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# 1 **Experimental coevolution of species interactions**

2 Michael A. Brockhurst<sup>1</sup> and Britt Koskella<sup>2</sup>

3

4 <sup>1</sup> Department of Biology, University of York, York, YO10 5DD, UK

5 <sup>2</sup> BioSciences, University of Exeter, Cornwall Campus, Tremough, TR10 9EZ, UK

6

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

18

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

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22

## 23 **The rise of experimental coevolution**

24 Naturalists have long recognised the importance of species interactions as a driving force of  
25 adaptation. Indeed, 19<sup>th</sup>-century evolutionary biologists often cited the conspicuous  
26 coadaptations of interspecific pollination and mimicry **mutualisms** as exemplars of  
27 evolution by natural selection. It is perhaps surprising then that coevolution, the process of  
28 reciprocal adaptation and counter adaptation by ecologically interacting species, was not  
29 studied in earnest until the mid-20<sup>th</sup> century. The first wave of empirical coevolution research  
30 was predominantly observational and field-based [1, 2]. Such studies inferred the action of  
31 reciprocal selection indirectly, typically from spatial patterns of trait co-variation between  
32 populations or by comparative and phylogenetic analyses of ecologically interacting clades.  
33 These early studies strongly suggested that coevolution was a central process driving natural  
34 selection and shaping the structure and function of communities, while never being able to  
35 provide unequivocal evidence of reciprocal evolutionary changes.

36

37 To overcome certain limitations of fieldwork - chiefly that the action of other sources of  
38 selection driving the observed patterns can never be ruled out - researchers have sought to  
39 bring the study of coevolution into the lab. Here, environments can be precisely controlled  
40 to exclude extraneous sources of selection, and the use of fast-growing organisms like  
41 microbes or classic lab-model animals, allows for the direct observation of coevolution in  
42 real time (Figure 1 & Box 1). Significantly, since many such experimental systems are  
43 amenable to cryogenic preservation, this allows experimenters to perform “**time-shifts,**” for  
44 instance, testing the performance of parasites against hosts from the evolutionary past or  
45 future (Figure 2). By analyzing these time-shifted interactions between coevolving species the  
46 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**

71 *The tempo and mode of antagonistic coevolution*

72 According to the **Red Queen hypothesis**, reciprocal selection arising from **interspecific**  
73 **antagonisms**, such as host-parasite interactions, should accelerate evolutionary rates  
74 through the need for continual adaptation and counter-adaptation [8, 9]. (The history of the  
75 use of the Red Queen metaphor is described in refs. [10, 11].) Recent tests of this prediction  
76 have compared evolutionary rates under coevolution against controls where coevolution is  
77 prevented, for example in the presence versus absence of an antagonist (Figure 1), and  
78 provide strong support for this hypothesis from a range of species interactions. When co-  
79 cultured, *Caenorhabditis elegans*, and a bacterial parasite, *Bacillus thuringiensis*, both exhibit greater  
80 molecular evolutionary change, assessed by microsatellites and gene content respectively,  
81 than do control populations of the nematode or bacterium propagated alone [12]. However,  
82 for parasite species in particular the complete removal of the host is an extreme  
83 environmental alteration, necessitating comparison of populations propagated *in vivo* with *in*  
84 *vitro* controls. A more subtle manipulation is to allow one antagonist to evolve while holding  
85 the other in evolutionary stasis, by regularly replacing its entire population with individuals of  
86 the ancestral genotype. By this approach, it has been demonstrated, using pooled whole-  
87 genome resequencing, that genomes of bacteriophage virus  $\Phi 2$  coevolving with the  
88 bacterium *Pseudomonas fluorescens* evolve at double the rate of  $\Phi 2$  populations evolving against  
89 a fixed, ancestral *P. fluorescens* genotype [13]. Similarly, whole genome analysis of *Escherichia*  
90 *coli* and the bacteriophage Q $\beta$  revealed increased mutational change in coevolving, relative to  
91 evolving populations of both host and parasite [14]. These studies strongly support the Red  
92 Queen view of interspecific antagonisms as a strong driver of evolutionary change and, for  
93 the first time, have allowed for direct tests of causation rather than correlation.

94

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  $\Phi 2$ ,

119 while initially dominated by ARD, becomes increasingly FSD-like through time [19]. This  
120 appears to arise because, after a certain point, the costs to individual genotypes of accruing  
121 additional mutations that further increase the breadth of infectivity or resistance were  
122 unviable. The increasing costs act to prevent fixation of super-generalist genotypes and  
123 progressively weaken the response to directional selection over time. These findings suggest  
124 that, at least in part, the prevailing mode of reciprocal selection is determined by the  
125 coevolutionary history of an association and more long-term studies are required to resolve  
126 this. There is now a clear need for the development of coevolutionary theory targeted at  
127 resolving the impact of mixed modes of reciprocal selection on coevolutionary processes and  
128 at understanding the genetic and ecological factors driving switches in the prevailing mode of  
129 reciprocal selection.

130

### 131 *Antagonistic coevolution and evolvability*

132 The pressure for continual innovation during **antagonistic coevolution** can, in theory,  
133 select for mechanisms that increase **evolvability**, particularly in hosts, since they are often  
134 assumed to possess less evolutionary potential than their parasites [20]. Greater genetic  
135 diversity within a population increases the efficacy of selection and, notwithstanding  
136 immigration, can be achieved through increased rates of mutation or recombination. Studies  
137 across a range of species interactions strongly support the hypothesis that antagonistic  
138 coevolution selects for evolvability in hosts. The evolution of **hypermutable** *P. fluorescens*  
139 genotypes, with defective DNA proofreading enzymes, was found to occur at a higher  
140 frequency in populations coevolving with phage  $\Phi$ 2 than those evolving alone [21]. Similarly,  
141 more spontaneous mutations were observed in *C. elegans* that had been coevolving with *B.*  
142 *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  $\Phi 2$  undergo an almost 10 $\times$  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  $\Phi 2$  [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

191 *Specificity of antagonistic coevolutionary interactions*

192 Key to our understanding of coevolutionary dynamics is the underlying genetic specificity of  
193 the interaction and the emergent patterns of interaction specificity. Experiments with  
194 bacteria and phage have revealed that coevolution can lead to a nested interaction structure  
195 [40, 41], such that hard to infect bacterial genotypes are infected by generalist but not  
196 specialist phage genotypes [42]. Moreover, coevolving bacteria phage populations can harbor,  
197 at any given time, a diverse mix of phenotypes, ranging from specialists to generalists [26, 40],  
198 which is dynamic and variable through time. Interestingly, coevolution itself appears to be  
199 crucial in shaping host-range of some phages. In  $\Phi 2$ , spontaneous host-range mutants  
200 selected to infect a novel host genotype evolved narrower host ranges than did phages with a  
201 history of coevolution against this host genotype [43]. Here, broad host ranges relied upon  
202 the accumulation of multiple adaptive mutations acquired through repeated rounds of  
203 selection for infectivity. Similarly, the evolution of particular resistant bacterial genotypes in  
204 coevolving populations of *E. coli* and  $\lambda$  were necessary for the subsequent evolution by  
205 phage of the ability to bind to a new host receptor, OmpF, which was found to require the  
206 stepwise accumulation of four adaptive mutations [44]. Both studies highlight the  
207 importance of historical contingency in determining the trajectory of coevolution.

208

209 In addition to the effects of limited mutational supply, the evolution of generalists can also  
210 be constrained by costs associated with resistance and infectivity mutations. Often such  
211 trade-offs are expected due to **antagonistic pleiotropy**. In the case of bacteria-phage  
212 coevolution, phages often bind to bacterial cell-surface proteins that perform important  
213 functions, such as nutrient uptake or motility, and mutations conferring resistance to phages  
214 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  $\phi 6$ , 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 *Ocosporea 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  $\Phi 2$  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

263 communities had faster growth rates and higher yields than ancestral communities. Time-  
264 shifted pairings confirmed that adaptations in each species contributed to community-level  
265 improvements in growth rate and yield. This study highlights the utility of experimental  
266 coevolution for understanding species interactions in general, and beyond antagonistic  
267 interactions, and furthermore demonstrates the need for more studies of mutualistic species  
268 interactions.

269

### 270 *Coevolution of complex communities*

271 While most experimental coevolution has employed pairs of species, species interaction  
272 networks in nature are often complex. Scaling experimental coevolution studies up to the  
273 community level is a key next step. A study of *P. syringae* coevolving with multiple phages  
274 found that bacterial hosts are able to evolve resistance against multiple phages  
275 simultaneously, but that they pay a higher cost for these multiple resistances when grown in  
276 the absence of phage [55]. Addition of a protist predator, *Tetrahymena thermophila*, to  
277 coevolving populations of *P. fluorescens* and  $\Phi 2$  impeded ARD coevolution between the  
278 bacteria and phage, and favoured the maintenance of coexisting resistance phenotypes  
279 specialized against one or other of these natural enemies [56]. Generalist bacterial resistance  
280 presumably did not evolve in these communities due to the existence of fitness trade-offs  
281 associated with multiple resistances. Networks of species interactions can also shape the  
282 evolution and stability of the community as a whole. Experimental communities of naturally  
283 co-occurring bacteria collected from holes in beech trees found that the interactions among  
284 these species were key to their ability to adapt to novel environments in the laboratory [57].  
285 These species, when propagated in communities, evolved more over 70 generations than  
286 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  $\Phi$ X174 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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526 **Box 1. When is it experimental coevolution?**

527 In a classic article, Janzen defined the term coevolution [72], which at the time had become  
528 broadly and imprecisely applied by researchers of species interactions. Janzen stressed the  
529 requirement for the demonstration of adaptations in both species arising from reciprocal  
530 selection before a pattern should be attributed to coevolution. This definition of coevolution  
531 based on evolutionary outcomes is valuable for distinguishing coevolved adaptations but is  
532 not useful for defining an experimental approach to the study of coevolution. We propose  
533 that the term “experimental coevolution” should be applied to experiments where either:  
534 (a.) interacting species are co-cultured *and* experimenters attempt to quantify evolutionary  
535 responses in both (or all if >2) interacting species; or (b.) interacting species are co-cultured  
536 *and* evolutionary responses of populations from coevolving treatments are compared to  
537 evolutionary responses of populations from control treatments where coevolution is  
538 prevented.

539 One of the most powerful aspects of experimental coevolution is that control  
540 treatments can be used to tease apart evolutionary change, based on adaptation to the abiotic  
541 environment and/or drift, from coevolutionary change. The exact approach depends on the  
542 system being used and the question being addressed, but one option is to compare the  
543 evolution of each species alone with the coevolution of the two. This approach can be used  
544 to tease apart selection imposed by abiotic versus biotic factors, for example by specifically  
545 identifying the responses to parasite-mediated selection. However, to specifically tease apart  
546 evolution in response to a biotic agent of selection from coevolutionary change requires the  
547 introduction of a “one-sided evolution” treatment, where one of the partners is held in  
548 evolutionary stasis while the other is allowed to evolve. This one-sided evolution treatment  
549 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

569 **Glossary of terms**

570 Antagonistic coevolution/Interspecific antagonism: Coevolution is the reciprocal adaptation  
571 and counter adaptation of species that interact ecologically. When the fitnesses of the two  
572 species are negatively correlated, such that an adaptation that increases fitness in one species  
573 decreases in fitness of the other species and *vice versa*, these species interactions are termed  
574 antagonistic.

575 Antagonistic pleiotropy: A situation where one gene underlies more than one trait, and  
576 where an adaptation that is advantageous in one biotic or abiotic environment is deleterious  
577 in another.

578 Arms Race Dynamics (ARD): A mode of antagonistic coevolution driven by directional  
579 selection whereby hosts and parasites respectively accumulate resistance or infectivity alleles  
580 through a series of recurrent selective sweeps. This process leads, through time, to an  
581 increase in the range of parasite genotypes hosts can resist and an increase in the range of  
582 host genotypes that parasites can infect.

583 Cophylogeny: An approach by which the macroevolutionary histories of two clades are  
584 compared, for example to determine whether evolutionary branching of one species is  
585 correlated with branching in another.

586 Evolutionary stasis: This occurs when a population remains genetically constant over time.  
587 This can be manipulated during experimental coevolution by continually replacing the  
588 population of one of the two partners with the ancestral genotype in order to prevent  
589 evolution in this species.

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

592 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.

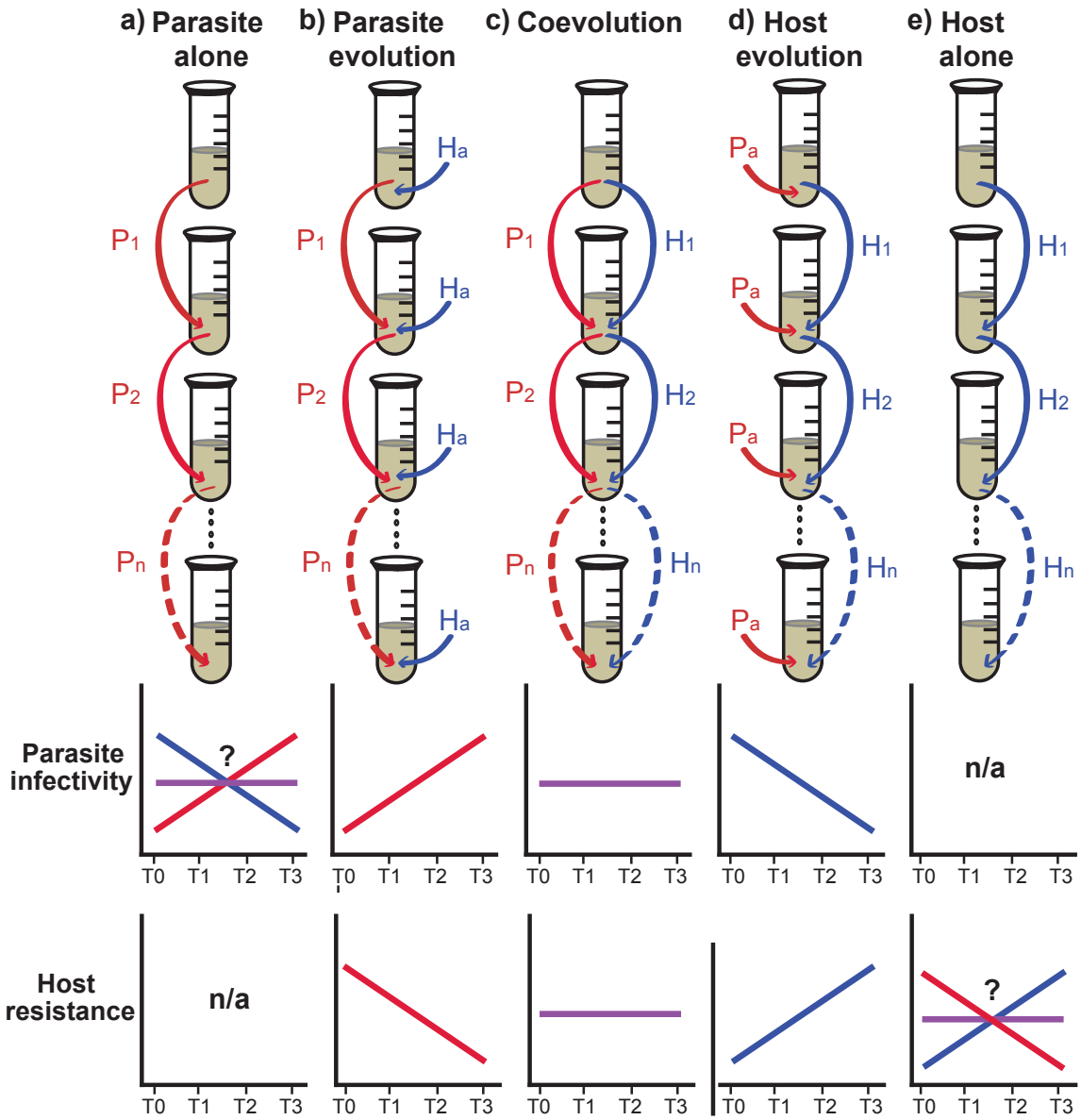
653 An illustrative example of techniques used to compare coevolution of two species (in this  
654 case, host and parasite) by examining changes in replicate experimental populations (or  
655 metapopulations, if connected by gene flow). A time shift experiment (a) can be performed  
656 across experimental 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
<b>Invertebrate victim</b>						
<i>C. elegans</i> - <i>B. thuringiensis</i>	Single species	•	✓	✓	✓	[12, 74]12, 73]
<i>C. elegans</i> - <i>S. marcescens</i>	Evolution	•	•	✓	✓	[75]
<i>P. antipodarum</i> - <i>Microphallus</i> sp.	Single species	•	✓	✓	✓	[[16, 76]
<i>T. castaneum</i> - <i>Noseum whitei</i>	Single species	✓	•	✓	✓	[[22, 27, 77]
<i>B. glabrata</i> - <i>S. mansoni</i>	Single species	•	✓	✓	✓	[[66, 78]
<i>D. Magna</i> - <i>O. bayeri</i>	Single species	•	•	✓	•	[51]
<b>Protist victim</b>						
<i>P. caudatum</i> - <i>H. undulata</i>	Single species	•	✓	✓	x	[79]
<b>Bacterial victim</b>						
<i>P. fluorescens</i> - phage Φ2	Evolution	✓	✓	✓	✓	[[40, 80, 81]
<i>P. aeruginosa</i> - phage PP7	None	✓	•	✓	x	[82]
<i>E. coli</i> - phage Qβ	Evolution	•	•	✓	✓	[83]
<i>E. coli</i> - phage T7	None	•	✓	✓	✓	[30]
<i>E. coli</i> - phage T4	Single species	•	•	✓	•	[84]
<i>E. coli</i> - phage PP01	None	•	•	✓	✓	[85]
<i>Synechococcus</i> sp. - phage RIM8	Single species	•	•	✓	✓	[86]
<i>S. marcescens</i> - <i>T. themophila</i>	Single species	•	•	✓	x	[[87, 88]

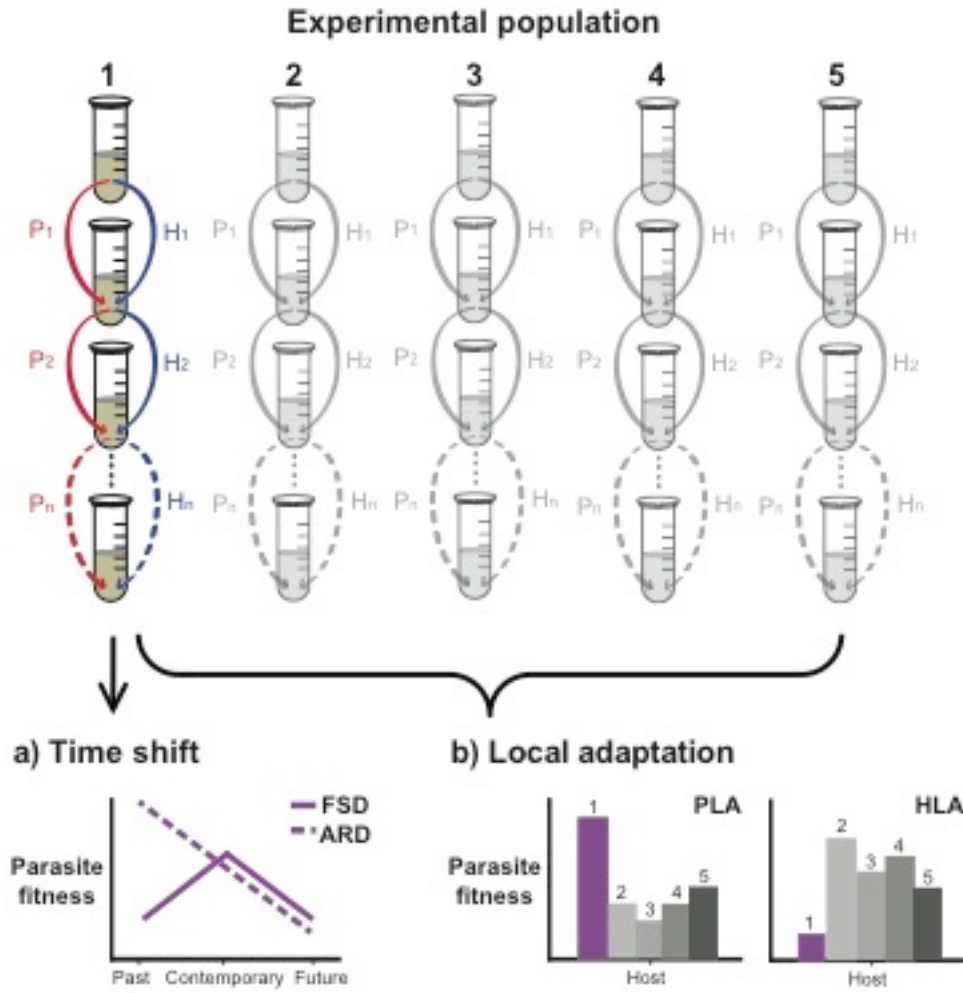
668

669 Table 1



670

671 Figure 1



672

673 Figure 2