

Testing for coevolutionary diversification: linking pattern with process

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Coevolutionary diversification is cited as a major mechanism driving the evolution of diversity, particularly in plants and insects. However, tests of coevolutionary diversification have focused on elucidating macroevolutionary patterns rather than the processes giving rise to such patterns. Hence, there is weak evidence that coevolution promotes diversification. This is in part due to a lack of understanding about the mechanisms by which coevolution can cause speciation and the difficulty of integrating results across micro- and macroevolutionary scales. In this review, we highlight potential mechanisms of coevolutionary diversification, outline approaches to examine this process across temporal scales, and propose a set of minimal requirements for demonstrating coevolutionary diversification. Our aim is to stimulate research that tests more rigorously for coevolutionary diversification.

What is coevolutionary diversification?

The idea that coevolution (see [Glossary](#)) is instrumental in creating new species has had a long history in evolutionary biology. Darwin was among the first to recognize the importance of coevolution among plants and insects [1,2], but it was the seminal work by Ehrlich and Raven [3] that brought this topic to center stage. After an extensive review of plant–butterfly interactions and proposing that coevolution of plant defenses and insect counter-defenses could lead to adaptive radiation, these authors concluded that: ‘[Our] most important overall conclusion is that the importance of reciprocal selective responses between ecologically closely linked organisms has been vastly underrated in considerations of the origins of organic diversity.’ ([3] p. 606). As an outcome of the intense interest generated by this paper, research on coevolution not only expanded, but also led to considerable confusion about what coevolution is. This confusion spurred Janzen [4] to clarify the concept, which served to refocus research

on appropriate tests of coevolution and led to significant advances in coevolutionary thinking in the ensuing decades [5–9].

We currently find ourselves facing a similar problem with the concept of coevolutionary diversification. Although studies on coevolutionary diversification abound, tests examining this process remain tenuous because we lack a framework for linking coevolutionary processes to phylogenetic patterns. This is especially true for plant–insect interactions, where the concept has been invoked to explain speciation at a range of levels: from between focal pairs of interacting genera [10–14] to all of angiosperm and insect diversification [15–17]. Here, we argue that evidence supporting an association between reciprocal natural selection and increased diversification is at best weak. Consequently, we combine research on plant–insect interactions and key findings from other taxonomic groups to propose approaches to resolve this important problem.

As has been done for coevolution, there is a need to further refine what coevolutionary diversification is and what constitutes support for this concept [9,18–20]. We define coevolutionary diversification as the process by which coevolution between two or more taxa increases

Glossary

Codiversification: speciation events in one lineage of species (e.g., plants) are correlated with speciation events in a second lineage of species (e.g., insect pollinators).

Coevolution: reciprocal natural selection that causes reciprocal evolutionary changes in the traits of two or more interacting species.

Cophylogeny: the degree to which two or more lineages share a similar phylogenetic topology.

Cospeciation: the matching of speciation events in two or more interacting taxa.

Escape-and-radiate coevolution: the process by which coevolutionary arm races between plants and their natural enemies causes the evolution of new plant defenses that lead to an adaptive radiation in the plant taxa, followed by the evolution of new insect counter-defenses that lead to an adaptive radiation of insects onto the plant radiation.

Geographic mosaic: differences in coevolved phenotypes or the degree of coevolution among populations within a coevolving species.

Phylogenetic tracking: a pattern in which speciation events in one lineage mirror speciation events in another lineage. Usually assumed that one lineage speciates first and is followed by speciation in the other.

Reciprocal transplant experiment: a method in which individuals are swapped among partner taxa and their fitness from interacting with their local and foreign partners is compared.

Speciation–extinction estimates: estimates of the speciation and extinction rates for a lineage that are usually derived from comparative phylogenetic analyses. The two rates together determine the net diversification of a lineage.

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Keywords: coevolution; speciation; microevolution; macroevolution; species interactions; reproductive isolation; divergent selection.

0169-5347/\$ – see front matter

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net diversification (speciation rate – extinction rate) in at least one of the interacting taxa (*sensu* [8,21]). Coevolutionary diversification is caused by processes at the microevolutionary level, but results in overall changes in the pattern of speciation among interacting species. Thus, studying coevolutionary diversification will require integrating results across different hierarchical scales, from within populations (microevolution) to among higher taxa (macroevolution), to examine the relation between coevolution and speciation.

Thompson [6,7] was among the first to suggest there are direct links between coevolution and speciation. In particular, he suggested that coevolutionary diversification is most likely in groups of interacting species that control the ovum of the gametes of a partner. Thompson pointed to specialized plant–pollinator interactions and maternally inherited intracellular symbionts as examples. Coevolution, especially among geographically subdivided populations, could change the ability of individuals from different locally adapted populations to mate successfully, which might in turn influence mating patterns in the other species. Thompson [7] also clarified Ehrlich and Raven's concept of coevolution [3] as 'escape-and-radiate coevolution'. In escape-and-radiate coevolution, coevolution happens at key moments to drive the evolution of plant defenses and insect counter-defenses, and then other speciation processes (e.g., allopatric speciation) come into play as plants and insects increase their geographic ranges or further subdivide resources.

Despite decades of study on coevolutionary diversification [7,9,21–24], there are three reasons why this concept and its role in generating biodiversity remain ambiguous. First, the process of speciation relies on mechanisms that enhance reproductive isolation among diverging populations [25], but there is no clear understanding of the mechanisms by which coevolution can cause such isolation. Second, tests of coevolutionary diversification have focused on elucidating expected phylogenetic patterns as opposed to the processes that generate such patterns (Box 1). Finally, it is unclear how coevolution at the microevolutionary level can lead to macroevolutionary patterns. Together, these gaps in knowledge hamper our ability to test rigorously for coevolutionary diversification.

In this review, we propose mechanisms by which coevolution can lead to reproductive isolation and outline how to test adequately for coevolutionary diversification. To address these problems, we need information on whether coevolving traits can lead directly or indirectly to reproductive isolation. We also need to determine the conditions under which coevolution leads to increased speciation among interacting lineages, especially when speciation might be episodic, as in escape-and-radiate coevolution. Thus, to understand diversification within coevolving lineages, it will be necessary to combine data from multiple evolutionary scales [26]. Below, we offer a framework that we hope will lead to rigorous and powerful tests of coevolutionary diversification.

Mechanisms of coevolutionary diversification

How might coevolution lead to differentiation among populations, reproductive isolation, and eventually speciation?

The framework for ecological speciation provides an excellent context for addressing this question [27,28]. Nasil [29] outlines three steps necessary for ecological speciation: (i) a source of divergent selection; (ii) a form of reproductive isolation; and (iii) a genetic mechanism linking selection to reproductive isolation. If we allow coevolution to be the source of divergent selection, then coevolutionary diversification is a special case of ecological speciation [8]. A major avenue of research in the study of coevolutionary diversification should be to focus on how and when coevolution is a source of divergent selection and whether selection on coevolving traits can be linked to reproductive isolation.

Coevolution as a source of divergent selection

Coevolution can cause divergent selection among populations through spatially variable patterns of selection [30–34]. In particular, the geographic mosaic theory of coevolution demonstrates that the strength and direction of reciprocal natural selection will be spatially variable and lead to different trait and interaction outcomes among populations [7,8,35]. For example, the arms race between Japanese camellia (*Camellia japonica*) and its weevil seed predator (*Curculio camelliae*) selects for increased weevil rostrum length and greater fruit pericarp thickness. This coevolutionary interaction produces striking variation in these traits across populations [36]. Similarly, research on divergent selection caused by coevolution among pine trees and their seed predators, the red crossbill (*Loxia curvirostris*) and red squirrel (*Sciurus vulgaris*), also highlights how populations of the same species can experience different selection pressures depending on the community context [96,97,98,99,100]. Specifically, a long series of detailed studies show that the coevolutionary trajectory of the beak of the red crossbill and the cone morphology of the lodgepole pine differ depending on the presence or absence of red squirrels.

Within populations, disruptive selection can split a population into two lineages [37]. Theoretical work suggests that such disruptive selection arises for many ecological interactions, particularly competition, and leads to the evolution of divergent phenotypes [38–41]. The best experimental evidence for disruptive selection stems from microbial systems, where coevolution can cause divergent phenotypes due to competition. Microcosm experiments starting from a single lineage [42], or multiple independent lineages [43], show that competition for limited resources can drive divergent evolution for the exploitation of new resources. The next step is to examine the relative roles of spatially variable selection and disruptive selection in divergence of groups of coevolving taxa in which coevolutionary diversification is suspected.

Coevolution and reproductive isolation

Despite progress in demonstrating that coevolution can cause divergent selection [33,35,41], it remains unclear how this translates into reproductive isolation among diverging populations. Theoretical work relies on examining how coevolution affects phenotypic variance of coevolving traits, in which increasing phenotypic variance is a proxy for diversification [8,38,39,44,45]. In one of the first models linking coevolution with reproductive isolation,

Box 1. Coevolutionary diversification and phylogenetic patterns

Coevolution is a process that is often studied by comparing phylogenetic trees and searching for concordant patterns of speciation. A common misconception is that one-to-one matching of speciation events, or cospeciation, is support for coevolution. Although concordant phylogenies suggest a common underlying process, this pattern alone is insufficient evidence to demonstrate coevolution, because there are several possible explanations, as discussed below.

(i) Vicariance

Interacting species often have similar geographic ranges and, therefore, will experience the same biogeographic events. The uprising of mountains, incursion of water, and glacial cycles are examples of events that can subdivide the ranges of species and facilitate speciation by restricting gene flow among populations (Figure 1A). Although the species are interacting and may even be coevolving, patterns of phylogenetic concordance are caused by common biogeographic processes rather than by reciprocal natural selection.

(ii) Phylogenetic tracking

In situations where one of the interacting species is more strongly dependent on its partner, matching phylogenetic patterns may arise as a result of the dependent partner tracking speciation events occurring in the independent partner (Figure 1B). Highly specialized interactions are predicted to have greater matching of speciation events, given that interacting species are restricted to one another.

(iii) Vertical transmission

Interactions that are transmitted vertically from parent to offspring are likely to exhibit concordant phylogenetic patterns. Similar to phylogenetic tracking, vertical transmission restricts opportunities for host shifts and, thus, speciation in the host is followed by speciation in the interacting partner (Figure 1C).

(iv) Coevolution

When coevolved traits directly impact the reproductive isolation of both partners, one potential outcome is cospeciation. Changes in coevolved traits could lead to reproductive isolation of the locally coevolving populations, resulting in matching speciation events. However, a more likely result is discordant phylogenies, where there are significant mismatches between interacting species (Figure 1D). This pattern is one that would be predicted for escape-and-radiate coevolution.

Ideally, studies identifying concordant phylogenies would first reject hypotheses i–iii before assuming that coevolution is the primary driver of speciation. Matching phylogenies resulting from coevolutionary selection may be relatively rare because this requires a link between the coevolving traits and reproductive isolation. Given that coevolutionary diversification is an increase in net diversification rate caused by reciprocal evolutionary change between interacting lineages, other phylogenetic patterns are also possible. Indeed, a prediction of escape-and-radiate coevolution is not cospeciation, but rather alternating bursts of speciation [13,18,19]. Within the bursts, mismatching of

speciation events is likely, and making specific predictions about the branching order is impossible. Thus, without additional data on reciprocal evolutionary selection, phylogenetic analysis of coevolving taxa is inadequate for demonstrating coevolutionary diversification.

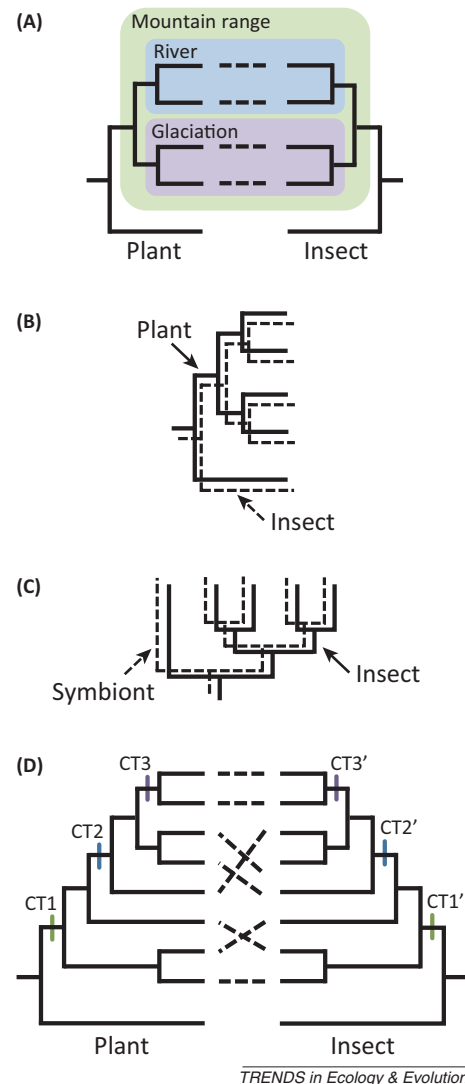


Figure 1. Examples of phylogenetic congruence between interacting lineages.

Kiester and colleagues [44] proposed that changes in the phenotypic variance of coevolving traits among geographically isolated populations, if coupled with sexual selection within pollinator species, can lead to speciation among plant and pollinator populations. They also argued that genetic drift is important in changing the evolutionary trajectory of coevolving traits among populations. Thus, divergence is the result of the interplay among coevolution, geographic isolation, genetic drift, and pollinator behavior. Hence, coevolution alone might not be sufficient to lead to reproductive isolation.

Coevolution can influence reproductive isolation either directly or indirectly via several mechanisms (Table 1). It can lead to reproductive isolation directly if coevolving traits simultaneously promote positive assortative mating.

These traits could be automatic isolating traits (i.e., magic traits), whereby the coevolving traits under selection also directly influence mate choice [46–49]. For example, larvae of the moth *Utethesia ornatrix* feed on *Crotalaria* defended by pyrrolizidine alkaloids [50]. Adult moths use sequestered *Crotalaria* alkaloids as mating cues and males transfer these toxins to females in their nuptial gift [51]. Although the details of the coevolutionary dynamics remain to be investigated [52], this example highlights the direct links that are possible between coevolving traits and mating preferences. Theoretical work has also demonstrated that disruptive selection can directly generate reproductive isolation if coevolution influences the propensity for mating among diverging phenotypes [53,54]. In coevolving host–pathogen populations, for example, coevolution

Table 1. Potential direct and indirect mechanisms of coevolutionary diversification

Type of mechanism	Consequence for reproductive isolation
Direct	
Coevolving trait(s) also determine(s) mate choice [49]	Coevolution can quickly lead to reproductive isolation
Coevolving trait(s) cause(s) correlated evolution in mating traits [60]	Coevolution can quickly lead to reproductive isolation
'Hybrids' or migrants have reduced fitness [58,79]	Reproductive isolation evolves secondarily via continued coevolution
Indirect	
Coevolution facilitates use of new environments (e.g., mutualistic partner that provides new service [92] or coevolution leads to greater competitive ability [93])	Reproductive isolation evolves via genetic drift, abiotic, and biotic selection
Coevolution leads to specialization (e.g., specialized taxa geographically restricted [94] or with limited dispersal [83])	Reproductive isolation evolves via genetic drift, abiotic, and biotic selection
Coevolution results in evolution of key innovation (e.g., novel defense mechanisms [23] or a trait facilitating mutualism with many species [95])	Reproductive isolation evolves via genetic drift, abiotic, and biotic selection

can lead to the evolution of assortative or disassortative mating depending upon the underlying genetic architecture [55,56].

Reproductive isolation among populations differing in coevolutionary trajectories can also directly evolve due to coevolution preventing the establishment of immigrants. That is, migrants can have incompatible phenotypes that interact poorly with the locally coevolved phenotypes of the resident population [30,57–60]. Thus, continued coevolution might generate selection that limits gene flow among populations and reduces mating between individuals with divergent coevolved phenotypes. This is different than the automatic isolating trait concept in that changes might occur in reproductive traits that are not directly involved in the coevolutionary process.

Coevolution can also influence reproductive isolation indirectly by initiating a cascade of evolutionary processes that lead to reproductive isolation. Given that coevolving populations can be geographically structured, coevolution in combination with isolation by distance and genetic drift can result in a mosaic of coevolved trait values across the range of an interaction [7,8,44,57,61,62]. These processes will facilitate the evolution of reproductive isolation given enough time. Escape-and-radiate coevolution involves such a scenario, in which coevolution can result in novel adaptations that alleviate a plant population from previous ecological constraints, enabling the plant to colonize a wider range of geographically separated environments [63]. The colonization of new environments restricts gene flow and promotes population divergence via allopatry, eventually causing speciation. If the original or newly interacting insect populations adapt to exploit the derived plant populations, they could themselves colonize the geographically separated populations and undergo a similar process of allopatric speciation [64].

Genetic mechanisms linking coevolution to reproductive isolation

The translation of divergent reciprocal selection into reproductive isolation among diverging populations will be partly determined by the genetic mechanisms underlying coevolving traits and traits conferring reproductive isolation [55]. We are just beginning to understand the genetic basis of coevolving traits, such as the genes involved in the biosynthesis of glucosinolates in the plant family

Brassicaceae [65], and the corresponding nitrile-specifier proteins used by pierid butterflies to detoxify glucosinolate breakdown products [23]. For plant–pollinator interactions, the genetic basis of key pollination traits, such as flower color, nectar production, pistil length, and anther length, has been shown to be physically linked in the genome of *Petunia* [66], suggesting the possibility of correlated evolution of changes in pollinator attraction and reproductive isolation. In some cases, such as for sympatric *Phlox* species, there is a direct relation between reproductive isolation and selection on flower color genes that control pollinator specificity and reduce hybridization [67]. Although we still require additional information about the underlying genetics of many coevolved traits, these examples illustrate the potential ties between traits that are important in species interactions and reproductive isolation.

Approaches to test for coevolutionary diversification

Testing for coevolutionary diversification will require data that integrate microevolutionary processes with macroevolutionary patterns to understand how coevolution affects divergence, speciation, and extinction in coevolving lineages. Admittedly, this is a challenging goal, but one that is critical for understanding the importance of coevolution to diversification. Previous verbal models provide a starting template for clarifying the conditions in which coevolutionary diversification is possible [3,7,10]. In addition, the dichotomy between direct and indirect mechanisms of coevolutionary diversification has implications for testing the role of coevolution in diversification (Table 1).

Rigorous tests of coevolutionary diversification require an explicit framework that incorporates both the process and the expected pattern. There are several different avenues that researchers can take to test for coevolutionary diversification (Table 2). Identifying the best set of key questions to ask will depend upon the study system and its limitations, but we emphasize that questions should be answered at both the micro- and macroevolutionary levels. More importantly, researchers need to acknowledge the difference in the power of tests that rely on correlative versus experimental approaches. Correlative approaches provide suggestive evidence of the role of coevolution [68], and are more convincing when combined with appropriate experimental tests. The combination of both approaches

Table 2. Approaches to test for coevolutionary diversification

Key questions	Evidence at microevolutionary scale	Evidence at macroevolutionary scale
Is there evidence for current, ongoing coevolution?	Reciprocal fitness effects on interacting species Reciprocal selection on specific traits and coadaptation	Not applicable
Tests of direct mechanisms of coevolutionary diversification		
Is there divergent selection on coevolving traits?	Suggestive: different trait classes within or among populations in one species correlated with trait classes in other species	Suggestive: significant differences in average trait values across taxa are correlated with average trait values in interacting taxa
	Strong: reciprocal transplants among trait classes result in reduced fitness of interacting species	Strong: reciprocal transplants among coevolved taxa show reduced fitness with new partners
Do coevolving traits influence mating success?	Suggestive: correlated evolution of coevolved trait(s) and reproductive trait(s)	Suggestive: changes in key reproductive characters correlated or functionally tied with changes in coevolved traits among taxa
	Strong: coevolved trait(s) influence mate choice Strong: assortative mating between different coevolutionary trait classes	
Does selection limit gene flow among differentiated coevolved phenotypes?	Strong: reciprocal transplants show local coevolutionary interactions are important in restricting gene flow	Suggestive: coevolving taxa speciate in sympatry
		Strong: hybrids have reduced fitness when interacting with coevolved partners of parent species Strong: reciprocal transplants among coevolved taxa show reduced fitness with new partners
Tests of indirect mechanisms of coevolutionary diversification		
Do coevolved traits cause changes in geographic range that facilitate allopatric divergence?	Suggestive: phylogeographic structure demonstrates isolation by distance, range expansion, and/or fragmentation associated with novel coevolved trait(s)	Suggestive: key innovation caused by coevolution at base of radiations in both interacting lineages
Does coevolution lead to diversification?	Suggestive: evidence of genetically distinct, coevolved ecotypes across the geographic range of the interaction	Strong: rates of speciation greater or extinction rates less for coevolved taxa when compared to sister taxa

will help in reaching firm conclusions about coevolutionary diversification.

The discussion below assumes that coevolution has been identified as an important selective force in a focal study system. This in itself is not a trivial task, given the data required to demonstrate reciprocal selection among interacting species. We direct readers to the recommendations of previous papers on how to test for coevolution [4,5,7,68,69]. Below, we focus on testing for coevolutionary diversification and propose approaches to address key questions about the process and patterns (Table 2).

Is there divergent selection on coevolving traits?

For coevolution to lead to diversification, coevolution must facilitate divergence within or among populations of interacting species. Testing for this outcome will depend on whether there is disruptive selection or spatially variable selection on coevolved traits. In the former, experiments demonstrating similar fitness of divergent phenotypes are needed. For example, Herron and Doebeli [70] demonstrated that disruptive selection generated divergent, stable ecotypes from an isogenic line of *Escherichia coli* bacteria competing for a common food source. In the latter case, experiments are needed that demonstrate that differences in coevolved traits are most beneficial when coevolved phenotypes are paired with the respective trait in the coevolved partner. For example, Anderson and Johnson [71] showed that variation in proboscis length across populations of the South African fly *Prosopea ganglbaueri* was positively correlated with spur length of the plant species that the fly pollinates. Such correlative studies are the first step in demonstrating a role of coevolution in population divergence. The addition of reciprocal transplant experiments

among interacting populations is a powerful approach to determine whether differences in coevolved traits among groups result in fitness reductions when paired with local versus foreign coevolved phenotypes [72–75]. Reciprocal transplant experiments can be applied at both the micro- and macroevolutionary levels to demonstrate experimentally the role of coevolution in trait divergence.

Do coevolving traits influence mating success?

Understanding whether coevolutionary selection leads to changes in reproductive isolation is critical. When diverging, coevolving traits are also used in mating (e.g., floral morphology), coevolution can lead directly to reproductive isolation. Addressing this point will require knowledge of mating systems and how coevolving traits are involved. Mating trials among different coevolved phenotypes and assessment of the number of progeny produced will provide estimates of how coevolved traits influence overall reproductive isolation. These assessments can occur at a variety of stages in reproduction (pre- versus post-mating) and experiments can be designed that cover the range of possible mechanisms that cause reproductive isolation [76].

Does coevolution limit gene flow among differentiated coevolved phenotypes?

When coevolutionary selection reduces the ability of migrants and hybrids to establish successfully in different populations, then the evolution of reproductive isolation can evolve to limit gene flow among populations. Reciprocal transplants among populations within species provide one means to examine the success of migrants in novel populations [77,78]. Estimates of establishment success of migrants can be combined with comparisons of the fitness

of migrants relative to residents [79] to provide important insight into how coevolution shapes patterns of gene flow below the species level. Results from reciprocal transplant experiments across species in coevolving lineages would corroborate the role of coevolution in reproductive isolation and speciation. We would expect that reciprocal transplants among species within coevolved clades would also result in reduced fitness of novel pairings. Similarly, we predict that hybrids between closely related species would have reduced fitness when interacting with the coevolved partners of either parent species because of incompatibilities in coevolved traits. Thus, there would be selection for the evolution of reproductive barriers. An additional approach would be to examine the geographic distribution of close relatives. In rare instances, it might be possible to determine whether coevolving taxa speciated in sympatry, which would strongly suggest that divergent coevolutionary selection led to the formation of new species. Taken together, the combination of micro- and macroevolutionary results provides strong evidence for testing whether coevolution was responsible for driving divergence in coevolving lineages.

Do coevolved traits cause changes in geographic range that facilitate allopatric divergence?

The previous questions focus on direct mechanisms of coevolutionary diversification, yet indirect mechanisms might also cause divergence. For instance, coevolutionary interactions might lead to the evolution of a key innovation in one of the interacting taxa, which enables a significant expansion in geographic range that subsequently isolate populations. Once populations are geographically isolated, the same processes that facilitate allopatric speciation, such as isolation by distance, genetic drift, and local adaptation to new environments, will create phenotypic and genetic divergence among coevolving populations. Thus, as an indirect mechanism of diversification, coevolution sets the stage for geographic isolation and subsequent divergence rather than coevolutionary selection directly favoring divergence. This indirect mechanism is one of the central components of escape-and-radiate coevolution, where coevolutionary processes facilitate range or niche expansion that enables taxa to diversify. A similar scenario can also occur when coevolved traits influence the acquisition of a new mutualist or increase competitive ability, enabling a species to expand its range [80]. By contrast, coevolution can also directly fragment populations of a widespread species if coevolution leads to population extinction, as in pathogen–host dynamics [81]. Fragmentation can also occur if coevolution creates highly specialized species that are dependent on one another. In this case, gene flow among populations can be impeded by large geographic gaps between populations of compatible partners [82,83]. Similarly, the geographic mosaic of coevolution that forms across the range of a species is another avenue through which coevolution combined with other processes can eventually lead to reproductive isolation and speciation.

Although phylogeographic and/or population genetic methods are available for assessing changes in demographic history and population divergence [84], understanding

how coevolved traits might have contributed to these patterns is more problematic. One approach is to compare demographic histories of coevolving and non-coevolving groups of populations. The expectation is that, if a coevolved trait led to changes in population genetic structure and demography, the estimates of demographic change in the coevolved group would differ from populations not involved in the coevolutionary interaction. Given that we cannot directly observe the history of changes in trait values and geographic distribution of an interaction, this approach necessarily relies on correlation and modeling rather than on direct experimentation, thus requiring additional evidence to provide a reliable test. For this reason, answers to additional key questions (Table 2) are paramount for assessing coevolutionary diversification.

Does coevolution lead to diversification?

As described above, coevolutionary diversification posits that reciprocal natural selection leads to a net increase in the number of species between interacting lineages. In most tests of coevolutionary diversification, researchers begin by testing for congruence in phylogenetic topologies among interacting lineages. Congruence can have several non-coevolutionary explanations, but if these alternative explanations are ruled out, this approach can help to demonstrate whether coevolution is linked to speciation (Box 1).

To test directly whether coevolution leads to higher diversification rates, we advocate the use of new comparative phylogenetic tools that examine how variation in ecological interactions or traits is related to speciation and extinction rates. These methods are generally referred to as the binary state speciation–extinction (BiSSE) method [85], and can incorporate discrete and continuous [86] trait and ecological data across a phylogenetic tree. For example, this method could be used in plant lineages that contain both wind- and insect-pollinated species (e.g., *Thalictrum*) to test how the presence and/or absence of biotic interactions affect net diversification in the plant lineage. A similar test could then be performed to examine how variation in pollinator traits affects diversification. One can also test whether variation in continuously varying coevolving traits, such as the concentration or diversity of glucosinolates in mustard plants and the activity of detoxification enzymes in pierid butterflies, is associated with altered diversification. Such tests would complement past research aimed at answering these questions [23,87], while providing a more direct and powerful test of how ecological interactions and variation in coevolving traits affect diversification.

Concluding remarks

The most comprehensive tests of coevolutionary diversification will be those in which the role of coevolution in population divergence can be linked to patterns of speciation in coevolving groups. This is a critical goal if we are to understand the importance of coevolution in diversification. The key questions listed in Table 2 can be answered by applying currently available approaches. Identifying sources of selection [88], reciprocal transplant studies [72–74], comparative phylogenetic analyses [89–91], and

speciation-extinction estimates [85,86] can all be integrated into tests of coevolutionary diversification. The major limitation will be determining which of these approaches can be used in a given system.

We conclude by advocating three main criteria to demonstrate coevolutionary diversification. First, coevolution must be important in facilitating divergence among populations within a coevolving species. Second, coevolving traits should influence reproductive isolation, either directly or indirectly, among diverging populations. Finally, coevolving lineages should have higher net diversification rates than non-coevolving lineages. We have no doubt that many researchers will find these criteria rather stringent, but if we are to determine whether coevolution ‘...has been vastly underrated in considerations of the origins of organic diversity’ [3], we need to move beyond elucidating patterns to identifying and distinguishing among the multiple processes that generate them. Only then will we be able to judge whether coevolutionary diversification is an important process in creating Darwin’s entangled bank.

Acknowledgments

We thank B. Anderson, C. Darwell, C. Smith, J. Thompson, and an anonymous reviewer for comments that greatly improved the manuscript. Our research is funded by Syracuse University (D.M.A. and K.A.S.) and NSERC Canada (M.T.J.J.).

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