

Cophylogenetic Methods to Untangle the Evolutionary History of Ecological Interactions

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Abstract

Myriad branches in the tree of life are intertwined through ecological relationships. Biologists have long hypothesized that intimate symbioses between lineages can influence diversification patterns to the extent that it leaves a topological imprint on the phylogenetic trees of interacting clades. Over the past few decades, cophylogenetic methods development has provided a toolkit for identifying such histories of codiversification, yet it is often difficult to determine which tools best suit the task at hand. In this review, we organize currently available cophylogenetic methods into three categories—pattern-based statistics, event-scoring methods, and more recently developed generative model-based methods—and discuss their assumptions and appropriateness for different types of cophylogenetic questions. We classify cophylogenetic systems based on their biological properties to provide a framework for empiricists investigating the macroevolution

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of symbioses. In addition, we provide recommendations for the next generation of cophylogenetic models that we hope will facilitate further methods development.

1. INTRODUCTION

All organisms are members of ecological communities and interact with individuals of other species throughout their lives. These interactions vary in terms of kind, strength, and intimacy, i.e., the degree of biological integration among associated organisms (Guimarães et al. 2007, Ollerton 2006). Modern microbiology, ecology, and molecular biology, as well as the advent of new sequencing technologies, have revealed the immense, often cryptic, diversity of symbioses (high-intimacy interactions). It is estimated that >40% of the lineages in the tree of life are symbionts in the broad sense—parasites, mutualists, and commensals intimately dependent on the lives of others (Dobson et al. 2008, Natl. Res. Council. 2007, Wang & Qiu 2006, Thompson 1994, Price 1980).

This diversity of coevolving symbiotic lineages is staggering in its extent and pervasiveness among Earth's ecosystems, ranging from gut bacteria in vertebrate megafauna to parasitic mistletoe plants on conifers and angiosperms; from chemosynthetic bacterial symbionts of deep-sea bivalves to galling, chewing, and leaf-mining herbivorous insects on angiosperms; from the mitochondria and chloroplasts of eukaryotes to green algae inhabiting the tissues of corals, sea slugs, lichens, and salamander eggs; and from specialized brood-pollination mutualisms like fig wasps and yucca moths to a mostly unmeasured diversity of mites and nematodes associated with most plants and animals (three motivating examples are shown in **Figure 1**; for foundational work on the study of coevolution, see Janzen 1980 and Thompson 1994, 2005). Fossil data and phylogenetic inferences suggest that interspecific interactions, especially symbioses, can persist for millions, and sometimes hundreds of millions, of years (e.g., Compton et al. 2010, Labandeira et al. 1994, McKinney 1995, Zeng & Wiens 2021), effectively coupling the evolutionary histories of even long-diverged clades. Furthermore, organisms engaged in nonsymbiotic and more diffuse interactions—e.g., via pollination, seed dispersal, and predation—are often phylogenetically

Symbioses:

interactions between two or more organisms in close physical or physiological association; symbioses may be mutualistic, antagonistic (in which case they are called parasitic), or commensalistic

Symbiont:

any organism engaged in symbiosis with another taxon; when used in opposition to host, refers to the smaller of two organisms engaged in symbiosis

Coevolution:

reciprocal natural selection in two or more interacting species

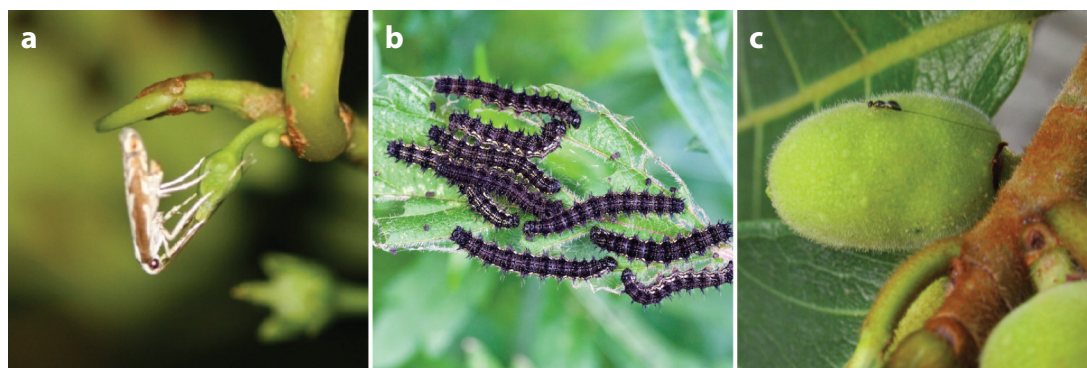


Figure 1

Examples of ecological interactions that provide biological motivation for this review. (a) Leafhopper moth (*Epicephala* sp.) pollinating its leafhopper host (*Glochidion grayanum*, syn. *Phyllanthus grayanus*), an example of a brood pollination mutualism (Tahiti, French Polynesia). (b) Parasitic caterpillar larvae of *Aglais urticae* on their host plant *Urtica dioica*. (c) A fig syconium (*Ficus popenoei*) and a nonpollinating parasitic female wasp (*Idarnes* sp.) that is an antagonist of the fig and fig wasp mutualism. Image in panel a reproduced from Hembry et al. (2012). Image in panel b provided by Niklas Janz. Image in panel c provided by Kevin Quintero.

conservative in the taxa with which they interact (Rezende et al. 2007, Thompson 1994). Such ecological interactions may also leave signatures in the macroevolutionary patterns of coupled lineages (**Figure 2**) (Hembry & Weber 2020, Jablonski 2008, Weber & Agrawal 2014).

Over a century ago Heinrich Fahrenholz (1913), a parasitologist and lice specialist, first proposed that the evolutionary history and taxonomy of parasites should closely reflect those of their hosts. The rise of molecular phylogenetics in the late twentieth century made it possible to test Fahrenholz's hypothesis within a statistical framework. Researchers found that while the topologies for many host and parasite trees may be similar, they rarely matched perfectly (e.g., Cruaud et al. 2012, Hafner & Nadler 1990) because events such as host switching and symbiont speciation can result in incongruencies between the evolutionary history of hosts and symbionts (**Figure 2**). These findings spurred biologists to invent cophylogenetic methods, a new class of computational approaches that untangle why ecologically linked lineages exhibit similar or different diversification patterns.

Cophylogenetic methods have historically been separated into two categories: global-fit methods that assess the overall congruence between two phylogenetic trees and event-based methods that map the symbiont phylogeny onto the host phylogeny using discrete events (Page 2003). Explicitly, global-fit methods use summary statistics to compare two complete phylogenetic patterns and measure cophylogenetic congruence (Balbuena et al. 2013, Hafner et al. 1994, Legendre et al. 2002), whereas event-based methods use predefined scoring systems (e.g., parsimony) to search for optimal arrangements of historical events (**Figure 2**) that can reconcile the topologies of two interacting clades (Brooks 1985, Conow et al. 2010, Page 1994, Ronquist 2003). However, since the terms event-based and global-fit were coined, newer approaches have proliferated that use generative models and statistical inference to shed light on the underlying evolutionary processes responsible for producing cophylogenetic data (e.g., Braga et al. 2020, Dismukes & Heath 2021, Huelsenbeck et al. 2000). While model-based methods can result in estimates of historical events, the use of explicit stochastic models accounting for historical associations sets them apart from other event-based methods. In this review, we adopt an alternative set of categories for cophylogenetic methods: (*a*) pattern-based methods that include approaches historically categorized as global-fit methods in addition to other previously uncategorized methods (Section 2.1), (*b*) event-scoring methods that are event based but model-free optimization methods (Section 2.2), and (*c*) generative model-based methods that use probabilistic models to describe the generating process underlying cophylogenetic patterns (Section 2.3). While all cophylogenetic methods are typically informed by the same data sources—two phylogenies and a matrix that denotes which tips interact—the methods differ widely in their assumptions and the degree to which they are suitable for different types of cophylogenetic systems or taxonomic levels. We also note that coevolution—reciprocal natural selection among taxa—is not necessarily, and may often not be, the process that creates phylogenetic congruence and that phylogenetic congruence can arise even in cases where there is no coevolution taking place between a pair of interacting clades (Thompson 2005). Because of these considerations, choosing which methods are appropriate for a given system and set of biological hypotheses is a difficult task.

This review has two primary objectives: (*a*) to help researchers understand which cophylogenetic methods are best suited to their cophylogenetic systems and questions and (*b*) to identify gaps in understanding or capability associated with our current methods for cophylogenetic analysis to stimulate future advances. We begin by summarizing recent developments among pattern-based and event-scoring methods, which have been reviewed in various contexts previously (Blasco-Costa et al. 2021, De Vienne et al. 2013, Page 2003, Stevens 2004), in addition to a newer emerging class of generative model-based cophylogenetic methods. We first define the major features of the cophylogeny problem and then survey assorted available pattern-based methods, event-scoring

Host: in parasitism and commensalism, the organism that the parasite or commensal exploits for resources; in mutualistic symbioses often used to refer to the larger of two interacting mutualists

Cophylogenetic method:

a computational framework that measures the support for one or more cophylogenetic hypotheses through the unified analysis of two phylogenies and the interactions among their taxa

Generative model:

a statistical model defined by a joint probability distribution that describes the process responsible for producing observed data

Cophylogenetic system:

a host phylogeny, a symbiont phylogeny, and the interactions between terminal taxa connecting the two trees

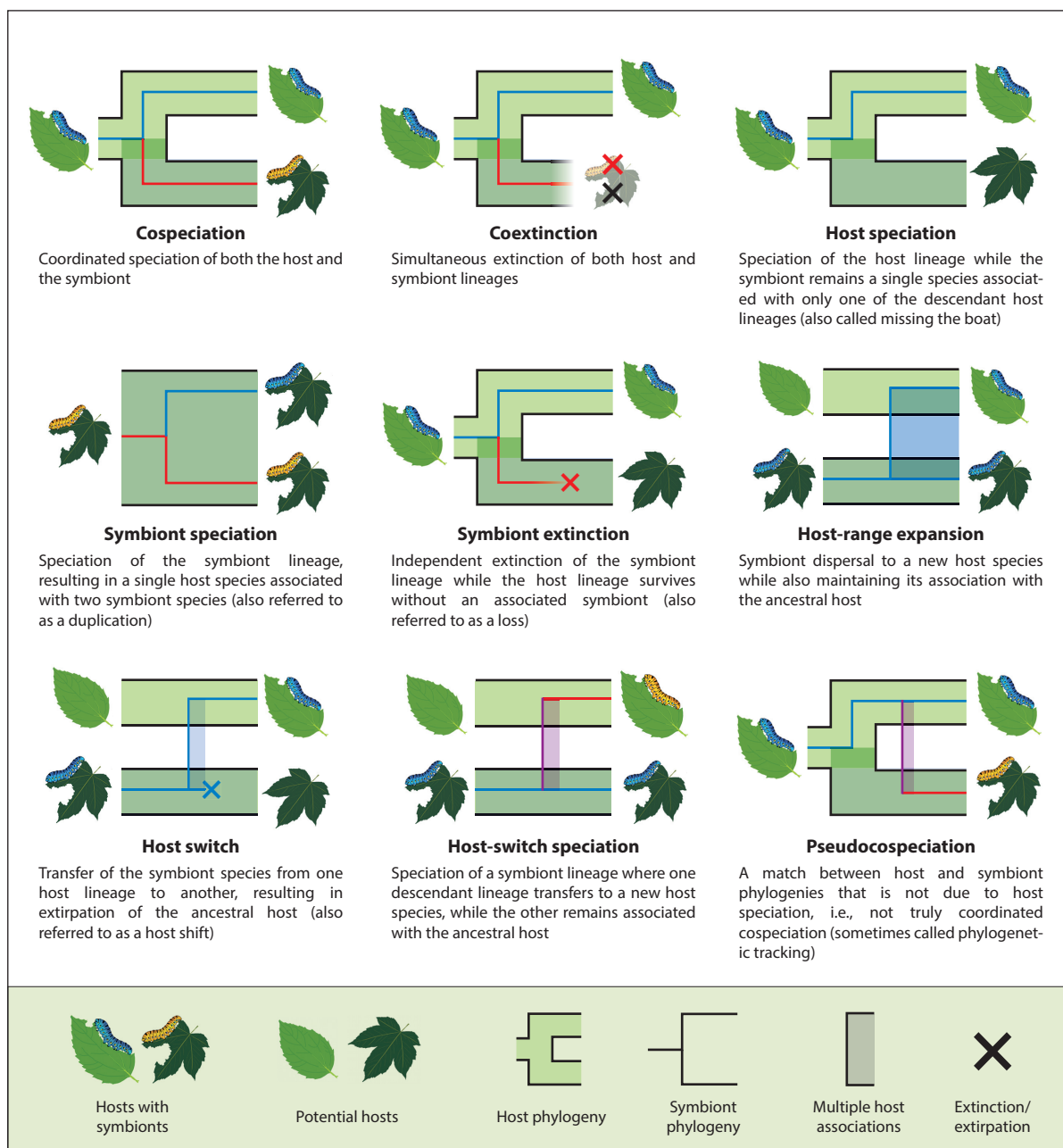


Figure 2

Cophylogenetic systems can generate a number of different event types or scenarios that can manifest in different patterns of the host and symbiont phylogenies. These are illustrated using a hypothetical host–parasite example. Figure adapted from Page (2003, figure 1.1).

methods, and generative model-based methods, while assessing the assumptions, strengths, and weaknesses of each method family. We continue by discussing the types of cophylogenetic systems and data, dividing these into broad categories that can be analyzed using similar methods. Lastly, we survey aspects of cophylogenetic methodology and analysis that are important, yet underexplored, areas of research in need of further study.

2. CURRENT METHODS FOR COPHYLOGENETIC DATA

Biologists expect that many intimate ecological interactions generate predictable cophylogenetic patterns of topologies, divergence times, and associations between two clades. In an extreme example, if symbiont lineages speciated always and only in response to the divergences of their host lineages, and never switched hosts afterward, one would expect to see identical (i.e., completely congruent) topologies and branch-length distributions for both hosts and symbionts (Brooks 1985, Fahrenholz 1913, Paterson & Gray 1997). Such a cophylogenetic history would generate a strong pattern of phylogenetic congruence, as pairs of random trees seldom match perfectly in topology (Felsenstein 1978). However, since even strongly associated clades do not exhibit total phylogenetic congruence, this raises the question, How can we measure the strength of a cophylogenetic signal using these imperfect patterns?

Phylogenetic comparative methods explain patterns of trait variation among taxa as an outcome of descent with modification. Cophylogenetic methods are similar, except they investigate how the presence or absence of species interactions may be correlated both within and between clades. Here, we focus on cophylogenetic methods that explain the distributions of ecological interactions between species pairs from two separate clades. These cophylogenetic methods look at the codistribution of taxon interactions, clade memberships, divergence times, and/or associated traits to ask whether the topological similarities between two phylogenies are due to a shared evolutionary history of ecological interaction. Exactly what questions a researcher asks depends on the biology of the cophylogenetic system, available data, and what available methods are suitable for producing meaningful insights (**Figure 3**).

Most cophylogenetic analyses require three pieces of information as input: two phylogenies and an interaction matrix. Time-calibrated phylogenies are generally preferred so that rates of evolutionary change or event times can be placed within a broader temporal and geological context, although trees with branches measured in relative units of time or in molecular substitution events can be used when data for calibrating trees to absolute time are unavailable. The interaction matrix defines which extant taxa from the two clades interact, as informed by field observations, experimental evidence, or previously published reports. Most methods assume that the recorded interactions within a clade are fundamentally analogous in kind. For example, many butterfly species parasitize one set of plants as larvae and pollinate a different set of plants as adults, but these antagonistic and mutualistic interactions evolve by different underlying mechanisms and should not be treated as directly comparable. Interaction matrix cells are usually recorded as discrete values (e.g., the presence or absence of host use), but some data sets record experimentally verified potential interactions or continuous values (e.g., feeding intensity). Some methods accommodate other forms of auxiliary information, such as chemical volatiles, geographical range data, taxon morphology, etc. Our overview of methods, however, focuses primarily on those analyzing two-clade presence/absence interaction data.

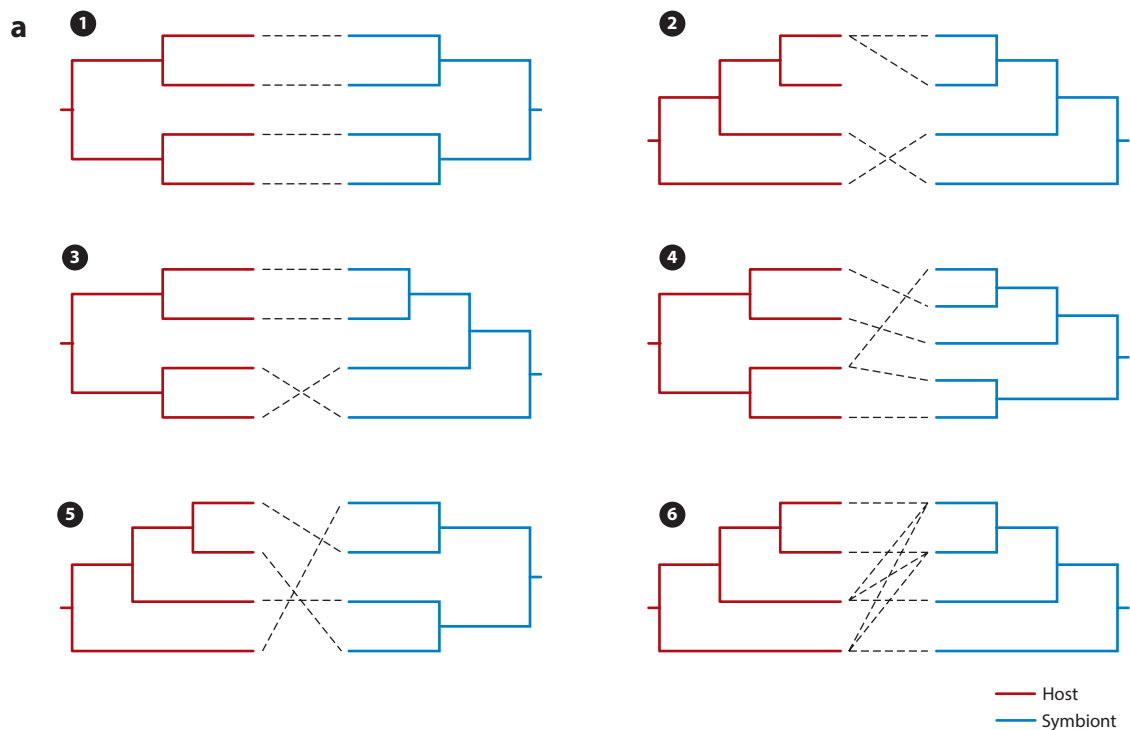
In this section, we describe three classes of methods: pattern-based methods, event-scoring methods, and generative model-based methods. Pattern-based methods decompose the host and symbiont phylogeny into phylogenetic distance matrices that can then be used to test the extent to which the interactions could have been produced due to chance alone. Event-scoring methods map

Phylogenetic congruence:

topological matching of two phylogenies of interacting clades above some statistical threshold

Cophylogenetic signal:

a measure of the congruence of evolutionary history between two interacting clades



Scenario	Typical number of interactions per host taxon	Typical number of interactions per symbiont taxon	Cophylogenetic signal	Host switching	Putative example	Reference(s)
1	1	1	High	Low	Pocket gophers and lice	Hafner et al. 1994
2	1+	1	High	High	<i>Coronaviridae</i> and mammals	Anthony et al. 2017
3	1	1	Low	Medium	<i>Anolis</i> and <i>Plasmodium</i>	Charleston & Perkins 2003
4	1+	1	Medium	High	Figs and fig wasps	Cruaud et al. 2012, Satler et al. 2019
5	1	1	Low	High	Columbidae and feather lice	Doña et al. 2017
6	1+	1+	High	Low	Angiosperms and Nymphalini	Janz et al. 2001

Figure 3

(a) Six possible cophylogenetic scenarios with host phylogenies in dark red (left) and symbiont phylogenies in light blue (right). Interactions are shown by dashed lines. (b) Putative examples of the cophylogenetic scenarios. We provide these simplified examples to illustrate that the biological processes generating macroevolutionary patterns vary substantially depending on the system and on the taxonomic scale (see also **Figure 4**). Thus, it makes little sense to treat all these data the same analytically.

a symbiont phylogeny onto a host phylogeny using the classic cophylogenetic events (e.g., host switching, symbiont speciation, cospeciation). Each of these events is assigned a cost to determine the lowest cost mapping. The newer generative models encompass and use probabilistic models to describe the processes that produce observed cophylogenetic patterns.

2.1. Pattern-Based Methods

Pattern-based methods test whether two interacting phylogenies are more similar than would be expected by chance. Methods in this family differ primarily in how they operationalize the terms similarity and chance. Similarity is generally defined through a test statistic that measures distance between two phylogenetic distance matrices whose rows and columns are ordered to match the associations between the host and symbiont clades. Chance is frequently defined in terms of a null distribution of cophylogenetic similarity scores for pairs of trees with randomly distributed interactions. Typically, cophylogenetic data are transformed into a simplified structure to measure similarity using classical statistical transformations and tests.

2.1.1. Distance-matrix tests. Hafner & Nadler (1990) used a Mantel test to show that the phylogenetic distance matrix elements for a pair of host and symbiont clades (pocket gophers and pocket gopher lice, in their study) are more correlated than expected by chance. To measure the significance of the correlation, the test also simulates a null distribution of permuted matrices without a cophylogenetic correlation structure. If the correlation between the two original distance matrices exceeds that for most permuted matrices, the null hypothesis of no cophylogenetic correlation may be rejected. Mantel tests are extremely simple to apply, but they may suffer from low statistical power (i.e., large trees are needed to reject the null hypothesis) and inflated Type-I error in phylogenetic settings (Harmon & Glor 2010).

In a subsequent study on gopher–louse interactions, Hafner et al. (1994) applied the Wilcoxon signed-rank test (Wilcoxon 1992) to test whether the lengths of congruent branches between two host and symbiont phylogenies were consistently shorter or longer for the symbiont clade. The sum of signed-rank scores is first computed for the original phylogenies, and a null distribution for the statistic is then simulated via permutation, as with the Mantel test of Hafner & Nadler (1990). In the case of Hafner et al. (1994), the Wilcoxon signed-rank test showed that the molecular branch lengths (measured in expected number of nucleotide substitutions per site) in the parasite tree significantly outranked (were consistently longer than) host molecular branch lengths, implying that the lice lineages experienced greater amounts of genetic change when compared to their corresponding host lineages.

Both the Mantel and Wilcoxon signed-rank tests can typically be applied only to data sets with extremely specialized (one-to-one) relationships that contain an equal number of taxa in both phylogenies. That said, Hommola et al. (2009) introduced a variant of the Mantel test that accommodates multiple interactions per parasite and/or host taxon. Nonetheless, distance matrix methods often require that some taxa and/or interactions must be artificially removed from the analysis to conform with the test requirements.

2.1.2. Global-fit tests. Many global-fit tests rely upon principal coordinate analysis to transform the host and symbiont phylogenetic distance matrices into new coordinate systems, so that the overall similarity between the phylogenies may be compared (though see Schardl et al. 2008, Hommola et al. 2009). As with the distance-matrix tests, these tests also require a host phylogeny, a symbiont phylogeny, and an interaction matrix as input; unlike distance matrix tests, however, the two phylogenies may differ in size, and symbionts and hosts may have multiple interactions.

The first published global-fit method, Parafit (Legendre et al. 2002), computes an eponymous global-fit statistic by transforming the phylogenetic distances for hosts and symbionts into separate principal coordinate systems that are then aligned through an interaction matrix. Parafit next measures the global fit of the cophylogenetic system using a linear algebra framework (Legendre et al. 1997). The global fit is maximized when the host and symbiont matrices are perfectly identical in branch lengths and topologies for interacting species and decreases as the two phylogenies

Null distribution:

a distribution of test statistic values that represent the expectations of a null hypothesis (e.g., under the hypothesis of no cospeciation)

Phylogenetic distance matrix:

a square, symmetric matrix with cell values that equal the phylogenetic distances for each pair of taxa in a phylogenetic tree

Tritrophic system:

a cophylogenetic system in which two clades do not interact with each other but both interact with a third clade; typically involving three trophic levels

Tanglegram:

a cophylogenetic alignment that rotates clades to minimize the number of crossed (tangled) interactions between tips

grow incongruent. Parafit has also been extended to test for cophylogenetic patterns between three phylogenies (tritrophic systems) (Mramba et al. 2013). Nooney et al. (2017) refined the tritrophic hypothesis-testing framework of Mramba et al. (2013), allowing it to potentially generalize to larger numbers of interacting clades and richer cophylogenetic topologies, including networks.

Whereas Parafit was designed to test for correlations between the host and symbiont phylogenies, the Procrustes approach to cophylogeny (PACo) (Balbuena et al. 2013) adapts this method to test for the dependence of parasite phylogeny on a host phylogeny. To do so, PACo uses Procrustean superimposition to align the phylogenetic distances among parasites to those for the corresponding host distances. Hutchinson et al. (2017) generalized PACo to allow for symmetrical dependencies between clades to model mutualistic host–symbiont relationships. Once both phylogenies share the same coordinate system, global fit of the cophylogenetic pattern is measured as the residual sum of squares, m^2 , with small values indicating greater congruence. Signal for global and interaction-specific methods are tested using permutation and jackknifing approaches.

Because global-fit scores can be difficult to interpret biologically, Balbuena et al. (2020) introduced the random tanglegram partitions (Random TaPas) method to measure cophylogenetic congruence in terms of patterns consistent with cospeciation and other cophylogenetic events that are implied by a tanglegram. Random TaPas subsamples tanglegrams from the full distribution of cophylogenetic interactions, computes a distribution of significance scores against those subsamples using other global-fit methods (Balbuena et al. 2013, Legendre et al. 2002, Schardl et al. 2008), and then measures whether frequencies of cospeciation exceed null expectations.

In addition to quantifying the global fit of cophylogenetic patterns, most global-fit methods (Balbuena et al. 2013, 2020; Legendre et al. 2002) can also measure the cophylogenetic significance of individual taxa and/or interactions.

2.1.3. Tree-shape tests. Tree-distance metrics, which are often used to measure differences among trees that share the same set of taxa—e.g., different Bayesian posterior tree samples—can also be applied to study symbiont tree congruence. The program COMPONENT (Page 1989), for example, measures how many taxa must be removed from the host and the symbiont phylogenies to produce a perfect cophylogenetic pattern. Because no single tree-shape metric perfectly measures all differences between trees, Avino et al. (2019) tested for cophylogenetic signal using a panel of 18 tree-shape metrics against data simulated under different cophylogenetic hypotheses. They found that data generated under different migration rates, speciation rates, and cophylogenetic event probabilities produced predictable tree-shape metrics; for example, cophylogenetic Robinson–Foulds distances (Robinson & Foulds 1979) predictably increased as host-switching rates in symbionts increased.

2.1.4. Take-home message. Pattern-based methods benefit from fact that they are not explicit event-based models. These methods remain computationally efficient for large data sets with many interactions, while still being able to identify patterns that are consistent with a wide range of hypotheses of codiversification. Because these methods are fast, it is common practice to apply multiple pattern-based methods to establish a consensus for the cophylogenetic signal. In doing so, the consensus is stronger if the signal is shared across the three types of methods outlined in Sections 2.1.1–2.1.3. Importantly, identifying significant signals for most pattern-based methods relies on null hypothesis testing. Null model definition, data set size, and the interpretation of a rejected null hypothesis should all be approached with extreme care. Because pattern-based methods do not provide an explicit generative model to produce cophylogenetic data sets, it is often difficult to interpret what the cophylogenetic test statistics mean biologically. Whether or not this meaning is relevant depends on the question at hand, which we discuss more in Section 3.

2.2. Event-Scoring Methods

Event-scoring methods attempt to reconcile discordance between host and symbiont trees. These have previously been called event-based methods; however, we have separated them here to draw a distinction between more pattern-based and parsimony methods (this section) and event-based methods that rely on probabilistic generative models (Section 2.3). Event-scoring methods seek to connect cophylogenetic data to the events that could have produced them but differ in their methodology. All event-scoring methods typically use a defined set of events to describe the possible ways the host and symbiont lineages evolved together. Reconstructed events—such as cospeciation, host-shifts, symbiont speciation (also called duplication), and symbiont extinction (also called loss)—are used to explain how the host and symbiont lineages evolved with respect to their shared cophylogenetic history (for definitions of these events, see **Figure 2**).

2.2.1. Brooks parsimony analysis. Brooks parsimony analysis (BPA) was one of the first quantitative methods to examine cophylogenetic systems in an algorithmic way. To do this, BPA treats each symbiont's relationship with specific hosts as a binary character of the host and then finds the most parsimonious mapping of that character onto the host tree (Brooks 1981). In this method, the symbiont phylogeny is recoded into a set of binary characters using additive binary coding to convert the cophylogenetic data set into a phylogeny with character states. To do this, half of these characters are used to record the presence or absence of the symbiont taxa (i.e., the tips of the symbiont phylogeny), and the remaining characters each represent interior nodes of the symbiont phylogeny. This mapping is then used to determine which events occur in which parts of the host and symbiont phylogenies. BPA is able to accommodate complexities such as hosts with multiple symbionts and makes few assumptions about the underlying process. For example, there is no *a priori* assumption that cospeciation is the most likely event to occur using BPA (Brooks et al. 2015). Patterns of homoplasy and homology under the original BPA method can be difficult to interpret, however, as it lacks important cophylogenetic event types (e.g., symbiont speciation) (Siddall & Perkins 2003). BPA has also been criticized widely for inaccurately counting events relating to lineage sorting and host switches (Brooks et al. 2004, Siddall & Perkins 2003).

2.2.2. Generalized parsimony reconciliation. Other event-scoring methods take a different approach to reconciling a symbiont tree with its host phylogeny using events, either attempting to maximize the number of cospeciation events (Page 1994) or assigning relative costs to each event and minimizing the total cost of all events (Charleston & Page 2002, Conow et al. 2010, Merkle & Middendorff 2005). Rather than determining events after the analysis, these methods define events and assign costs to each event *a priori*. Specialized algorithms are used to map the symbiont phylogeny onto the host phylogeny until a lowest-cost mapping is found. Assigning sensible costs for events is crucial; for example, with a no-cost cospeciation event, one assumes that cospeciation is quite likely to occur in their particular system. Indeed, most of these methods assign cospeciation a low cost by default, which implies that, if users do not change the default costs, cospeciation (i.e., concordant host and symbiont phylogenies) is the most likely event to occur. As others have noted (Brooks et al. 2015, De Vienne et al. 2013), this can be a flawed assumption, as concordance can be produced in a number of different ways—some cophylogenetic and some not.

One notable challenge researchers face when applying generalized parsimony reconciliation methods is choosing and specifying values for event costs for their study system. Methods have recently been introduced to assist in determining event costs (Baudet et al. 2015, Santichaivekin et al. 2021). The approach by Baudet et al. (2015) uses a simple birth–death model to generate simulated data and approximate Bayesian computation to determine appropriate costs for events.

This, however, comes with the caveat that the costs depend on the assumptions of the simulation model (for example, by default there is no host extinction in the model used in Baudet et al. 2015). Additionally, these methods can produce more than one optimal solution to mapping the symbiont tree onto the host tree, and in some cases this number of solutions can be exceedingly high (Hypša 2006), making it difficult to interpret the results. Despite their challenges, these methods are very computationally efficient and can be used in combination with multiple data sources such as biogeographic data (Merkle & Middendorf 2005) and time-calibrated phylogenies (Conow et al. 2010).

2.2.3. Finding events without cophylogenetic methods. Evolutionary biologists in some cases have sought evidence for the events inferred by event-scoring methods (cospeciation, duplication, host-shifts, and extinction) using cophylogenetic methods in tandem with noncophylogenetic approaches, such as biogeographic or phylogenetic comparative methods (Althoff et al. 2012, Hembry et al. 2013), or without cophylogenetic methods altogether (Luo et al. 2017, Smith et al. 2008). The choice to use multiple lines of evidence or noncophylogenetic methods may be motivated by concern over how to rigorously assign costs to unique events of interest (Luo et al. 2017), concern over how to distinguish phylogenetic tracking from true cospeciation (Althoff et al. 2012), or a focus on a phylogenetic scale that is not amenable to cophylogenetic analyses [e.g., a single putative cospeciation event (Smith et al. 2008)]. The use of multiple lines of evidence to identify events can be a powerful approach for testing hypotheses about cophylogenetic history, but it also testifies to the limitations inherent in using some currently available event-scoring methods on their own.

2.2.4. Take-home message. Event-scoring methods reconstruct the historical sequence of events that produced a cophylogenetic pattern, but they are heavily reliant on user-specified costs or post hoc hypotheses. Recent work has introduced useful and highly efficient methods to determine meaningful event costs. Reconciliation analysis methods have seen widespread use and theoretical development in recent years (Althoff et al. 2012, Drinkwater & Charleston 2014, Drinkwater et al. 2016, Flynn & Moreau 2019).

2.3. Generative Model-Based Methods

Understanding the evolutionary forces responsible for producing observed cophylogenetic patterns is the ultimate goal of researchers investigating the codiversification of interacting clades. To this end, statistical models that describe how host–symbiont associations change over time to generate present-day interactions are extremely useful. Building on previous work that introduced statistical tests for cophylogenetic congruence (Huelsenbeck et al. 1997), the study by Huelsenbeck et al. (2000) was among the first to describe a probabilistic model capable of generating cophylogenetic data. Their model, in which a Poisson process generates host-switch events, assumes a strict one-to-one matching of hosts and symbionts and is appropriate for interactions that exhibit such highly specialized partnerships. Though strong assumptions limit the range of cophylogenetic questions that this model can address, Huelsenbeck et al. (2000) nevertheless laid the foundation for future development of generative models for inferring cophylogenetic processes.

Newer developments in model-based approaches for cophylogenetic analysis take inspiration from two other types of models that describe correlated evolution: (*a*) the evolution of gene trees within species trees and (*b*) phylogenetic diversification driven by biogeographical processes. Such models can provide reasonable analogs to cophylogenetic patterns of species interactions, as demonstrated by recent studies applied to host–symbiont systems to explicitly estimate cophylogenetic parameters (Braga et al. 2020, 2021; Groussin et al. 2017; Satler et al. 2019).

2.3.1. Modeling species interactions. Two recent studies exemplify different strategies for modeling the evolution of host–symbiont interactions. In the first (Satler et al. 2019), the process producing cophylogenetic patterns between sympatric Panamanian strangler figs and their pollinating fig wasps was modeled as the combination of four evolutionary processes: host switching, cospeciation, symbiont speciation, and symbiont extinction. This approach characterizes the evolutionary process similarly to the event-scoring methods (Section 2.2) but in a probabilistic framework. The second example study (Braga et al. 2020) models gains and losses of specific host taxa along the symbiont phylogeny to produce the observed present-day associations among parasitic Nymphalini butterfly species and angiosperm families. One major difference in the methods used by these studies is the treatment of events. Specifically, in Satler et al. (2019), the process happens along the host tree and the events are necessary to map the symbiont tree onto the host tree, whereas in Braga et al. (2020) the process happens along the symbiont tree, and hosts are modeled as characters that evolve interdependently because evolutionary distances between hosts affect the probability of host gains.

It may be reasonable to assume that tightly linked obligate mutualisms—like fig trees and their wasp pollinators—are governed by a process that resembles the evolution of gene families within species phylogenies. Satler et al. (2019) used this reasoning to apply an existing method employing a combined gene-tree and species-tree model to elucidate the shared histories of host and mutualist lineages. Association patterns between genes and species are generated by a diversification process that includes codivergence, gene duplication, gene loss, and gene transfer (Szöllősi et al. 2012, 2013). For a system like the fig and fig wasp mutualism, these events are analogous to cospeciation, symbiont speciation, symbiont extinction, and host switching. Satler et al. (2019) applied a gene-family evolution model [using the program for amalgamated likelihood estimation (ALE) (Szöllősi et al. 2012, 2015)] to reveal that a history of frequent host switching was responsible for the current associations observed in Panamanian strangler figs and their pollinators. The application of these models to the codiversification of mutualistic systems was initially introduced by Groussin et al. (2017) to estimate cospeciation and host switching in the evolutionary associations of mammals and their gut microbes. These studies (Groussin et al. 2017, Satler et al. 2019) demonstrate the potential for model-based approaches to yield deeper insights into the macroevolution of ecological interactions.

Braga et al. (2020) developed an approach for understanding host-repertoire evolution in clades of parasitic lineages that uses models adapted from the field of historical biogeography to describe species-area distributions (Landis et al. 2013, Ree et al. 2005). In this method, each parasitic lineage has a host repertoire, which is the set of possible hosts a parasitic species can exploit. When assessing ecological interactions using a biogeography-based model, the host repertoire corresponds to the set of possible areas that make up the geographic range of a species (Braga et al. 2020). Under this model, a parasitic lineage has a realized and a fundamental host repertoire, such that a range expansion to a new host species begins with a gain in the parasite's ability to exploit the new host (the new host becomes part of the fundamental host repertoire), followed by the parasite's switch to using the new host (the new host species is part of the realized host repertoire); these nonhost, potential, and realized associations evolve through an ordered two-step process, imbuing the host repertoire with macroevolutionary memory (Goldberg & Foo 2020, Janz et al. 2001). In addition, phylogenetic distance-dependent host-range expansion rates are modeled to account for how closely related any new host is to all hosts currently used by a parasite. By modeling changes in the assemblage of host species exploited along the symbiont lineages, this method enables estimation of ancestral host repertoire, providing insights into the evolution of ecological interactions over time (Braga et al. 2021).

Host repertoire: the set of host taxa with which a symbiont associates or interacts

While these few applications of generative models highlight the potential of probabilistic methods for yielding significant insights into cophylogenetic patterns, models should be selected carefully, with full awareness of their assumptions, limitations, and advantages. Certain models are clearly more suitable for analyzing certain types of cophylogenetic systems than others. For example, it may not be appropriate to adapt a gene-family evolution model to study the codiversification of host-parasite systems, since these models do not allow hosts to escape an association with a parasite (i.e., a species must be associated with at least one gene). Moreover, models of gene trees within species trees restrict symbiont species to only one host-species associate at a time. While there are workarounds for this limitation [e.g., a symbiont with two hosts can be represented as two sister lineages in the symbiont tree, as in Satler et al. (2019)], besides being biologically unrealistic in many systems, it is unclear whether or how such transformations bias parameter estimates. The approach of Braga et al. (2020) was designed to allow symbionts to have multiple hosts in their repertoires at any given time, making this model suitable for studying the associations of generalist symbionts. However, their implementation does not allow host diversification and is currently appropriate only for systems where all host taxa are older than the symbiont clade. This restriction also means that analyses under this model do not estimate parameters associated with host evolution, though it may be possible to flip the focus to evaluate the symbiont repertoire of a clade of hosts.

Generative models and statistical inference are powerful tools for understanding how evolutionary processes influence ecological interactions. Very few studies, however, have investigated the accuracy and consistency of model-based cophylogenetic analysis. Host-symbiont association data generated under complex macroevolutionary models are essential if we want to understand the performance and limitations of all cophylogenetic methods. Recently introduced simulation tools (e.g., Braga et al. 2020, Dismukes & Heath 2021, Maliet et al. 2020) for generating cophylogenetic data under explicit cophylogenetic models will yield deeper knowledge about the ways in which generative models can be applied to these questions. For instance, Braga et al. (2020) used simulated data to show that their host-repertoire model could reliably estimate the true simulating rates of host-repertoire evolution, including the effect of host phylogenetic distance on host gain rates. Expanding the range of methods and statistical models evaluated using simulated data will be an important advancement for the field of cophylogenetics.

2.3.2. Take-home message. The types of interspecies interactions existing in nature are extraordinarily diverse, and a one-size-fits-all model is neither possible nor desirable. Nevertheless, explicit models of codiversification and species interactions are necessary to gain greater understanding of the evolutionary forces responsible for generating present-day host-symbiont associations. Critically, future generative models must be motivated by the biological systems they seek to describe, with clearly defined assumptions and outcomes. Furthermore, methods integrating additional ecological (Clayton et al. 2015), biogeographical (Althoff et al. 2012, Hembry et al. 2013), genomic (Cai et al. 2021), and paleontological (de Baets et al. 2021) data have the potential to enhance cophylogenetic analyses. Such data can provide more context for past and present host-symbiont associations by placing them in an environment, a space, and a time.

Designing biologically motivated models conditioned on a wide range of data sources, however, inherently leads to complex, parameter-rich processes. Statistical inference under complex macroevolutionary models is challenging to execute (e.g., requiring detailed specification files and long analysis times). When complete, such analyses result in numerous parameter estimates that may be highly uncertain and/or poorly identifiable, making the output difficult to summarize and interpret. For this reason, it is essential that researchers applying these methods carefully consider the assumptions of the model and methods, clearly define their *a priori* hypotheses, and present

results that adequately communicate parameter uncertainty. With great models comes great responsibility.

2.4. Methods Summary

The three categories of cophylogenetic methods discussed in Section 2 consider different types of cophylogenetic systems and interactions. **Table 1** lists software tools and approaches that have

Table 1 Summary of reviewed cophylogenetic methods

Method	System	Phylogenetic data	Interactions	Reference(s)
Pattern-based statistics				
Mantel test	Symmetric	Topology, branch lengths, and divergence times	One:one	Hafner & Nadler 1990
Wilcoxon test	Symmetric	Topology, branch lengths, and divergence times	One:one	Hafner et al. 1994
Parafit	Symmetric	Topology, branch lengths, and divergence times	Many:many	Legendre et al. 2002
MRCAlink	Symmetric	Topology, branch lengths, and divergence times	Many:many	Schardl et al. 2008
PACo	Directional, symmetric	Topology, branch lengths, and divergence times	Many:many	Balbuena et al. 2013, Hutchinson et al. 2017
Random TaPas	Directional, symmetric	Topology and branch lengths	Many:many	Balbuena et al. 2020
Event-scoring methods				
BPA	Directional	Topology	Many:many	Brooks 1981, Brooks 1990
TreeMap	Directional	Topology	One:many	Page 1994
Jane	Directional	Topology, branch lengths, and divergence times	One:many	Conow et al. 2010
Tarzan	Directional	Topology and divergence times	One:many	Merkle & Middendorf 2005
COALA	Directional	Topology, branch lengths, and divergence times	One:many	Baudet et al. 2015
Jungles	Directional	Topology, branch lengths, and divergence times	One:many	Charleston 1998
eMPress	Directional	Topology, branch lengths, and divergence times	One:many	Santichaivekin et al. 2021
DIVA	Directional	Topology	Many:many	Ronquist 1995
CoRe-PA	Directional	Topology and divergence times	One:many	Merkle et al. 2010
Generative model-based methods				
Bayesian host switching	Directional	Topology and divergence times	One:one	Huelsenbeck et al. 2000
DEC	Directional	Topology and divergence times	Many:many	Ree et al. 2005
ALE	Directional	Topology and divergence times	One:many	Szöllősi et al. 2012, 2015
Host repertoire evolution	Directional	Topology and divergence times	Many:many	Braga et al. 2020

Each method differs in how it treats interactions [directional (e.g., parasite on host), symmetric (e.g., plant and pollinator)], the types of phylogenetic data it can use [topology, topology and branch lengths, topology and divergence times, or all three types], and the number of interactions it permits per host and symbiont taxon (assumed numbers of host-per-symbiont to symbiont-per-host interactions: one:one, one:many, and many:many). Abbreviations: ALE, amalgamated likelihood estimation; BPA, Brooks parsimony analysis; COALA, coevolution assessment by a likelihood-free approach; CoRe-PA, cophylogeny reconstruction using parsimony analysis; DEC, dispersal-extinction-cladogenesis; DIVA, dispersal-vicariance analysis; PACo, Procrustes approach to cophylogeny; Random TaPas, random tanglegram partitions.

implemented methods we have reviewed. When choosing a method to analyze cophylogenetic data, it is important to consider the nature of the ecological interactions, the phylogenetic data available, and the number of taxa with which symbionts and hosts associate.

Generative model-based methods and pattern-based methods both make use of probabilities in evaluating data. Event-scoring methods make use of parsimony scores. A major advantage of using probabilities rather than parsimony scores is the ability to evaluate uncertainty. Parsimony scores are limiting in the sense that they allow for the possibility of multiple lowest cost mappings with no way to determine which of these is the most plausible.

All of the cophylogenetic methods we described in Section 2 are inference methods, in the sense they allow biologists to reconstruct how relationships between hosts and symbionts evolved, whether certain cophylogenetic relationships are phylogenetically conserved or clustered, and at what rates various cophylogenetic events occurred. However, only generative model-based methods can simulate synthetic data sets. Making use of simulated data enables detailed analysis of the performance of cophylogenetic inference under realistic evolutionary scenarios. Such studies determine how accurately each method can estimate certain values from cophylogenetic data, how robust they are to different types of error, and the computational resources and time they require. When designing cophylogenetic studies, researchers can combine this information with detailed knowledge of the biological properties of their system and explicit *a priori* hypotheses to choose the most appropriate methods that will lead to reliable conclusions.

3. CLASSIFYING COPHYLOGENETIC SYSTEMS

The cophylogenetic methods toolbox contains a wide variety of approaches. Because these methods differ in their assumptions, it should come as no surprise that some methods are more applicable to certain classes of cophylogenetic problems over others. In this section, we characterize how distinct types of cophylogenetic systems interface with currently available cophylogenetic methods to guide biologists toward selecting the right tools for the job in their own research. **Table 1** summarizes how we view which methods are appropriate for which systems and data sets. In this section, we first describe the different properties of cophylogenetic systems that may impact their patterns and dynamics of codiversification. Second, we describe qualities of data sets. Third, we review which system and data-set properties are most congruent with which cophylogenetic methods. Finally, we discuss situations for which cophylogenetic methods are inappropriate and call for better awareness of what cophylogenetic biology and the rest of evolutionary ecology can learn from each other.

3.1. Properties of Cophylogenetic Systems

Evolutionary ecologists classify species interactions (of which cophylogenetic systems are a subset) along a variety of axes (Ollerton 2006, Thompson 1994). Four of these axes represent properties that have important consequences for patterns and processes of codiversification, as well as for choice of cophylogenetic methods: types of interaction [e.g., antagonism (including parasitism), mutualism, commensalism], whether one or both taxa depend on the interaction, degree of specialization (number of partner taxa), and transmission ecology (vertical or horizontal). **Figure 4** uses a hypothetical system to illustrate various ways in which ecological associations may be distributed among host and symbiont taxa.

3.1.1. Types of interactions. Symbiotic interactions between pairs of clades—or other interactions with high biological intimacy, in which individuals of one taxon spend much or all of their lives in close physical or physiological proximity to individuals of the other taxon (Ollerton

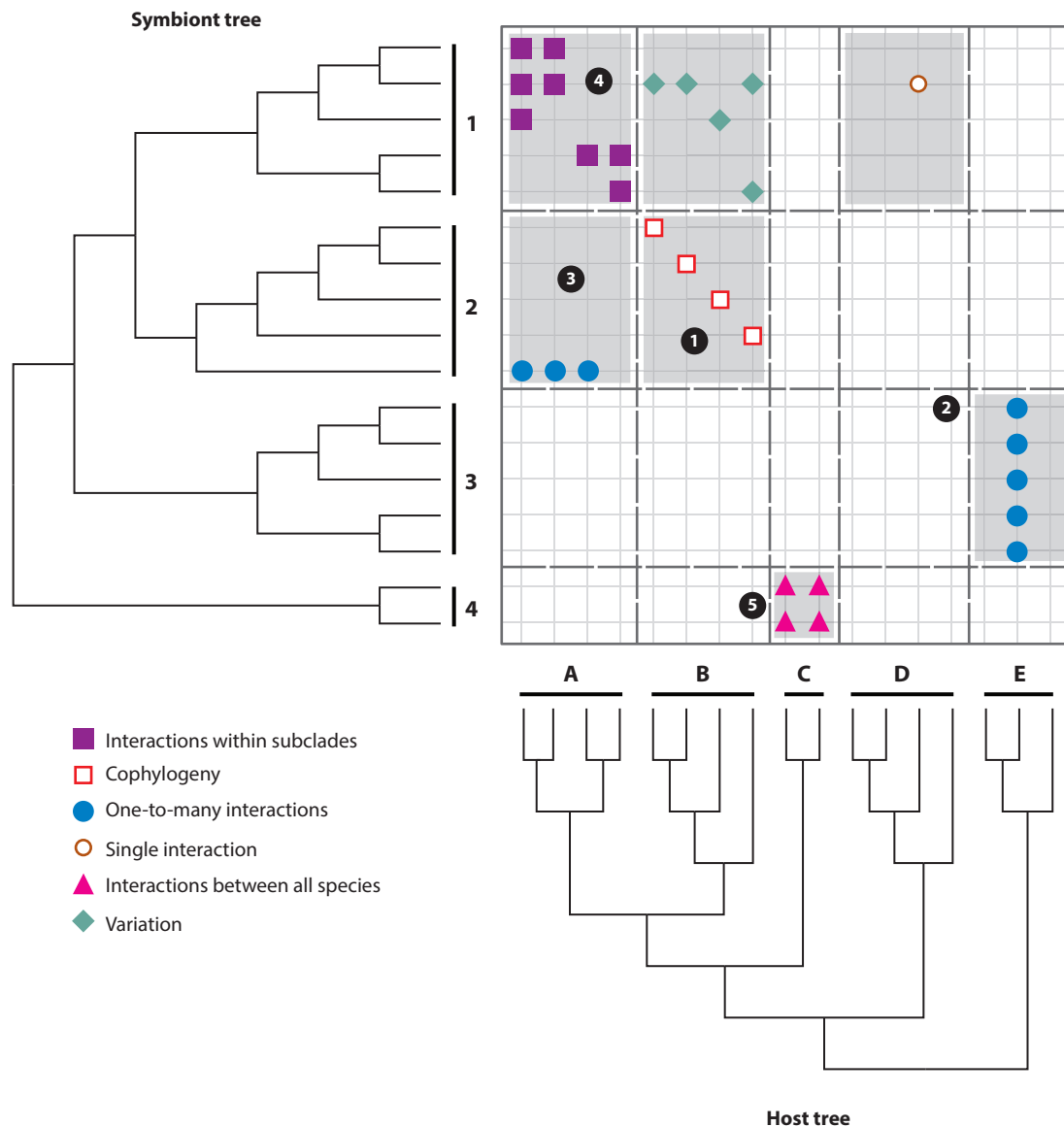


Figure 4

Hypothetical cophylogenetic system showing the different ways species–species interactions might be distributed within clade–clade interactions. The symbiont tree is split into four clades (1–4), and the host tree contains five clades (A–E). In the interaction matrix, species–species interactions are represented by different point shapes, while clade–clade interactions are represented by gray-shaded rectangles. Specialization can be seen at several levels. ① Species–species: Within interaction between clades 2 and B, each symbiont species interacts with a unique host species. ② Clade–species: All species in clade 3 are specialized to the same species in clade E. ③ Species–clade: A single species in clade 2 interacts with several species within clade A. ④ Subclade–subclade: Each subclade within clade 1 only interacts with one subclade of A. ⑤ Clade–clade: All species within clades 4 and C interact. All but one of the interactions of the sister clades 1 and 2 are with hosts from the sister clades A and B, and the exception is a taxonomically rare interaction, where a single species–species interaction occurs between clades 1 and D. Only the interactions between clades 2 and B show a perfect cophylogenetic pattern (considering only tree topology). When data are summarized at the clade level, different amounts of data (species–species interactions) are reduced to one clade–clade interaction. This might be desirable or necessary in some situations (e.g., to compensate for uneven sampling effort or to reduce data set size for tractability), but it adds an assumption about data distribution.

2006)—are the most tractable for cophylogenetic analyses. Depending on the benefits to individuals of each interacting clade, these interactions can be classified as antagonism (usually parasitism in cophylogenetic systems), commensalism, or mutualism. Although these three classes are each fascinating in their own right, we primarily consider whether a given biological interaction's underlying processes of diversification are or are not compatible with the assumptions of different cophylogenetic estimation methods.

How two groups of symbionts interact changes which cophylogenetic methods can be applied to their study. Parasitic and commensal interactions are unidirectional; i.e., the survival of one symbiont depends upon that of its host but not vice versa. Mutualistic interactions are bidirectional in the sense that survival of both symbionts is positively affected by the interaction. (Note, however, that while both symbionts benefit, dependence in mutualisms may be asymmetric if one symbiont is more dependent on the interaction than is the other.) A symmetric method applied to a unidirectional host–parasite system would unintentionally search for the possibility that parasite phylogeny influenced host phylogeny (but for cases where parasites do influence host speciation, see Shoemaker et al. 1999). A unidirectional method applied to a symmetric plant–pollinator system, on the other hand, would require that either the plant or the pollinator diversification was not influenced by the diversification of its partner clade. Applying a unidirectional method to a symmetric system twice, with each symbiont playing the role of host, can help identify mutually compatible bidirectional inferences, however. In a similar vein, symbiont loss (extinction) may be more biologically realistic in a parasitic or commensalistic system but less so in a given mutualistic system.

Not all systems can be categorized so easily, so applying multiple methods could be both appropriate and instructive. Systems are often defined with respect to a specific trait and/or life history stage, even though the same species may interact in a variety of different ways depending on which trait or life history stage is considered. Interactions between butterflies and angiosperms could potentially be defined as parasitic or mutualistic, depending on whether the question concerns larval herbivory of caterpillars on host-plant tissues versus adult pollination of flowers. Similarly, the traits, behaviors, and genes facilitating such ecological interactions might involve either chemical defenses and counterdefenses in the first case or cues and anatomical features in the second case. One goal of our guide is to aid the practicing biologist in defining the appropriate scope for the ecological interactions, taxa, and traits to be studied productively using cophylogenetic methods.

3.1.2. Specialization and generalization. Interacting species in a cophylogenetic system often vary in terms of the number of species with which they interact (**Figure 4**). At one extreme, all species might have one-to-one interactions (specialists, e.g., interactions between clades 2 and B in **Figure 4**) and at another extreme, all species may interact with many species (generalists, e.g., clade 1 in **Figure 4**). Systems in which the average level of specialization is high [in the language of species interaction network ecology, degree k is low for most taxa (Bascompte & Jordano 2013)] are most amenable to the assumptions of cophylogenetic analyses. By contrast, there is a threshold above which too many species are too generalized to avoid violating the assumptions of cophylogenetic analyses, and alternative methods should be considered in these cases (Hembry & Weber 2020).

3.1.3. Vertical and horizontal transmission. Life history traits may also skew how cophylogenetic variation is generated through either codiversification or host-switching events. The most consequential of these for cophylogenetic analyses is whether transmission of symbionts to hosts across generations is vertical (parent to offspring, such as the mitochondria of eukaryotes or *Buchnera* symbionts of aphids) or horizontal (symbionts can switch hosts in each generation, as is the

case for leafflower moths with leafflowers and *Symbiodinium* algae that are symbiotic with corals). In systems where vertical transmission is the rule, cospeciation (contemporaneous codivergence) may be assumed to be common; indeed, some of the clearest phylogenetically congruent systems fall into this category (Hayward et al. 2021). In systems with horizontal transmission, the opposite is the case. Methods assuming that the horizontal transmission of symbionts via host switching is rare may then be prone to overpenalizing incongruencies in cophylogenetic patterns.

3.1.4. Other properties of cophylogenetic systems. The interaction data that are used to power cophylogenetic methods are generally based on the presence or absence of actual interactions that are observed in the field. However, it is possible that many species have the potential to interact but are unable to do so due to mitigating ecological or geographical circumstances. For example, if two similar host species, A and B, are geographically allopatric, and a parasite species is sympatric with and uses only host A (but not host B), the parasite may realize its potential to use host B if the two entered geographical sympatry. Although binary representations of the presence and absence of interactions are often used in cophylogenetics to simplify data representation and methodological complexity, most ecological interactions are, in truth, more accurately represented by complex sets of phenotypic interactions that are governed by large numbers of quantitative traits.

3.2. Data-Set Properties

3.2.1. Taxonomic rank and missing taxa. Cophylogenetic data sets must be carefully defined in an appropriate manner for the question of study. The scale and scope of taxon sets for each symbiont clade is often determined by several practical factors. Ideally, most cophylogenetic studies would use species-level (or even individual-level) data sets, but (a) data with such fine-scale taxonomic resolution are often not available or are difficult to obtain and (b) computational methods generally become less efficient as the number of taxa increases. If data are missing, it is important to consider the specific data points that are missing. For example, if the data set for a clade of highly vagile parasites contains only North American, but not South American, taxa, cophylogenetic methods may underestimate the degree of host switching and/or generalization in the system.

It is also necessarily easier to assemble cophylogenetic data sets that encode symbiotic associations between higher taxonomic ranks (between genera or families) rather than between lower-rank taxa (between species or populations). That said, data associated with higher ranks can variably misrepresent the degree of generalization or specialization in different lineages, which can in turn bias how methods perform. Event-scoring and generative model-based methods that are explicitly designed to model species-level interactions should be used with species-level taxa whenever possible; it is probably better to use only one or a few species-level taxa to represent family-level variation rather than lump all interactions together for a single family-level taxon representative. Pattern-statistic methods are also influenced by taxon sampling and the choice of taxonomic rank, but it is harder to characterize exactly how so based on first principles, since they lack a mechanism for generating data sets.

3.2.2. Phylogenetic estimates. All cophylogenetic methods require topologies for both clades. Regarding branch length estimates, many pattern-based methods are solely informed by cophylogenetic distance matrices and may be applied to unrooted phylogenies for clocklike genes. Event-scoring methods that rely on parsimony, in contrast, make no use of branch-length information and require only topology. Time-calibrated phylogenies are preferred, if not required, for several event-scoring and most generative model-based methods that reconstruct the chronology of

cophylogenetic events. Phylogenetic error, in terms of both topology and branch lengths, can easily lead cophylogenetic estimates astray, e.g., by inflating the required number of host-switching events to explain incongruence. Topologies with polytomies that represent uncertainty may be appropriate for pattern-statistic methods but less so for event-scoring and generative model-based methods. Applying the method of choice to a Bayesian posterior of trees can help assess the extent to which the results are sensitive to phylogenetic uncertainty, as well as uncertainty in divergence-time estimates if the phylogenies are time-calibrated. Balbuena et al. (2020) and Pérez-Escobar et al. (2015) have developed specific approaches for dealing with phylogenetic uncertainty using both simulated and empirical data.

3.3. When Cophylogenetic Methods Are Not Appropriate

Many systems in which two clades interact are not suited for analysis with cophylogenetic methods. The interactions may be too generalized at the species level or too clearly phylogenetically incongruent to meet the assumptions of cophylogenetic methods. Accordingly, it has long been recognized in evolutionary ecology that many situations in which species interactions influence diversification or in which trait coevolution occurs cannot be detected using cophylogenetic methods (Thompson 1994, 2005). Indeed, in some, if not many, cases, coevolution between two clades should not result in phylogenetic congruence (Thompson 2005, Poisot 2015). There is a rich and burgeoning literature on ways to use phylogenetic comparative methods to detect the signature of species interactions in macroevolutionary data in situations where cophylogenetic approaches are not appropriate (for reviews, see Harmon et al. 2019, Hembry & Weber 2020, Weber et al. 2017). Cophylogenetic research and research applying phylogenetic comparative methods to the role of species interactions in macroevolution have largely proceeded independently of one another. However, these subfields are very closely related, and recent model-based methods development (e.g., Braga et al. 2020) begins to blur the distinction between them. We suggest that recent developments in each field may usefully inform the other in light of recent attention toward elucidating the role of ecological interactions among taxa in macroevolution.

4. CONCLUSION

Cophylogenetic methodology has advanced tremendously over the past several decades, and yet there is still far to go (Brooks 1985, Hafner et al. 1994, Page 1994). Today's cophylogenetic methods are varied in their assumptions, in what types of data they analyze, and in what types of estimates they produce. Yet, it is as critical as ever for practicing biologists to carefully weigh which features of a method are most appropriate to study the question at hand. It is apparent to us that cophylogenetic studies do not yet benefit from the conveniences of many other macroevolutionary analysis frameworks—such as those relying on standard molecular models or phylogenetic comparative methods—because cophylogenetics currently lacks one-size-fits-all methods. The reasons why adequate methods are lacking largely boil down to our imperfect understanding of the evolutionary processes that generate cophylogenetic patterns, taxon sampling limitations, the inherent statistical and combinatorial complexity of cophylogenetic metrics and models, and the poor computational scalability of our inference methods for those approaches. As a result, every method typically must compromise something biological (realism), statistical (complexity or generality), or computational (speed or scalability) for it to be useful. In this review, we attempt to equip readers with a framework to locate where their data sets and hypotheses reside in the tangled frontier of cophylogenetics, navigate it safely, and use these methods productively and rigorously.

When do we care about cophylogenetic patterns versus processes? It depends. Although there has been a strong tradition in phylogenetic comparative methods and macroevolution research to make claims only about pattern (Hembry & Weber 2020, Losos 2011, Revell et al. 2008), many of the goals in cophylogenetic research explicitly aim to draw conclusions about process. For example, it remains unclear to what extent true, contemporaneous cospeciation—a focal event that many cophylogenetic methods seek to identify—occurs. This ambiguity is not due to the state of cophylogenetic methods alone, as contemporaneous cospeciation can be challenging to demonstrate using additional lines of evidence or entirely noncophylogenetic approaches (e.g., population genomics). Certainly, there is value in knowing that two clades show a pattern of coarse phylogenetic congruence and that their evolutionary history has probably shaped their contemporary interaction patterns in some way. But we would argue that much of the motivation for cophylogenetic investigation is the explicit testing of hypotheses about the processes that generate these compelling patterns.

In this light, the recent development of model-based approaches in the field is especially important. Only a few model-based cophylogenetic approaches for simulating data (e.g., Dismukes & Heath 2021) and inferring historical processes (e.g., Blasco-Costa et al. 2021, Braga et al. 2020, Satler et al. 2019) are currently available; a richer set of simulation tools, as well as studies using simulations to understand the performance of methods, are needed for the field to blossom. Cophylogenetic methods development is still lagging in terms of model design and performance assessment when compared with macroevolutionary methods from similar fields.

Given the extremely high-dimensional nature of cophylogenetic systems, and the inherent difficulty of defining closed-form likelihood equations for the appropriate generative models, we anticipate that likelihood-free deep-learning methods will soon increase in popularity. Flexible, efficient, and mechanistic generative models for simulating large numbers of training data sets are essential for training neural networks. That said, many challenges remain in terms of how to most efficiently structure cophylogenetic data for method input, how to define and/or select the best cophylogenetic summary statistics, and how to identify the limits of reasonable inference. Testing hypotheses of global cophylogenetic congruence might be feasible, but can we expect deep learning, or any new-fangled method, to correctly reconstruct all historical cospeciation and host-switching events with high confidence?

Our understanding of how intertwined symbiotic lineages diversify remains limited by the hypotheses we have considered, the data we have gathered, and the methods that we can employ. We are still far from knowing the limits of what cophylogenetic inferences can and cannot do. To stimulate future research toward this knowledge, we list a number of challenges to consider that have not been fully solved by current cophylogenetic approaches.

Many such challenges concern how to define a general conceptual basis for framing and exploring cophylogenetic questions, such as:

- How do we distinguish true cospeciation from pseudocospeciation, and how common is each?
- How do we properly measure and model realized versus fundamental partner repertoires that facilitate and prevent host switching and host-range expansion in symbioses?
- What roles do preadaptation (Donoghue & Sanderson 2015) and macroevolutionary memory (Goldberg & Foo 2020) play in the gain and loss of species interactions?
- How important is functional trait evolution to the gain/loss of interactions—for example, using trait-matching (Nuismer & Harmon 2015) for plant-pollinator systems (Muchhala & Thomson 2009)?

Other challenges primarily concern how to develop new methodological frameworks that can accurately test the hypotheses or measure the quantities that inform how cophylogenetic systems evolve:

- How do we model interactions among more than two clades, e.g., among figs, pollinating fig wasps, and antagonistic galling wasps (Wang et al. 2019)?
- How can we better combine cophylogenetic analyses with other lines of evidence such as morphological, chemical, ecological, genomic, biogeographic, or paleontological data (de Baets et al. 2021)?
- How do we perform inference under complex models, with large numbers of states, traits, and interspecific biotic interactions (Quintero & Landis 2020)?
- How well do these methods perform in terms of accuracy, robustness, etc. for different kinds of data sets and under different assumptions?
- What role does extinction play in inferring ancestral reconstructions accurately and in inducing coextinction events (Rezende et al. 2007)?
- How can we more rigorously quantify the phylogenetic conservatism of symbioses in cases where cophylogenetic methods themselves are not appropriate?

Even this short list should make it apparent that cophylogenetics research still brims with fascinating problems needing further study. We anticipate that progress in the field will spring forth, much as it has in the past, from a productive and stable mutualistic system of generations upon generations of scientists—with varied specialties in ecology, evolution, genetics, statistics, computer science, and more—who have delivered cophylogenetics to its current state and will continue to advance it well into the future.

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Errata

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