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Review

Measuring Coevolutionary Dynamics in Species-Rich Communities

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Identifying different types of coevolutionary dynamics is important for understanding biodiversity and infectious disease. Past work has often focused on pairs of interacting species, but observations of extant communities suggest that coevolution in nature occurs in networks of antagonism and mutualism. We discuss challenges for measuring coevolutionary dynamics in species-rich communities, and we suggest ways that established approaches used for two-species interactions can be applied. We propose ways that such data can be complemented by genomic information and linked back to extant communities via network structure, and we suggest avenues for new theoretical work to strengthen these connections. Quantifying coevolution in species-rich communities has several potential benefits, such as identifying coevolutionary units within networks and uncovering coevolutionary interactions among pathogens of humans, livestock, and crops.

Highlights

Coevolutionary interactions between species can display various types of temporal dynamics that impact on key properties such as biodiversity and infectious disease in different ways.

Recent theory and observations of natural communities reveal how coevolution plays out in complex networks of species.

Experimental research has tended to focus on pairwise coevolution, but there has been a recent surge in experiments with microorganisms in species-rich communities.

We discuss challenges/approaches for quantifying coevolutionary processes in experimental species-rich communities, as well as new theoretical avenues, with the aim of helping biologists to untangle coevolving interactions in complex ecological networks.

Why Is Measuring Coevolutionary Dynamics in Diverse Communities Important?

Coevolution (see Glossary) results from reciprocal selection imposed on each other by interacting species. Coevolution can result from antagonistic interactions, as in predator–prey or host–parasite interactions, or from mutualistic interactions, as in plant–pollinator interactions. Because it is a major driver of evolutionary change, coevolution is central to explaining the evolutionary maintenance of sex [1], evolutionary diversification [2,3], and key aspects of infectious disease such as virulence and host range [4,5]. The outcome of coevolution for these properties depends on the types of **coevolutionary dynamics** that are at play.

A particularly important distinction is between directional and non-directional or fluctuating dynamics [4,6,7]. Directional coevolution, frequently referred to as an 'arms-race' dynamic, results in escalatory adaptation and counter-adaptation over time, such as increasing mean host resistance and parasite infectivity [8] or increasing antiherbivore defense in plants and herbivore counter-adaptation [9,10]. Such dynamics are expected to involve successive selective sweeps, and may purge genotypic diversity within each species over time. There can nevertheless be polymorphisms at individual timepoints, or stable polymorphisms depending on the nature of fitness costs (e.g., 'trench warfare' dynamics [11]). By contrast, fluctuating selection favors different phenotypes at different times, resulting in cycles of increasing and decreasing frequency of alleles involved in the interaction [12–15]. For example, in host–parasite systems, if different host/parasite genotypes are specialized to resist/infect particular genotypes of the opposing species, negative frequency-dependent selection may cause genotype frequencies to oscillate over time without directional change in the average resistance/infectivity [16,17]. Unlike directional dynamics, fluctuating dynamics resulting from negative frequency-dependent selection are expected to maintain genotypic and phenotypic diversity over time within populations of interacting species and their communities [18].

It has long been recognized that coevolution in nature does not occur in isolated pairwise interactions but instead takes place in complex **ecological networks** of interactions among many

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species [7,19–22]. This web of interactions potentially includes indirect effects [23,24], where one species influences another by changing the abundance of a third species that they both interact with. There can also be higher-order interactions, where changes in the population density of one species influence another species by altering the per capita effect of a third species [25,26]. Theory has revealed ways that coevolution may shape community structure and coexistence [5], including the temporal dynamics of how interspecific fitness interactions evolve [27–29]. Empirical data on the structure of extant communities [30–35] have allowed observed interspecific interactions to be interpreted in the context of coevolutionary models. In some cases, selection imposed by multiple interspecific interactions embedded in complex communities has been measured, such as selection for resistance of plants to various herbivore species [36–39], changing networks of interactions among plants and insect pollinators [40], and selection on host range of multi-host plant pathogens [41]. These studies have revealed fundamental insights into how selection imposed by interspecific interactions acts in diverse communities, which species are likely to be coevolving, and which traits are involved. However, it has remained challenging to characterize and quantify temporal coevolutionary dynamics (e.g., separating directional vs. fluctuating selection) in species-rich, natural communities directly (by experimental comparison between ancestral and evolved populations, rather than by observation of extant communities). There are practical challenges associated with doing so. For example, the timescale over which coevolution occurs is often – although not always – longer than a realistic experiment or field study.

The main practical obstacles to observing and quantifying coevolutionary dynamics have been circumvented in a large number of experimental studies by using simplified microbial communities. Such work has almost exclusively focused on two-species communities (e.g., [8,42]). These communities, such as a bacterium coevolving with a viral parasite (bacteriophage), have two key advantages for studying coevolutionary dynamics. First, they coevolve sufficiently rapidly that the dynamics can be observed in real time. Second, the effects of changing genotype frequencies within each species on the fitness effects of interspecific interactions can be directly measured. Samples of organisms such as bacteria and viruses can be frozen at various timepoints and later thawed and incubated in different combinations in controlled conditions. This permits a **time-shift** approach to measuring coevolutionary dynamics, where selection on interspecific interactions is inferred by measuring how changes in the genotypic composition of each species (across sampled timepoints) influence fitness effects resulting from interspecific interactions [43]. Such data have been used extensively for pairwise coevolutionary interactions, including real-time coevolution experiments [8,42,44–46] and analysis of resurrected samples of dormant hosts and microparasites from nature [16]. It is unclear whether the same approach can be extended to more species-rich communities.

We ask here whether tools used to infer pairwise coevolutionary dynamics can be adapted or extended to communities of more than two species. We focus initially on experimental designs (e.g., [8,16,47]) and quantitative interpretation (e.g., [43,48–51]) employing **interspecific interaction matrices** that describe variation of interspecific interactions as a function of time from the perspective of component species, such as time-shift analyses. We use examples from antagonistic host–parasite coevolution because coevolutionary dynamics have often been measured in this context, and this is where some important applications arise. We then suggest ways that such data can be strengthened by genomic analysis, connected back to community-level properties, and driven forward by new theoretical work (e.g., [35,52]).

Progress here would be beneficial because it would allow us to describe and quantify coevolutionary dynamics (e.g., distinguish between directional and fluctuating selection) in more realistic communities than in typical real-time coevolution experiments. This is a necessary step toward

Glossary

Coevolution: reciprocal adaptive evolution of interacting species.
Coevolutionary dynamics: reciprocal changes in allele frequencies and associated phenotypes in each interacting species over time that result from coevolution.
Ecological network: species interactions described as a network where species are generally nodes and interactions are links. Examples of past approaches to describing ecological networks include food webs, networks of host–parasite communities, and mutualistic networks such as those between plants and their pollinators.
Interspecific interaction matrix: in the context of phenotypic data about host–parasite coevolutionary dynamics, a matrix containing information about the strength of interspecific interaction – such as the fitness decrease for a host species upon infection by a parasite species – in various combinations of samples of each species, taken from different timepoints or locations (Box 1).
Time-shift: an experimental approach in which population samples from interacting species collected at multiple timepoints are 'put together' in different combinations. The type of coevolutionary dynamics is inferred from how changes in the genetic composition of each species (over sampled timepoints) affect interspecific interactions (e.g., how the fitness of a host species is affected by parasites from the past, present, and future).

connecting the large, but separate, bodies of knowledge on (i) coevolutionary dynamics in simplified experimental communities, and (ii) the structure of diverse communities that are assumed to result from coevolution, but where the dynamics have not been observed directly. Methodologically, there are implications for designing real-time coevolution experiments using model systems that increasingly incorporate additional species (e.g., [46,53–55]). There are also applied areas where quantifying coevolutionary dynamics in communities is relevant. Coevolution among pathogens infecting the same hosts in agricultural or medical contexts potentially influences important properties such as virulence [56–58]. Coevolution could also influence the community-level properties of microorganisms in applications such as bioremediation [59,60] and cheese making [61], with potential downstream effects for product quality or yield.

Approaches to Measuring Coevolutionary Dynamics in Experiments

Most experimental observations of two-species coevolutionary dynamics involve measuring reciprocal fitness effects in combinations of population samples taken from different times or places. The basic principle here is that we can quantify coevolution by measuring how changes in genetic composition within the component species (across sampled timepoints; often compiled into an interspecific interaction matrix) influence the fitness effects of interspecific interactions (Box 1 and Figure 1).

Time-shift analysis and related approaches using interaction matrices have identified whether pairs of species in natural or experimental communities are coevolving (e.g., myxoma virus and rabbits in Australia [62]) and what type of dynamics are in play (e.g., bacteria and bacteriophages *in vitro* [17,45]). For example, the dynamics in Figure 1 are consistent with directional selection for increasing host resistance and parasite infectivity ('arms-race' dynamics). Each species is better-adapted to samples of the other species from the past than the future. By contrast, fluctuating selection could result in hosts or parasites being better adapted to contemporary ('present') samples of the other species than to those from other timepoints ('past' and 'future') [16,17]. Fluctuating selection can also result in fluctuations in average interspecific fitness effects [12,13], which are better inferred from changes of mean interspecific fitness effects across time from the perspective of each species separately [63], rather than from time-shifts. Such experiments and analyses have been extensively reviewed for pairwise coevolution [43,48–50,64]. Applying this approach to interactions among more than two species presents several challenges including (i) increasing experimental scale, (ii) identifying which species impose selection on each other and which do not, and (iii) disentangling pairwise interactions from those involving more than two species (e.g., indirect effects and higher order interactions). A first step to overcoming

Box 1. Estimating Interspecific Interaction Matrices for Pairwise Coevolution

First, we collect population samples of each species at multiple timepoints, for example, by cryo-freezing them during an experiment and later thawing them, or resurrecting dormant lifecycle stages from the past. For a given pair of population samples (one from each species), we can assay fitness for each sample (one of the species) on its own and in the presence of the other sample (the other species). We can then compile these data into an interaction matrix that gives some measure of the interspecific fitness effects (e.g., the effect of species 1 on species 2 fitness) across different combinations of species-1 time and species-2 time (see Figure 1B in main text). We can also make a corresponding matrix giving the interspecific fitness effects in the other direction (the effect of species 2 on species 1 fitness) at the same combinations of timepoints. In practice, reciprocal interspecific fitness effects are often approximated for both species simultaneously from a single fitness-related measure such as pathogen infectivity or host resistance (e.g., via plaque assays in bacteria–bacteriophage systems), and for multiple clones or genotypes within each population sample. If these interspecific fitness effects result in adaptive changes in genotype frequencies within both species (i.e., coevolution), we expect the average interspecific fitness effects themselves to change over time as a result. We can test this by analyzing how they vary as a function of the timepoint combinations across the interaction matrix (see Figure 1C,D in main text). Interaction matrices can also be compiled using samples taken from multiple locations, rather than timepoints, to analyze other properties such as local adaptation (higher average interspecific fitness effects in sympatric compared to allopatric combinations of samples).

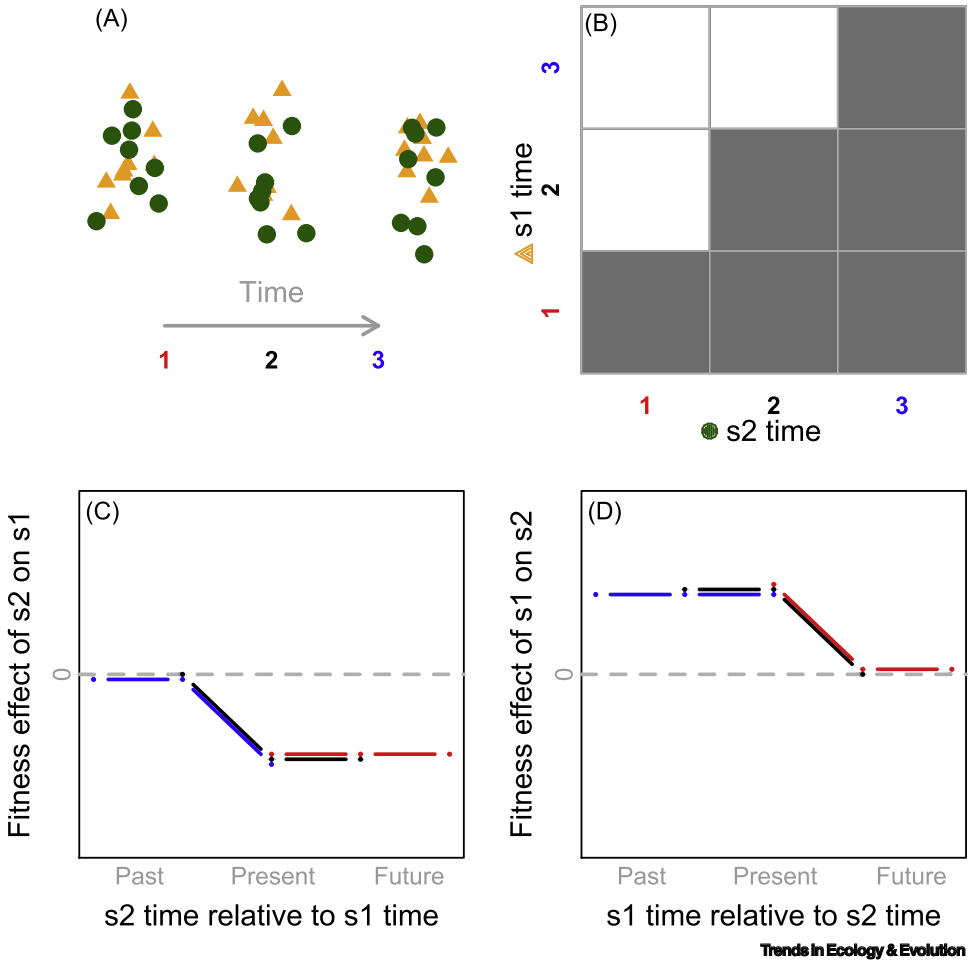


Figure 1. Inferring Coevolution Between Two Species. (A) In an experiment or field study, samples of species 1 (s1, orange points) and species 2 (s2, green points) are taken from various timepoints. The different points here indicate different individuals within each population. (B) After measuring the interspecific fitness effects at each combination of timepoints, we can compile this data into an interaction matrix. This matrix shows the effect of s2 on s1; darker cells show stronger negative effects (here, s2 is a parasite). (C) The matrix plotted as a function of the difference between s2 time and s1 time ('time-shift'); each line represents a sample of s1 from a different timepoint (red = 1, black = 2, blue = 3). This shows changes in s2 over time impose selection on s1, which is relatively susceptible to fitness reductions caused by s2 from the future compared to the past. (D) Plotting the effect of s1 on s2 fitness in the same way (with each line representing a different point in s2 time; red = 1, black = 2, blue = 3) shows that changes in s1 over time also impose selection on s2 (for simplicity, we assume here the effect of s1 on s2 fitness is the additive inverse of the effect of s2 on s1). A score of zero indicates no fitness effect of one species on the other.

these challenges is to compile interspecific interaction matrices for species-rich communities, and there are multiple possible ways of doing so (Box 2 and Figure 2).

Inferring Coevolution from Data about Interactions in Species-Rich Communities

If we compile information about interspecific interactions in species-rich communities (Box 2 and Figure 2), how do we then quantify the dynamics? In particular, how do we separate directional from fluctuating selection? Measuring multiple pairwise interactions (Figure 2B and Box 2) and bundling some species together (Figure 2C and Box 2) both produce 2D interaction matrices. We can therefore apply the same quantitative approaches as have been used for two-species communities. These generally rely on the principle that, under directional/fluctuating coevolution,

Box 2. Practicalities of Inferring Coevolutionary Dynamics in Species-Rich Communities

Coevolution in species-rich communities can be partially described by using the same procedure as for pairwise interactions (Box 1 and see Figure 1 in main text), but for several species pairs (see Figure 2B in main text). This reveals who is coevolving with whom and associated pairwise dynamics. This approach has the disadvantage of requiring a larger number of assays than for a single species pair. If this makes experiments impractical, possible solutions include (i) reducing the size of each interaction matrix (sample fewer timepoints), (ii) estimating only part of each interaction matrix (typically, only combinations that are necessary for time-shift analysis (e.g., [99]), or (iii) focusing on interactions among a subset of species (e.g., [55]). A more important limitation is this approach does not identify interactions involving more than two species at a time (e.g., indirect effects or higher-order interactions), and therefore does not solve the fundamental challenge of moving beyond pairwise interactions.

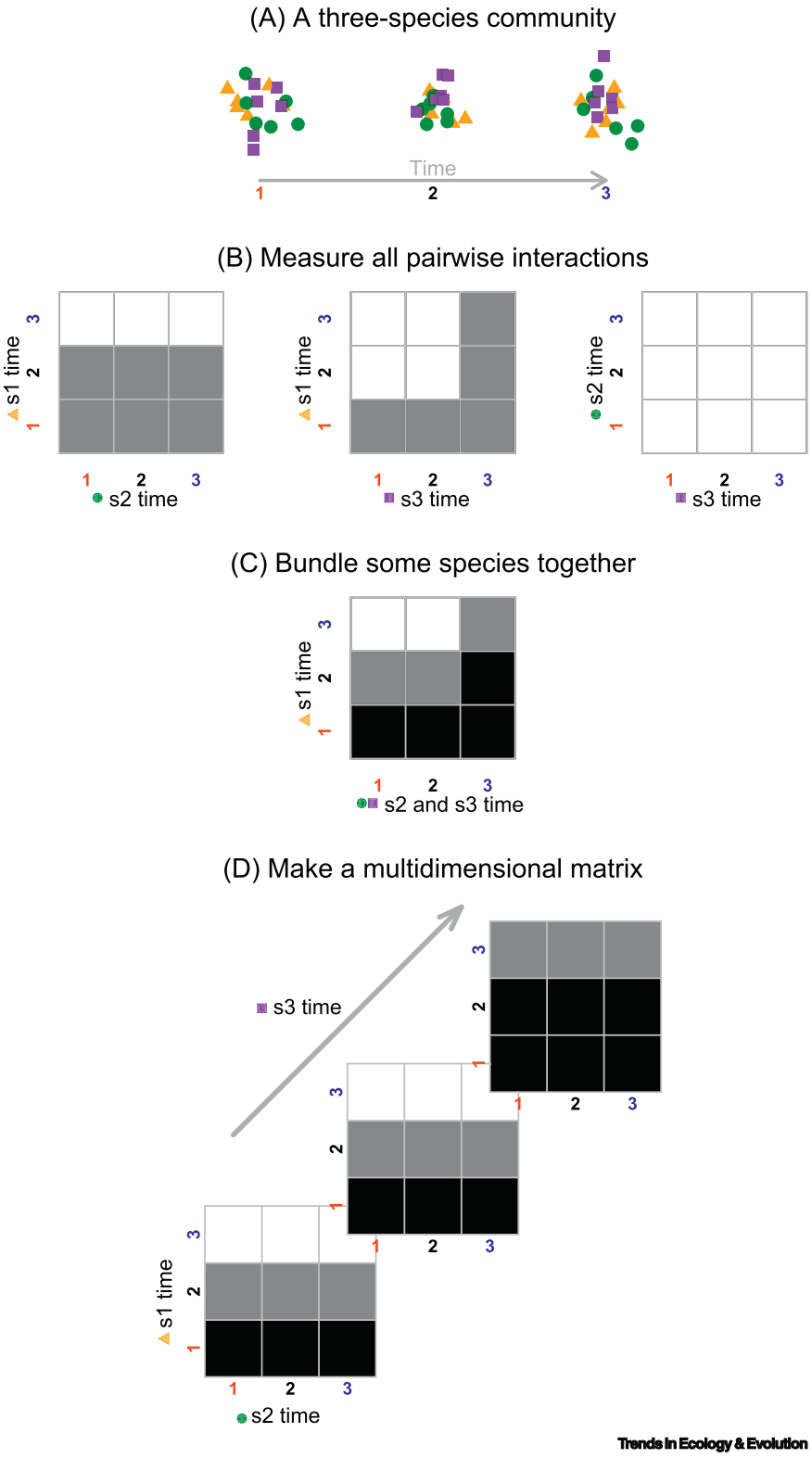
A second approach is to bundle some component species together, treating them as a single unit in the experimental design and analysis. Species could be grouped by taxon (e.g., viruses) or natural history (e.g., pathogens), before collecting data as for a two-species community. Instead of individual species, one or both dimensions of the interaction matrix represent groups ('bundles') of species (see Figure 2C in main text). The advantage of this approach is it does not require a large increase in experimental scale compared to the two-species scenario. The key limitation is that bundling obscures selection imposed by individual species within groups. That is, although it accounts for coevolutionary interactions involving more than two species, it does not separate them from component pairwise interactions. This approach is therefore most informative for community-level coevolution if we additionally estimate some interaction matrices for individual species pairs, thus allowing us to compare dynamics between interacting species pairs in the presence and absence of additional species. This approach can also reveal the effect of increasing species diversity within groups (e.g., parasites) for coevolution with another species (e.g., hosts) [71].

A third approach is to make an interaction matrix with as many dimensions as there are species (see Figure 2D in main text). For example, with three species, we can compile an interaction matrix for each species that gives the combined fitness effect of interactions with the other two species at each combination of s1 time, s2 time, and s3 time (see Figure 2D in main text). Because such interaction matrices are fully factorial, they allow us to test whether changes in genetic composition within each species have fitness effects for the other species (using a similar type of linear model as with only two species; see Figure S5 in the online supplemental information). In the example in Figure 2D in main text, changes in s2 over time do not affect selection on s1, but changes in s3 over time do. Thus, despite not measuring every pairwise interaction, this approach can provide information about coevolutionary interactions among individual pairs of species. The number of assays becomes unrealistic with more than a few species (see Figure S1 in the online supplemental information), but, unlike the other two approaches, such matrices potentially reveal coevolutionary interactions among all three species. For example, if evolution of s2 were to modify the coevolutionary interaction between s1 and s3, the effect of changing s3 genetic composition over time for s1 fitness would depend on changes in s2 (i.e., the fitness effect of interspecific interactions for s1 would depend on the s2 time × s3 time interaction). A limitation is that such matrices do not reveal pairwise ecological interactions, such as competition or cooperation, because this requires assaying the species in each pair alone and in the pairwise mix [100].

changes in genetic composition over time will cause interspecific fitness effects to change monotonically or fluctuate. These different types of temporal dynamics can be inferred using linear models of interspecific fitness effects as a function of time from the perspective of each species (e.g., species-1 time and species-2 time), or the difference between them (time-shift, as in the examples in Figure 1C,D and Figures S2–S4 in the online supplemental information), fitted as either continuous (directional) or categorical (directional or fluctuating) predictors (Box 2 and Figure S5 in the online supplemental information) [8,16,17,49,63,65]. Alternatively, we could infer directional selection by calculating the ratio of directional change compared to the total change over time [66]. This approach has the advantage of not assuming a particular type of directional association. Unlike a linear model, it produces a single value for each time-series, making hypothesis testing within individual communities more challenging. When the interaction matrix has more than two dimensions (Figure 2D), we can use a similar linear modeling approach as in the two-species case. We would take the interspecific fitness effect for one species as the response variable, and time from the perspective of component species (e.g., species-1 time, species-2 time, species-3 time) as categorical or continuous predictors.

Genomics Approaches

Pairing assessments of phenotypic change with next-generation sequencing permits a fuller characterization of evolutionary rate and diversification. This combination of approaches has been used elegantly in studies of antagonistic coevolution between two species [67–69].



(See figure legend at the bottom of the next page.)

Incorporating sequencing can allow us to begin investigating the molecular mechanisms that underpin coevolution in species-rich communities. Metasequencing can help to monitor changes in community structure and diversity over time or space, and this is particularly valuable if species are not culturable or are difficult to separate in culture. For example, Sierocinski *et al.* [70] compared the structure and productivity of individual methanogenic communities with their relative contributions to mixed communities (initiated with samples of multiple individual communities). In doing so, they inferred evidence of coselection on groups of taxa from coevolved communities. At the level of individual species within complex communities, resequencing ancestral and evolved lineages can reveal the impact of community diversity on the evolutionary rate, diversification, and modes of selection of species during coevolution. Paterson *et al.* [2] developed a bioinformatic approach for a pairwise host–parasite system whereby genetic distances were compared between ancestral and evolved lineages (i.e., evolutionary rate), and were compared among lineages within treatments (variation in evolutionary trajectories). Betts *et al.* [71] applied this method to a more species-rich type of community, assessing the impact of coevolution in a host–multiparasite community. They experimentally coevolved a bacterial host (*Pseudomonas aeruginosa*) with five lytic bacteriophages, and subsequently resequenced bacteria at the end and middle of the experiment. By comparing the number and identity of SNPs in bacterial populations across lineages and treatments relative to the ancestor, they found that populations coevolving with highly diverse parasite communities evolved more rapidly and experienced greater genetic divergence among populations and from the ancestral strain.

Resequencing evolved lineages can also pinpoint mutations in genes underlying generalist or specialist mechanisms at the interface of species interactions. One approach is to focus on genomic changes in two interacting populations in the community (perhaps involving a species that is dominant across replicates and treatments, or a species whose genome is well-annotated). At the end of the experiment by Betts *et al.* [71], the authors used SNP data to compute a network of host–parasite gene interactions for each population with one particularly dominant phage parasite. For all host–parasite gene combinations, they calculated the number of communities/replicates in which substitutions in those genes co-occurred. The authors then drew connections between those host and parasite genes across all communities forming a network. The two most common gene co-occurrences were between a phage infectivity gene and two host generalist-resistance genes. Although this approach does not tackle coevolutionary dynamics *per se*, it highlights genomic changes that are linked in interacting species in the community. Network plots of gene interactions such as these could potentially be applied to multiple combinations of species within complex communities. They could help to resolve genes at the interface of coevolutionary associations that have been observed in the same experiment via phenotypic data or predicted based on correlated changes in abundance across species (e.g., from metagenome data).

Figure 2. Approaches to Inferring Coevolution in a Three-Species Community. (A) We sample species 1–3 (s1, s2, s3) over time. In (B–D) we assume that s2 and s3 are parasites of s1, and that there are the same changes in susceptibility of s1 to s2 and s3 over time in each case. Upon successful infection, s1 suffers a fitness decrease (darker cells) whereas s2 or s3 gain a fitness increase (we assume for simplicity this equals the additive inverse of the fitness decrease for the host). Upon simultaneous infection by both parasites, we assume that the benefit for each parasite is unchanged compared to single infections and that the host suffers a decrease equal to the sum of the single-infection effects. (B) We can analyze every pairwise interaction and plot them as in Figure 1, giving in each interaction matrix the fitness effect of interactions with the species on the *x* axis for the species on the *y* axis. This reveals s1 evolves to become less susceptible to s2 (left), and s2 does not counteradapt; s1 also evolves reduced susceptibility to s3 (middle) and s3 counteradapts; s2 and s3 do not interact or coevolve (right). (C) Analyzing the same community with s2 and s3 bundled together reveals directional changes in susceptibility of host species s1 and infectivity of the parasite species bundle s2&s3). (D) To make a three-species matrix, at every combination of timepoints (s1 time, s2 time, s3 time) we assay each species alone and in the three-species mix (note that we do not necessarily assay pairwise mixes by this approach); only effects of s2&s3 for s1 are shown. Corresponding time-shift plots are given in Figures S2–S4 in the online supplemental information.

Going Back to Nature

The previous approaches are derived from experimental studies. The advantage is that they explicitly monitor the process of coevolution. The limitation is that, as noted throughout this review, they have traditionally focused on pairs of species. Observations of natural communities, by contrast, address the community context through characterizing the structure of the species interaction networks, but are mainly static descriptors. One big challenge in coevolutionary studies is to bridge between these two approaches. Whether this is possible largely hinges on the question of whether one can infer the type of coevolutionary dynamics from the structure of these networks.

In this regard, Thompson [7] assumed that the coevolutionary forces shaping antagonistic and mutualistic networks are different, and that these differences should leave a signal in the resulting network of interactions. Specifically, antagonistic systems would be driven by coevolutionary alternation (i.e., selection favoring parasites attacking less defended hosts), whereas mutualistic systems would result from the combination of coevolutionary matching (the length of a pollinator's proboscis matches that of a plant's corolla) and convergence (e.g., convergence in the traits of flowers pollinated by butterflies rather than by bees) [7]. Theoretical work has shown that, indeed, mutualistic networks are more nested whereas antagonistic networks tend to be more compartmentalized [72]. In such cases, a nested network is that in which the more specialist species interact only with proper subsets of those species interacting with the more generalists [30] (Figure 3A). A compartmentalized network, in turn, would describe a network organized into compartments, where a compartment would be a group of hosts and parasites that interact frequently among themselves but show few interactions with the species in other compartments [73] (Figure 3B). However, the nature of these models is ecological, and the above differences in network structure are driven by differences in network stability, not by coevolutionary selection. Models of coevolutionary dynamics have also shown a signal on the resulting network structure depending on the mechanism of coevolution between species [74]. Specifically, a nested structure is most likely to result when coevolutionary selection is weak and the interaction is described by a mechanism of phenotype threshold rather than matching (i.e., a pollinator will interact with the plant provided that its proboscis is long enough) [74].

The above evidence of coevolution shaping network structure is based on theoretical work. Moving now toward experimental evidence, recent work on bacteria–phage infection networks has shown that the network of interactions between coevolving bacteria and phages becomes more nested over time (as defined above) under arms-race dynamics ([75]; see however [76]).

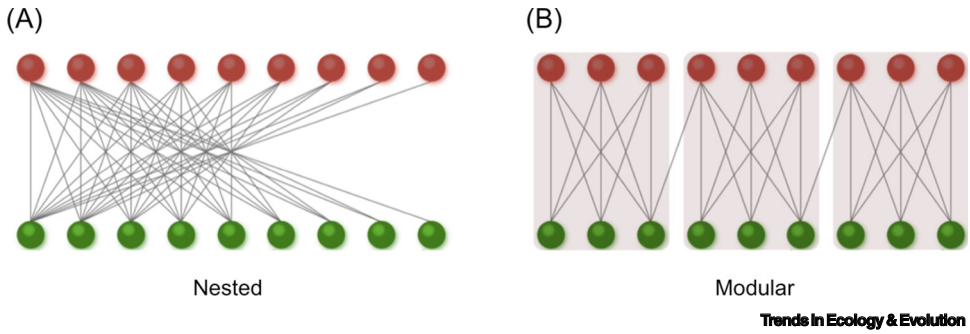


Figure 3. Complex Communities Can Be Represented as Interaction Networks. In this figure nodes represent species (or strains) of a given set such as plants (or hosts) in green and pollinators (or parasites) in red. A link between two nodes represents an interaction that can be either mutualistic or antagonistic depending on the system. Two contrasting network structures are nestedness (A) and modularity (B) (refer to the text for definitions of both).

This is still very preliminary, but it supports the notion that coevolutionary selection indeed affects the architecture of the resulting network of interactions, thus allowing us to bridge between the two main approaches to coevolution.

Advances from Theoretical Work and Potential New Directions

As with most empirical research, theoretical models of coevolution have primarily focused on isolated interactions between two species. There are few theoretical predictions for how coevolution proceeds in complex communities (but see references above), even though the notion of coevolution at a community-level or as a community-dependent process is well established [7,21,77–79]. It is, however, not especially surprising given that the main challenge for theoreticians mirrors that faced by empiricists: it is generally easier to study two-species interactions than species-rich systems. For empiricists, including more species requires a greater number of controls, treatments, and assays, making the challenge largely a question of available resources and time. But for theoreticians the challenge is typically more fundamental because models with more species require more assumptions, more parameters, and higher dimensionality, which can make systems computationally burdensome, make parameterization difficult, restrict analytic techniques or tractability, and limit the generality of predictions.

Much of the existing theoretical work on coevolution in communities has concentrated either on niche partitioning, where disruptive selection promotes non-overlapping species distributions in niche space [80–84], or on methods for distinguishing between pairwise and 'diffuse' coevolution [85]. The latter is more relevant here because traits under selection in the niche-partitioning literature typically govern indirect rather than direct interactions among coevolving species. Building on earlier definitions [19,37,39], Strauss *et al.* [78] established three criteria for coevolution to be considered pairwise: (i) traits governing interactions in different species that are under selection must be genetically uncorrelated, (ii) the strength or direction of selection for traits by one species must not be altered by the presence or absence of other species in the community, and (iii) the community composition does not affect genetic variances or covariances for a trait under selection. For example, if an allele that confers resistance to one parasite affects susceptibility to another, or if coinfection changes the strength or direction of selection compared to a single infection, or if the expression of resistance changes with community composition, then coevolution is 'diffuse'. These criteria and associated methods are useful for determining whether simulated or experimental coevolution is pairwise or not, but crucially they do not inform how coevolution proceeds in communities over time (e.g., directional vs. fluctuating selection). Moreover, these papers seem to hold a dichotomic view in which coevolution should lead to either highly specific one-on-one interactions or to diffuse assemblages that are intractable to analysis. If anything, the network approach has shown that there are general patterns even in complex communities.

Recently, theoretical studies have begun to use mathematical models to explore the actual dynamics of coevolution in simple three-species communities [27,28,86,87]. This work shows how the transition from bipartite to tripartite interactions can qualitatively and quantitatively change coevolutionary outcomes, and highlights that species interactions can depend on the wider ecological context. For example, symbionts can evolve along the parasitism–mutualism continuum depending on the presence/absence of other species [88], and the costs/benefits of plant–pollinator interactions depend on ecological factors such as interaction frequency [89]. There have also been theoretical studies of coevolution in larger networks of species interactions, as noted above, but these have generally been limited to mutualistic communities [35,52,74,90]. One important lesson from this theoretical work is that indirect effects resulting from species that only interact through common partners can be as important in shaping trait evolution as effects

driven by directly interacting partners [35]. This reinforces the view that bringing a community approach to coevolution is important. There are several key areas where future theoretical research can contribute (see Outstanding Questions). For example, only a small number of three-species systems have been modeled in coevolutionary frameworks, and there has been little or no work on multivictim/multiexploiter coevolution, hyperparasitism, and vector-borne diseases.

Concluding Remarks and Future Perspectives

Measuring coevolutionary dynamics among more than two species is challenging. However, classical approaches involving interspecific interaction matrices can be extended to this task, particularly if they are combined with genomic analyses and interpreted in light of new theoretical work. Although we have used several examples of three-species communities, the same approaches can be scaled up to larger numbers. A key challenge is to incorporate eco-evolutionary feedbacks into datasets from species-rich communities [20]. In this direction, population dynamics can potentially be accounted for in experimental designs that do not standardize population sizes across samples from different timepoints (such that variation across interaction matrices reflects changing genetic composition and population dynamics).

Future research on community-level coevolution could open up new applications (see Outstanding Questions). First, in bacteriophage therapy [91], bacteriophages are often applied as multispecies cocktails. The relevance of coevolution between target bacteria and these parasite communities remains unclear [92,93], although it is amenable to experimental observation [71] and could influence treatment effectiveness and durability. Second, in the human gastrointestinal microbiota, pathogens interact with multiple resident species [94]. It is not yet clear whether they coevolve with these resident species within individual hosts/patients, but laboratory-based research on simplistic microbiota reveals potential consequences for virulence [53]. Improved understanding of coevolutionary interactions within individual patients could have implications for predicting infection outcomes and optimizing microbiota-based therapies, such as fecal microbial transplants [95]. Third, coinfection by multiple parasite species is common in humans, livestock, and agriculture [57,58]. Although parasite species can hybridize in this context [96], they might also coevolve. How does coevolution within such communities, and over longer timescales, with/without host evolution, affect treatment/infection outcomes? Understanding these connections could inform interventions such as targeted treatment of coinfected individuals or manipulation of host genetic diversity in the case of crop plants [97,98].

Outstanding Questions

Do pairwise species interactions observed in isolation, such as in real-time coevolution experiments, show similar dynamics in species-rich communities? This question is crucial for translating direct observations of coevolution in experiments back to complex communities.

How do different types of coevolutionary dynamics (e.g., arms-race dynamics, different types of fluctuating selection dynamics) affect network structure? Understanding this connection would enable us to draw coevolutionary inferences from observed network structure (e.g., nestedness vs. modularity).

Are pairwise and community-wide coevolutionary interactions associated with different types of dynamics? The answer will help in devising appropriate experimental designs and quantitative approaches for coevolution experiments in species-rich communities.

How do eco-evolutionary feedbacks influence coevolution in species-rich communities? Such feedbacks are known to impact on pairwise coevolution and can mediate (directly and indirectly) the strength of selection resulting from species interactions. Accounting for population dynamics as well as for changes in genotype frequencies will enhance our understanding of what drives coevolution in communities.

Can we gain general insights into coevolutionary dynamics in communities from qualitative information about individual species or groups? For instance, do antagonistic groups of species tend to have similar dynamics regardless of whether the constituent species are parasites, predators, or competitors, or do these distinctions matter for predicting the dynamics?

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Supplemental Information

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