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Experimental coevolution of species interactions

Michael A. Brockhurst[1] and Britt Koskella[2]

[1] Department of Biology, University of York, York, YO10 5DD, UK

[2] BioSciences, University of Exeter, Cornwall Campus, Tremough, TR10 9EZ, UK

Coevolution, the process of reciprocal adaptation and counter-adaptation between ecologically interacting species, affects almost all 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. 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.

Keywords: experimental evolution; coevolution; species interactions; host-parasite; mutualism

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The rise of experimental coevolution

Naturalists have long recognised the importance of species interactions as a driving force of adaptation. Indeed, 19th-century evolutionary biologists often cited the conspicuous coadaptations of interspecific pollination and mimicry **mutualisms** 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 co-variation 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, while never being 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 lab. Here, environments can be precisely controlled to exclude extraneous sources of selection, and the use of fast-growing organisms like microbes or classic lab-model animals, allows for the direct observation of coevolution in real time (Figure 1 & Box 1). Significantly, since many such experimental systems are amenable to cryogenic preservation, this allows 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, while time-shifts

47 are possible in certain field systems [4], a crucial advantage of laboratory coevolution
48 experiments is that control lineages, propagated under identical environmental conditions
49 but where a given species is absent or where one species is held in **evolutionary stasis**, can
50 also be established (Figure 1). Comparison of coevolving lineages against control lineages
51 allows unequivocal identification of adaptations that evolved in response to reciprocal
52 selection, i.e. those adaptations that are present only in coevolving lineages.
53
54 Coevolution experiments were first pioneered using simple microbial communities in the
55 1970s [5-7]. While these kinds of microbial associations remain the most intensively studied
56 due to their ease of propagation, the experimental coevolution approach has recently been
57 extended to a much wider range of species interactions involving more complex host
58 organisms such as snails, beetles, bees and worms (Table 1). Moreover, whereas early studies
59 largely focused on antagonisms, in part due to the intensity of reciprocal selection and rapid
60 evolution generated by such interactions, today experimental coevolution researchers are
61 studying other forms of species interaction, such as mutualisms. Experimental coevolution is
62 providing causal tests of longstanding coevolutionary hypotheses, and also revealing novel
63 facets of the coevolutionary process that are not captured or predicted by current theory. In
64 this article we do not aim to provide an exhaustive account of experimental coevolution
65 research but rather to review the key areas in which experimental coevolution has advanced
66 our understanding of the coevolutionary process, identify the main gaps in our knowledge
67 for future research, and highlight the ways in which coevolutionary research can be of
68 applied importance.
69
70 **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 refs. [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 exhibit greater molecular evolutionary change, assessed by microsatellites and gene content respectively, than do 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 allowed for direct tests of causation rather than correlation.

95 The process of rapid reciprocal adaptation inherent to antagonistic coevolution can be
96 driven by at least two contrasting modes of reciprocal selection. Specifically, frequency
97 dependent selection, where changing allele frequencies in host and parasite populations are
98 driven by parasite-mediated selection against common host resistance alleles; or directional
99 selection, where recurrent selective sweeps of novel host resistance and parasite infectivity
100 alleles occur through time, leading to increases in a parasite's **host range** and the subsequent
101 host resistance traits. These possibilities have been termed **Fluctuating Selection**
102 **Dynamics** (FSD) and **Arms Race Dynamics** (ARD), respectively [3, 15]. Distinguishing
103 these dynamics requires either time-shifts to detect contrasting patterns of phenotypic
104 evolution in host resistance and parasite infectivity traits (Figure 2), or alternatively, direct
105 estimation of temporal change in the frequencies of resistance and infectivity alleles, or of
106 linked genetic markers.
107
108 Experimental coevolution has revealed evidence for the operation of both of these modes of
109 reciprocal selection. A response to frequency-dependent selection by parasites has been
110 observed by tracking host genotypic markers in coevolving laboratory populations of the
111 freshwater snail, *Potamopyrgus antipodarum*, infected by a sterilizing trematode parasite,
112 *Microphallus sp.* [16]. However, several other studies reveal signatures of both FSD and ARD
113 within the same coevolving population, suggesting that these contrasting modes of selection
114 are not mutually exclusive. For example, genotypic data from *C. elegans – B. thuringiensis*
115 coevolution experiments suggest that different host loci are under different modes of
116 selection; perhaps reflecting that the infection/resistance process comprises multiple steps of
117 interaction, each with independent genetic bases [12, 17, 18]. Furthermore, patterns of
118 phenotypic and molecular evolution suggest that the interaction between *P. fluorescens* and Φ2,

while 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 super-generalist 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, since they are often assumed to possess 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 those evolving alone [21]. Similarly, more spontaneous mutations were observed in *C. elegans* that had been coevolving with *B. thuringiensis* compared to parasite-free controls [12]. For sexual host populations,

143 recombination offers another potential escape from coevolving parasites. Populations of the
144 flour beetle, *Tribolium castaneum*, coevolving with a microsporidian parasite, *Noseum whitei*,
145 displayed higher rates of meiotic recombination than both parasite-free controls [22] and
146 populations exposed to an insecticide [23]. Similarly, higher rates of outcrossing have been
147 observed in populations of *C. elegans* coevolving against the bacterial parasite *Serratia*
148 *marcescens* relative to populations where the bacterium was held in evolutionary stasis [24].
149 Moreover, the rate of host population extinction was higher in coevolving populations where
150 *C. elegans* outcrossing was prevented compared to populations where outcrossing was
151 possible. While host evolvability has been well studied, the effect of antagonistic coevolution
152 on parasite evolvability has not been addressed and provides a fruitful avenue for future
153 studies particularly in sexually recombining parasites.
154
155 *Antagonistic coevolution as a driver of diversification and divergence*
156 Antagonistic coevolution can lead to higher levels of within-population polymorphism
157 through either the transient coexistence of contending alleles undergoing selective sweeps or
158 the operation of negative frequency-dependent selection. Several bacteria-phage coevolution
159 studies reveal antagonistic coevolution as a driver of phenotypic and genetic diversification
160 in both bacteria and phage [13, 25, 26]. Similarly, populations of *T. castaneum* coevolving with
161 *N. whitei* harbor significantly more allelic diversity than parasite-free control populations [27].
162 The intense selection associated with antagonistic coevolution can also drive divergence
163 among populations, as each takes a subtly different coevolutionary trajectory. Experimentally
164 coevolving populations of phage Φ2 undergo an almost 10× higher level of between-
165 population genomic divergence, compared to populations evolving against an evolutionarily
166 fixed bacterial population [13]. Correspondingly, phage-mediated selection lead to greatly

167 increased allopatric diversity (i.e., diversity among populations) among experimentally
168 coevolved *P. fluorescens* populations [28].
169
170 Among-population divergence of parasite infectivity and host resistance traits can also be
171 detected using local adaptation assays, whereby, for example, parasite performance is
172 compared against their sympatric and allopatric host genotypes (Figure 2). These
173 experiments reveal a wide range of local adaptation patterns across various species
174 interactions including parasite local adaptation, host local adaptation or lack of local
175 adaptation (Table 1). Crucially, however, these studies allow explicit tests of theoretical
176 predictions on the effects of key ecological and life-history parameters on the evolution of
177 local adaptation. For instance, several studies of bacteria-phage metapopulations have
178 revealed that moderate parasite dispersal drives the evolution of parasite local adaptation [29-
179 31] (for detailed reviews of the parasite local adaptation literature see refs. [32, 33]). Among-
180 population divergence of coevolving species interactions can be further enhanced if there
181 exists environmental heterogeneity among patches [34, 35]. For example, variation in
182 productivity between populations drives the evolution of greater parasite local adaptation in
183 populations of *P. fluorescens* and Φ2 [36]. Between-population divergence of traits at the
184 coevolutionary interface, i.e., resistance and infectivity, can be accompanied by correlated
185 divergence in other phenotypic traits, such as colony morphology and biofilm formation in
186 bacteria coevolving with phages [28, 37, 38]. Moreover, recent evidence from experimental
187 populations of *T. castaneum* and *N. whitei* suggest that between population divergence caused
188 by antagonistic coevolution can even drive the correlated evolution of reproductive isolation,
189 and therefore could play a role in speciation [39].
190

Specificity of antagonistic coevolutionary interactions

Key to our understanding of 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

215 can often come at a cost of increased susceptibility to another; experimentally evolved
216 *Prochlorococcus* hosts that were resistance to one phage genotype showed increased
217 susceptibility to another phage genotype [47]. Correspondingly, mutations allowing host-
218 range expansion in phages are also frequently associated with trade-offs, leading to impaired
219 growth on the original host. For example, during experimental host range expansion of
220 phage ϕ6, spontaneous mutants able to infect novel hosts were found to be less infective to
221 their native hosts [48]. However, surprisingly few studies have attempted to explicitly
222 determine how costs of multiple resistance and infectivity mutations accumulate and interact
223 through time during experimental coevolution (although see [49]) and correspondingly how
224 this shapes coevolutionary dynamics and trajectory [50].
225
226 **Emerging directions in experimental coevolution**
227 The major contributions of experimental coevolution thus far have been to provide direct
228 evidence of the tempo and mode of antagonistic coevolutionary dynamics, the role of
229 antagonistic coevolution in increasing diversity within and among populations, including the
230 role of parasitism in maintaining sexual recombination, and the structure of specificity in
231 coevolving antagonistic interactions. But as the field matures it is taking some exciting new
232 directions; in what follows, we outline several promising emerging research directions.
233
234 *Experimental coevolution in 'real-world' environments*
235 While an original motivation behind laboratory coevolution experiments was to exclude the
236 confounding selection pressures of complex natural environments, there is currently a shift
237 towards performing experiments in more naturalistic 'real world' environments. Such studies
238 are valuable, particularly when performed using well-studied species associations, as they

239 reveal ecological constraints on coevolution imposed by natural environments. Moreover,
240 such studies can guide analysis of natural communities. Zbinden and coauthors (2008)
241 infected populations of *Daphnia magna* with the microsporidian parasite *Octosporea bayeri* under
242 natural conditions in field mesocosms to examine the evolution of host resistance and
243 associated life-history changes and demonstrated rapid evolution with some associated costs
244 of evolved resistance [51]. Gomez & Buckling [52] have performed experimental
245 coevolution of *P. fluorescens* and Φ2 in soil microcosms, where in contrast to previous lab
246 studies in rich liquid media, the coevolutionary dynamics follow FSD rather than ARD
247 during the early stage of coevolution. This is likely to have been caused by much higher costs
248 of resistance mutations in soil compared to liquid media thereby weakening the response of
249 bacteria to directional selection.
250
251 *Experimental coevolution of other forms of species interaction*
252 Several researchers have begun to apply the experimental coevolution approach to study
253 other forms of species interaction beyond antagonisms; in particular, mutualisms. This is an
254 important step because such interactions are widespread in nature and, while antagonistic
255 coevolution can promote diversification, theory suggests that those species interactions in
256 which there is no cost to **phenotypic matching** (e.g. mutualistic interactions) may actually
257 hinder diversification [53]. Hillesland et al. (2009) have demonstrated the rapid evolution of
258 trait complementarity in an experimentally imposed obligate **syntrophic mutualism** [54].
259 They co-cultured a sulphate reducing bacterium, *Desulfovibrio vulgaris*, and a methanogenic
260 archaeon, *Methanococcus maripaludis*, on lactate, where the two players had to collaborate to
261 perform an energy yielding reaction. Communities initially underwent large population
262 density fluctuations, but stabilized after around 300 generations. These coevolved

communities had faster growth rates and higher yields than 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

While most experimental coevolution has employed 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 bacterial hosts are able to evolve resistance against multiple phages simultaneously, but that they pay 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 favoured the maintenance of coexisting resistance phenotypes specialized against one or other of these natural enemies [56]. Generalist bacterial resistance presumably 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 found 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

287 waste products generated from another species within the community. Indeed, **interspecific**
288 **facilitation** was a common outcome of coevolution in these competitive communities.
289 Future work will certainly allow great insight to the assembly, structure, function, and
290 dynamics of communities.
291
292 *Cophylogeny and cospeciation*
293 Early work on coevolution utilized macroevolutionary patterns to infer microevolutionary
294 processes (e.g. [58]), for example by comparing phylogenies of species pairs to look for co-
295 speciation. However, while frequently cited as evidence for coevolution it cannot be ruled
296 out that the same biogeographical or ecological process that drove speciation among one
297 species was responsible, independently, for speciation of the other [59]. Similarly, divergence
298 among lineages of one species might lead to subsequent divergence in the other (i.e.,
299 concordant phylogeny) but may also lead to the evolution of more generalist interaction
300 networks or "escape" of one player if the new lineage no longer interacts with the other
301 player [60]. Although there exists theory predicting when diversification of one species might
302 lead to diversification of the other (e.g., [61]), there is little data testing the validity of these
303 predictions. Combining experimental coevolution with phylogenetic methods has great
304 potential to reveal the underlying dynamics that lead both to codiversification and the
305 breakdown of **cophylogeny** patterns [62]. Towards this goal, several experimental evolution
306 studies have created known phylogenies through population splitting and then attempted to
307 infer their structure from genome sequences of viruses at the nodes. Experiments with
308 bacteriophages ΦX174 and phi-6 have demonstrated that the high degree of convergent
309 evolution and reversions made phylogenetic reconstruction incapable of accurately
310 explaining the evolutionary history of the phage [63, 64]. By revealing whether convergence

311 is a general phenomenon of viral evolution, further studies could inform use of molecular
312 epidemiology in tracking viral outbreaks. More generally, long-term experimental
313 coevolution holds great promise in testing whether codivergence and/or cospeciation among
314 interacting species is the exception or the rule.
315
316 **Concluding remarks and potential for application**
317 Overall, experimental evolution has afforded remarkable strides forward in our
318 understanding of population-level responses to selection, the underlying genetics of
319 adaptation, and the limits of evolution [65]. Although still in its infancy, experimental
320 *co*evolution has great potential for informing our understanding of community stability,
321 species invasions, and the spread of disease, and as such holds promise in more applied
322 fields, most notably human health. Experimental coevolution techniques have already been
323 successfully applied to understand the evolution of human parasites: Webster et al. (2007)
324 found that experimental coevolution of the human parasite, *Schistosoma mansoni,* with
325 different genotypes of the intermediate host snail, *Biomphalaria glabrata,* led to rapid
326 adaptation to the snails but also altered infectivity on the definitive host [66]. Furthermore, it
327 is now abundantly clear that our own microbiota determine key aspects of our physical and
328 mental health, and experimental coevolution could play a critical part in testing how these
329 microbial communities evolve and change over time, both as a function of microbe-microbe
330 interactions and of host-microbe interactions [67]. The efficacy and long-term implications
331 of **phage therapy** for controlling bacterial pathogens and the use of probiotics for
332 promoting healthy gut flora is also ripe for experimental coevolution testing, and good
333 headway is already being made using experimental evolution of bacteria in response to
334 phages [55, 68-70] and to test evolution of bacteria in the gut [71]. Expanding this research

335 to explore the coevolutionary implications of these treatments is a clear next step and
336 experimental coevolution could be fruitfully employed to select for stable microbial
337 consortia with desirable traits for use in probiotics.
338
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343
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Box 1. When is it experimental coevolution?

In a classic article, Janzen defined the term coevolution [72], 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 should 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: (a.) interacting species are co-cultured *and* experimenters attempt to quantify evolutionary responses in both (or all if >2) interacting species; or (b.) interacting species are co-cultured *and* evolutionary responses of populations from coevolving treatments are compared to 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 specifically tease apart 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 to the coevolution treatment to determine which evolutionary

550 changes are the result of an evolutionary response to the biotic agent versus a result of
551 coevolutionary interactions.

552 As experimental (co)evolution proceeds, fitness of the (co)evolving populations can
553 be measured over time to determine, for example, whether parasites become more or less
554 prudent on their hosts and whether hosts evolve towards complete resistance. In coevolving
555 populations, fitness can be measured both on the ancestral antagonist populations, allowing
556 for observation of absolute changes in population fitness, and on the coevolved antagonist.
557 As illustrated in figure 1, this latter relative fitness might not change over time, as the other
558 species is responding to any adaptations and countering. Finally, for many experimental
559 evolution systems, populations from each time point can be frozen and later resurrected to
560 perform time shifts in which the fitness of one species can be tested on populations of the
561 other from the past (i.e. populations which have not yet responded to any new adaptations),
562 the same time point, or from the future (i.e. populations that have potentially already
563 responded to any new adaptations). Note however, that for frequency-dependent selection,
564 populations may be unfit on past populations of the antagonist if, for example, they have
565 moved on to infect/resist common types in the contemporary antagonist populations.
566
567
568

Glossary of terms

Antagonistic coevolution/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 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 an adaptation that is advantageous in one biotic or abiotic environment is deleterious in another.

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 hosts can resist and an increase in the range of host genotypes that parasites can infect.

Cophylogeny: 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: This 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 genotype in order to prevent evolution in this species.

Evolvability: The ability of a population to generate genetic diversity thereby allowing it to respond to selection.

Host-range: The subset of hosts that a parasite can successfully infect. Note that the known

593 host range for a given parasite is necessarily determined by the reference panel against which
594 it has been tested and that parasite performance can vary within a given host range, such that
595 the parasite performs better on some hosts than others.
596 Fluctuating Selection Dynamics: A mode of antagonistic coevolution driven by negative-
597 frequency dependent selection whereby parasites evolve to infect common host genotypes,
598 thereby favouring rare host alleles, which subsequently become common, leading to
599 sustained oscillations in host and parasite allele frequencies. FSD does not lead to the
600 evolution of broader parasite host ranges or increasing host resistance through time.
601 Hypermutable**:** Strains of bacteria with mutation rates far in excess of the wild-type; these
602 typically arise through mutations altering mismatch repair enzymes.
603 Interspecific facilitation: A scenario whereby one species enhances the fitness or growth of
604 another either directly, for example by increasing the availability of nutrients, or indirectly,
605 for example by reducing competition or predation. Facilitatory interactions can benefit either
606 one or both participants, and in the latter case are considered to be interspecific **mutualisms**.
607 Mutualisms**:** Mutually beneficial species interactions, which in reality are often mutually
608 exploitative interactions but where net benefits accrue to both parties.
609 Phage therapy: The use of bacteriophage viruses to control the growth and/or harmfulness
610 of pathogenic bacteria.
611 Phenotypic matching: The clustering of or correlation between traits governing a
612 coevolutionary interaction, such that the common phenotype in the local populations of one
613 partner is matched by the reciprocal trait in the other.
614 Red Queen hypothesis: The idea that, for antagonistic species interactions, the relative
615 fitness of each antagonist does not increase over time, despite continual adaptation, due to
616 the counteracting adaptations of their opponent. This hypothesis was later formalized to

617 describe the potential role of coevolving parasites in generating an advantage for sexual
618 recombination.
619 Syntrophic mutualism: A form of microbial mutualism where the transfer of metabolites
620 between species is essential for growth.
621 Time-shift experiment: Studies in which samples of coevolving populations are collected
622 through time (either artificially by cryogenic freezing, or naturally by the deposition of resting
623 stages) and then resurrected to challenge against coevolving partners from past,
624 contemporary and future time-points.
625

626 **Table and Figure Legends:**

627 Table 1. The experimental systems of antagonistic experimental coevolution**.**

628 Examples of study systems used and approaches taken using experimental coevolution so far.
629 Although this list is not exhaustive, it is representative of the types of systems for which this
630 approach has proven successful due, in part, to ease of use in the laboratory, short
631 generation times (although note exceptions below), cryogenic preservation and large
632 population sizes. Broadening the taxonomic range of study systems employed in
633 experimental coevolution is an important future challenge to explore the generality of the
634 patterns observed thus far. Moreover, it is clear that even for existing study systems there is
635 work to be done in terms of employing the full range of assays available (i.e., both time-shift
636 and local adaptation assays) and in terms of simultaneously analyzing the evolution of both
637 victim and exploiter species.

638

639

640 Figure 1. The experimental designs of experimental coevolution.

641 A simplified illustration of experimental coevolution of host and parasite, where one can
642 compare single species evolution (controlling for both adaptation to lab conditions and drift),
643 one-sided experimental evolution (*i.e.,* one species evolving in response to another which is
644 unable to respond) and experimental coevolution, where it is possible to directly measure
645 evolutionary change of one species in response to the other and any reciprocal adaptations
646 that occur. Line graphs represent one scenario of evolutionary change in parasite populations
647 (top) or host populations (bottom) over the course of the experiment. In the case of a
648 parasite or host evolving alone, adaptation to the lab environment and/or drift could result
649 in increased success against the host/parasite, decreased success against the host/parasite, or
650 no change in fitness.

651

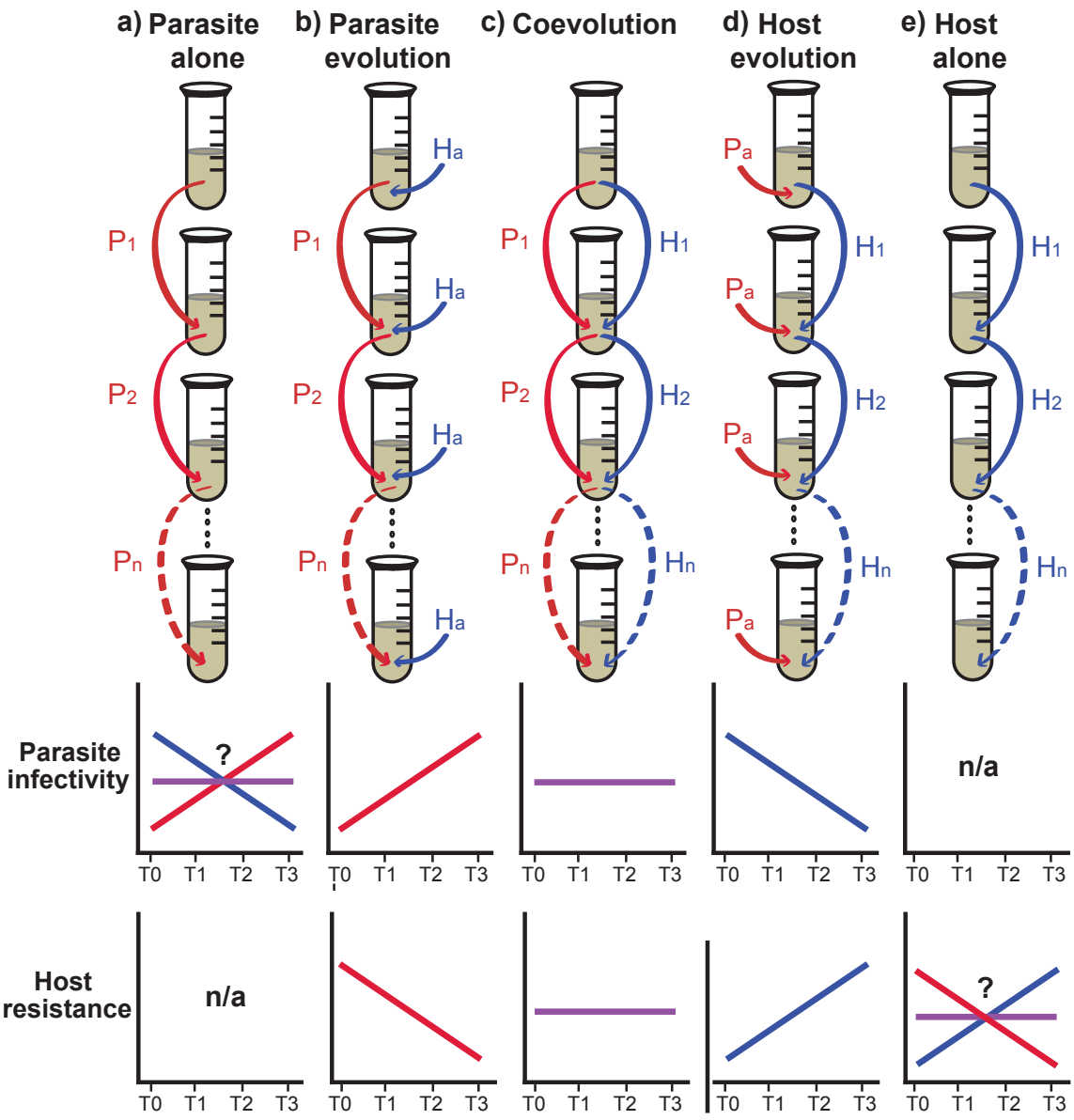
652 Figure 2. Approaches to quantifying reciprocal adaptation.

653 An illustrative example of techniques used to compare coevolution of two species (in this
654 case, host and parasite) by examining changes in replicate experimental populations (or
655 metapopulations, if connected by gene flow). A time shift experiment (a) can be performed
656 across experimental time within each population by comparing the fitness of one player
657 against the other from past, contemporary or future time points. This method can give
658 unique insight into the coevolutionary dynamic underlying the change. For example, a
659 scenario in which fitness is lowest against populations from the future and highest against
660 those from the past might indicate arms race dynamics with directional selection whereas a
661 pattern of peak fitness against contemporary populations or those from only the recent past
662 is more in line with negative frequency dependent selection. However, note that the exact
663 pattern will depend on the lag in evolutionary response of one player against the other [73].

664 A local adaptation experiment (b) compares performance of parasites against their sympatric
665 hosts with their performance against allopatric hosts; higher parasite performance against
666 sympatric versus allopatric hosts indicates that parasites are locally adapted.
667

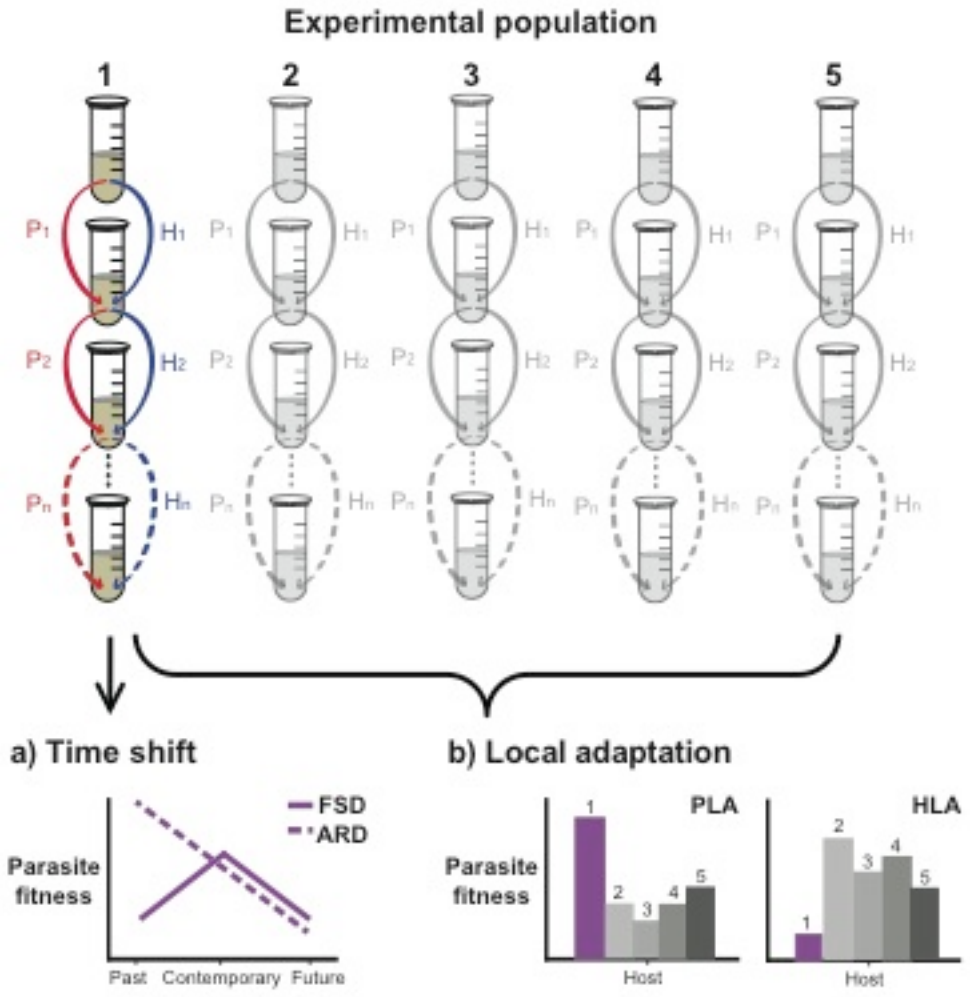
Model system	Control treatment	Time shift	Local adaptation	Victim change?	Exploiter change?	Reference
Invertebrate victim						
C. elegans - *B. thuringiensis*	Single species	•	✔	✔	✔	[12, 74]12, 73]
C. elegans - *S. marcescens*	Evolution	•	•	✔	✔	[75]
P. antipodarum - *Microphallus sp.*	Single species	•	✔	✔	✔	[[16, 76]
T. castaneum - *Noseum whitei*	Single species	✔	•	✔	✔	[[22, 27, 77]
B. glabrata - *S. mansoni*	Single species	•	✔	✔	✔	[[66, 78]
D. Magna - *O. bayeri*	Single species	•	•	✔	•	[51]
Protist victim						
P. caudatum - *H. undulata*	Single species	•	✔	✔	✗	[79]
Bacterial victim						
P. fluorescens - phage Φ2	Evolution	✔	✔	✔	✔	[[40, 80, 81]
P. aeruginosa - phage PP7	None	✔	•	✔	✗	[82]
E. coli - phage Qβ	Evolution	•	•	✔	✔	[83]
E. coli - phage T7	None	•	✔	✔	✔	[30]
E. coli - phage T4	Single species	•	•	✔	•	[84]
E. coli - phage PP01	None	•	•	✔	✔	[85]
Synechococcus sp. - phage RIM8	Single species	•	•	✔	✔	[86]
S. marscesens - *T. themophila*	Single species	•	•	✔	✗	[[87, 88]

Table 1



670

671 Figure 1



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673 Figure 2