Review

Experimental coevolution of species interactions

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Coevolution, the process of reciprocal adaptation and counter-adaptation between ecologically interacting species, affects most organisms and is considered a key force structuring biological diversity. Our understanding of the pattern and process of coevolution, particularly of antagonistic species interactions, has been hugely advanced in recent years by an upsurge in experimental studies that directly observe coevolution in the laboratory. These experiments pose new questions by revealing novel facets of the coevolutionary process not captured by current theory, while also providing the first empirical tests of longstanding coevolutionary ideas, including the influential Red Queen hypothesis. In this article, we highlight emerging directions for this field, including experimental coevolution of mutualistic interactions and understanding how pairwise coevolutionary processes scale up within species-rich communities.

The rise of experimental coevolution

Naturalists have long recognized the importance of species interactions as a driving force of adaptation. Indeed, 19th-century evolutionary biologists often cited the conspicuous co-adaptations of interspecific pollination and mimicry mutualisms (see Glossary) as exemplars of evolution by natural selection. It is perhaps surprising then that coevolution, the process of reciprocal adaptation and counter-adaptation by ecologically interacting species, was not studied in earnest until the mid-20th century. The first wave of empirical coevolution research was predominantly observational and field based [1,2]. Such studies inferred the action of reciprocal selection indirectly, typically from spatial patterns of trait covariation between populations or by comparative and phylogenetic analyses of ecologically interacting clades. These early studies strongly suggested that coevolution was a central process driving natural selection and shaping the structure and function of communities, although were never able to provide unequivocal evidence of reciprocal evolutionary changes.

To overcome certain limitations of fieldwork (chiefly that the action of other sources of selection driving the observed patterns can never be ruled out), researchers have sought to bring the study of coevolution into the

Glossary

Antagonistic coevolution and/or interspecific antagonism: coevolution is the reciprocal adaptation and counter-adaptation of species that interact ecologically. When the fitnesses of the two species are negatively correlated, such that an adaptation that increases fitness in one species decreases in the fitness of the other species and vice versa, these species interactions are termed 'antagonistic'.
Antagonistic pleiotropy: a situation where one gene underlies more than one trait, and where one trait is beneficial whereas the other is deleterious in a given environment.
Arms race dynamics (ARD): a mode of antagonistic coevolution driven by directional selection whereby hosts and parasites, respectively accumulate resistance or infectivity alleles through a series of recurrent selective sweeps. This process leads, through time, to an increase in the range of parasite genotypes that hosts can resist and an increase in the range of host genotypes that parasites can infect.
Co-phylogeny: an approach by which the macroevolutionary histories of two clades are compared, for example, to determine whether evolutionary branching of one species is correlated with branching in another.
Evolutionary stasis: occurs when a population remains genetically constant over time. This can be manipulated during experimental coevolution by continually replacing the population of one of the two partners with the ancestral genotypes to prevent evolution in this species.
Evolvability: ability of a population to generate genetic diversity, thereby enabling it to respond to selection.
Fluctuating selection dynamics (FSD): a mode of antagonistic coevolution driven by negative frequency-dependent selection, whereby parasites evolve to infect common host genotypes, thereby favoring rare host alleles, which subsequently become common, leading to sustained oscillations in host and parasite allele frequencies. FSD does not lead to the evolution of broader parasite host ranges or increasing host resistance through time.
Host range: subset of hosts that a parasite can successfully infect. Note that the known host range for a given parasite is necessarily determined by the reference panel against which it has been tested and that parasite performance can vary within a given host range, such that the parasite performs better on some hosts than on others.
Hypermutable: strains of bacteria with mutation rates far in excess of the wild type; these typically arise through mutations altering mismatch repair enzymes.
Interspecific facilitation: a scenario in which one species enhances the fitness or growth of another either directly, for example, by increasing the availability of nutrients, or indirectly, for example, by reducing competition or predation. Facilitatory interactions can benefit either one or both participants and, in the latter, are considered to be interspecific mutualisms.
Mutualisms: mutually beneficial species interactions, which in reality are often mutually exploitative interactions but where net benefits accrue to both parties.
Phage therapy: use of bacteriophage viruses to control the growth and/or harmfulness of pathogenic bacteria.
Phenotypic matching: clustering of, or correlation between, traits governing a coevolutionary interaction, such that the common phenotype in the local populations of one partner is matched by the reciprocal trait in the other.
Red Queen hypothesis: the idea that, for antagonistic species interactions, the relative fitness of each antagonist does not increase over time, despite continual adaptation, due to the counteracting adaptations of their opponent. This hypothesis was later formalized to describe the potential role of coevolving parasites in generating an advantage for sexual recombination.
Syntrophic mutualism: form of microbial mutualism where the transfer of metabolites between species is essential for growth.
Time-shift experiment: studies in which samples of coevolving populations are collected through time (either artificially by cryogenic freezing, or naturally by the deposition of resting stages) and then resurrected to challenge against coevolving partners from past, contemporary, and future time points.

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laboratory. Here, environments can be precisely controlled to exclude extraneous sources of selection, and the use of fast-growing organisms such as microbes or classic model animals, enables the direct observation of coevolution in real time (Figure 1 and Box 1). Significantly, given that many such experimental systems are amenable to cryogenic preservation, this enables experimenters to perform 'time shifts', for instance, testing the performance of parasites against hosts from the evolutionary past or future (Figure 2). By analyzing these time-shifted interactions between coevolving species, the temporal dynamics of coevolution can be directly estimated [3]. Moreover, although time shifts are possible in certain field systems [4], a crucial advantage of laboratory coevolution experiments is that control lineages, propagated under identical environmental conditions but where a given species is absent or where one species is held in evolutionary stasis, can also be established (Figure 1). Comparison of coevolving lineages against control lineages enables unequivocal identification of adaptations that evolved in response to reciprocal selection, that is, those adaptations that are present only in coevolving lineages.

Coevolution experiments were first pioneered using simple microbial communities during the 1970s [5–7]. Although these kinds of microbial association remain the most intensively studied due to their ease of propagation, the experimental coevolution approach has recently been extended to a wider range of species interactions involving more complex host organisms, such as snails, beetles, bees, and worms (Table 1). Moreover, whereas early studies largely focused on antagonisms, in part due to the intensity of reciprocal selection and rapid evolution generated by such interactions, today experimental coevolution researchers are studying other forms of species interaction, such as mutualisms. Experimental coevolution is providing causal tests of longstanding coevolutionary hypotheses, and also revealing novel facets of the coevolutionary process that are not captured or predicted by current theory. In this article, we do not aim to provide an exhaustive account of experimental coevolution research; instead, we review the key areas in which experimental coevolution has advanced understanding of the coevolutionary process, identify the main gaps in knowledge for future research, and highlight the ways

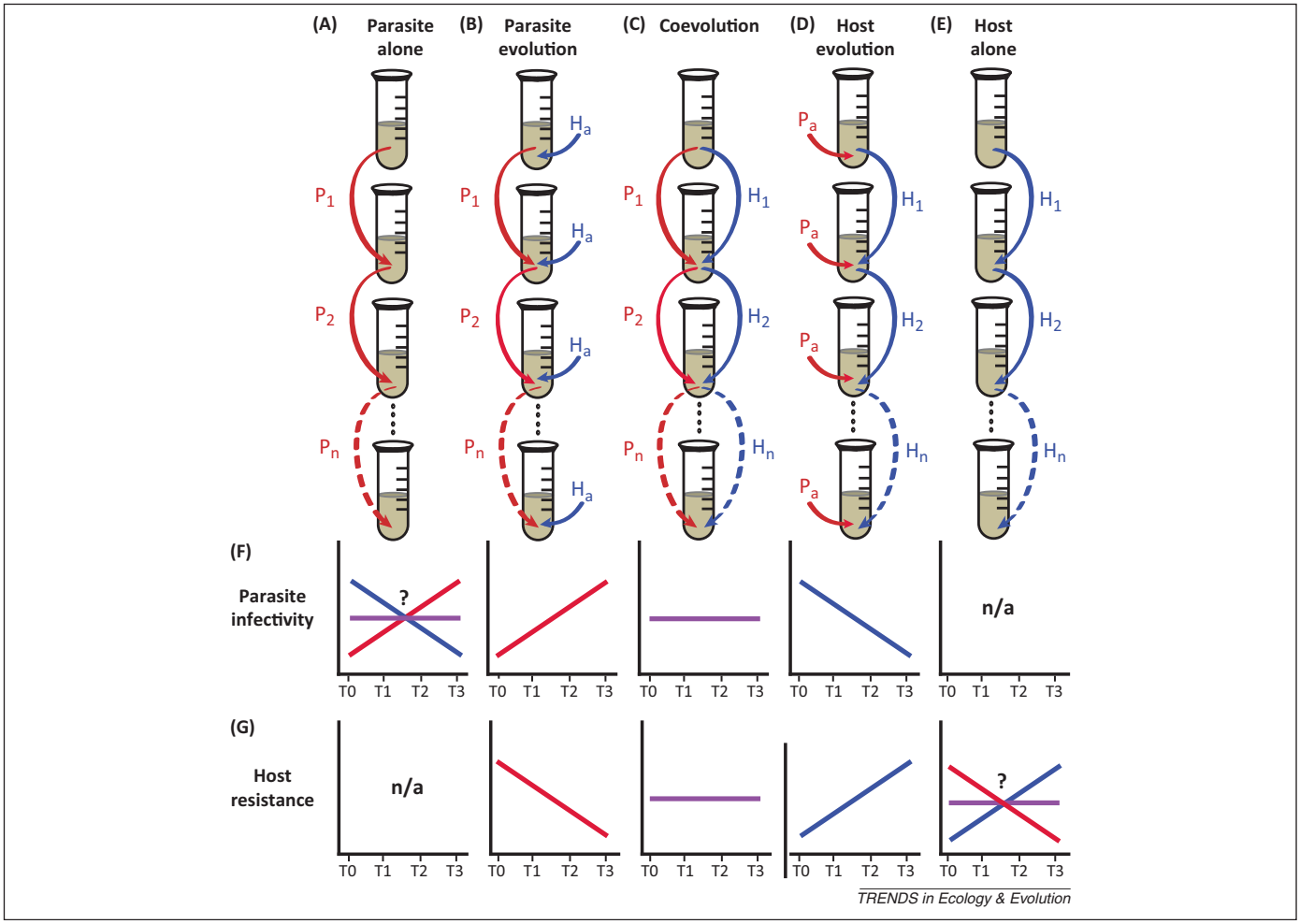

Figure 1. The experimental designs of experimental coevolution. A simplified illustration of experimental coevolution of host and parasite, where one can compare single-species evolution **(A,E)** (controlling for both adaptation to laboratory conditions and drift), one-sided experimental evolution **(B,D)** (i.e., one species evolving in response to another that is unable to respond, e.g., the ancestral type, Pa or Ha) and experimental coevolution **(C)**, where it is possible to measure directly evolutionary change of one species in response to the other and any reciprocal adaptations that occur. Line graphs represent one scenario of evolutionary change in parasite populations **(F)** or host populations **(G)** over the course of the experiment. In the case of a parasite or host evolving alone, adaptation to the laboratory environment and/or drift could result in increased success against the host and/or parasite, decreased success against the host and/or parasite, or no change in fitness.

Box 1. When is it experimental coevolution?

In a classic article, Janzen defined the term 'coevolution' [71], which at the time had become broadly and imprecisely applied by researchers of species interactions. Janzen stressed the requirement for the demonstration of adaptations in both species arising from reciprocal selection before a pattern could be attributed to coevolution. This definition of coevolution based on evolutionary outcomes is valuable for distinguishing coevolved adaptations, but is not useful for defining an experimental approach to the study of coevolution. We propose that the term 'experimental coevolution' should be applied to experiments where either: (i) interacting species are co-cultured and experimenters attempt to quantify evolutionary responses in both (or all if >2) interacting species; or (ii) interacting species are co-cultured and evolutionary responses of populations from coevolving treatments are compared with evolutionary responses of populations from control treatments where coevolution is prevented.

One of the most powerful aspects of experimental coevolution is that control treatments can be used to tease apart evolutionary change, based on adaptation to the abiotic environment and/or drift, from coevolutionary change. The exact approach depends on the system being used and the question being addressed, but one option is to compare the evolution of each species alone with the coevolution of the two. This approach can be used to tease apart selection imposed by abiotic versus biotic factors, for example, by specifically identifying the responses to parasite-mediated selection. However, to tease apart specifically evolution in response to a biotic agent of selection from coevolutionary change requires the introduction of a 'one-sided evolution' treatment, where one of the partners is held in evolutionary stasis while the other is allowed to evolve. This one-sided evolution treatment can be directly compared with the coevolution treatment to determine which evolutionary changes are the result of an evolutionary response to the biotic agent versus a result of coevolutionary interactions.

As experimental (co)evolution proceeds, fitness of the (co)evolving populations can be measured over time to determine, for example, whether parasites become more or less prudent on their hosts and whether hosts evolve towards complete resistance. In coevolving populations, fitness can be measured both on the ancestral antagonist populations, enabling observation of absolute changes in population fitness, and on the coevolved antagonist. As illustrated in Figure 1 (main text), this latter relative fitness might not change over time, because the other species is responding to any adaptations and countering. Finally, for many experimental evolution systems, populations from each time point can be frozen and later resurrected to perform time shifts in which the fitness of one species can be tested on populations of the other from the past (i.e., populations that have not yet responded to any new adaptations), the same time point, or from the future (i.e., populations that have potentially already responded to any new adaptations). Note, however, that for frequency-dependent selection, populations may be unfit on past populations of the antagonist if, for example, they have moved on to infect and/or resist common types in the contemporary antagonist populations.

in which coevolutionary research can be of applied importance.

Experimental coevolution of antagonistic species interactions

The tempo and mode of antagonistic coevolution

According to the Red Queen hypothesis, reciprocal selection arising from interspecific antagonisms, such as host–parasite interactions, should accelerate evolutionary rates through the need for continual adaptation and counter-adaptation [8,9]. (The history of the use of the Red Queen metaphor is described in [10,11].) Recent tests of this prediction have compared evolutionary rates under coevolution against controls where coevolution is prevented, for example, in the presence versus absence of an antagonist (Figure 1), and provide strong support for this hypothesis from a range of species interactions. When co-cultured, *Caenorhabditis elegans* and a bacterial parasite, *Bacillus thuringiensis*, both exhibited greater molecular evolutionary change, assessed by microsatellites and gene content respectively, than did control populations of the nematode or bacterium propagated alone [12]. However, for parasite species in particular, the complete removal of the host is an extreme environmental alteration, necessitating comparison of populations propagated *in vivo* with *in vitro* controls. A more subtle manipulation is to allow one antagonist to evolve while holding the other in evolutionary stasis, by regularly replacing its entire population with individuals of the ancestral genotype. By this approach, it has been demonstrated, using pooled whole-genome resequencing, that genomes of bacteriophage virus Φ2 coevolving with the bacterium *Pseudomonas fluorescens* evolve at double the rate of Φ2 populations evolving against a fixed, ancestral *P. fluorescens* genotype [13]. Similarly, whole-genome analysis of *Escherichia coli* and the bacteriophage Qβ revealed increased mutational change in coevolving, relative to evolving, populations of both host and parasite [14]. These studies strongly support the Red Queen view of interspecific antagonisms as a strong driver of evolutionary change and, for the first time, have enabled direct tests of causation rather than correlation.

The process of rapid reciprocal adaptation inherent to antagonistic coevolution can be driven by at least two contrasting modes of reciprocal selection. Specifically, frequency-dependent selection, where changing allele frequencies in host and parasite populations are driven by parasite-mediated selection against common host resistance alleles; or directional selection, where recurrent selective sweeps of novel host resistance and parasite infectivity alleles occur through time, leading to increases in the host range of the parasite and the subsequent host resistance traits. These possibilities have been termed 'fluctuating selection dynamics' (FSD) and 'arms race dynamics' (ARD), respectively [3,15]. Distinguishing these dynamics requires either time shifts to detect contrasting patterns of phenotypic evolution in host resistance and parasite infectivity traits (Figure 2), or direct estimation of temporal change in the frequencies of resistance and infectivity alleles, or of linked genetic markers.

Experimental coevolution has revealed evidence for the operation of both of these modes of reciprocal selection. A response to frequency-dependent selection by parasites has been observed by tracking host genotypic markers in coevolving laboratory populations of the freshwater snail, *Potamopyrgus antipodarum*, infected by a sterilizing trematode parasite, *Microphallus* sp. [16]. However, several other studies reveal signatures of both FSD and ARD within the same coevolving population, suggesting that these contrasting modes of selection are not mutually exclusive. For example, genotypic data from *C. elegans*–*B. thuringiensis* coevolution experiments suggest that different host loci are under different modes of selection;

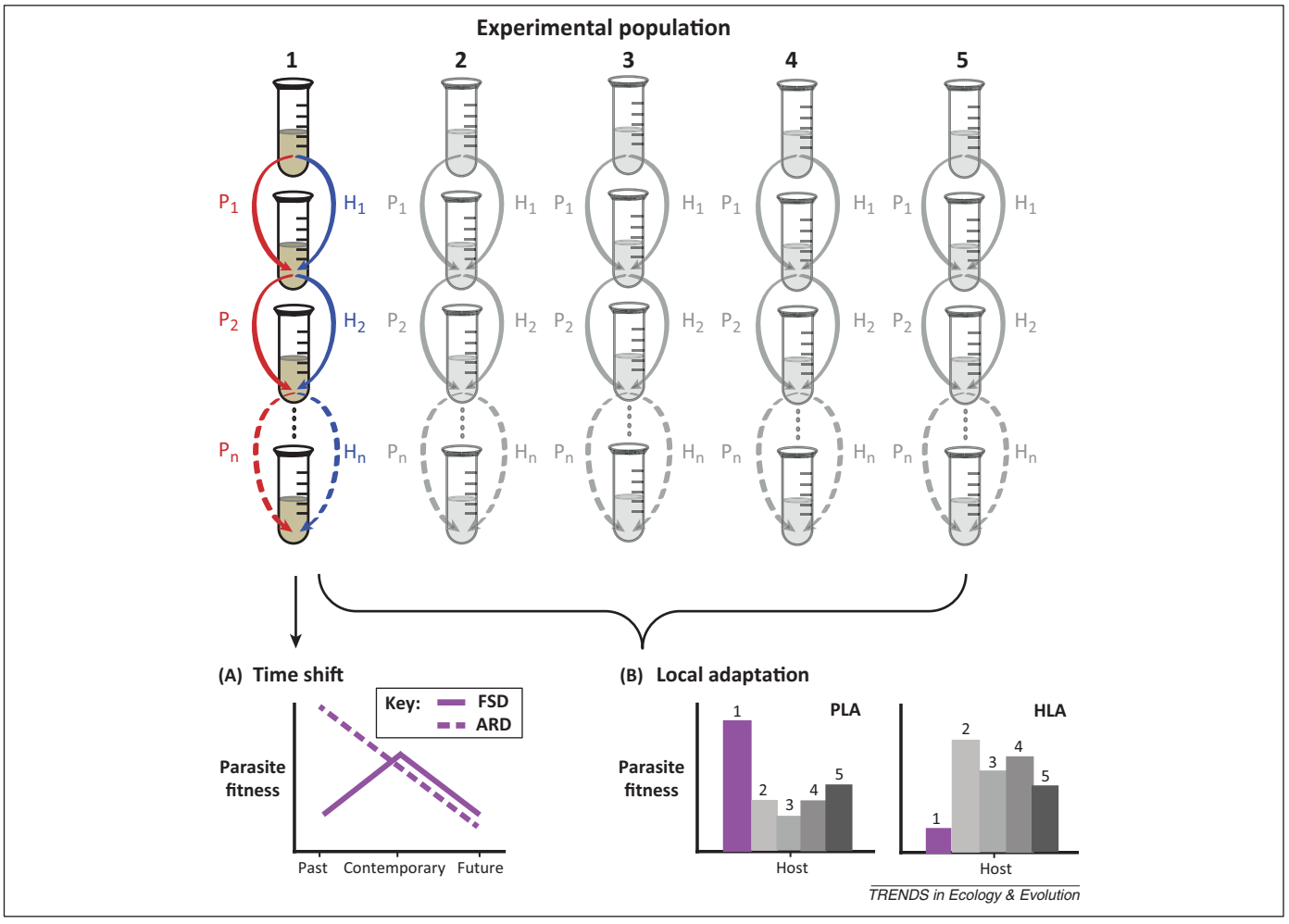

Figure 2. Approaches to quantifying reciprocal adaptation. An illustrative example of techniques used to compare the coevolution of two species (in this case, host and parasite) by examining changes in replicate experimental populations (or metapopulations, if connected by gene flow). A time shift experiment **(A)** can be performed across experimental time within each population by comparing the fitness of one player against the other from past, contemporary, or future time points. This method can give unique insight into the coevolutionary dynamic underlying the change. For example, a scenario in which fitness is lowest against populations from the future and highest against those from the past might indicate arms race dynamics with directional selection, whereas a pattern of peak fitness against contemporary populations or those from only the recent past is more in line with negative frequency-dependent selection. However, the exact pattern will depend on the lag in evolutionary response of one player against the other [72]. A local adaptation experiment **(B)** compares the performance of parasites against their sympatric hosts with their performance against allopatric hosts; higher parasite performance against sympatric versus allopatric hosts indicates that parasites are locally adapted.

perhaps reflecting that the infection–resistance process comprises multiple steps of interaction, each with independent genetic bases [12,17,18]. Furthermore, patterns of phenotypic and molecular evolution suggest that the interaction between *P. fluorescens* and Φ2, although initially dominated by ARD, becomes increasingly FSD-like through time [19]. This appears to arise because, after a certain point, the costs to individual genotypes of accruing additional mutations that further increase the breadth of infectivity or resistance were unviable. The increasing costs act to prevent fixation of supergeneralist genotypes and progressively weaken the response to directional selection over time. These findings suggest that, at least in part, the prevailing mode of reciprocal selection is determined by the coevolutionary history of an association and more long-term studies are required to resolve this. There is now a clear need for the development of coevolutionary theory targeted at resolving the impact of mixed modes of reciprocal selection on coevolutionary processes and at understanding the genetic and ecological factors driving switches in the prevailing mode of reciprocal selection.

Antagonistic coevolution and evolvability

The pressure for continual innovation during antagonistic coevolution can, in theory, select for mechanisms that increase evolvability, particularly in hosts, because they are often assumed to have less evolutionary potential than their parasites [20]. Greater genetic diversity within a population increases the efficacy of selection and, notwithstanding immigration, can be achieved through increased rates of mutation or recombination. Studies across a range of species interactions strongly support the hypothesis that antagonistic coevolution selects for evolvability in hosts. The evolution of hypermutable *P. fluorescens* genotypes, with defective DNA proofreading enzymes, was found to occur at a higher frequency in populations coevolving with phage Φ2 than in those evolving alone [21]. Similarly, more spontaneous mutations were observed in *C. elegans* that had been coevolving with *B. thuringiensis* compared with parasite-free controls [12]. For sexual host populations, recombination offers another potential escape from coevolving parasites. Populations of the flour beetle, *Tribolium castaneum*, coevolving with a microsporidian parasite,

Table 1. The experimental systems of antagonistic experimental coevolution[a]

Model system	Control treatment	Time shift	Local adaptation	Victim change?	Exploiter change?	Refs
Invertebrate victim						
Caenorhabditis elegans–Bacillus thuringiensis	Single species	•	✓	✓	✓	[12,73]
C. elegans–Serratia marcescens	Evolution	•	•	✓	✓	[74]
Potamopyrgus antipodarum–Microphallus sp.	Single species	•	✓	✓	✓	[16,75]
Tribolium castaneum–Noseum whitei	Single species	✓	•	✓	✓	[22,27,76]
Biomphalaria glabrata–Schistosoma mansoni	Single species	•	✓	✓	✓	[65,77]
Daphnia magna–Octosporea bayeri	Single species	•	•	✓	•	[51]
Protist victim						
Paramecium caudatum–Holospora undulata	Single species	•	✓	✓	×	[78]
Bacterial victim						
Pseudomonas fluorescens–phage Φ2	Evolution	✓	✓	✓	✓	[40,79,80]
Pseudomonas aeruginosa–phage PP7	None	✓	•	✓	×	[81]
Escherichia coli–phage Qβ	Evolution	•	•	✓	✓	[14]
E. coli–phage T7	None	•	✓	✓	✓	[30]
E. coli–phage T4	Single species	•	•	✓	•	[82]
E. coli–phage PP01	None	•	•	✓	✓	[83]
Synechococcus sp.–phage RIM8	Single species	•	•	✓	✓	[26]
Serratia marcescens–Tetrahymena thermophila	Single species	•	•	✓	×	[84,85]

[a]Examples of study systems used and approaches taken using experimental coevolution so far. Although this list is not exhaustive, it is representative of the types of system for which this approach has proven successful due, in part, to ease of use in the laboratory, short generation times cryogenic preservation, and large population sizes. Broadening the taxonomic range of study systems used in experimental coevolution is an important challenge to explore the generality of the patterns observed thus far. Moreover, it is clear that, even for existing study systems, there is work to be done in terms of using the full range of assays available (i.e., both time-shift and local adaptation assays) and in terms of simultaneously analyzing the evolution of both victim and exploiter species. '•' represent situations where the experiment has not yet been performed.

Noseum whitei, displayed higher rates of meiotic recombination compared with both a parasite-free control [22] and a population exposed to an insecticide [23]. Similarly, higher rates of outcrossing have been observed in populations of *C. elegans* coevolving against the bacterial parasite *Serratia marcescens* relative to populations where the bacterium was held in evolutionary stasis [24]. Moreover, the rate of host population extinction was higher in coevolving populations where *C. elegans* outcrossing was prevented compared with populations where outcrossing was possible. Although host evolvability has been well studied, the effect of antagonistic coevolution on parasite evolvability has not been addressed and provides a fruitful avenue for future studies, particularly in sexually recombining parasites.

Antagonistic coevolution as a driver of diversification and divergence

Antagonistic coevolution can lead to higher levels of within-population polymorphism through either the transient coexistence of contending alleles undergoing selective sweeps or the operation of negative frequency-dependent selection. Several bacteria–phage coevolution studies reveal antagonistic coevolution as a driver of phenotypic and genetic diversification in both bacteria and phage [13,25,26]. Similarly, populations of *T. castaneum* coevolving with *N. whitei* harbored significantly more allelic diversity than did parasite-free control populations [27]. The intense selection associated with antagonistic coevolution can also drive divergence among populations, because each takes a subtly different coevolutionary trajectory. Experimentally coevolving populations of phage Φ2 undergo an almost 10-fold higher level of between-population genomic divergence, compared with populations evolving against an evolutionarily fixed bacterial population [13]. Correspondingly, phage-mediated selection led to greatly increased allopatric diversity (i.e., diversity among populations) among experimentally coevolved *P. fluorescens* populations [28].

Among-population divergence of parasite infectivity and host resistance traits can also be detected using local adaptation assays, whereby, for example, parasite performance is compared against their sympatric and allopatric host genotypes (Figure 2). These experiments reveal a wide range of local adaptation patterns across various species interactions, including parasite local adaptation, host local adaptation, or lack of local adaptation (Table 1). Crucially, however, these studies enable explicit tests of theoretical predictions on the effects of key ecological and life-history parameters on the evolution of local adaptation. For instance, several studies of bacteria–phage metapopulations have revealed that moderate parasite dispersal drives the evolution of parasite local adaptation [29–31] (for detailed reviews of the parasite local adaptation literature see [32,33]). Among-population divergence of coevolving species interactions can be further enhanced if environmental heterogeneity exists among patches [34,35]. For example, variation in productivity between populations drives the evolution of greater parasite local adaptation in populations of *P. fluorescens* and Φ2 [36]. Between-population divergence of traits at the coevolutionary interface (i.e., resistance and infectivity) can be accompanied by correlated divergence in other phenotypic traits, such as colony morphology and biofilm formation in bacteria coevolving with phages [28,37,38]. Moreover, recent evidence from experimental populations of *T. castaneum* and *N. whitei* suggest that between-population divergence caused by antagonistic coevolution can even drive the correlated evolution of reproductive isolation and, therefore, could play a role in speciation [39].

Specificity of antagonistic coevolutionary interactions

Key to understanding coevolutionary dynamics is the underlying genetic specificity of the interaction and the emergent patterns of interaction specificity. Experiments with bacteria and phage have revealed that coevolution can lead to a nested interaction structure [40,41], such that hard-to-infect bacterial genotypes are infected by generalist but not specialist phage genotypes [42]. Moreover, coevolving bacteria–phage populations can harbor, at any given time, a diverse mix of phenotypes, ranging from specialists to generalists [26,40], which is dynamic and variable through time. Interestingly, coevolution itself appears to be crucial in shaping host range of some phages. In Φ2, spontaneous host-range mutants selected to infect a novel host genotype evolved narrower host ranges than did phages with a history of coevolution against this host genotype [43]. Here, broad host ranges relied upon the accumulation of multiple adaptive mutations acquired through repeated rounds of selection for infectivity. Similarly, the evolution of particular resistant bacterial genotypes in coevolving populations of *E. coli* and λ were necessary for the subsequent evolution by phage of the ability to bind to a new host receptor, OmpF, which was found to require the stepwise accumulation of four adaptive mutations [44]. Both studies highlight the importance of historical contingency in determining the trajectory of coevolution.

In addition to the effects of limited mutational supply, the evolution of generalists can also be constrained by costs associated with resistance and infectivity mutations. Often, such trade-offs are expected due to antagonistic pleiotropy. In the case of bacteria–phage coevolution, phages often bind to bacterial cell-surface proteins that perform important functions, such as nutrient uptake or motility, and mutations conferring resistance to phages typically impair these functions [45,46]. In addition, evolved resistance against one phage can often come at a cost of increased susceptibility to another; experimentally evolved *Prochlorococcus* hosts that were resistance to one phage genotype showed increased susceptibility to another phage genotype [47]. Correspondingly, mutations allowing host-range expansion in phages are also frequently associated with trade-offs, leading to impaired growth on the original host. For example, during experimental host-range expansion of phage Φ6, spontaneous mutants able to infect novel hosts were found to be less infective to their native hosts [48]. However, surprisingly few studies have attempted to determine explicitly how costs of multiple resistance and infectivity mutations accumulate and interact through time during experimental coevolution (although see [49]) and, correspondingly, how this shapes coevolutionary dynamics and trajectory [50].

Emerging directions in experimental coevolution

The major contributions of experimental coevolution thus far have been to provide direct evidence of the tempo and mode of antagonistic coevolutionary dynamics, the role of antagonistic coevolution in increasing diversity within and among populations, including the role of parasitism in maintaining sexual recombination, and the structure of specificity in coevolving antagonistic interactions. However, as the field matures, it is taking some exciting new directions; in what follows, we outline several promising emerging research directions.

Experimental coevolution in 'real-world' environments

Although an original motivation behind laboratory coevolution experiments was to exclude the confounding selection pressures of complex natural environments, there is currently a shift towards performing experiments in more naturalistic 'real-world' environments. Such studies are valuable, particularly when performed using well-studied species associations, because they reveal ecological constraints on coevolution imposed by natural environments. Moreover, such studies can guide analysis of natural communities. Zbinden and coauthors infected populations of *Daphnia magna* with the microsporidian parasite *Octosporea bayeri* under natural conditions in field mesocosms to examine the evolution of host resistance and associated life-history changes; the authors demonstrated rapid evolution with some associated costs of evolved resistance [51]. Gomez and Buckling [52] performed experimental coevolution of *P. fluorescens* and Φ2 in soil microcosms, where, in contrast to previous laboratory studies in rich liquid media, the coevolutionary dynamics followed FSD rather than ARD during the early stage of coevolution. This is likely to have been caused by higher costs of resistance mutations in soil compared with liquid media, thereby weakening the response of bacteria to directional selection.

Experimental coevolution of other forms of species interaction

Several researchers have begun to apply the experimental coevolution approach to study other forms of species interaction beyond antagonisms; in particular, mutualisms. This is an important step because such interactions are widespread in nature and, although antagonistic coevolution can promote diversification, theory suggests that those species interactions in which there is no cost to phenotypic matching (e.g., mutualistic interactions) hinder diversification [53]. Hillesland *et al.* demonstrated the rapid evolution of trait complementarity in an experimentally imposed obligate syntrophic mutualism [54]. They co-cultured a sulfate-reducing bacterium, *Desulfovibrio vulgaris*, and a methanogenic archaeon, *Methanococcus maripaludis*, on lactate, where the two players had to collaborate to perform an energy-yielding reaction. Communities initially underwent large population density fluctuations, but stabilized after approximately 300 generations. These coevolved communities had faster growth rates and higher yields compared with ancestral communities. Time-shifted pairings confirmed that adaptations in each species contributed to community-level improvements in growth rate and yield. This study highlights the utility of experimental coevolution for understanding species interactions in general, and beyond antagonistic interactions, and furthermore demonstrates the need for more studies of mutualistic species interactions.

Coevolution of complex communities

Whereas most experimental coevolution studies have used pairs of species, species interaction networks in nature are often complex. Scaling experimental coevolution studies

up to the community level is a key next step. A study of *P. syringae* coevolving with multiple phages found that the bacterial hosts were able to evolve resistance against multiple phages simultaneously, but that they paid a higher cost for these multiple resistances when grown in the absence of phage [55]. Addition of a protist predator, *Tetrahymena thermophila*, to coevolving populations of *P. fluorescens* and Φ2 impeded ARD coevolution between the bacteria and phage, and favored the maintenance of coexisting resistance phenotypes specialized against one or other of these natural enemies [56]. Generalist bacterial resistance did not evolve in these communities due to the existence of fitness trade-offs associated with multiple resistances.

Networks of species interactions can also shape the evolution and stability of the community as a whole. Experimental communities of naturally co-occurring bacteria collected from holes in beech trees showed that the interactions among these species were key to their ability to adapt to novel environments in the laboratory [57]. These species, when propagated in communities, evolved more over 70 generations than when grown in monoculture, and adapted to fill different niches, for example, to utilize the waste products generated from another species within the community. Indeed, interspecific facilitation was a common outcome of coevolution in these competitive communities. Future work will certainly enable great insight into the assembly, structure, function, and dynamics of communities.

Co-phylogeny and co-speciation

Early work on coevolution utilized macroevolutionary patterns to infer microevolutionary processes (e.g., [58]), for example, by comparing phylogenies of species pairs to look for co-speciation. However, although frequently cited as evidence for coevolution, it cannot be ruled out that the same biogeographical or ecological process that drove speciation among one species was responsible, independently, for speciation of the other [59]. Similarly, divergence among lineages of one species might lead to subsequent divergence in the other (i.e., concordant phylogeny), but may also lead to the evolution of more generalist interaction networks or 'escape' of one player if the new lineage no longer interacts with the other player [60]. Although there exists theory predicting when diversification of one species might lead to diversification of the other (e.g., [61]), there are few data testing the validity of these predictions. Combining experimental coevolution with phylogenetic methods has great potential to reveal the underlying dynamics that lead both to co-diversification and the breakdown of co-phylogeny patterns [59]. Towards this goal, several experimental evolution studies created known phylogenies through population splitting and then attempted to infer their structure from genome sequences of viruses at the nodes. Experiments with bacteriophages ΦX174 and phi-6 demonstrated that the high degree of convergent evolution and reversions made phylogenetic reconstruction incapable of accurately explaining the evolutionary history of the phage [62,63]. By revealing whether convergence is a general phenomenon of viral evolution, further studies could inform use of molecular epidemiology in tracking viral outbreaks. More generally, long-term experimental coevolution holds great promise in testing whether co-divergence and/or co-speciation among interacting species is the exception or the rule.

Concluding remarks and potential for application

Overall, experimental evolution has afforded remarkable strides forward in understanding population-level responses to selection, the underlying genetics of adaptation, and the limits of evolution [64]. Although still in its infancy, experimental coevolution has great potential for informing understanding of community stability, species invasions, and the spread of disease and, as such, holds promise in more applied fields, most notably human health. Experimental coevolution techniques have already been successfully applied to understand the evolution of human parasites: Webster *et al.* found that experimental coevolution of the human parasite, *Schistosoma mansoni*, with different genotypes of the intermediate host snail, *Biomphalaria glabrata*, led to not only rapid adaptation to the snails, but also altered infectivity on the definitive host [65]. Furthermore, it is now clear that one's own microbiota determine key aspects of one's physical and mental health, and experimental coevolution could play a critical part in testing how these microbial communities evolve and change over time, both as a function of microbe–microbe interactions and of host–microbe interactions [66]. The efficacy and long-term implications of phage therapy for controlling bacterial pathogens and the use of probiotics for promoting healthy gut flora is also ripe for experimental coevolution testing, and good headway is already being made using experimental evolution of bacteria in response to phages [55,67–69] and to test evolution of bacteria in the gut [70]. Expanding this research to explore the coevolutionary implications of these treatments is a clear next step and experimental coevolution could be fruitfully used to select for stable microbial consortia with desirable traits for use in probiotics.

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