

Phylogeny, ecology, and the coupling of comparative and experimental approaches

Marjorie G. Weber and Anurag A. Agrawal

Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA

Recent progress in the development of phylogenetic methods and access to molecular phylogenies has made comparative biology more popular than ever before. However, determining cause and effect in phylogenetic comparative studies is inherently difficult without experimentation and evolutionary replication. Here, we provide a roadmap for linking comparative phylogenetic patterns with ecological experiments to test causal hypotheses across ecological and evolutionary scales. As examples, we consider five cornerstones of ecological and evolutionary research: tests of adaptation, tradeoffs and synergisms among traits, coevolution due to species interactions, trait influences on lineage diversification, and community assembly and composition. Although several scenarios can result in a lack of concordance between historical patterns and contemporary experiments, we argue that the coupling of phylogenetic and experimental methods is an increasingly revealing approach to hypothesis testing in evolutionary ecology.

Why integrate phylogenies and experiments?

The joint application of comparative phylogenetic and experimental methods has been advocated since at least the 1990s as a way to generate and evaluate causal hypotheses in evolutionary ecology [1–3]. Merging these two approaches can be particularly revealing because of the complementary insights they provide: phylogenetic comparative methods allow for the identification of broad-scale patterns across many taxa over long periods of time, whereas experimental manipulations allow for tests of mechanistic hypotheses implicated in driving those patterns. Furthermore, the joint use of phylogenetic and experimental methods can address common interpretational drawbacks of using one method alone. For example, when used in isolation, comparative phylogenetic studies stop short of rigorously evaluating the ecological mechanisms suggested by their results. Conversely, experimental results can be interpreted as general patterns without being placed in a broader evolutionary framework. We revisit the call for integration of comparative phylogenetics and experiments, and discuss the potential of this merger to facilitate novel links between historical patterns and

ecological processes given the significant methodological leaps made over the last decade. Rather than broadly survey across all empirical and comparative approaches, we focus specifically on the integration of manipulative ecological experiments (used to investigate causal arguments) with recently developed phylogenetic comparative analyses (that allow for historical inference), because their joint use represents a growing frontier in evolutionary ecology and has not been extensively dealt with in a previous review.

We begin with a general conceptual framework for integrating manipulative experiments with comparative phylogenetics. However, because the methods, benefits and challenges associated with coupling these approaches change with the hypothesis being addressed, we consider five major areas in evolutionary ecology: tests of adaptation, trait tradeoffs and synergisms, coevolution and cospeciation, trait influences on lineage diversification, and ecological community structure.

A conceptual roadmap for integrative, reciprocal hypothesis testing

When used in isolation, phylogenetic and experimental approaches can each generate hypotheses that are then testable using the alternative approach (Figure 1) [2–5]. For example, phylogenetic patterns can suggest the existence of a causal mechanism (i.e., selection) that can then be investigated using manipulative experiments on contemporary populations (Figure 1a). A phylogenetic framework can also help researchers to design these experiments, allowing for powerful, evolutionarily replicated tests (Figure 1b, Box 1). Similarly, experimental results can generate evolutionary hypotheses that are testable using phylogenetic comparative methods (Figure 1c,d), such as when an ecological process is hypothesized to result in a specific macroevolutionary pattern.

Despite the benefits of integrating approaches, the reconciliation of results from contemporary and historical studies can present logistical and interpretational challenges. For example, the comparison of experimental results across multiple species within a clade often involves a common garden design, which can lead to variable results simply because species are removed from the ecological context in which they evolved. Furthermore, interpretational issues arise when experimental and comparative results conflict (Figure 1e), because conflicting results can reflect a

Corresponding author: Weber, M.G. (mgw58@cornell.edu).

Keywords: community ecology; comparative biology; coevolution and cospeciation; convergence; correlated evolution; key innovation; phylogenetic field experiment; phylogenetic signal; species coexistence; adaptive radiation.

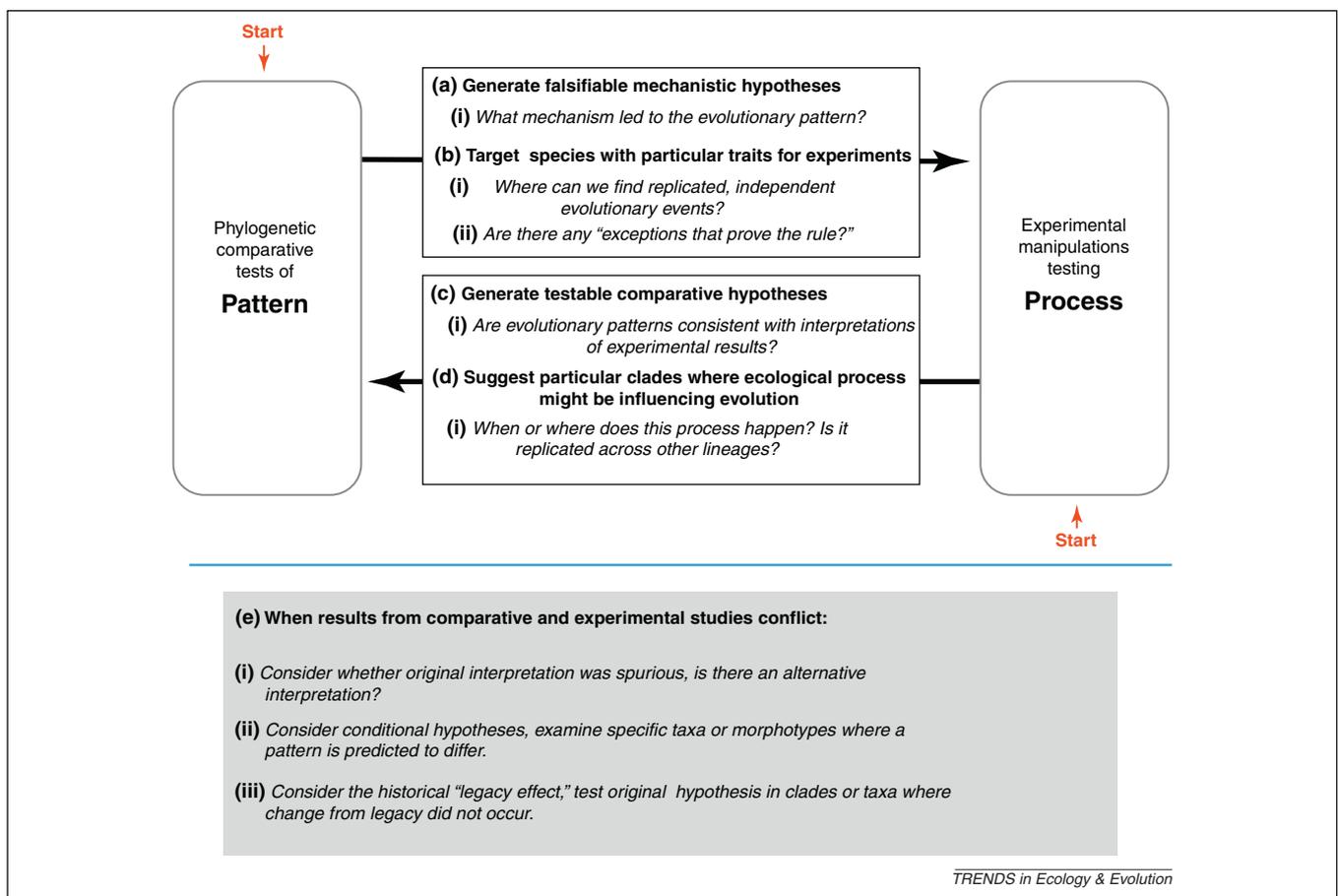


Figure 1. A schematic describing iterative hypothesis testing using phylogenetic patterns and experiments. Whether we begin with tests of pattern or process, results can lead to studies of the other type.

true rejection of the original hypothesis or can be the result of changes in the strength or direction of key forces over space or time (the legacy effect [3]). Indeed, many factors that potentially influence population dynamics shift over time or space, such as species range, habitat type, ecological interactions, and genotypic makeup. Regardless, the rejection of a historically derived hypothesis using current populations remains informative, because it suggests that the hypothesized process is not at play in the contemporary system. In this way, lack of concordance between historical and contemporary data can clarify a causal hypothesis, thereby allowing researchers to ask how current and historical populations differ or to formulate alternative hypotheses that better explain previous results [6].

Tests of adaptation through trait–environment associations

A great deal of research in evolutionary ecology is focused on identifying the adaptive value of traits under different conditions. Adaptive hypotheses are commonly investigated using a phylogenetic framework, whereby researchers ask whether traits and environments are evolutionarily correlated across a phylogeny. Indeed, comparative methods for identifying phylogenetic patterns consistent with adaptation have become increasingly rigorous and accessible over the last decade. Models of character evolution are becoming more sophisticated, and are now easily

implemented using open access statistical programs [7]. Bayesian and maximum likelihood methods are available to evaluate the fit of phylogenetic, character and habitat data to models of character evolution in which, for example, traits and habitat are non-independent [8,9], in which traits are evolving according to different selective optima in different environments [10,11], or in which rates of phenotypic evolution differ among clades in a phylogeny [12,13]. These methods are being increasingly applied to a broad range of traits and taxonomic groups. Nonetheless, although phylogenetic patterns can be consistent with an adaptive signature, they do not adequately address causal hypotheses on their own, as they fail to evaluate the role of implicated selective agents. Pairing these studies with experiments that clarify the costs and benefits of traits in different environments can shed light on adaptive interpretations of phylogenetic patterns. However, if these cost–benefit experiments were presented in isolation, they could not be generalized or interpreted in a historical context (for examples of experiments that were interpreted differently using a historical framework, see [14]).

Consider crypsis, for example, which is generally considered an adaptation to avoid predation. In *Timema* walking sticks, a dorsal stripe is hypothesized to confer crypsis for insects on plants with needle-like leaves (Figure 2a) [15]. Phylogenetic comparative analyses of stripe evolution across *Timema* were consistent with this

Box 1. Replication in causal hypothesis testing using phylogenies and experiments

Replication is crucial for identifying causal relationships at all levels of biological organization. First, because organisms and the environments they live in are not static, but rather plastic, variable and evolving, replication is essential if we are to capture accurate measures of biological phenomena. Second, replication allows for the use of statistical inference in biological research, both in manipulative experiments and correlational analyses. Although the use of replication is standard in traditional ecological studies, the importance of replication in phylogenetic hypothesis testing is debated, an incongruence between disciplines that perhaps occurs because many important evolutionary events truly occur only once. However, single evolutionary events are not sufficient tests of causal hypotheses on their own, and more rigorous evaluation requires integration with other types of information, such as targeted experiments or the identification of evolutionary replication if it exists.

In research on key innovations, for example, studies continue to implicate one-time instances of trait evolution as causal agents in accelerated diversification rates [95]. There are severe limitations to this single-case approach. For instance, evolutionary transitions in a particular trait are almost always associated with other changes in additional traits or with transitions in the ecology of organisms (e.g., radiation into a novel habitat) and without replication or experimentation it is impossible to distinguish between these confounding effects [61]. Powerful statistical methods have recently been devel-

oped to directly test if and where on a phylogeny a shift in diversification rate occurred [56]. However, although these approaches can reject the underlying hypothesis, they only circumstantially address the causes of shifts in diversification rate and do not address the relative importance of multiple factors associated with these shifts. Indeed, hypothesis testing in biology typically focuses on rejecting the null hypothesis, which is difficult in phylogenetic comparative studies without experimentation and replication.

In cases of unique apomorphies, studies are restricted to one-time evolutionary events and thus necessarily lack replication. In these situations, rigorous models that explicitly incorporate interactions between factors have potential for building strong evidence for evolutionary links in the absence of replication. In addition, experimental and case-study-based mechanistic evidence for associations between traits and diversification can shed light on these relationships.

To summarize, it is not possible to determine cause and effect in phylogenetic studies without experimentation and replication. Because phylogenetic analyses are focused on examining evolutionary patterns rather than process, if there are confounding factors, then cause (and the relationship itself) becomes questionable. However, experiments addressing causal hypotheses coupled with evolutionary independence gained through explicit statistical consideration of phylogeny can provide increased evidence for causal explanations.

adaptive hypothesis, revealing that the origin of dorsal stripes was evolutionarily correlated with shifts onto plants with needle-like leaves (Figure 2c). However, experimental manipulations using extant walking sticks were ultimately needed to evaluate whether crypsis (the proposed mechanism) was present and likely to be driving the trait–environment association. Experimental comparisons of predation rates in closely related striped and non-striped *Timema* were conducted, confirming that dorsal stripes confer crypsis and protection from predators. These studies support the interpretation of the phylogenetic pattern (Figure 2b) [16], thus providing a clear example of how the integration of comparative and experimental approaches can reinforce an otherwise speculative adaptive hypothesis (for other examples, see [17–20]).

Experimental tests of adaptive hypotheses often take the form of reciprocal transplants or manipulations using closely related but divergent species pairs. For example, Fine *et al.* conducted reciprocal transplants using ten pairs of closely related Amazonian tree species that differed in their soil habitat (nutrient-poor sand vs nutrient-rich clay) to test the hypothesis that plant defensive strategy is adapted to resource availability [21,22]. Plant species repeatedly differentiated in their chemistry over evolutionary time, and species from low-nutrient soils had greater levels of defensive chemicals than species from high-nutrient habitats [21,22]. In this case, however, the two environments differed along both biotic (herbivore) and abiotic (resource) axes and neither the trait–environment correlation nor the transplant experiment was sufficient to determine the ecological basis of trait differentiation. Experimental manipulations were ultimately needed, and confirmed that plants performed equally well in both habitats in the absence of herbivores, implicating habitat-specific herbivory, rather than resource availability *per se*, as the mechanism driving the relationship, a conclusion that was unreachable in the absence of integration.

Maladaptation and invasion success

With *a priori* information from both approaches, it is possible to design studies that merge comparative approaches and manipulative experiments to test a targeted adaptive hypothesis. For example, *Viburnum* spp. shrubs in Europe evolved with a damaging beetle herbivore, whereas North American species have, until recently, lived beetle-free. Consistent with an adaptive hypothesis, a phylogenetic field experiment on 16 species of *Viburnum* demonstrated that North American species have consistently (and convergently) lower defenses against beetles than their non-native congeners [23]. Experiments further confirmed that lower plant defenses were crucial for the success of the invasive insect pest, resulting in North American species being more susceptible than species from the insect's native range. Thus, the integration of historical patterns with ecological experiments revealed how a trait–environment mismatch can cause the proliferation of pests, potentially driving a species invasion.

Tradeoffs, synergisms and trait interactions over time

Many hypotheses in ecology and evolutionary biology address covariation among multiple traits. For example, life-history theory predicts that progeny size and number should tradeoff because of the allocation of limiting resources [24]. Other traits are predicted to show negative correlations for adaptive reasons: when one trait is employed, the other is disfavored by natural selection [25]. By contrast, when two traits function additively or synergistically, we expect natural selection to favor their correlated evolution. Evolutionary changes in one trait are also sometimes predicted to be dependent on changes in another trait. For example, gregariousness in caterpillars was hypothesized to originate after warning coloration, because gregariousness is only thought to be advantageous in visibly non-palatable animals [26]. Such sequential events are also important when one trait is

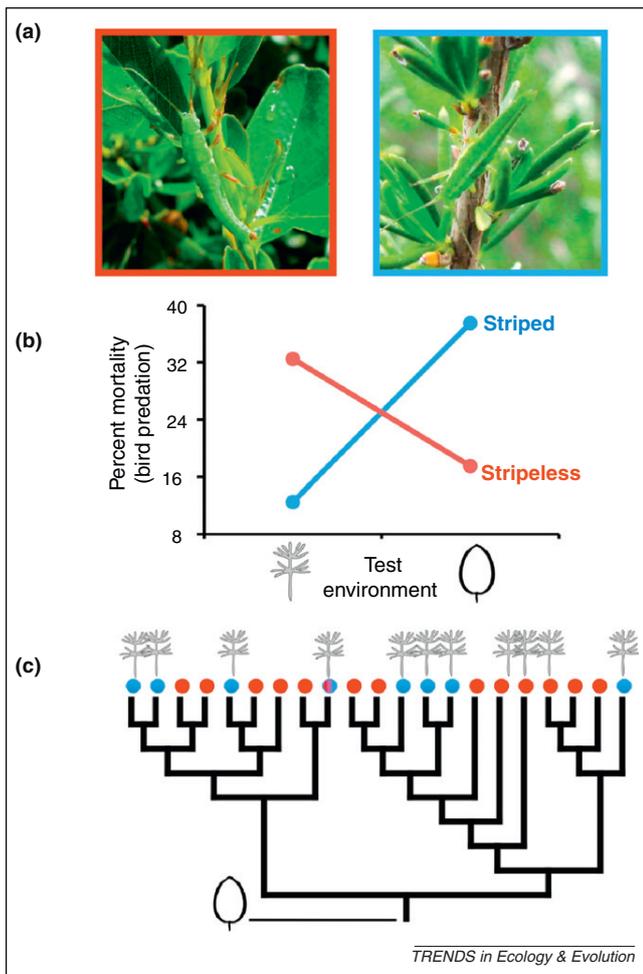


Figure 2. Dorsal stripe morphology as an adaptation to predation in *Timema* walking sticks. (a) Two morphs of *T. cristinae* on their respective host plants: striped (blue outline) and stripeless (red outline) (photographs by Aaron Comeault). (b) A manipulative ecological experiment measuring selection on individuals with and without stripes in both habitats [16]. In the presence of bird predators, the striped morph has higher fitness on plants with needle-like leaves, whereas the stripeless morph has higher fitness on broad leaves. Fitness of the morphs is equivalent on the two plant types in the absence of predators [93]. (c) To test for the broad-scale consequences of such divergent selection, a phylogenetic study of all 21 species of *Timema* was conducted. *Timema* walking sticks evolved a dorsal stripe five or six times independently, each time associated with a shift to needle-like leaves. Species with dorsal stripes are marked with a blue dot; those without stripes are marked in red. Use of broadleaf host plants is ancestral, with repeated host shifts to plants with needle-like leaves associated with the evolution of a dorsal stripe. Species with members that shifted to needle-like leaves are marked with grey plants above tips (bottom panel redrawn from [15]).

hypothesized to evolve by modification of another ecologically relevant trait [27].

Comparative studies of trait–trait interactions frequently employ phylogenetic methods to test whether traits are evolutionarily correlated. Indeed, new methods have recently been developed to test for phylogenetic patterns that are consistent with evolutionary non-independence of multiple traits [10,28,29]. In general, the same phylogenetic comparative methods used to evaluate adaptive signatures (discussed above) are used to identify patterns consistent with trait–trait correlations. Despite the increased sophistication of these methods, however, interpretation of evolutionary correlations on their own is exceedingly difficult because correlated evolution can be caused by several different processes, including selection

for particular trait combinations and genetic or developmental constraints [30,31]. Coupling of phylogenetic comparative results to experiments testing whether various trait pairings differentially influence fitness can distinguish between these scenarios. For example, to address why pollination and defense traits frequently show correlated evolution in plants, Herrera *et al.* experimentally asked whether individuals possessing particular combinations of these traits had higher fitness [32]. They found repeatable, non-additive fitness effects of the traits, consistent with the interpretation that selection is driving correlated evolution (for another example, see [33]).

Many ecological studies experimentally demonstrate how multiple traits function together in particular populations, but fail to test whether these ecological interactions are persistent or powerful enough to influence long-term evolution. Phenotypic or genetic (e.g., gene silencing) manipulations of multiple traits using a full-factorial design are particularly powerful because the statistical interaction term (in analysis of variance) indicates whether traits have an additive or non-additive effect. When traits have their greatest ecological impact together, and if these interactions affect individual fitness, they may evolve in a positively correlated fashion, a hypothesis that is testable using the phylogenetic comparative methods cited above. For example, by merging experimental and phylogenetic tests, we found that plant traits providing food and housing rewards to arthropod bodyguards exhibit complementary ecological effects and were evolutionarily correlated, consistent with the hypothesis that the ecological benefit of having both types of traits drives their evolutionary overlap [34].

Using exceptions to prove the rule

A novel approach to studying ecological and evolutionary trait interactions is to generate hypotheses based on cases in which the evolution of two traits that frequently evolve together has become decoupled (Figure 3). Lineages that

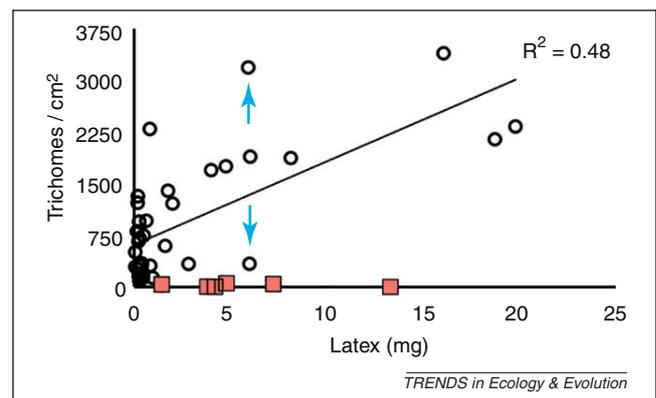


Figure 3. Deviations from correlated evolution can decouple traits that are hypothesized to be intact ecologically [35], suggesting species to target in future experimental studies. An example of correlated evolution between foliar trichome densities and latex production (mg of latex exuded when cut) across 44 species of milkweed (*Asclepias* spp.) (data from [94]). If an ecological process is hypothesized to drive an evolutionary correlation between two traits, then species or lineages that deviate from that correlation are also expected to deviate ecologically. Note that six species (in red) are excluded from the regression; although these species have few trichomes, they are the only species with leaf-surface wax crystals, leading to the hypothesis that wax crystals function ecophysiologicaly as trichomes [94], which was tested using experiments. In addition, blue arrows indicate potential target species for ecological experiments because of their deviation from the overall pattern.

deviate from correlated evolution can then be targeted in experiments that test hypotheses about the cause of the original correlation. Arnqvist and Rowe [35] used this approach in water striders to evaluate whether correlations in male and female secondary sexual morphology were driven by an evolutionary arms race between the sexes. They compared comparative phylogenetic results with outcomes of experimental matings, and found that species whose traits deviate from correlated evolution also had imbalanced behavioral interactions (with one sex having the behavioral upper hand), thereby supporting the evolutionary arms race hypothesis. Examples such as this, which creatively make use of exceptions to, or deviations from, evolutionary patterns to design rigorous experimental tests, are remarkably rare and yet hold tremendous potential for progress in evolutionary ecology. We predict that this approach will prove particularly promising given recent increases in access to large online organismal trait databases (e.g., The Worldwide Leaf Economics Spectrum [36], TRY – A Global Database of Plant Traits [37]).

Coevolution and cospeciation

Coevolution (i.e., reciprocal evolution between species leading to diversification) has long been suspected in systems where species interact with high specificity [38]. In general, specialized species interactions are hypothesized to result in coevolution when they increase the ecological or geographic structure of populations, thereby promoting differentiation [39]. For example, interactions between South Hills crossbills and Rocky Mountain lodgepole pine were hypothesized to result in specialized beak morphology and vocalization [40], which in turn promoted assortative mating and nearly complete reproductive isolation between bird populations [41]. Coevolution is a provocative hypothesis, but it is exceedingly difficult to test whether specific interactions were important in the evolution of diversity. However, we gain confidence in coevolutionary claims when patterns are presented alongside experimental evidence of ecological factors implicated in driving coevolution, such as specialization and differentiation.

Traditionally, parallel phylogenies have been used to identify potential cases of coevolution. Parallel phylogenies represent highly specific associations between clades of organisms, with early diverging species in one clade associating with basal species of the other clade, and progressively derived taxa similarly sharing an association (resulting in phylogenies that, when held side by side, appear as mirror images). Indeed, groups such as vertebrate hosts and their parasitic lice [Figure 4] [42], plants and their pollinating seed parasites [43], and metabolically codependent symbionts [44] have parallel phylogenies. Nonetheless, this pattern, termed cospeciation, is also caused by joint vicariance of both groups, and ecological interactions between the species need not be invoked. In other words, species can have parallel phylogenies because they have similar biogeographical histories (due to habitat sharing) rather than because of specific coevolutionary interactions (e.g., yuccas and yucca moths [45]).

Other aspects of phylogenetic congruence can be useful in inferring whether ecological interactions drove cospeciation. First, time-calibrated phylogenies can be used to

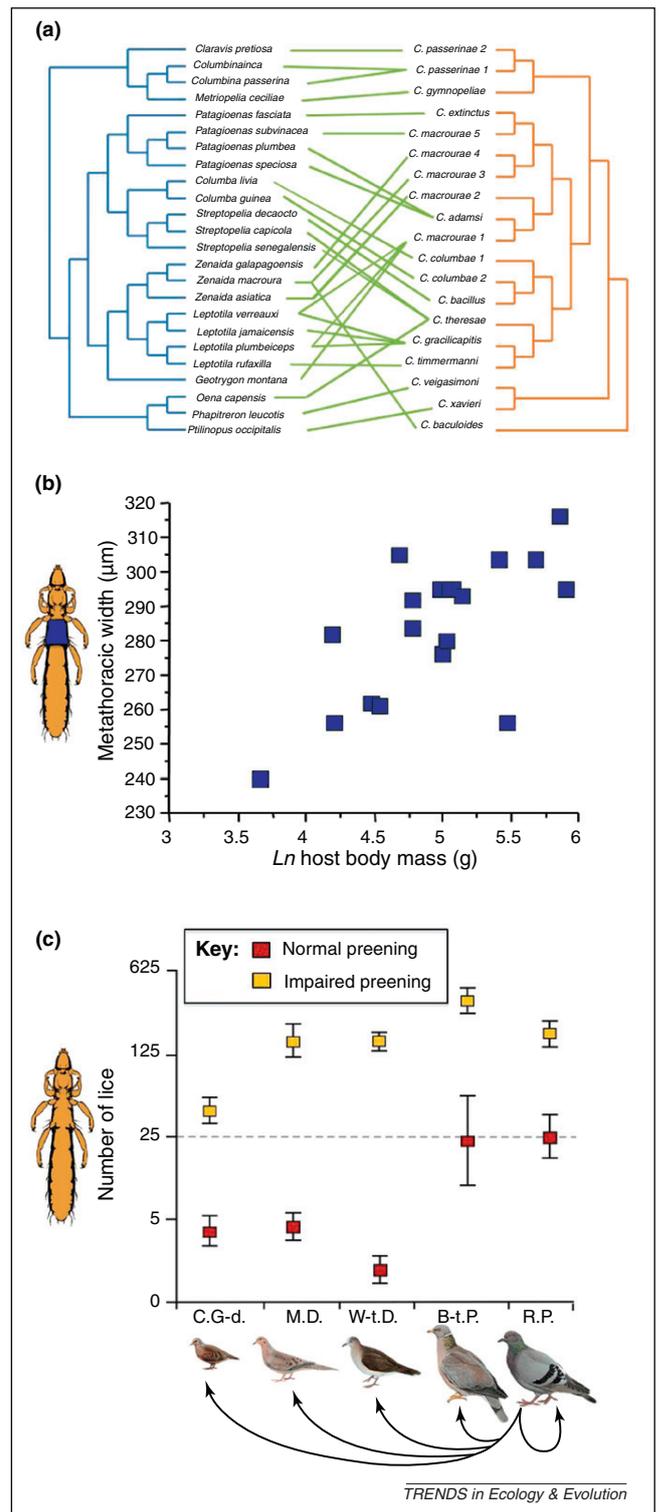


Figure 4. Merging of phylogenetic and experimental approaches in the study of host-parasite macroevolution. Despite being able to feed and proliferate on all birds, feather lice are preened from birds that are unmatched for size, suggesting that host defense reinforces cospeciation by preventing host switches. (a) Phylogenies of Columbiform birds and their feather lice in the genus *Columbicola*, showing a pattern of cospeciation. Green lines show host-parasite associations. (b) Host and parasite body size show correlated evolution, suggesting that physical constraints might be a driver of cospeciation. (c) Population sizes (mean±SE) of lice (*C. columbae*) transferred to novel host species. The dotted line represents the native host (rock pigeon, R.P., *Columba livia*). Host abbreviations: C.G-d., common ground-dove (*Columbina passerina*); M.D., mourning dove (*Zenaida macroura*), W-t.D., white-tipped Dove (*Leptotila verreauxi*), B-t.P., band-tailed pigeon (*Patagioenas fasciata*). Modified from [42] courtesy of the authors.

elucidate the temporal sequence of divergences, potentially ruling out coevolution. For example, in associations between leaf-cutter ants and lepiotaceous fungi, fungal lineages diverged well before ants, with ants acquiring fungi relatively recently [46]. Thus, ants might be evolutionarily tracking fungi or fungi might have subsequently spread through the ant lineages, but it is unlikely that reciprocal interaction was involved in cospeciation. This approach holds particular promise because methods for calibrating phylogenies using fossil or geographic data have greatly improved in the last decade, and it is now possible to estimate calibrated phylogenies while incorporating uncertainty in both node dating and tree topology using Bayesian inference [47]. Furthermore, patterns of congruence between phylogenies generally involve some element of incongruence, which itself can be informative. For example, partner switching could pinpoint instances in which species deviated from an overall pattern of coevolution and inform further investigations into the drivers of host shifts. However, although multiple lines of phylogenetic evidence can be consistent with coevolutionary hypotheses, integration with ecological information is required for researchers to implicate ecological factors as drivers of coevolutionary patterns.

Experimental studies can be a powerful tool for testing coevolutionary interpretations of phylogenetic patterns (see examples in [48]). For example, if coevolution is hypothesized to cause phenotypic matching between interacting species (e.g., the correspondence in body size between birds and their louse parasites [42]) (Figure 4), then reciprocal transplant experiments can confirm host switching is indeed limited by matched traits. For birds and lice, both body size and defensive preening behavior were shown to be important in maintaining specificity (Figure 4b,c) [42]. In addition, if coevolution is occurring, tradeoffs in fitness are expected when specialized species interact with close relatives of their usual host. Experimental evidence of these tradeoffs (e.g., host use in parasites, reward collection in mutualists and the ability to resist competitors) is also consistent with specialization contributing to divergence. Although experiments do not necessarily imply that the interactions contributed to divergence *per se*, they can rigorously evaluate whether other lines of evidence are consistent with a pattern of cospeciation being driven by an ecological interaction and whether phenotypes constrain the range of available partners (for other examples, see [49–51]).

Trait influences on lineage diversification

Organismal traits are frequently implicated in influencing the species richness of particular clades, either positively (in the case of key innovations or adaptive radiations) or negatively (in the case of evolutionary dead-ends). Examples of these traits range from nectar spurs and self-fertilization in plants [52,53] to incisor growth in rodents [54]. Recently, a surge of powerful comparative phylogenetic methods have been developed to address whether a given trait is associated with changes in clade diversification rates, such as model comparison approaches that utilize maximum likelihood and Bayesian methods to evaluate if and where on a phylogeny diversification rates might have shifted [55–57]. However, although these methods can test

for evolutionary patterns consistent with hypotheses linking traits with diversification rate shifts, they do not evaluate causation or address hypothesized mechanisms (Box 1) [58]. Thus, experimental manipulations are ultimately needed to evaluate whether specific causal relationships are present [59].

Traits are hypothesized to influence diversification rates via mechanisms such as changes in reproductive or ecological specialization, changes in population density, and escape from competition via invasion into new adaptive zones [60]. Each of these mechanisms are testable using experiments under the right conditions. Nonetheless, long generation times or slow rates of evolution can pose substantial logistical challenges to applying experimentation in this way [61]. In some cases, empirical tests of mechanistic hypotheses linking traits to species diversity have been pursued, and several traits (such as nectar spurs [62,63], sexual dichromatism [64,65] and viviparity [66,67]) have been evaluated using a combination of both phylogenetic and experimental methods.

For traits not involved in mate choice and reproduction, the links between key innovations and mechanisms of population differentiation are less clear and rarely experimentally explored. For example, plant defense theory led Farrell *et al.* to hypothesize that defensive canals (carrying latex or resin) promote increased speciation rates in plants [68]. The hypothesized mechanism for this association was that latex decreases herbivore pressure, which in turn allows for larger population sizes and lower risk of extinction. To evaluate this hypothesis, Farrell *et al.* tested for a macroevolutionary association between defensive canals and increased diversification rates using sister clade comparisons, and found that in 13 out of 16 plant lineages, clades with canals had more species than their sister clades without canals. However, a number of ecological and evolutionary processes could account for this pattern [17,60,69], and thus targeted experiments are needed to test whether additional evidence supports the proposed ecological mechanisms. Experimental manipulations could address whether the presence of latex does decrease herbivore pressure, and whether decreasing herbivore pressure alters population sizes or diversification, key steps that are necessary if the hypothesis of Farrell *et al.* is correct. These tests could include intraspecific selection experiments or experiments that follow population-level impacts of a transgenic trait manipulation. Although studies have linked the production of latex to reduced herbivory and plant fitness [70], little work has focused on the putative link between latex and increased population density or rates of genetic differentiation.

Diversifying approaches to studying diversification

Many traits implicated in influencing clade diversification rates still remain to be investigated using both experimental and modern comparative methods. For example, altered beak morphology promotes assortative mating and reproductive isolation in some bird populations [41], and associative learning influences the genetic differentiation of apple maggot flies [71], both of which could increase diversification rates. Phylogenetic tests could be used to address whether these traits are associated with altered

diversification rates. Alternatively, population variation can be used to elucidate whether a trait is associated with varied population structure, size or geographic range [72]. Coupling this approach with transplant experiments could demonstrate the importance of a trait in colonization, establishment and success in novel environments, moving beyond the realm of correlates as evidence for diversification hypotheses.

Phylogenetic structure in ecological communities

Understanding the assembly and subsequent structure of communities has been a central pursuit of ecology. Research traditions were established early in this field, including approaches that utilized experimental manipulations of species interactions [73] and those that evaluated patterns generated by historical processes [74]. Both approaches argued that the relatedness of the species that make up a community is non-random and cited species interactions (often competition) as a major driver of community structure.

The increased availability of molecular phylogenies has led to a resurgence of interest in the relationship between species relatedness and patterns of co-occurrence in communities [75,76]. It is now possible to incorporate information on trait evolution and the relatedness of species in a community [77,78] when generating hypotheses about the forces driving community assembly (Figure 5). For example, when ecologically relevant traits are phylogenetically conserved or show a strong phylogenetic signal [79] and species in a community are ‘overdispersed’ (the co-occurrence of more distantly related species than expected at random), negative ecological interactions among phenotypically similar relatives are frequently hypothesized to have driven community assembly (Figure 5) [80]. However, several different processes can result in the same trait evolution and phylogenetic community structure pattern [81], and thus experiments are ultimately needed to evaluate whether these hypothesized processes are consistent with the ecological dynamics currently operating in a community.

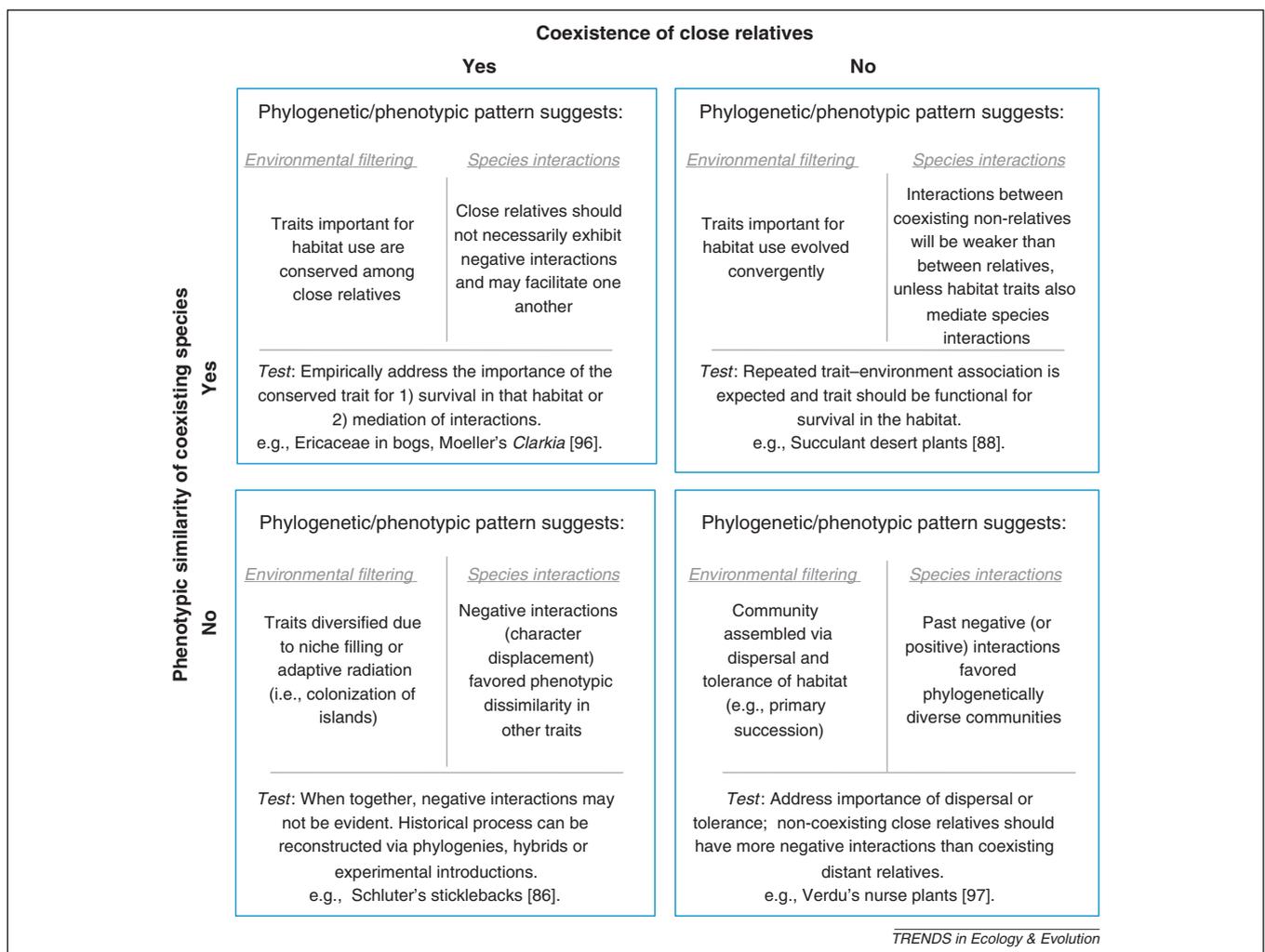


Figure 5. Information about the relatedness and phenotypic similarity of species in a community can provide insights into processes frequently hypothesized to drive community composition [72,80,81,86,88,96,97]. Under certain scenarios, traits that are relevant to environmental filtering (i.e., are necessary for species to live in particular abiotic environments) can show different patterns than traits that are related to species interactions. Note that only the ‘no–yes’ pairings involve trait evolution during diversification: in the bottom-left, trait evolution is frequently hypothesized to be driven by species interactions, and in the top-right by adaptation to the environment. In both cases the no–yes traits are predicted to show a weaker phylogenetic signal than the like pairings (yes–yes and no–no). The like pairings focus on assembly of communities once the species have evolved, presumably in allopatry. Experimental tests on extant communities can clarify the mechanisms that generate community structure.

A phylogenetic signal for species interactions is suggestive, but not definitive evidence, that species interactions influenced community structure [79]. For example, in a meta-analysis of experiments, Cahill *et al.* found that competition between eudicot plant species is more intense among close relatives compared to distant relatives [82]. In manipulative experiments, plant competition is reduced with increasing phylogenetic distance (i.e., more distantly related species are more productive when grown together) [83,84]. For herbivory and disease, it is similarly the case that closely related plants often share parasites, which could potentially lead to overdispersion [85]. However, positive species interactions can also have a phylogenetic signal [80], and negative interactions between close relatives should not be assumed *a priori*. Studies that match patterns of co-occurrence with experimental manipulations testing for increased negative interactions among related species can close the loop between pattern and process, demonstrating the importance of species interactions in the generation of community structure [81].

Rather than phylogenetic overdispersion, phylogenetic clustering (closely related species coexisting more often than expected) is often hypothesized to reflect habitat filtering or character displacement among coexisting close relatives, especially when paired with certain patterns of trait evolution (Figure 5). Unlike the general prediction outlined above, here experiments using closely related species are expected to reveal relatively low levels of competition or survivorship in reciprocal transplant experiments. To demonstrate whether these processes lead to clustering, experimental reconstruction of the history of events can be a powerful approach. For example, Schluter experimentally demonstrated that closely related competitor species drove natural selection towards divergent ecologies in a target population of threespine sticklebacks [86]. Similarly, experimental work has demonstrated that competition leads to diversifying selection in microbes [87]. Coupling these experiments with reciprocal transplants could identify the relative effects of different processes, such as environmental filtering, facilitation and competition, in driving phylogenetic clustering.

Convergent evolution and traits with low phylogenetic signal [88] can also generate non-intuitive links between species interactions and phylogenetic community structure (Figure 5) [80]. For example, in plant communities, herbivore pressure is frequently hypothesized to drive phenotypic overdispersion [89,90] because herbivores often host-shift onto chemically similar plant species. More generally, overdispersion of defense phenotypes suggests that apparent competition (negative interactions between species via shared enemies) is important in generating community structure. Although tests of this idea have not been conducted, the experiments should be straightforward. Among co-occurring species, those that are phenotypically similar are predicted to experience stronger enemy-mediated interactions than those that are phenotypically dissimilar, regardless of phylogenetic relatedness [89–91].

An expanding frontier in evolutionary ecology

Ultimately, deep historical events can never be directly observed or manipulated, and there are thus no definitive

means by which we can directly test many evolutionary hypotheses. Instead, evolutionary ecologists must be willing to integrate multiple lines of evidence as they evaluate the plausibility of their causal hypotheses given all the information (see the ‘evolutionary detective’ described by Losos [92]). Here we have argued that integration of phylogenetic analyses and manipulative experiments is a revealing and rapidly growing approach to evaluating hypotheses that link evolutionary patterns (e.g., clade diversification rates, correlated character evolution, cospeciation, phylogenetic community structure) with mechanistic causes (e.g., population fragmentation, ecological tradeoffs, specialization, species interactions). Experimental approaches such as reciprocal transplants and phenotypic manipulations can elucidate ecological processes that are operating for a single (or small number of) species at a given place and time, but the generality and long-term evolutionary consequences of such an ecological scenario remain unclear. In comparative phylogenetic analyses, a large-scale evolutionary pattern can suggest hypotheses about ecological processes, but the analyses themselves do not address specific mechanisms and experimental methods are ultimately required to evaluate causal scenarios and avoid evolutionary ‘storytelling’. The combined application of phylogenetic and experimental methods can greatly enhance the process of testing and refining hypotheses, and such integration should be increasingly utilized in the pursuit of strong inference in evolutionary ecology.

Acknowledgements

We thank Susan Cook-Patton for extensive discussions during the inception of this project. Jeannine Cavender-Bares, Jonathan Losos, Dale Clayton, Michael Donoghue, Monica Geber, Mike Sanderson, Harry Greene, Irby Lovette, the Cornell ‘phylo-fun group’ and Catherine Wagner provided helpful discussion or comments; we are also grateful to Sarah Bush and Patrik Nosil for providing material for the figures. Our research and laboratory (www.herbivory.com) are supported by an NSF Graduate Research Fellowship (M.G.W.) and NSFDEB-1118783 (A.A.A.).

References

- McLennan, D.A. (1991) Integrating phylogeny and experimental ethology: from pattern to process. *Evolution* 45, 1773–1789
- Brooks, D.R. and McLennan, D.A. (1991) *Phylogeny, Ecology, and Behavior*, University of Chicago Press
- Losos, J.B. (1996) Phylogenies and comparative biology, stage II: testing causal hypotheses derived from phylogenies with data from extant taxa. *Syst. Biol.* 45, 259–260
- Jackson, R.B. *et al.* (2002) Linking molecular insight and ecological research. *Trends Ecol. Evol.* 17, 409–414
- Scheiner, S. (2010) Toward a conceptual framework for biology. *Q. Rev. Biol.* 85, 293–318
- Edwards, E.J. and Smith, S.A. (2010) Phylogenetic analyses reveal the shady history of C4 grasses. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2532–2537
- Development Core Team, R. (2010) *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing
- Pagel, M. (1994) Detecting correlated evolution on phylogenies – a general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* 255, 37–45
- Pagel, M. and Meade, A. (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am. Nat.* 167, 808–825
- Butler, M.A. and King, A.A. (2004) Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am. Nat.* 164, 683–695
- Beaulieu, J.M. *et al.* (2012) Modeling stabilizing selection: expanding the Ornstein–Uhlenbeck model of adaptive evolution. *Evolution* <http://dx.doi.org/10.1111/j.1558-5646.2012.01619.x>

- 12 Revell, L.J. *et al.* (2012) A new phylogenetic method for identifying exceptional phenotypic diversification. *Evolution* 66, 135–146
- 13 Slater, G.J. *et al.* (2012) Fitting models of continuous trait evolution to incompletely sampled comparative data using approximate Bayesian computation. *Evolution* 66, 752–762
- 14 Autumn, K. *et al.* (2002) Integrating historical and mechanistic biology enhances the study of adaptation. *Q. Rev. Biol.* 77, 383–408
- 15 Sandoval, C.P. and Crespi, B.J. (2008) Adaptive evolution of cryptic coloration: the shape of host plants and dorsal stripes in *Timema* walking-sticks. *Biol. J. Linn. Soc.* 94, 1–5
- 16 Sandoval, C.P. (1994) Differential visual predation on morphs of *Timema cristinae* (Phasmatodeae, Timemidae) and its consequences for host-range. *Biol. J. Linn. Soc.* 52, 341–356
- 17 McPeck, M.A. (1996) Linking local species interactions to rates of speciation in communities. *Ecology* 77, 1355–1366
- 18 Losos, J.B. *et al.* (2001) Experimental studies of adaptive differentiation in Bahamian *Anolis* lizards. *Genetica* 112, 399–415
- 19 Wilson, P. *et al.* (2004) A multivariate search for pollination syndromes among penstemons. *Oikos* 104, 345–361
- 20 Castellanos, M.C. *et al.* (2004) 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. *J. Evol. Biol.* 17, 876–885
- 21 Fine, P.V.A. *et al.* (2004) Herbivores promote habitat specialization by trees in Amazonian forests. *Science* 305, 663–665
- 22 Fine, P.V.A. *et al.* (2006) The growth–defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* 87, S150–S162
- 23 Desurmont, G. *et al.* (2011) Evolutionary history predicts plant defense against an invasive pest. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7070–7074
- 24 Messina, F.J. and Fox, C.W. (2001) Offspring size and number. In *Evolutionary Ecology: Concepts and Case Studies* (Fox, C.W. *et al.*, eds), pp. 113–127, Oxford University Press
- 25 Agrawal, A.A. *et al.* (2010) Tradeoffs and adaptive negative correlations in evolutionary ecology. In *Evolution After Darwin: the First 150 Years* (Bell, M. *et al.*, eds), pp. 243–268, Sinauer Associates
- 26 Sillen-Tullberg, B. (1988) Evolution of gregariousness in aposematic butterfly larvae: a phylogenetic analysis. *Evolution* 42, 293–305
- 27 Armbruster, W.S. *et al.* (1997) Do biochemical exaptations link evolution of plant defense and pollination systems? Historical hypotheses and experimental tests with *Dalechampia* vines. *Am. Nat.* 149, 461–484
- 28 Revell, L.J. and Collar, D.C. (2009) Phylogenetic analysis of the evolutionary correlation using likelihood. *Evolution* 63, 1090–1100
- 29 Hansen, T.F. *et al.* (2008) A comparative method for studying adaptation to a randomly evolving environment. *Evolution* 62, 1965–1977
- 30 Harvey, P.H. and Pagel, M.D. (1991) *The Comparative Method in Evolutionary Biology*, Oxford University Press
- 31 Armbruster, W.S. and Schwaegerle, K.E. (1996) Causes of covariation of phenotypic traits among populations. *J. Evol. Biol.* 9, 261–276
- 32 Herrera, C.M. *et al.* (2002) Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. *Proc. Natl. Acad. Sci. U.S.A.* 99, 16823–16828
- 33 Armbruster, W.S. (2002) Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. *J. Evol. Biol.* 15, 468–486
- 34 Weber, M.G. *et al.* (in press) Phylogenetic and experimental tests of interactions among mutualistic plant defense traits in *Viburnum* (Adoxaceae). *Am. Nat.*
- 35 Arnqvist, G. and Rowe, L. (2002) Antagonistic coevolution between the sexes in a group of insects. *Nature* 415, 787–789
- 36 Wright, I.J. *et al.* (2004) The worldwide leaf economics spectrum. *Nature* 428, 821–827
- 37 Kattge, J. *et al.* (2011) TRY – a global database of plant traits. *Global Change Biol.* 17, 2905–2935
- 38 Ehrlich, P.R. and Raven, P.H. (1964) Butterflies and plants: a study in coevolution. *Evolution* 18, 586–608
- 39 Schluter, D. (2000) *The Ecology of Adaptive Radiation*, Oxford University Press
- 40 Edelaar, P. and Benkman, C.W. (2006) Replicated population divergence caused by localized coevolution? A test of three hypotheses in the red crossbill–lodgepole pine system. *J. Evol. Biol.* 19, 1651–1659
- 41 Smith, J.W. and Benkman, C.W. (2007) A coevolutionary arms race causes ecological speciation in crossbills. *Am. Nat.* 169, 455–465
- 42 Clayton, D.H. *et al.* (2003) Host defense reinforces host–parasite cospeciation. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15694–15699
- 43 Smith, C.I. *et al.* (2008) Distinguishing coevolution from covariation in an obligate pollination mutualism: asynchronous divergence in Joshua tree and its pollinators. *Evolution* 62, 2676–2687
- 44 Clark, M.A. *et al.* (2000) Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. *Evolution* 54, 517–525
- 45 Althoff, D.M. *et al.* (2012) Geographic isolation trumps coevolution as a driver of yucca and yucca moth diversification. *Mol. Phylogenet. Evol.* 62, 898–906
- 46 Mikheyev, A.S. *et al.* (2010) Comparative dating of attineant and lepidoteaceous cultivar phylogenies reveals coevolutionary synchrony and discord. *Am. Nat.* 175, E126–E133
- 47 Drummond, A. and Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214
- 48 Thompson, J. (2005) *The Geographic Mosaic of Coevolution*, University of Chicago Press
- 49 Toju, H. and Sota, T. (2006) Imbalance of predator and prey armament: geographic clines in phenotypic interface and natural selection. *Am. Nat.* 167, 105–117
- 50 Miller, G.T. and Pitnick, S. (2002) Sperm–female coevolution in *Drosophila*. *Science* 298, 1230–1233
- 51 Koskella, B. and Lively, C.M. (2007) Advice of the rose: experimental coevolution of a trematode parasite and its snail host. *Evolution* 61, 152–159
- 52 Takebayashi, N. and Morrell, P.L. (2001) Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. *Am. J. Bot.* 88, 1143–1150
- 53 Hodges, S.A. (1997) Floral nectar spurs and diversification. *Int. J. Plant Sci.* 158, S81–S88
- 54 Wilson, R.W. (1951) Evolution of the early tertiary rodents. *Evolution* 5, 207–215
- 55 Rabosky, D.L. (2006) LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. *Evol. Bioinform.* 2, 247–250
- 56 Moore, B.R. and Donoghue, M.J. (2009) A Bayesian approach for evaluating the impact of historical events on rates of diversification. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4307–4312
- 57 FitzJohn, R.G. (2010) Quantitative traits and diversification. *Syst. Biol.* 59, 619–633
- 58 Armbruster, W. and Muchhala, N. (2009) Associations between floral specialization and species diversity: cause, effect, or correlation? *Evol. Ecol.* 23, 159–179
- 59 Donoghue, M.J. (2005) Key innovations, convergence, and success: macroevolutionary lessons from plant phylogeny. *Paleobiology* 31, 77–93
- 60 Heard, S.B. and Hauser, D.L. (1995) Key evolutionary innovations and their ecological mechanisms. *Hist. Biol.* 10, 151–173
- 61 Barraclough, T.G. *et al.* (1998) Revealing the factors that promote speciation. *Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci.* 353, 241–249
- 62 Hodges, S.A. and Arnold, M.L. (1995) Spurring plant diversification: are floral nectar spurs a key innovation? *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* 262, 343–348
- 63 Fulton, M. and Hodges, S.A. (1999) Floral isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* 266, 2247–2252
- 64 Barraclough, T.G. *et al.* (1995) Sexual selection and taxonomic diversity in passerine birds. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* 259, 211–215
- 65 Uy, J.A.C. *et al.* (2009) Plumage and song differences mediate species recognition between incipient flycatcher species of the Solomon Islands. *Evolution* 63, 153–164
- 66 Schrader, M. and Travis, J. (2008) Testing the viviparity-driven-conflict hypothesis: parent–offspring conflict and the evolution of reproductive isolation in a poeciliid fish. *Am. Nat.* 172, 806–817
- 67 Slowinski, J.B. and Guyer, C. (1993) Testing whether certain traits have caused amplified diversification: an improved method based on a model of random speciation and extinction. *Am. Nat.* 142, 1019–1024
- 68 Farrell, B.D. *et al.* (1991) Escalation of plant defense: do latex and resin canals spur plant diversification. *Am. Nat.* 138, 881–900

- 69 Rabosky, D.L. (2009) Ecological limits and diversification rate: alternative paradigms to explain the variation in species richness among clades and regions. *Ecol. Lett.* 12, 735–743
- 70 Agrawal, A.A. (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evol. Ecol. Res.* 7, 651–667
- 71 Feder, J.L. *et al.* (1994) Host fidelity is an effective pre-mating barrier between sympatric races of the apple maggot fly. *Proc. Natl. Acad. Sci. U.S.A.* 91, 7990–7994
- 72 Wagner, C.E. and McCune, A.R. (2009) Contrasting patterns of spatial genetic structure in sympatric rock-dwelling cichlid fishes. *Evolution* 63, 1312–1326
- 73 Connell, J.H. (1961) Influence of interspecific competition and other factors on distribution of the barnacle *Chthamalus stellatus*. *Ecology* 42, 710–723
- 74 Diamond, J.M. (1975) Assembly of species communities. In *Ecology and Evolution of Communities* (Cody, M.L. and Diamond, J.M., eds), pp. 342–444, Belknap Press of Harvard University Press
- 75 Cavender-Bares, J. *et al.* (2009) The merging of community ecology and phylogenetic biology. *Ecol. Lett.* 12, 693–715
- 76 Strauss, S.Y. *et al.* (2006) Exotic taxa less related to native species are more invasive. *Proc. Natl. Acad. Sci. U.S.A.* 103, 5841–5845
- 77 Ingram, T. and Shurin, J.B. (2009) Trait-based assembly and phylogenetic structure in northeast Pacific rockfish assemblages. *Ecology* 90, 2444–2453
- 78 Sargent, R.D. and Ackerly, D.D. (2008) Plant–pollinator interactions and the assembly of plant communities. *Trends Ecol. Evol.* 23, 123–130
- 79 Burns, J.E. and Strauss, S.Y. (2011) More closely related species are more ecologically similar in an experimental test. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5302–5307
- 80 Cavender-Bares, J. *et al.* (2004) Phylogenetic overdispersion in Floridian oak communities. *Am. Nat.* 163, 823–843
- 81 Vamosi, S.M. *et al.* (2009) Emerging patterns in the comparative analysis of phylogenetic community structure. *Mol. Ecol.* 18, 572–592
- 82 Cahill, J.F. *et al.* (2008) Does phylogenetic relatedness influence the strength of competition among vascular plants? *Perspect. Plant Ecol. Evol. Syst.* 10, 41–50
- 83 Cadotte, M.W. *et al.* (2008) Evolutionary history and the effect of biodiversity on plant productivity. *Proc. Natl. Acad. Sci. U.S.A.* 105, 17012–17017
- 84 Burns, J.H. and Strauss, S.Y. (2011) More closely related species are more ecologically similar in an experimental test. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5302–5307
- 85 Futuyma, D.J. and Agrawal, A.A. (2009) Macroevolution and the biological diversity of plants and herbivores. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18054–18061
- 86 Schluter, D. (2003) Frequency dependent natural selection during character displacement in sticklebacks. *Evolution* 57, 1142–1150
- 87 Meyer, J.R. and Kassen, R. (2007) The effects of competition and predation on diversification in a model adaptive radiation. *Nature* 446, 432–435
- 88 Arakaki, M. *et al.* (2011) Contemporaneous and recent radiations of the world's major succulent plant lineages. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8379–8384
- 89 Becerra, J.X. (2007) The impact of herbivore–plant coevolution on plant community structure. *Proc. Natl. Acad. Sci. U.S.A.* 104, 7483–7488
- 90 Kursar, T.A. *et al.* (2009) The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18073–18078
- 91 Becerra, J.X. (1997) Insects on plants: macroevolutionary chemical trends in host use. *Science* 276, 253–256
- 92 Losos, J.B. (2009) *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*, University of California Press
- 93 Nosil, P. (2007) Divergent host plant adaptation and reproductive isolation between ecotypes of *Timema cristinae* walking sticks. *Am. Nat.* 169, 151–162
- 94 Agrawal, A.A. *et al.* (2009) Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias* spp.): chemistry, ecophysiology, and insect behaviour. *New Phytol.* 183, 848–867
- 95 Marazzi, B. and Sanderson, M.J. (2010) Large-scale patterns of diversification in the widespread legume genus *Senna* and the evolutionary role of extrafloral nectaries. *Evolution* 64, 3570–3592
- 96 Moeller, D.A. (2004) Facilitative interactions among plants via shared pollinators. *Ecology* 85, 3289–3301
- 97 Valiente-Banuet, A. and Verdu, M. (2007) Facilitation can increase the phylogenetic diversity of plant communities. *Ecol. Lett.* 10, 1029–1036