UNIVERSITY of York

This is a repository copy of *Experimental coevolution of species interactions*.

White Rose Research Online URL for this paper: <u>https://eprints.whiterose.ac.uk/77995/</u>

Version: Submitted Version

Article:

Brockhurst, Michael orcid.org/0000-0003-0362-820X and Koskella, Britt (2013) Experimental coevolution of species interactions. Trends in Ecology & Evolution. pp. 367-375. ISSN 0169-5347

https://doi.org/10.1016/j.tree.2013.02.009

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk https://eprints.whiterose.ac.uk/

Experimental coevolution of species interactions 1 Michael A. Brockhurst¹ and Britt Koskella² 2 3 4 ¹ Department of Biology, University of York, York, YO10 5DD, UK 5 ² 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 20 Published in Trends in Ecology and Evolution 28:367-375 doi: 10.1016/j.tree.2013.02.009 21

23 The rise of experimental coevolution

24 Naturalists have long recognised the importance of species interactions as a driving force of 25 adaptation. Indeed, 19th-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 studied in earnest until the mid-20th century. The first wave of empirical coevolution research 29 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

To overcome certain limitations of fieldwork - chiefly that the action of other sources of 37 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 49 also be established (Figure 1). Comparison of coevolving lineages against control lineages 50 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 Φ^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.

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 Φ_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 experimentally168 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 Φ 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].

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 λ 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

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 $\phi 6$, 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

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. But as the field matures it is taking some exciting new directions; in what follows, we outline several promising emerging research directions.

233

234 Experimental coevolution in 'real-world' environments

While 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, 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 $\Phi 2$ 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 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 allow great insight to the assembly, structure, function, and
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 coevolution 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 to explore the coevolutionary implications of these treatments is a clear next step and experimental coevolution could be fruitfully employed to select for stable microbial consortia with desirable traits for use in probiotics.

338

339 Acknowledgements

We are grateful to the reviewers for constructive comments on a previous version of this manuscript. This work was supported by a project grant (NE/H005080/1) to MAB and a research fellowship (R16150) to BK from the Natural Environment Research Council (UK).

343

344 Cited literature

- 345 1 Janzen, D.H. (1966) Coevolution of Mutualism between Ants and Acacias in Central
- 346 America. *Evolution* 20, 249-&
- 2 Ehrlich, P.R. and Raven, P.H. (1964) Butterflies and Plants a Study in Coevolution. *Evolution* 18, 586-608
- 349 3 Gaba, S. and Ebert, D. (2009) Time-shift experiments as a tool to study antagonistic
- 350 coevolution. *Trends Ecol. Evol.* 24, 226-232
- 351 4 Decaestecker, E., et al. (2007) Host-parasite 'Red Queen' dynamics archived in pond
- 352 sediment. Nature 450, 870-U816
- 353 5 Horne, M.T. (1970) Coevolution of Escherichia-Coli and Bacteriophages in Chemostat
- 354 Culture. *Science* 168, 992-&
- 6 Cowlishaw, J. and Mrsa, M. (1975) Co-Evolution of a Virus-Alga System. *Appl Microbiol* 29,
 234-239
- 357 7 Chao, L., et al. (1977) Complex Community in a Simple Habitat Experimental-Study with
- 358 Bacteria and Phage. *Ecology* 58, 369-378

- 359 8 Van Valen, L. (1973) A new evolutionary law. Evol. Theory 1, 1-30
- 360 9 Bell, G. (1982) The Masterpiece of Nature: The Evolution and Genetics of Sexuality. University of
- 361 California Press
- 362 10 Wilkinson, D.M. (2000) Running with the Red Queen: refelctions on 'Sex versus non-sex
- 363 versus parasite'. Oikos 91, 589-596
- 364 11 Brockhurst, M.A. (2011) Sex, Death and the Red Queen. Science 333, 166-167
- 365 12 Schulte, R.D., et al. (2010) Multiple reciprocal adaptations and rapid genetic change upon
- 366 experimental coevolution of an animal host and its microbial parasite. P Natl Acad Sci USA
- **367** 107, 7359-7364
- 368 13 Paterson, S., et al. (2010) Antagonistic coevolution accelerates molecular evolution. Nature
- **369** 464, 275-278
- 370 14 Kashiwagi, A. and Yomo, T. (2011) Ongoing Phenotypic and Genomic Changes in
- 371 Experimental Coevolution of RNA Bacteriophage Q β and Escherichia coli. *PLoS Genet.*
- 372 15 Gandon, S., et al. (2008) Host-parasite coevolution and patterns of adaptation across time
- 373 and space. J Evolution Biol 21, 1861-1866
- 374 16 Koskella, B. and Lively, C.M. (2009) Evidence for Negative Frequency-Dependent
- 375 Selection During Experimental Coevolution of a Freshwater Snail and a Sterilizing
- 376 Trematode. *Evolution* 63, 2213-2221
- 377 17 Fenton, A., et al. (2012) Two-step infection processes can lead to coevolution between
- 378 functionally independent infection and resistance pathways. *Evolution* 66, 2030-2041
- 379 18 Agrawal, A.F. and Lively, C.M. (2003) Modelling infection as a two-step process
- 380 combining gene-for-gene and matching-allele genetics. P Roy Soc Lond B Bio 270, 323-334
- 381 19 Hall, A.R., et al. (2011) Host-parasite coevolutionary arms races give way to fluctuating
- 382 selection. *Ecol. Lett.* 14, 635-642

- 20 Gandon, S. (2002) Local adaptation and the geometry of host-parasite coevolution. *Ecol. Lett.* 5, 246-256
- 385 21 Pal, C., et al. (2007) Coevolution with viruses drives the evolution of bacterial mutation
- 386 rates. *Nature* 450, 1079-1081
- 387 22 Kerstes, N., et al. (2012) Antagonistic experimental coevolution with a parasite increases
- 388 host recombination frequency. BMC Evol. Biol. 12, 18
- 389 23 Greeff, M. and Schmid-Hempel, P. (2010) Influence of co-evolution with a parasite,
- 390 Nosema whitei, and population size on recombination rates and fitness in the red flour
- 391 beetle, Tribolium castaneum. Genetica 138, 737-744
- 392 24 Morran, L.T., et al. (2011) Running with the Red Queen: Host-Parasite Coevolution
- 393 Selects for Biparental Sex. *Science* 333, 216-218
- 394 25 Forde, S.E., et al. (2008) Coevolution drives temporal changes in fitness and diversity
- across environments in a bacteria-bacteriophage interaction. *Evolution* 62, 1830-1839
- 396 26 Marston, M.F., et al. (2012) Rapid diversification of coevolving marine Synechococcus
- 397 and a virus. P Natl Acad Sci USA 109, 4544-4549
- 398 27 Bérénos, C., et al. (2011) Antagonistic coevolution with parasites maintains host genetic
- 399 diversity: an experimental test. Proc. R. Soc. Lond. B Biol. Sci. 278, 218-224
- 400 28 Buckling, A. and Rainey, P.B. (2002) The role of parasites in sympatric and allopatric host
- 401 diversification. *Nature* 420, 496-499
- 402 29 Morgan, A.D., et al. (2005) The effect of migration on local adaptation in a coevolving
- 403 host-parasite system. *Nature* 437, 253-256
- 404 30 Forde, S.E., et al. (2004) Adaptation varies through space and time in a coevolving host-
- 405 parasitoid interaction. Nature 431, 841-844
- 406 31 Vogwill, T., et al. (2010) How does spatial dispersal network affect the evolution of

- 407 parasite local adaptation? *Evolution; international journal of organic evolution* 64, 1795-1801
- 408 32 Hoeksema, J.D. and Forde, S.E. (2008) A meta-analysis of factors affecting local
- 409 adaptation between interacting species. Am Nat 171, 275-290
- 410 33 Greischar, M.A. and Koskella, B. (2007) A synthesis of experimental work on parasite
- 411 local adaptation. *Ecol Lett* 10, 418-434
- 412 34 Thompson, J.N. (1994) The Coevolutionary Process. University of Chicago Press
- 413 35 Thompson, J.N. (2005) The geographic mosaic of coevolution. University of Chicago Press
- 414 36 Lopez Pascua, L., et al. (2012) Abiotic heterogeneity drives parasite local adaptation in
- 415 coevolving bacteria and phages. J. Evol. Biol. 25, 187-195
- 416 37 Brockhurst, M.A., et al. (2004) The effect of spatial heterogeneity and parasites on the
- 417 evolution of host diversity. P Roy Soc Lond B Bio 271, 107-111
- 418 38 Vogwill, T., et al. (2011) Coevolving parasites enhance the diversity-decreasing effect of
- 419 dispersal. *Biol Letters* 7, 578-580
- 420 39 Bérénos, C., et al. (2012) Antagonistic coevolution accelerates the evolution of
- 421 reproductive isolation in Tribolium castaneum. Am Nat 180, 520-528
- 422 40 Poullain, V., et al. (2008) The evolution of specificity in evolving and coevolving
- 423 antagonistic interactions between a bacteria and its phage. *Evolution; international journal of*
- 424 organic evolution 62, 1-11
- 425 41 Scanlan, P.D., et al. (2011) Genetic basis of infectivity evolution in a bacteriophage. Mol
- 426 *Ecol* 20, 981-989
- 427 42 Flores, C.O., et al. (2011) Statistical structure of host-phage interactions. P Natl Acad Sci
- 428 USA 108, E288-E297
- 429 43 Hall, A.R., et al. (2011) Bacteria-Phage Coevolution and the Emergence of Generalist
- 430 Pathogens. Am Nat 177, 44-53

- 431 44 Meyer, J.R., et al. (2012) Repeatability and Contingency in the Evolution of a Key
- 432 Innovation in Phage Lambda. *Science* 335, 428-432
- 433 45 Bohannan, B.J.M., et al. (1999) Epistatic interactions can lower the cost of resistance to
- 434 multiple consumers. *Evolution* 53, 292-295
- 435 46 Brockhurst, M.A., et al. (2005) The effect of a bacteriophage on diversification of the
- 436 opportunistic bacterial pathogen, Pseudomonas aeruginosa. P R Soc B 272, 1385-1391
- 437 47 Avrani, S., et al. (2012) Virus-host swinging party in the oceans: Incorporating biological
- 438 complexity into paradigms of antagonistic coexistence. *Mobile Genetic Elements* 2, 88-95
- 439 48 Duffy, S., et al. (2006) Pleiotropic costs of niche expansion in the RNA bacteriophage $\Phi 6$.
- 440 *Genetics* 172, 751-757
- 441 49 Buckling, A., et al. (2006) Antagonistic coevolution with parasites increases the cost of
- 442 host deleterious mutations. *P R Soc B* 273, 45-49
- 443 50 Fenton, A. and Brockhurst, M.A. (2007) Epistatic Interactions Alter Dynamics of
- 444 Multilocus Gene-for-Gene Coevolution. Plos One 2, -
- 445 51 Zbinden, M., et al. (2008) Experimental evolution of field populations of Daphnia magna
- 446 in response to parasite treatment. J. Evol. Biol. 21, 1068 1078
- 52 Gomez, P. and Buckling, A. (2011) Bacteria-Phage Antagonistic Coevolution in Soil. *Science* 332, 106-109
- 449 53 Jeremy B. Yoder and Scott L. Nuismer (2010) When Does Coevolution Promote
- 450 Diversification? *Am Nat* 176, 802-817
- 451 54 Hillesland, K.L. and Stahl, D.A. (2010) Rapid evolution of stability and productivity at the
- 452 origin of a microbial mutualism. Proceedings of the National Academy of Sciences 107, 2124-2129
- 453 55 Koskella, B., et al. (2012) The costs of evolving resistance in heterogeneous parasite
- 454 environments. Proc. R. Soc. Lond. B Biol. Sci. 279, 1896-1903

- 455 56 Friman, V.P. and Buckling, A. (2012) Effects of predation on real-time host-parasite
- 456 coevolutionary dynamics. *Ecol Lett*
- 457 57 Lawrence, D., et al. (2012) Species Interactions Alter Evolutionary Responses to a Novel
- 458 Environment. PLoS Biol 10, e1001330
- 459 58 Dougherty, E.C. (1949) The phylogeny of the nematode family Metastrongylidae Leiper,
- 460 [1909]: a correlation of host and symbiote evolution. *Parasitology* 39, 222-234
- 461 59 Weber, M.G. and Agrawal, A.A. (2012) Phylogeny, ecology, and the coupling of
- 462 comparative and experimental approaches. Trends in Ecology & amp; Evolution 27, 394-403
- 463 60 Thompson, J.N. (2005) The Geographic Mosaic of Coevolution. The University of Chicago
- 464 Press
- 465 61 Best, A., et al. (2010) The Evolution of Host-Parasite Range. Am Nat 176, 63-71
- 466 62 Weber, M.G. and Agrawal, A.A. (2012) Phylogeny, ecology, and the coupling of
- 467 comparative and experimental approaches. *Trends in Ecology and Evolution* 27, 394-403
- 468 63 Bull, J.J., et al. (1997) Exceptional Convergent Evolution in a Virus. Genetics 147, 1497-
- **469** 1507
- 470 64 Turner, P.E., et al. (2012) Evolutionary genomics of host-use in bifurcating demes of
- 471 RNA virus phi-6. Bmc Evol Biol 12, 153
- 472 65 Buckling, A., et al. (2009) The Beagle in a bottle. Nature 457, 824-829
- 473 66 Webster, J., et al. (2007) Is host-schistosome coevolution going anywhere? BMC Evol. Biol.
- 474 7,91
- 475 67 Van den Abbeele, P., et al. (2011) The host selects mucosal and luminal associations of
- 476 coevolved gut microorganisms: a novel concept. FEMS Microbiol. Rev. 35, 681-704
- 477 68 O'Flynn, G., et al. (2004) Evaluation of a Cocktail of Three Bacteriophages for Biocontrol
- 478 of Escherichia coli O157:H7. Appl. Environ. Microbiol. 70, 3417-3424

- 479 69 Hall, A.R., et al. (2012) Effects of Sequential and Simultaneous Applications of
- 480 Bacteriophages on Populations of Pseudomonas aeruginosa In Vitro and in Wax Moth
- 481 Larvae. Appl. Environ. Microbiol. 78, 5646-5652
- 482 70 Barbas, A.S., et al. (2009) Altering and Assessing Persistence of Genetically Modified E.
- 483 coli MG1655 in the Large Bowel. Exp. Biol. Med. 234, 1174-1185
- 484 71 Lee, S.M., et al. (2010) Adaptation in a Mouse Colony Monoassociated with Escherichia
- 485 coli K-12 for More than 1,000 Days. Appl. Environ. Microbiol. 76, 4655-4663
- 486 72 Janzen, D.H. (1980) When is it coevolution? *Evolution* 34, 611-612
- 487 73 Blanquart, F. and Gandon, S. (2013) Time-shift experiments and patterns of adaptation
- 488 across time and space. *Ecol Lett* 16, 31-38
- 489 74 Schulte, R.D., et al. (2011) Host-parasite local adaptation after experimental coevolution
- 490 of Caenorhabditis elegans and its microparasite Bacillus thuringiensis. *Proc. R. Soc. Lond. B*491 *Biol. Sci.* 278, 2832-2839
- 492 75 Morran, L.T., et al. (2012) TEMPORAL DYNAMICS OF OUTCROSSING AND
- 493 HOST MORTALITY RATES IN HOST-PATHOGEN EXPERIMENTAL
- 494 COEVOLUTION. *Evolution*, no-no
- 495 76 Koskella, B. and Lively, C.M. (2007) Advice of the rose: Experimental coevolution of a
- 496 trematode parasite and its snail host. *Evolution* 61, 152-159
- 497 77 Bérénos, C., et al. (2009) Evolution of host resistance and trade-offs between virulence
- 498 and transmission potential in an obligately killing parasite. J. Evol. Biol. 22, 2049-2056
- 499 78 Webster, J.P. and Woolhouse, M.E.J. (1998) Selection and Strain Specificity of
- 500 Compatibility between Snail Intermediate Hosts and Their Parasitic Schistosomes. Evolution
- 501 52, 1627-1634
- 502 79 Lohse, K., et al. (2006) Experimental evolution of resistance in Paramecium caudatum against

- 503 the bacterial parasite Holospora undulate Evolution 60, 1177-1186
- 504 80 Buckling, A. and Rainey, P.B. (2002) Antagonistic coevolution between a bacterium and a
- 505 bacteriophage. Proc. R. Soc. Lond. B Biol. Sci. 269, 931-936
- 506 81 Brockhurst, M.A., et al. (2003) Population mixing accelerates coevolution. Ecol. Lett. 6,
- **507** 975-979
- 508 82 Brockhurst, M., Buckling, A, Rainey, PB (2006) Spatial heterogeneity and the stability of
- 509 host-parasite coexistence. J. Evol. Biol. 19, 374-379
- 510 83 Kashiwagi, A. and Yomo, T. (2011) Ongoing Phenotypic and Genomic Changes in
- 511 Experimental Coevolution of RNA Bacteriophage Q β and Escherichia coli. *PLoS Genet.* 7,
- 512 e1002188
- 513 84 Bohannan, B. and Lenski, R. (1997) Effect of resource enrichment on a chemostat
- 514 community of bacteria and bacteriophage. *Ecology* 78, 2303-2315
- 515 85 Mizoguchi, K., et al. (2003) Coevolution of bacteriophage PP01 and Escherichia coli
- 516 O157: H7 in continuous culture. Appl. Environ. Microbiol. 69, 170-176
- 517 86 Marston, M.F., et al. (2012) Rapid diversification of coevolving marine Synechococcus
- 518 and a virus. Proceedings of the National Academy of Sciences 109, 4544-4549
- 519 87 Friman, V.-P. and Laakso, J. (2011) Pulsed-Resource Dynamics Constrain the Evolution
- 520 of Predator-Prey Interactions. Am Nat 177, 334-345
- 521 88 Friman, V.P., et al. (2008) Availability of prey resources drives evolution of predator-prey
- 522 interaction. Proc. R. Soc. Lond. B Biol. Sci. 275, 1625-1633
- 523
- 524

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

569 Glossary of terms

570 <u>Antagonistic coevolution/Interspecific antagonism</u>: 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 <u>Antagonistic pleiotropy:</u> 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 <u>Arms Race Dynamics (ARD)</u>: 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 <u>Cophylogeny</u>: 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 <u>Evolutionary stasis</u>: 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 <u>Evolvability</u>: The ability of a population to generate genetic diversity thereby allowing it to
591 respond to selection.

592 <u>Host-range</u>: The 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 others.

596 <u>Fluctuating Selection Dynamics</u>: 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 <u>Hypermutable</u>: Strains of bacteria with mutation rates far in excess of the wild-type; these
 602 typically arise through mutations altering mismatch repair enzymes.

<u>Interspecific facilitation</u>: A scenario whereby 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 case are considered to be interspecific mutualisms.
 <u>Mutualisms</u>: Mutually beneficial species interactions, which in reality are often mutually
 exploitative interactions but where net benefits accrue to both parties.

609 <u>Phage therapy</u>: The use of bacteriophage viruses to control the growth and/or harmfulness
610 of pathogenic bacteria.

611 <u>Phenotypic matching</u>: 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 <u>Red Queen hypothesis</u>: 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 sexual618 recombination.
- 619 <u>Syntrophic mutualism</u>: A form of microbial mutualism where the transfer of metabolites620 between species is essential for growth.
- 621 <u>Time-shift experiment</u>: 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 <u>Table 1. The experimental systems of antagonistic experimental coevolution</u>.

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

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	•	~	~	v	[[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	•	~	~	X	[79]
Bacterial victim						
P. fluorescens - 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]
Synechococcus sp phage RIM8	Single species	•	•	~	~	[86]
S. marscesens - T. themophila	Single species	•	•	~	×	[[87, 88]

669 Table 1







673 Figure 2