any entity through time that occurs simultaneously with a branching process. As such, there are many other applications for these models. PCMs are increasingly used in fields other than evolutionary biology, such as linguistics, community ecology,
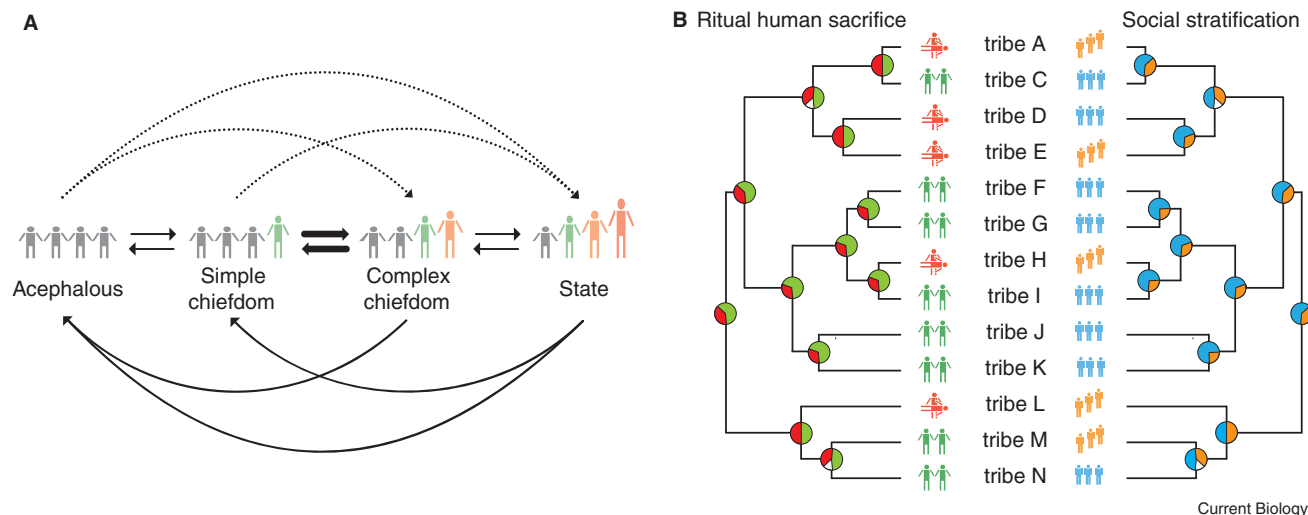
Figure 3. Applications of PCMs to anthropological questions.
(A) Transitions between different political states, with thick solid arrows indicating high transition rates, narrow solid arrows indicating moderate to low rates, and thin dotted arrows representing extremely low rates (or virtually zero transition). (B) Correlated evolution between ritual human sacrifice and social stratification with pie charts at nodes representing probabilities of different states at these ancestral nodes. Figures simplified and adapted from Currie *et al.* (2010) and Watts *et al.* (2016).

anthropology and paleobiology. One important point is that PCMs require external evidence to first estimate the branching structure, and this is not always available.

Traditionally, fields such as linguistics and anthropology have used and developed their own methods for comparing different linguistic and cultural attributes. Modern techniques developed for phylogenetics have been applied successfully to construct phylogenetic trees of existing languages in the exact same way as making species phylogenetic trees based on genes. Interestingly, with phylogenies built on linguistic similarity, we can use PCMs to analyze cultural data, which, like species data, are likely to be non-independent.

Here, we introduce two examples of where PCMs are applied to cultural data to resolve major questions in anthropology. First, Currie and colleagues tackled the evolution of the human political system using data on political complexity from 84 Austronesian societies and the 'phylogenetic' relationship inferred from their languages. Political complexity was categorized into four forms of political organization: acephalous society (no leaders), simple chiefdom (one leader), complex chiefdom (more than one leader) and state (centralized political

bureaucracy). Before this study, there was no quantitative analysis of how political complexity evolved. The authors showed, with the use of PCMs, that the political system evolved step by step to greater complexity and never skipped intermediate steps, although a more complex form can collapse into a less complex form in a non-sequential manner (Figure 3A). This study is among the earliest applications of PCMs to this type of data and it demonstrates that PCMs can capture unique elements of human cultural evolution that earlier methods in anthropology could not.

As a second example, Watts and colleagues questioned the functionality of human sacrifice for which records have been found in many early civilizations. One hypothesis (the 'social control hypothesis') suggests that human sacrifice legitimizes hierarchical classes, as the higher class demonstrates the ultimate authority by taking life, although this hypothesis remained anecdotal prior to this work. Watts and colleagues employed PCMs to test this hypothesis by utilizing data on ritual human sacrifice and the existence of social stratification from 93 Austronesian societies and the corresponding language-based phylogenetic tree. The results from PCMs clearly demonstrated a close link between human sacrifice and the

evolution of class-based societies, suggesting that human sacrifice promoted and maintained such social organization. Clearly, any other human religious practices could be investigated with the power of PCMs. Given recent success in applying PCMs to other disciplines, as we have just discussed, we expect to see more cross-disciplinary uses of PCMs based on evolutionary thinking.

Caveats and the future of PCMs
The goal of PCMs is ambitious: to explain the evolution of Earth's diversity. The power of these methods has improved recently due to the fast growth in both data resources (both trait and molecular) and computational power to build larger and better phylogenies. Moreover, computational resources have also facilitated new classes of models, which were not computationally viable even a few years ago. However, relative to the grand ambition of the goal, these methods, while exciting, are still preliminary and the difficult problem of accurately estimating uncertainty has not been fully solved.

PCMs are statistical models. As the statistician, George Box famously put it "All models are wrong but some are useful". We should be aware of the limitations and assumptions of statistical models in general, all of

which apply to PCMs. In reconstructing the past, there are (at least) three specific sources of uncertainty that are worthy of special attention.

Tree uncertainty. There is uncertainty from the building of the phylogeny. Tree-building methods are not perfect and often are used with data constraints, especially limited sampling of both genomes and species. As such, there is always uncertainty about phylogeny. Species can be misplaced in a phylogenetic tree, ancestral nodes can be wrongly inferred, or more subtly, but more commonly, branch lengths are incorrect. If we are interested in species diversification and what traits promote or inhibit such diversification, it is not difficult to see how these mistakes in phylogenetic trees can influence our inference.

Trait uncertainty. For most PCMs, we use trait values representative for particular species. However, traits are measured with error. In addition, what constitutes 'representative' is a difficult issue. Often, a value from a single population is used and, not uncommonly, some trait values come from a single observation. Another relevant point is that trait variation within species can be very large. Think of our own species — what is a representative value for human height?

Model uncertainty. When we investigate trait evolution, we assume a certain model of evolution — most often, the Brownian motion model. However, a trait can evolve quite differently from such a simple model and there may be heterogeneity in the tempo and mode among the branches of the tree. Although approaches are now available to test among competing models and to represent process heterogeneity in a limited way, there is no guarantee that any of the current generation of models are adequate in capturing the true complexity of trait evolution through space and time.

To have the appropriate confidence in our ability to infer events and processes from the deep past, we must both estimate and combine these uncertainties properly. However, dealing with all of these uncertainties simultaneously is still beyond the scope of the current generation of methods, with a few notable exceptions. That said, the appeal of the lofty goal — the promise of

explaining key aspects of the evolution of life — will drive the field forward. Although we are still a long way from achieving that goal, it is nonetheless an exciting time for PCMs. Further, these shortcomings have not stopped PCMs from providing us with important new insights into the evolutionary secrets of life, including the history of mankind.

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Gamma oscillations and photosensitive epilepsy

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Certain visual images, even in the absence of motion or flicker, can trigger seizures in patients with photosensitive epilepsy. As of yet, there is no systematic explanation as to why some static images are likely to provoke seizures, while others pose little or no risk. Here, we examined the neurophysiology literature to assess whether the pattern of neural responses in healthy visual cortex is predictive of the pathological responses in photosensitive epilepsy. Previous studies have suggested that gamma oscillations (30–80 Hz) measured in human visual cortex may play a role in seizure generation [1,2]. Recently, we and others have shown that increases in gamma band power can come from two very different cortical signals, one that is *oscillatory* (with a narrow peak between 30 Hz and 80 Hz), and another that is *broadband* [3]. The oscillatory signal arises from neuronal synchrony in the local population, while the broadband signal reflects the level of asynchronous neuronal activity, and is correlated with multiunit spiking [4]. These two responses have different biological origins and different selectivity for image properties. Here, we followed up on the previous proposals [1,2] to ask whether the image features that increase seizure likelihood in photosensitive epilepsy are linked to narrowband gamma oscillations specifically, or are associated with any kind of increase in visual activity. Based on published work, we compared pairs of image classes on a number of dimensions, and show that the type of image that elicits larger narrowband gamma oscillations in healthy visual cortex is also more likely to provoke seizures or pre-seizure activity in patients with photosensitive epilepsy. In contrast, images that elicit larger broadband, multiunit, or fMRI responses are much less predictive of seizure activity. We propose that a risk factor for seizures in patients with photosensitive epilepsy