apoptotic cells with or without stimulation with lipopolysaccharide (LPS). Stimulation of TLR4 with LPS was not sufficient to trigger inducible phagosome maturation (Fig. 3E). Simultaneous phagocytosis of apoptotic cells and bacteria showed that they did not colocalize, which suggested that they were internalized into separate phagosomes (Fig. 3F). Particle size was not a factor, because simultaneous phagocytosis of apoptotic cells and *Saccharomyces cerevisiae*, which also engages TLRs (*8*), did not result in colocalization either (Fig. 3F). At 1 hour and within the same cell, only phagosomes containing bacteria colocalized with LysoTracker at the inducible rate, and the rate of maturation of apoptotic cell–containing phagosomes was unaffected (Fig. 3G). Thus, the TLR-induced signal appeared to emanate from phagosomes containing cargo that engaged TLRs. Those phagosomes matured at an inducible rate, whereas phagosomes containing apoptotic cells matured at a constitutive rate. This rate was not influenced by the activation of TLR signaling within the same cell from a different phagosome that carried bacteria, or from the plasma membrane where the TLR was engaged by a nonparticulate ligand like LPS. This suggested that phagosome maturation was stimulated by a TLR signal that was spatially confined such that only phagosomes containing cargo that engaged TLRs were subject to inducible maturation.

Which TLR signaling pathway is responsible for inducing phagosome maturation? p38 mitogen-activated protein kinase (MAPK), which is activated by TLRs (*8*) (fig. S11), can modulate the rate of endocytic traffic by regulating activity of guanyl-nucleotide dissociation inhibitor (GDI) on Rab proteins (*21*, *22*). Specific p38 inhibitors impaired the ability of macrophages to efficiently phagocytose *E. coli*, as compared with controls (Fig. 4A). Furthermore, in the presence of p38 inhibitors, but not inactive control, no significant colocalization of *E. coli* and LysoTracker was detected, which indicated a block in phagosome maturation (Fig. 4, B and C). Thus, MyD88-dependent activation of p38 is involved in TLR-induced phagosome maturation.

Finally, many intracellular pathogens evade phagolysosomal fusion in macrophages by using a variety of strategies (*23*). Our findings identify TLR-MyD88-p38 signaling as a potential pathway that may be targeted to avoid phagolysosomal fusion.

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Supporting Online Material

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Species Interactions and the Evolution of Sex

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The Red Queen hypothesis posits that sex has evolved in response to the shifting adaptive landscape generated by the evolution of interacting species. Previous studies supporting the Red Queen hypothesis have considered a narrow region of parameter space and only a subset of ecological and genetic interactions. Here, we develop a population genetics model that circumscribes a broad array of ecological and genetic interactions among species and derive the first general analytical conditions for the impact of species interactions on the evolution of sex. Our results show that species interactions typically select against sex. We conclude that, although the Red Queen favors sex under certain circumstances, it alone does not account for the ubiquity of sex.

Interactions between species are as diverse as they are pervasive. Whether between malaria and humans or pollinating bees and orchids, species interactions are unique in their capacity to spur evolutionary change, with each species responding to changes in others. The result is a continual process of adaptation and counteradaptation where each species evolves continually to keep abreast of interacting species. It is this dynamic property of coevolving systems that forms the basis for the Red Queen hypothesis, which states that sexual reproduction is maintained because it improves a species' ability to respond to a changing biotic environment (*1*–*7*).

Previous studies have explored the Red Queen hypothesis through simulation and found that sex can be favored over evolutionary time (*1*, *3*–*8*). Yet it is difficult to discern the generality of these results for two reasons. First, most of these simulations have focused on a single type of ecological interaction governed by a particular set of genetic rules. Second, because of the large number of parameters in these models, simulations have explored a narrow region of parameter space, focusing on very strong selection, two selected loci, and comparing complete sexuality to complete asexuality. Strong selection, in particular, is known to be favorable to the Red Queen hypothesis (*7*, *9*–*12*). We developed a genetic model of species interactions that allows us to predict when the frequency of sex within a species should rise or fall over evolutionary time. Analytical predictions are derived under the assumption of weak selection per locus, and these results are extended to cases of strong selection through the use of deterministic simulations.

We investigated the Red Queen in its broadest sense and modeled the coevolutionary dynamics between species that interact in any manner that affects fitness reciprocally. The species might be host and parasite, predator and prey, mutualists, or competitors. To begin, we assume that there are two haploid species and that the outcome of their interac-

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tion depends on two genes with two alleles in each species (*A*/*a* and *B*/*b*). We then extend our results to interactions involving multiple species and multiple loci. We consider a fitness framework that, on appropriate choice of parameters, describes most genetic models used in previous studies of the Red Queen hypothesis (Fig. 1 and table S1). To determine when coevolution between species favors the evolution of sex, we track the fate of modifier alleles (at locus *M*) that alter either the probability of sexual versus asexual reproduction and/or the rates of recombination among loci. For the Red Queen hypothesis to explain the maintenance of sex within a species, interactions among species must generate genetic associations that prevent the spread of modifier alleles that decrease the extent of sex and/or recombination. Recursions were developed on the basis of a life cycle involving selection, followed by reproduction and then genotype-independent mortality of the adults at rate d_i for species i [Supporting Online Material (SOM) Text]. Population sizes were assumed large (no drift) and were held constant by setting the recruitment rate of offspring to the total mortality rate. Our model allows for different generation times in the two species ($d_1 \neq d_2$), with either semelparous ($d_i = 1$) or iteroparous ($d_i < 1$) life cycles. We do not include a cost of sex; extending our model to include a cost of sex is straightforward, but doing so would only further reduce the parameter space in which sex is maintained.

We derived mathematically tractable approximations to the coevolutionary dynamics by extending a methodology (*9*) developed for one-species models (SOM Text). These approximations require that the frequency of sex and recombination be greater than the forces generating genetic associations (e.g., epistasis) and that the per-locus strength of selection is weak. Under these circumstances, there is a separation of time scales such that allele frequencies slowly change whereas linkage disequilibria rapidly approach a steady state that is a function of current allele frequencies. This steady state, known as quasi-linkage equilibrium (QLE), can be determined analytically.

We find that, at QLE, the Red Queen generally favors modifier alleles that reduce the rate of sex or recombination within a population (Table 1) (SOM Text). Of the models described in Fig. 1, the evolution of increased sex and recombination occurred only with additive selection among loci (AMAM), which served as a positive control because additivity induces weak negative epistasis that favors recombination in one-species models (*9*). In every other case, modifier alleles that decrease the frequency of sex and recombination spread, whether the species interactions result in cyclic dynamics or in the spread of alleles to fixation (as in an evolutionary "arms race"). As long as QLE holds, the results are not sensitive to the relative generation times of the two species. Neither are the results altered by increasing the number of loci or the number of interacting species (SOM Text).

Why does the Red Queen select against sex? The Red Queen is thought to favor genetic mixing because sex creates rare gene combinations from common ones, which can improve a species' ability to keep apace of co-evolving species. In keeping with this view, the genetic associations within a population often do impede evolution [cases in Table 1 with negative v_1, which measures the effect of linkage disequilibria on genetic variance (*9*)]. However, epistasis in fitness generated by species interactions (measured by E_1, the departure from multiplicative fitness interactions) is typically too strong relative to selection (Table 1), such that the rare gene combinations produced by sex have very low fitness, on average. Consequently, the primary outcome of sex and recombination is the

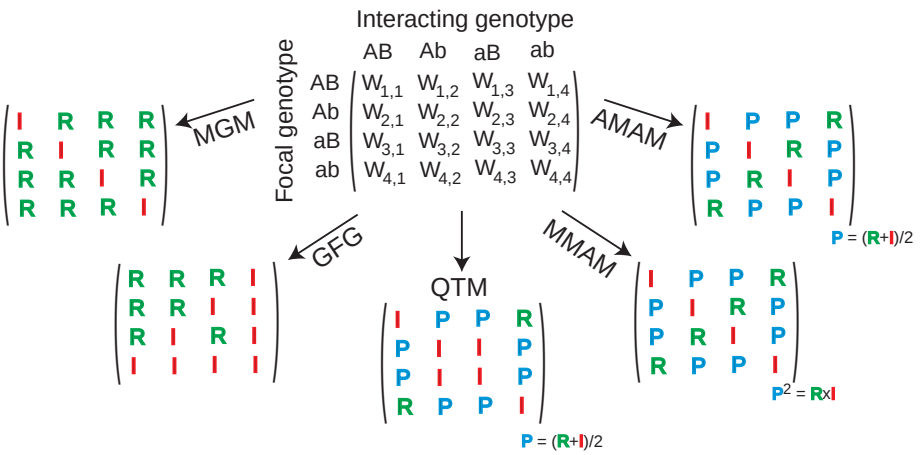

Fig. 1. Fitness effect of species interactions. A general fitness matrix (center) describes the fitness, $W_{j,k}$, of an individual of genotype *j* from the "focal" species interacting with a species consisting of genotype *k*. Competitive interactions decrease fitness in both species, mutualistic interactions increase fitness, and antagonistic interactions increase fitness in a parasite or predator but decrease fitness in a host or prey. The form of the matrix used (table S1) reduces to several models of interest, where *R* represents the fitness of resistant individuals, *I* represents the fitness of nonresistant individuals, and *P* represents the fitness of partially resistant individuals. (i) Matching-genotypes model (MGM) (*1*, *2*, *4*, *7*). Hosts resist parasites unless each parasite allele matches an allele in the host. MGM has been used to describe hosts with an immune system, which can recognize parasites that differ antigenically. (ii) Gene-for-gene (GFG) (*19*). Parasites carrying virulence alleles (*a* or *b*) infect all hosts, whereas parasites carrying avirulence alleles (*A* or *B*) are resisted by hosts carrying matching resistance alleles. GFG interactions are considered prevalent in plants (*8*). (iii) Quantitative trait model (QTM) (*3*). Parasites infect hosts that are phenotypically similar, with alleles *A* and *B* contributing additively to the phenotype. (iv) Multiplicative matching-alleles model (MMAM). Matching the interacting species causes an independent (multiplicative) change in fitness at each locus. MMAM serves as a negative control because the frequency of sex and recombination remains constant when selection acts multiplicatively and there is no source of linkage disequilibrium. (v) Additive matching-alleles model (AMAM) (*3*). Matching the interacting species causes an additive change in fitness at each locus. AMAM serves as a positive control because higher recombination evolves in one-species models with additive fitnesses (*9*). The fitness matrix also allows selection that is independent of species interactions (e.g., fixed costs of resistance).

Table 1. Genetic associations and the evolution of sex at QLE. We summarize QLE results for species interactions that harm a species (e.g., host, prey, or competitor) and for species interactions that benefit a species (e.g., parasite, predator, or mutualist) (*9*). The QLE results predict whether linkage disequilibrium between selected loci increase (↑) or decrease (↓) the additive genetic variance in fitness (v_1) and estimate the form of epistasis between selected loci (E_1). E_1 has the same sign as v_1 at QLE and can be strong (S, of the same order as selection per locus) or weak (W, of smaller order than selection). The frequency of sex column indicates whether modifier alleles that increase the frequency of sex and/or recombination spread (↑) or decline over time (↓). Zeros indicate no effect.

	Harmful interactions			Beneficial interactions		
	v_1	E_1	Frequency of sex	v_1	E_1	Frequency of sex
MMAM	0	0	0	0	0	0
AMAM	↓	W	↑	↓	W	↑
MGM	↓	S	↓	↑	S	↓
GFG	↓	S	↓	↑	S	↓
QTM	↑	S	↓	↓	S	↓

breaking apart of fit gene combinations to produce less fit recombinant genotypes.

We can gain insight into when the Red Queen might favor sex by considering conditions that prevent the attainment of QLE. When genetic mixing is infrequent relative to selection, the disequilibria can depart substantially from the steady-state values predicted on the basis of the current form of epistasis. To determine whether sex and recombination are then favored, we numerically iterated the recursions describing host-parasite interactions with one modifier locus and two interaction loci over a broad range of parameter values, tracking the change in frequency of a modifier allele in the host over 10,000 generations. We set each recombination rate and strength of selection to 0.005, 0.05, and 0.5; set mortality rates in the host to 25%, 50%, 75%, and 100%; allowed both strong and weak modifiers (5*x* and 5/3*x* ranges in recombination); and considered seven models of species interactions. In the gene-for-gene (GFG) model, costs of resistance and costs of virulence were set at 0%, 5%, and 15% of the strength of selection imposed by species interactions. The total number of parameter combinations examined was 13,608. As expected, sex and recombination were often favored in the additive model (AMAM, 76%), which exhibits weak epistasis (the positive control). The matching-genotypes model (MGM) was the next most favorable to sex (39%), followed by the GFG models ($GFG_{0\%\ costs}$, 10%; $GFG_{5\%\ costs}$, 27%; and $GFG_{15\%\ costs}$, 6%), the quantitative model (QTM, 5%), and finally the multiplicative model (MMAM, the negative control), which never favored recombination (0%). Even when sex and recombination were favored, the average change in the modifier frequency over 10,000 generations was typically small (table S1). Of those cases that favored sex and recombination, the majority involved the strongest selection coefficient and/or the lowest rate of recombination examined (SOM Text).

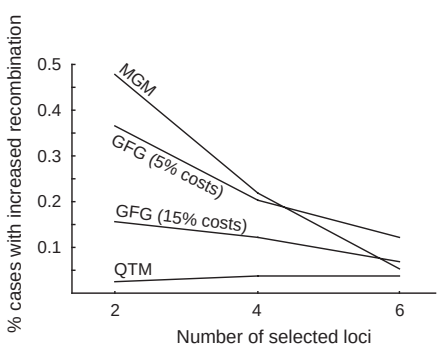

Fig. 2. Increasing the number of loci decreases the proportion of simulations in which sex is favored. Results are obtained by iterating the deterministic recursions and are averaged over a large number of parameter combinations (SOM Text). Sex was favored if modifiers causing more genetic mixing increased in frequency during the simulations.

We next increased the number of interaction loci in those coevolutionary scenarios that led to sustained cyclical dynamics (MGM, QTM, $GFG_{5\%\ costs}$, and $GFG_{15\%\ costs}$). The fraction of cases in which sex and recombination were favored dropped precipitously as the number of selected loci increased (Fig. 2). This trend is consistent with the expectation that increasing the number of loci decreases the per-locus strength of selection, making it more likely that the dynamics reach QLE, where sex and recombination are selected against.

The key issue, however, is not the number of cases that favor the evolution of sex but whether these cases lie within a biologically relevant region of parameter space. A critical evaluation thus requires that we know how often species interactions are characterized by strong selection per locus and a low effective rate of recombination. Strong selection requires (i) a high incidence of species interactions, (ii) that these interactions have a large effect on fitness, (iii) that these fitness effects are tightly correlated with genotype, and (iv) that the genotypic basis of these effects is a function of more than one but not many genes that are polymorphic in both species. Interactions that strongly affect fitness and have a high incidence are known in some species (*10*, *13*), but the number of underlying loci remains unknown. In plants, disease resistance is often determined by multiple loci, each of which explains only a small proportion of variation in resistance (*14*), suggesting weak selection per locus. Furthermore, variation in tolerance weakens the correlation between plant resistance traits and fitness (*15*). Indeed, a history of long-period cycles (weak selection) is required to explain polymorphism data at the disease-resistance locus, *Rpm1*, in *Arabidopsis* (*16*). More data are needed to determine how often all four requirements for strong selection are satisfied.

In contrast to strong selection, there are some clear cases of species with little genetic mixing. For instance, many plant resistance genes can be tightly linked (*17*), and several well-studied interactions involve individuals that infrequently, if ever, undergo sex, e.g., (*10*, *13*). In such cases, the Red Queen could be an important driving force for the evolution of increased sex and recombination. However, our simulations demonstrate that, in the absence of strong selection, sex and recombination will only be favored when the effective rate of recombination is very low. Thus, although the Red Queen might explain the origin of a small amount of sexual reproduction or the maintenance of infrequent sex in species that primarily reproduce asexually, it is unlikely to explain why so many species have so much sex.

Our result that the Red Queen typically favors lower amounts of sex and recombination contrasts with previous studies (*3*–*6*), which have focused on strong selection. Strong selection is particularly conducive to the evolution of sex because it can generate rapid fluctuations in epistasis that, under the right conditions, lead to a mismatch between epistasis and genetic associations (*7*), so that recombination creates rather than destroys currently favorable gene combinations. Yet even species interactions with a strong effect on individual fitness induce weak selection per locus if interactions occur in only a small fraction of the total population, if the success of an interaction depends weakly on genotypic matching, or if the interaction depends on many loci. These results show that, when a species must constantly evolve to stay abreast of surrounding species, the Red Queen does not maintain high amounts of sex and recombination unless species interactions induce strong selection per locus.

Although the Red Queen, on its own, typically favors less sex, it might play a role in the evolution of sex in combination with other processes such as mutation or random genetic drift (*11*). Synergistic effects between mechanisms favoring the evolution of sex have been found in models incorporating both mutation accumulation and species interactions (*12*, *18*), suggesting that the Red Queen might be less impotent with the right partner.

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