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Running with the Red Queen: Host-parasite coevolution selects for biparental sex

Levi T. Morran[1,*], **Olivia G. Schmidt**[1], **Ian A. Gelarden**[1], **Raymond C. Parrish II**[1], and **Curtis M. Lively**[1]

[1]Department of Biology, Indiana University, 1001 E. Third St., Bloomington, IN 47405

Abstract

Most organisms reproduce through outcrossing, even though it comes with significant costs. The Red Queen hypothesis proposes that selection from coevolving pathogens facilitates the persistence of outcrossing in spite of these costs. We utilized experimental coevolution to test the Red Queen hypothesis, and found that coevolution with a bacterial pathogen (*Serratia marcescens*) resulted in significantly more outcrossing in mixed mating experimental populations of the nematode *Caenorhabditis elegans*. Furthermore, we found that coevolution with the pathogen rapidly drove obligately selfing populations to extinction, while outcrossing populations persisted through reciprocal coevolution. Thus, consistent with the Red Queen hypothesis, coevolving pathogens can select for biparental sex.

Keywords

outcrossing; self-fertilization; coevolution; *C. elegans*; *S. marcescens*; Red Queen hypothesis

Outcrossing (i.e., mating between different individuals) is the most prevalent mode of reproduction among plants and animals. The maintenance of outcrossing on such a large scale strongly suggests that there is a selective advantage for outcrossing relative to self-fertilization or asexual reproduction. Nonetheless, the prevalence of outcrossing is puzzling, because it often incurs costs that are not associated with uniparental modes of reproduction (1–3). For example, many outcrossing species produce males that facilitate outcrossing, but are incapable of bearing offspring themselves, resulting in the “cost of males.” Every male takes the place of an offspring-bearing progeny (female or hermaphrodite) that could have been produced (2). The systematic loss of offspring-bearing progeny can reduce the numerical contribution of a lineage by as much fifty percent (2). Therefore, the selective benefits of outcrossing must more than compensate for this fitness deficit to achieve a high frequency in nature.

One selective benefit of outcrossing, relative to self-fertilization, is the capability to produce offspring with greater fitness under novel environmental conditions (4, 5). Outcrossing can increase fitness and accelerate a population’s rate of adaptation to novel conditions by permitting genetic exchange between diverse lineages, promoting genetic variation among offspring, and allowing beneficial alleles to be quickly assembled into the same genome (6, 7). In contrast, obligate selfing can impede adaptation by preventing genetic exchange, which results in the loss of within-lineage genetic variation, and ultimately confines beneficial alleles to a single lineage (8, 9). Under novel environmental conditions, the benefits of outcrossing can compensate for the cost of male production, but these benefits

[*]corresponding author: lmorran@indiana.edu Phone: 812-855-3282.

may be short lived (5). Outcrossing is less likely to be favored after populations adapt to a novel environment, as genetic exchange becomes less imperative, or perhaps even deleterious (8, 9). Hence, the long-term maintenance of outcrossing would seem to require that populations are constantly exposed to novel environmental conditions.

The Red Queen hypothesis provides a possible explanation for the long-term maintenance of outcrossing. Specifically, under the Red Queen hypothesis, coevolutionary interactions between hosts and pathogens might generate ever-changing environmental conditions, and thus favor the long-term maintenance of outcrossing relative to self-fertilization (10) or asexual reproduction (11, 12). The reason is that hosts are under selection to evade infection by the pathogen, while the pathogen is selected to infect the hosts. Assuming that some form of genetic matching between host and pathogen determines the outcome of interactions, pathogen genotypes that infect the most common host genotypes will be favored by natural selection (11, 13). This may produce substantial and frequent change in pathogen populations, thus rapidly changing the environment for the host population. Under these conditions, outcrossing can facilitate rapid adaptation by generating offspring with rare or novel genotypes, which are more likely to escape infection by coevolving pathogens (10–13). Conversely, selfing and asexual reproduction generate offspring with little or no genetic diversity, thus impeding the adaptive process and leaving them highly susceptible to infection by coevolving pathogens (10–13).

The Red Queen hypothesis has been empirically supported in studies of natural snail populations, which show that sexual reproduction is more common where parasites are common and adapted to infect the local host population (14, 15). Outcrossing also seems to reduce the degree of infection relative to biparental inbreeding and asexual reproduction in fish (16). Finally, the capability of antagonistic interactions to drive rapid evolutionary change has also been determined for several different systems (17–20). Nonetheless, direct controlled tests for the effect of coevolution on the maintenance of sex have proven difficult, as they require biological systems in which host and pathogen populations can coevolve for multiple generations in a manner that selects for increased infectivity by a pathogen as well as increased resistance (or enhanced avoidance) by the host. Further, the host species should exhibit genetic variation in its degree of outcrossing. Thus we chose to examine the nematode, *Caenorhabditis elegans*, and its pathogenic bacteria, *Serratia marcescens,* which exhibit these desired properties.

Populations of the host species, *C. elegans*, are composed of males and hermaphrodites. The hermaphrodites can reproduce through either self-fertilization or by outcrossing with males (21). Although usually low (<1% to 30%) (22), outcrossing rates can be genetically manipulated to produce either obligately selfing (5, 23) or obligately outcrossing (5, 24) populations. The pathogen, *S. marcescens* 2170, is highly virulent and capable of exerting strong selection on *C. elegans*. When consumed, live *S. marcescens* can produce a systemic infection that kills the nematode within 24 hours (25). This interaction has a heritable genetic basis (26), which allows for a potential response to selection. Moreover, *C. elegans* populations are capable of evolving greater fitness in response to *S. marcescens* exposure (5); and *S. marcescens* can evolve greater infectivity when successful infection of *C. elegans* is its only means of proliferation. Selection for increased infectivity can be imposed by propagating only those bacterial cells that have been harvested from the carcasses of hosts, which were killed by the bacteria within twenty-four hours of exposure. Therefore, the *C. elegans*/*S. marcescens* system can be used to generate antagonistic coevolution when a host population and a pathogen population are repeatedly passaged under selection together, thus permitting a direct test of the Red Queen hypothesis.

We used experimental coevolution in the *C. elegans*/*S. marcescens* system to test the prediction that antagonistic coevolution between host and pathogen populations can maintain high levels of outcrossing despite the inherent cost of males. We used obligately selfing, wildtype, and obligately outcrossing populations of *C. elegans* with a CB4856 genetic background (5). Whereas the reproductive modes of the obligately selfing and obligately outcrossing populations are genetically fixed, the wildtype populations can reproduce by either selfing or outcrossing (the baseline outcrossing rate is approximately 20–30% (5)); and the rate of outcrossing can respond to selection (5). Prior to the experiment, we mutagenized five independent replicate populations of each mating type (obligate selfing, wildtype, obligate outcrossing) by exposing them to ethyl methanesulfonate (EMS) to infuse novel genetic variation in each population. The five replicate populations were then passaged under three different parasite treatments (Table S1): **control** (no exposure to *S. marcescens*), **evolution** (repeated exposure to a fixed, non-evolving strain of *S. marcescens*), and **coevolution**. The **coevolution** treatment involved repeated exposure (30 host generations) to a potentially coevolving population of *S. marcescens*, which was under selection for increased infectivity. *S. marcescens* Sm2170 served as the ancestral strain in the **coevolution** treatment, as well as the fixed strain in the **evolution** treatment.

The results were consistent with the Red Queen hypothesis. In the **coevolution** treatment, all of the obligately selfing populations became extinct within twenty generations (Fig S1). However, none of the obligately selfing populations went extinct in either the **evolution** treatment or in the **control** treatment. In addition, all of the obligately outcrossing and wildtype populations persisted throughout the experiment in all three treatment types (Fig S1). Thus, extinction was only observed in obligately selfing hosts when confronted with coevolving pathogens.

We also found that the presence of coevolving *S. marcescens* selected for and maintained high levels of outcrossing in wildtype *C. elegans* populations (Fig. 1). Over the first eight generations of the experiment, outcrossing rates increased from 20% to over 70% in both the **evolution** and **coevolution** treatments (Fig. 1: $F_{2,11} = 8.26$, $P = 0.006$). However, the wildtype populations consistently exposed to a fixed population of *S. marcescens* (**evolution** treatment) exhibited a steady decline in outcrossing rates after this initial increase, eventually returning to control levels of outcrossing (Fig. 1), as previously observed (5). In contrast, populations in the **coevolution** treatment consistently maintained high levels of outcrossing throughout the experiment, relative to the **control** treatment (Fig. 1; $F_{1,12} = 209.5$, $P < 0.0001$). Coevolution with *S. marcescens,* therefore, favored the evolution and long-term maintenance of higher rates of outcrossing.

As also predicted by the Red Queen hypothesis, outcrossing hosts adapted to changes in the pathogen population, while selfing apparently prevented an adaptive counter response. The ancestral strain of the obligately selfing hosts suffered higher mortality rates when exposed to bacteria from the **coevolution** treatment than when exposed to either the ancestral bacteria (Fig 2A; $c > a$: $F_{1,71} = 21.2$, $P < 0.0001$) or to the non-coevolving control bacteria (Fig. 2A; $c > b$: $F_{1,71} = 31.9$, $P < 0.0001$). Therefore, the bacteria in the **coevolution** treatment evolved greater infectivity in response to selection. Further, the obligately selfing hosts did not adapt to the evolution of increased infectivity in the bacteria, as the bacteria from the **coevolution** treatment induced greater levels of mortality against the worms after ten generations of coevolution than against the ancestral hosts (Fig 2A; $f > c$: $F_{1,71} = 69.2$, $P < 0.0001$). Finally, a more than three-fold increase in mortality was observed in the obligately selfing hosts in only ten generations (Fig 2A; $f > a$: $F_{1,71} = 173.7$, $P < 0.0001$), which could explain why these hosts were driven to extinction.

The pathogens that coevolved with the wildtype and obligate outcrossing populations also evolved greater infectivity (Fig 2B,C; $i > h$: $F_{1,104} = 69.5$, $P < 0.0001$, $i > g$: $F_{1,104} = 32.9$, $P < 0.0001$, $o > n$: $F_{1,60} = 141.1$, $P < 0.0001$, $o > m$: $F_{1,60} = 50.9$, $P < 0.0001$). However, the wildtype and obligately outcrossing populations adapted to the changes in their respective coevolving pathogen populations. Specifically, both the wildtype and obligately outcrossing populations exhibited lower mortality rates against the pathogens with which they were currently evolving, than did their ancestors (Fig. 2B,C; $i > l$: $F_{1,104} = 27.9$, $P < 0.0001$, $o > r$: $F_{1,60} = 166.2$, $P < 0.0001$), thus indicating reciprocal coevolution in the outcrossing host populations. Whereas, the obligate selfing populations in the **coevolution** treatment became more infected over time (Fig. 2A), the wildtype populations maintained the same level of infectivity over the course of the experiment (Fig. 2B; $g = l$: $F_{1,104} = 0.35$, $P = 0.554$), while the obligate outcrossing populations were significantly less infected at the end of the experiment relative to the beginning (Fig. 2C; $m > r$: $F_{1,60} = 33.1$, $P < 0.0001$). Coupled with the maintenance of high outcrossing rates in the coevolving wildtype populations (Fig. 1), these results demonstrate the ability of antagonistic coevolution to continually generate novel environmental conditions under which outcrossing is favored and populations persist when interacting with a virulent pathogen.

A recent host/pathogen coevolution study in *C. elegans* further supports the conclusion that low levels of outcrossing impede the rate of adaptive evolution. The *C. elegans* hosts in this previous study appear to have primarily reproduced via self-fertilization, and did not evolve significantly greater resistance to a coevolving pathogen over 48 generations of selection (27). Contrary to our study, however, greater outcrossing rates did not evolve in these mixed-mating populations in response to the pathogen. It may be that higher levels of genetic variation and/or a greater level of pathogen virulence in our study account for the difference in outcomes.

In summary, we found that obligately selfing lineages were driven to extinction when confronted with a coevolving parasite. These results are consistent with the macroevolutionary aspects of the Red Queen hypothesis, as originally formulated by Van Valen (28). We also found that the presence of a coevolving pathogen selected for and maintained high levels of outcrossing in mixed-mating populations, whereas elevated levels of outcrossing were not maintained in populations where the pathogen was not coevolving. These results are consistent with the microevolutionary predictions of the Red Queen. Taken together, the results demonstrate that sex can facilitate adaptation to novel environments, but the long-term maintenance of sex requires that the novelty does not wear off.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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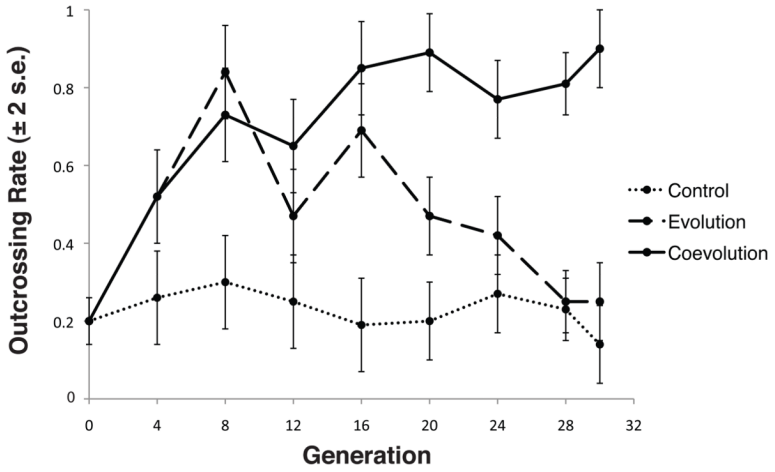

Fig. 1.

Wildtype outcrossing rates over time. Outcrossing rates in wildtype populations were not manipulated and free to evolve during the experiment. The wildtype populations were exposed to three different treatments: **control** (no *S. marcescens*; dotted line), **evolution** (fixed strain of *S. marcescens*; dashed line), and **coevolution** (coevolving *S. marcescens*; solid line) for thirty generations. Error bars represent two standard errors of the mean (SE).

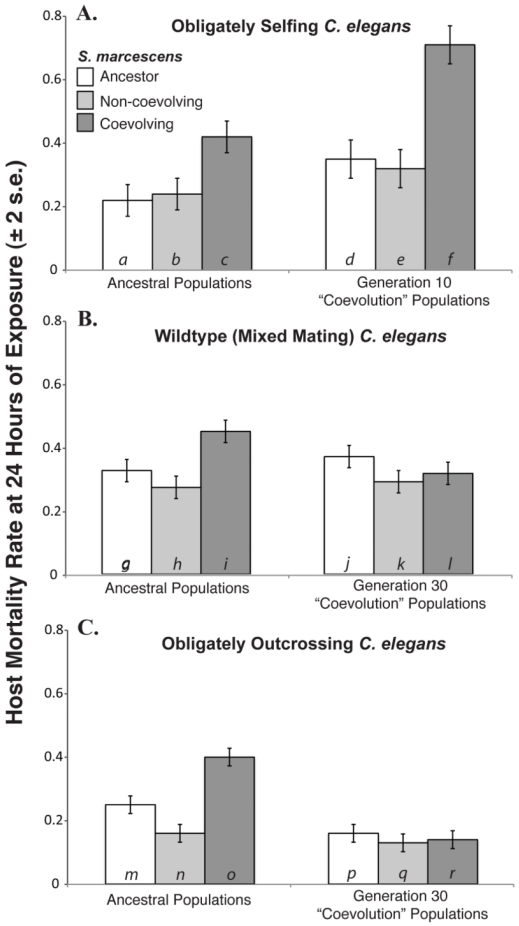

Fig. 2.

Coevolutionary dynamics of hosts and pathogens. We exposed hosts evolved under the **coevolution** treatment and their ancestral populations (prior to coevolution) to three pathogen populations: an **ancestor** strain (ancestral to all *S. marcescens* used in this study)**,** a **non-coevolving** strain (evolved without selection)**,** and their respective **coevolving** strain (coevolving with the host population). We evaluated host mortality after twenty-four hours of exposure to the pathogens and present the means across the replicate host populations. **A.** Three obligately selfing *C. elegans* populations persisted beyond ten host generations in the **coevolution** treatment. These populations were assayed prior to extinction. **B.** All five wildtype *C. elegans* populations in the **coevolution** treatment and their ancestors were assayed at the endpoint of the experiment (thirty host generations). **C.** All five obligately outcrossing *C. elegans* populations in the **coevolution** treatment and their ancestors were assayed at the endpoint of the experiment. Error bars represent two standard errors of the mean (SE).