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Parasites and deleterious mutations: interactions influencing the evolutionary maintenance of sex

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Abstract

The restrictive assumptions associated with purely genetic and purely ecological mechanisms suggest that neither of the two forces, in isolation, can offer a general explanation for the evolutionary maintenance of sex. Consequently, attention has turned to pluralistic models (i.e. models that apply both ecological and genetic mechanisms). Existing research has shown that combining mutation accumulation and parasitism allows restrictive assumptions about genetic and parasite parameter values to be relaxed while still predicting the maintenance of sex. However, several empirical studies have shown that deleterious mutations and parasitism can reduce fitness to a greater extent than would be expected if the two acted independently. We show how interactions between these genetic and ecological forces can completely reverse predictions about the evolution of reproductive modes. Moreover, we demonstrate that synergistic interactions between infection and deleterious mutations can render sex evolutionarily stable even when there is antagonistic epistasis among deleterious mutations, thereby widening the conditions for the evolutionary maintenance of sex.

Introduction

The widespread occurrence of sexual reproduction across many taxa is a puzzling observation to the evolutionary biologist. Asexual mutants should have a reproductive advantage because of the cost of sex (Maynard Smith, 1978; Bell, 1982). Many theories have been put forward to explain how sexual reproduction can be maintained despite this cost, and their very existence indicates that none has so far proved entirely satisfying. The prominent ecological explanation is that sex is an adaptation that helps resist parasitism – a specific case of the Red Queen hypothesis (Hamilton, 1980). However, parasite transmissibility and virulence must be very high to overcome the cost of sex (May & Anderson, 1983; Howard & Lively, 1994) implying that parasitism alone cannot provide a general explanation for the ubiquity of sexual reproduction. One of the main genetic explanations points to the purging of deleterious mutations (Muller, 1964; Kondrashov, 1988, 1993). These arguments require genomic mutation rates that are higher than typically observed (Keightley & Eyre-Walker, 1999; Lynch *et al.*, 1999; Bataillon, 2000; Fry, 2004; Haag-Liautard *et al.*, 2007). Moreover, a specific genetic theory, the mutational deterministic hypothesis (Kondrashov, 1988, 1993), requires synergistic epistasis among deleterious mutations. However, experimental tests for epistasis in a range of taxa show that this is by no means a common situation (deVisser *et al.*, 1996; Elena & Lenski, 1997; Elena, 1999; Wloch *et al.*, 2001; Rivero *et al.*, 2003; Cooper *et al.*, 2005; Martin *et al.*, 2007). Often no epistasis or antagonistic epistasis is observed and, importantly, there is variance in epistasis. Although some theoretical research has questioned the importance of epistasis for the evolutionary fate of sexual reproduction (Howard & Lively, 1998; Keightley & Otto, 2006), there is a large body of theory in which its role is pivotal. Consequently, as well as understanding the direct effect of interactions between infection and deleterious mutations on the evolutionary

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maintenance of sex, we also aim to examine how they can modulate the role of epistasis.

Pluralistic models, in the sense that different mechanisms and their interactions work at the same time, have gained prominence as a potential way to overcome the problem that any given explanation comes with limiting caveats (West *et al.*, 1999). Although ecological or genetic arguments are insufficient to explain the prevalence of sex on their own, in combination the associated limiting assumptions can be relaxed (Howard & Lively, 1994). A leading example, coevolving parasites and deleterious mutations, further provides an explanation for the advantage to sexual reproduction (rather than clonal succession), as asexual clones still have to contend with the accumulation of deleterious mutations (Howard & Lively, 1994). Previous modelling work in this area has tended to assume that host fitness effects caused by infection and deleterious mutations act independently (Howard & Lively, 1994, 1998, 2002). However, there has been some empirical work suggesting that there are interactions between infection and deleterious mutations (Cooper *et al.*, 2005; Young *et al.*, 2009) and between infection and host inbreeding depression (Stevens *et al.*, 1997; Coltman *et al.*, 1999; Carr & Eubanks, 2002; Haag *et al.*, 2003; Hayes *et al.*, 2004; Ivey *et al.*, 2004; Ivey & Carr, 2005; Ilmonen *et al.*, 2008). Inbreeding depression can be used as an indirect measure of deleterious mutation load under a restricted set of assumptions (Bataillon, 2000). It is difficult to predict the effect of interactions between these processes on the evolution of mating systems. Importantly, it remains to be demonstrated to what extent they increase the likelihood of the maintenance of sexual reproduction, especially in situations where there is no epistasis or antagonistic epistasis among deleterious mutations.

Here, we investigate the effects of interactions between parasitism and deleterious mutations on the maintenance of sexual reproduction using an individual-based model. We examine when an extant sexual population can resist invasion by a small, monoclonal asexual population using a realistic range of ecological and genetic parameter values.

Methods

Our methods closely follow those of Howard & Lively (1994) so that we can compare our results. Each haploid host genome consists of two unlinked di-allelic loci that relate to parasite recognition. There are another 500 loci that can accumulate deleterious mutations. The host population is assumed to have a carrying capacity of 1000 individuals. Simulations with a host population size of 10 000 yielded qualitatively very similar results to those presented here. Parasites are also haploid and reproduce sexually. There are two parasite generations per host generation. In every parasite generation, each host is exposed to a parasite with probability T (transmission probability, which is unaffected by host or parasite genotypes). The genotype of the parasite is chosen with a probability equal to that genotype's frequency in the parasite population (initially, these are all assumed to be equal). Infection genetics are based on a matching alleles (MA) model so that the parasite genotype has to match the host genotype at both loci to infect the host. Effectively, the model describes a classical case of host–parasite coevolution where host and parasite allele frequencies are under antagonistic frequency-dependent selection. The MA model used here is known to generate appreciable frequency-dependent selection (Agrawal & Lively, 2002).

If infection occurs, the parasite joins the pool of surviving parasites whose sexually produced offspring form the next parasite generation. If the parasite does not infect the host (either because of failure to transmit or incompatible infection genetics) it dies. There is a small mutation rate (0.03 per locus per generation) between allelic forms in the parasite to maintain all parasite genotypes (nominally AB, Ab, aB and ab) in the population.

Host fitness is determined by two components: fitness due to the number of deleterious mutations (w_m) and fitness due to infection status (w_p). The parameter s measures the strength of selection against deleterious mutations, so that in the absence of epistasis, a host with k deleterious mutations has $w_m = (1-s)^k$. The parameter v measures the parasite virulence and takes values from 0 (harmless) to 1 (lethal). Accordingly, an infected host has a component of fitness due to infection status given by $w_p = (1-v)$ and for an uninfected host $w_p = 1$. The probability of surviving to reproductive age is given by the overall fitness $w = w_m \times w_p$.

To consider the effects of interactions, two other parameters are included in the fitness function. Epistasis between deleterious mutations is measured by the parameter β such that $w_m = (1-s)^{k^{\exp(\beta)}}$, and is simply $(1-s)$ in the case where $k = 1$. In this way, $\beta = 0$ models the case where there is no epistasis; $\beta < 0$ models antagonistic epistasis (interactions among deleterious alleles cause a reduction in fitness which is less than would be expected if there were no interaction); $\beta > 0$ models synergistic epistasis (interactions among deleterious alleles cause a reduction in fitness which is greater than would be expected if there were no interaction). We used this function to model epistasis because other commonly used functions, such as $w_m = e^{-(\alpha s k + \frac{\beta}{2}k^2)}$, have the undesired property of leading to increased fitness as the number of deleterious mutations increases when epistasis is antagonistic. Similar functions have previously been used to describe epistasis (Lenski *et al.*, 1999; Wilke & Adami, 2001). A second parameter, ε, models the interaction between the two fitness components. The full fitness function is given by $w = (w_m \times w_p)^{\varepsilon}$ for infected hosts and $w = w_m$ for uninfected hosts. A value of $\varepsilon = 1$ models the case where there

is no interaction; $\varepsilon < 1$ models an antagonistic interaction; $\varepsilon > 1$ models a synergistic interaction.

The fitness function gives the probability of surviving to reproductive age. Hosts that survive to reproductive age are selected at random to be part of the reproducing population. Asexual hosts have a brood of 20 offspring. These are identical to the mother except in the case of deleterious mutation accumulation (which occurs at a rate of U per haploid genome per generation). When a sexual host is selected, another sexual host is randomly selected for cross-fertilization. Consequently, each sexual individual produces a lifetime average of ten embryos and achieves ten cross-fertilizations through male function, embodying the two-fold cost of sex (Howard & Lively, 1998). Free recombination at all loci is assumed. These offspring also acquire deleterious mutations at rate U. Reproducing individuals are selected until the number of broods equals the number of adults. From the 'bank' of offspring, 1000 individuals are randomly selected to form the next host generation. In cases where the population produces < 1000 individuals, all offspring form the next generation.

Initially, the extant sexual population is assumed to be at mutation-selection balance and with all parasite recognition genotypes equally represented in the population. In general, assumptions are chosen to be advantageous to asexuals so as to obtain conservative measures for the maintenance of sex and to control for extraneous sources of variability. Accordingly, the model scenario is the spontaneous emergence of a small (20 individuals) monoclonal asexually reproducing population. This is sufficiently small to capture the essence of an invasion scenario while ensuring the asexual population is not rapidly eliminated by stochastic effects. This asexual population is assigned the minimum probable number of deleterious mutations, $k_{\min} = \bar{k} - 2\sqrt{\bar{k}}$, where $\bar{k}$ is the average number of deleterious mutations in the sexual population (Charlesworth, 1990; Howard & Lively, 2002). The parameter $k_{\min}$ is used as an estimate of the minimum expected value from the distribution of 'mutation numbers' in the sexual population, and it is possible that none of the sexual individuals actually carry exactly k mutations.

Computer simulations are run until one type of reproductive mode goes to fixation or for 300 host generations, in which case the simulation is recorded as having coexistence of reproductive modes (representative cases of coexistence were further investigated after 10 000 host generations and their outcome is mentioned in the results section). For any given parameter set, the majority outcome of a set of replications is recorded. As stated, the basic model life cycle is very close to that used by Howard & Lively (1994). The differences are that we consider epistasis among deleterious mutations and interactions between deleterious mutations and infection in determining fitness, and we make a slight modification of the selection process: here, both components of fitness only affect host viability (i.e. surviving to reproductive age), whereas in Howard & Lively (1994) deleterious mutations affected viability while infection affected host fertility. This allows simpler implementation of our extended model. For the parasite traits (transmissibility and virulence), we explore the full parameter space. Genetic parameters are varied between $U = 0.5$ and 1.0 (genomic mutation rate) and $s = 0.0125$ and 0.05 (strength of selection against deleterious mutations). These are within the range of estimates from a variety of experiments (Bataillon, 2000; Haag-Liautard *et al.*, 2007). The epistasis parameter (β) is varied between −0.2 and +0.2, and the parameter controlling the interaction between infection and mutations (ε) is varied between 0.5 and 1.5. This allows a large spectrum of interactions to be captured (strong, weak, synergistic, antagonistic and cases with no interactions).

Because strong selection favours sex (Howard & Lively, 1994; Barton, 1995; Dybdahl & Lively, 1998; Peters & Lively, 1999; West *et al.*, 1999), we include a method for distinguishing if the mechanisms of epistasis and interactions between deleterious mutations and infection are directly selecting for sex or if they are simply increasing the strength of selection.

To do this, we calculate 'realized' values of strength of selection (RS), epistasis (RE), virulence (RV) and strength of interaction between deleterious mutations and infection (RI). Calculation of these quantities follows examples from other studies (Kimura & Maruyama, 1966; Agrawal, 2001) and in our system the quantities of interest are given by

$$\text{Realized selection (RS)} = \sum_k F_k \frac{\bar{W}_k - \bar{W}_{k+1}}{\bar{W}_k},$$

where F_k is the frequency of individuals carrying k deleterious mutations. The term $\bar{W}_k = (1 - p_k)W_{k,\text{Uninf}} + p_k W_{k,\text{Inf}}$ where p_k is the prevalence of infection among individuals with k mutations and$W_{k,\text{Inf}}$ and $W_{k,\text{Uninf}}$ represent the fitness of an individual with k mutations who is or is not infected, respectively.

$$\text{Realized epistasis (RE)} = \sum_k F_k \frac{\bar{W}_k \bar{W}_{k+2} - \bar{W}_{k+1}^2}{\bar{W}_k^2}$$

Realized strength of interaction between deleterious mutations and infection

$$\text{(RI)} = \sum_k F_k \frac{W_{k,\text{Uninf}} W_{k+1,\text{Inf}} - W_{k+1,Uninf} W_{k,Inf}}{W_{k,Uninf}}$$

$$\text{Realized virulence (RV)} = \sum_k F_k \frac{W_{k,\text{Uninf}} - W_{k,\text{Inf}}}{W_{k,\text{Uninf}}}$$

We calculate these quantities for a purely sexual population (size 1000, starting at mutation-selection balance, and in the presence of parasites) for 51 treatments (Appendix S1), which correspond to invasion simulations carried out in a separate part of this study.

We then relate the probability (Q) of resisting invasion by an asexual clone in the latter studies to these realized values to establish their effects (over and above their contribution to increasing the overall strength of selection) on the maintenance of sex. Formally, Q is the probability that sex goes to fixation under the invasion scenario outlined above. We use time-averaged values of RS, RE, RV and RI (over 500 host generations), which are stable with small fluctuations caused by stochasticity in the mean number of mutations per individual. We also verified that these time-averaged quantities are consistent across multiple replications.

Results

As expected because of the cost of sex, in models with no parasites and no deleterious mutations, the asexual population always displaces the sexual population and goes to fixation. This is also the case in models with no parasites but with deleterious mutations ($U = 1.0$, $s = 0.025$). In the absence of deleterious mutations ($U = 0.0$), sex only goes to fixation for combinations of high parasite transmissibility ($T = 0.8$–1.0) and extreme virulence ($v = 1.0$), i.e. for 3% of the transmissibility-virulence parameter space (Fig. 1). However, combinations of intermediate values of T and v may predict coexistence between sexually and asexually reproducing individuals over significant (300 generations) time scales (Fig. 1). Numerical simulations showed that in such cases reproductive modes coexisted for at least 10 000 host generations, indicative of genuine long-term coexistence.

When deleterious mutations and parasites are both present (although in the absence of epistasis ($\beta = 0$) and in the absence of any interaction between mutations and infection ($\varepsilon = 1$)), the region of parameter space in which sexual reproduction goes to fixation is greatly increased from 0% in the 'no parasites' model and 3% in the 'no mutations' model to 13–32% (for $U = 0.5$–1.0 and $s = 0.0125$–0.025) (Fig. 2). These results are in qualitative agreement with Howard & Lively (1994). Numerical simulations showed that cases of coexistence after 300 host generations in the model with parasites and mutation accumulation did not maintain this coexistence after 10 000 host generations. The majority of replicates (around 75%) resolved to fixation of sexual reproduction with the remaining replicates giving rise to fixation of asexual reproduction.

It is important to note that the assumption that the asexual invaders are monoclonal is crucial in predicting an advantage to sex. The observed 32% of parasite transmissibility-virulence parameter space in which sex is predicted to go to fixation (Fig. 2d), shrinks to just 3% in the case where the 20 asexual invaders are composed

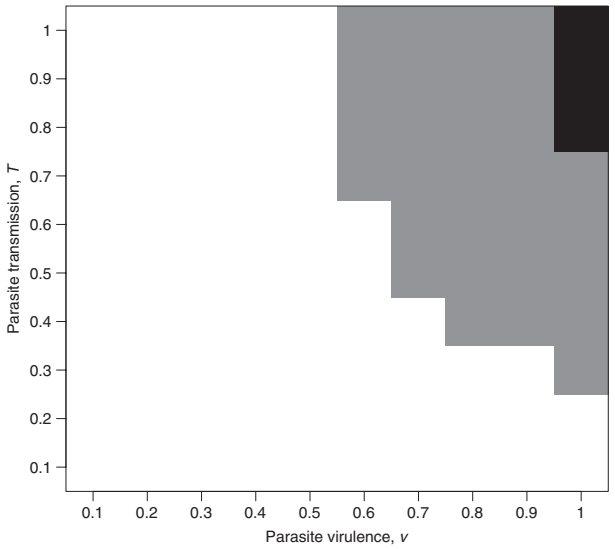

Fig. 1 Fixation of reproductive modes in a model with parasites but without deleterious mutations. For each combination of parasite transmission probability (T) and virulence (v), the majority outcome from ten replications of the model is shown. Black and white squares indicate fixation of sex and asex, respectively. Grey squares indicate that the majority outcome was coexistence between both reproduction modes for 300 host generations. Model details are given in 'Methods'.

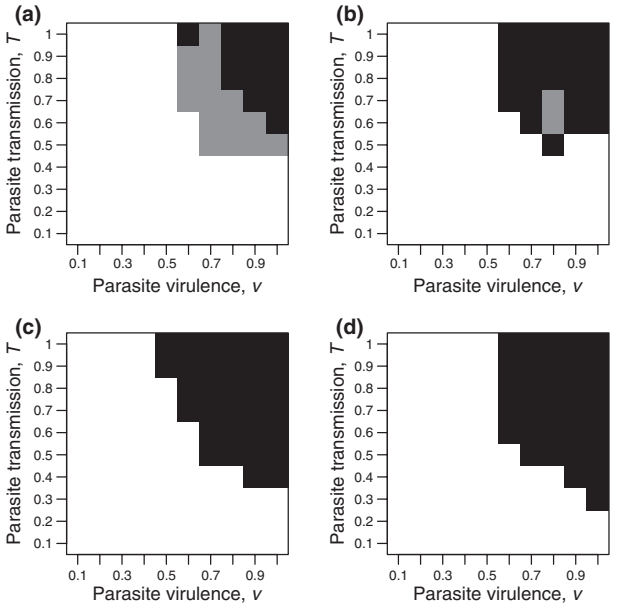

Fig. 2 Fixation of reproductive modes in a model with parasites and deleterious mutations. The colour of each square represents the majority outcome of ten replications of the model (as indicated in Fig. 1) for particular values of parasite transmission probability (T) and virulence (v). Results also depend on the genomic mutation rate (U) and the strength of selection against deleterious mutations (s) which take the values (a) $U = 0.5$, $s = 0.0125$; (b) $U = 1.0$, $s = 0.0125$; (c) $U = 0.5$, $s = 0.025$; (d) $U = 1.0$, $s = 0.025$.

of equal numbers of two of the four possible genotypes. Moreover, the 3% of parameter space that still predicts fixation of sexual reproduction corresponds to very high values of parasite transmission and virulence. Although concomitant invasion of sexual populations by several asexual clones may occur in situations where asexual lineages have already independently fixed in different populations and invade another population at the same time, it is unlikely that several asexual clones arise spontaneously in any given site (e.g. through rare meiosis-suppressing mutations). Monoclonal or multiclonal invasions most likely occur at different stages of the invasion process, the first during the initial stage, the latter being more relevant to the subsequent spread of asexual reproduction.

Both synergistic epistasis ($\beta > 0$) and a synergistic interaction between infection and mutation ($\varepsilon > 1$) promote the evolutionary maintenance of sex. For mutation parameters $U = 1.0$ and $s = 0.025$, and with no interactions ($\beta = 0.0$, $\varepsilon = 1.0$), sexual reproduction is evolutionarily stable for 32% of the transmissibility-virulence ($T - v$) parameter space (Fig. 2d). This rises to 55% for strong synergistic epistasis and to 58% for a strong synergistic interaction between infection and mutation (Fig. 3). When these two processes act in concert, the advantage to sex increases further, rising to cover 84% of $T - v$ parameter space (Fig. 3). Importantly, a sufficiently strong synergistic interaction between infection and mutation can offset antagonistic epistasis among deleterious mutations. This is shown in Fig. 3 where sex is maintained in 18% of $T - v$ parameter space in spite of strong antagonistic epistasis (whereas it is 0% with antagonistic epistasis only).

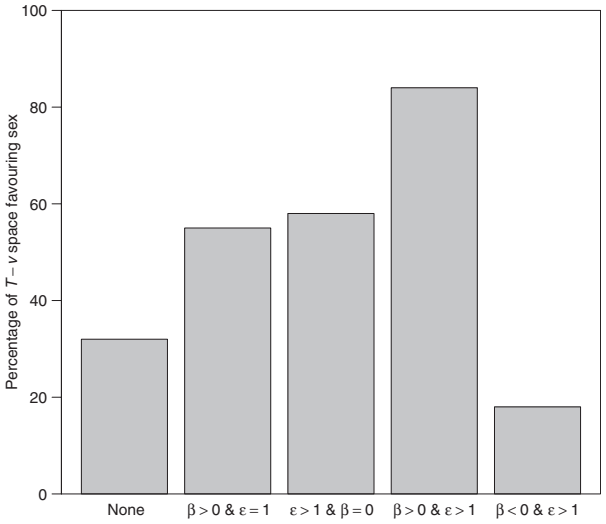

Fig. 3 Percentage of parasite transmissibility-virulence ($T - v$) parameter space in which fixation of sexual reproduction is the majority outcome of ten replications of the model, as a function of strength of epistasis among deleterious mutations (β) and interaction between infection and mutation (ε). The bar chart shows five different treatments in which a core set of parameters are held constant ($U = 1.0$, $s = 0.025$, $T = 0.5$, $v = 0.5$) and β and ε are systematically varied. From left to right: no interactions, $\beta = 0.0$, $\varepsilon = 1.0$; synergistic epistasis, $\beta = 0.2$, $\varepsilon = 1.0$; synergistic interaction between infection and mutation, $\beta = 0.0$, $\varepsilon = 1.5$; both synergistic epistasis and a synergistic interaction between infection and mutation, $\beta = 0.2$, $\varepsilon = 1.5$; antagonistic epistasis and a synergistic interaction between infection and mutation, $\beta = -0.2$, $\varepsilon = 1.5$.

The strength of epistasis (β) and of interactions between infection and mutation (ε) necessary to render sexual reproduction evolutionarily stable depend on both genetic and infection-related parameter values (Fig. 4). This is because of the way in which the two processes combine to change host fitness (Fig. 4a). Relative to a default parameter set of $U = 1.0$, $s = 0.025$, $T = 0.5$, $v = 0.5$ (Fig. 4b) increasing the genomic mutation rate (Fig. 4c, $U = 2.0$), strength of selection (Fig. 4d, $s = 0.05$), the transmission probability (Fig. 4e, $T = 0.8$) and parasite virulence (Fig. 4f, $v = 0.8$), all increase the region of $\beta - \varepsilon$ parameter space for which sex is maintained. Note that in all simulations, asexual reproduction is predicted to go to fixation in the absence of interactions ($\beta = 0$, $\varepsilon = 1$) except in cases of high virulence (probability of host death if infected = 0.8, Fig. 4f). Also in these simulations, sex can go to fixation for part of the parameter space corresponding to antagonistic epistasis ($\beta < 0$) provided that the interaction between infection and mutation is of sufficient synergistic strength. We also verified that these results were robust to increases in population size ($N = 10000$) for both a constant invading population size of 20 individuals (i.e. same number of invaders) and for an invading population size of 2% of the total population size (i.e. constant frequency of invaders). Further, we found qualitatively similar results to those presented in Fig. 4 when the invading population was composed of equal numbers of two asexual clones (differing in their parasite-recognition loci).

To obtain a more mechanistic understanding of our results, we looked at how parameter variations affected the realized strength of selection (RS), epistasis (RE), strength of interaction between deleterious mutations and infection (RI), and virulence (RV) (see Appendix S1 for detailed results). Among these parameters, RS and RV are the most straightforward as they compare the fitness of individuals differing by one deleterious mutation or by whether they are infected or not, respectively. In the absence of epistasis and parasites RS = s, and in the absence of deleterious mutations RV = v (results not shown; the latter holds even in the presence of deleterious mutations, as long as there are no interactions, i.e. $\varepsilon = 1$ – see details below). Looking at the results generated by all parameter combinations, we find that RS is not a good indicator of Q (a narrow range of RS values is associated with the full range of Q – Fig. 5a). Although an increase in s does increase the value of RS (Fig. 6), the association between RS and Q is only consistent in the

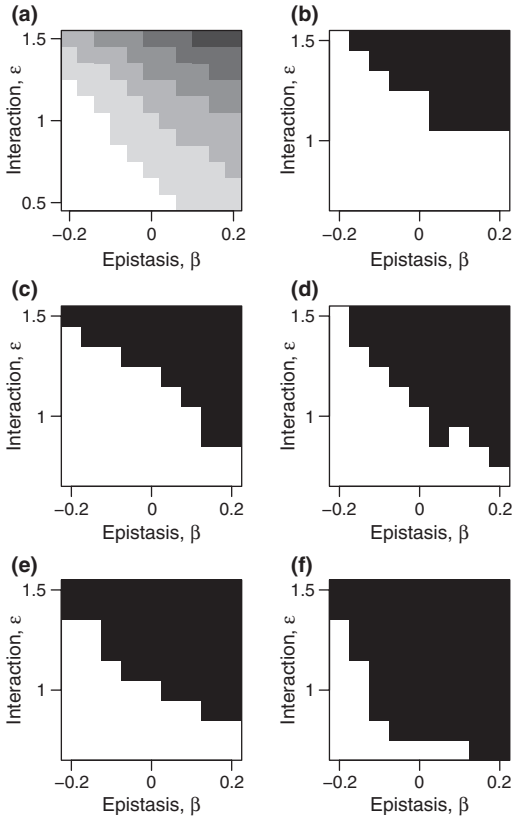

Fig. 4 Fixation of reproductive modes as a function of strength of epistasis among deleterious mutations (β) and interaction between infection and mutation (ε). Plot (a) is an example of how the parameters ε and β affect the fitness (w) of an individual. The example considers an individual with 40 deleterious mutations (the mean number of mutations per individual expected under mutation-selection balance for $U = 1.0$ and $s = 0.025$) that is also infected with a parasite of virulence $v = 0.5$. Greyscale indicates $0.0 \leq w < 0.1$ (white); $0.1 \leq w < 0.2$(light grey); $0.2 \leq w < 0.3$ (medium grey); $0.3 \leq w < 0.4$ (dark grey); $0.4 \leq w < 0.5$ (very dark grey); $0.5 \leq w < 0.6$ (black). For comparison, the same individual, if affected only by mutations or parasites (and with $\beta = 0$, $\varepsilon = 1$) would have a fitness of 0.36 or 0.5, respectively. Plots (b–f) show majority outcomes of ten replications of the model, which are either fixation of sexual (black squares) or asexual (white squares) reproduction. Default parameter values were used in (b) $U = 1.0$, $s = 0.025$, $T = 0.5$, $v = 0.5$; and in the other plots (c–f), parameters are the same except (c) $U = 2.0$; (d) $s = 0.05$; (e) $T = 0.8$; (f) $v = 0.8$.

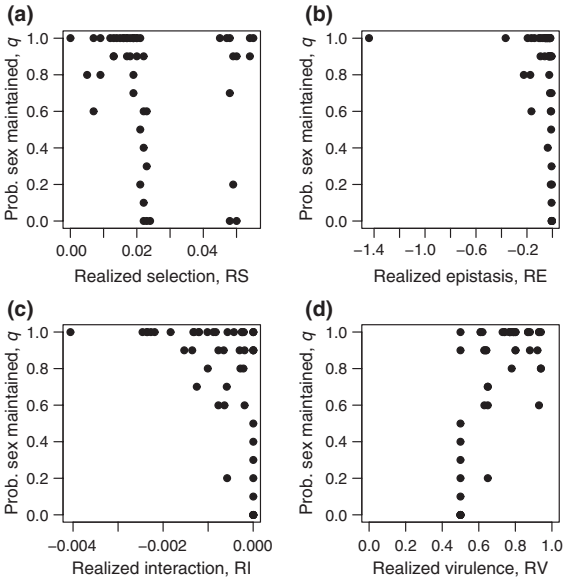

Fig. 5 The association of the probability of sexual reproduction going to fixation following an asexual invasion, Q, with (a) realized strength of selection, RS; (b) realized epistasis, RE; (c) realized strength of interaction between deleterious mutations and infection, RI; and (d) realized virulence, RV.

presence of interactions i.e. $\varepsilon > 1$ (Fig. 7a, closed circles vs. open squares). In these cases, Q always increases with s when it can.

In contrast, we see a strong association of Q with RV, with Q rising sharply as RV increases (Fig. 5d). In the absence of interactions, increases in RV simply mirror the increase in virulence, v (Appendix S1), and indeed RV = v. When $\varepsilon > 1$ then RV > v, the increase in RV is partly driven by the interaction parameter, ε (Fig. 6), indicating that the interaction between fitness effects of deleterious mutations and infection is manifested as additional virulence.

The sign of RI depends on the relative values of $W_{k,\mathrm{Uninf}}/W_{k,\mathrm{Inf}}$ and $W_{k+1,\mathrm{Uninf}}/W_{k+1,\mathrm{Inf}}$ (alternatively, $W_{k+1,\mathrm{Inf}}/W_{k,\mathrm{Inf}}$ and $W_{k+1,\mathrm{Uninf}}/W_{k,\mathrm{UInf}}$) and results in negative values of RI for $\varepsilon > 1$. Similarly, as we are focusing here on synergistic epistasis, RE is also negative. As the magnitude of both RE and RI increases, there is an increase in Q (Fig. 5b, c). The increase in the magnitude of RE is partly driven by the epistasis parameter, β, as expected, but also by parameters v and ε (Fig. 6). In other words, virulence (v) and strength of interaction between infection and deleterious mutations (ε) are converted into realized epistasis: the most extreme values of RE are obtained for high virulence and ε (Fig. 5b and Appendix S1). We see a strong association of Q with RV, with Q rising sharply as RV increases (Fig. 5d). In the absence of interactions, increases in RV simply mirror the increase in virulence, v (Appendix S1).

The realized strength of interaction between infection and deleterious mutations, RI, is the other parameter that affects Q, and Q increases as the magnitude of RI increases (Fig. 5c). Not surprisingly, RI is directly affected by the strength of interaction between infection and deleterious mutations (ε) (Fig. 6). However, when $\varepsilon > 1$, the magnitude of RI also increases with s (Fig. 7b). In this case, whenever Q can increase (i.e. $Q < 1$) then it does. This represents 4/8 cases; the other cases have $Q = 1.0$ from the outset. This analysis indicates that the effects of an increase in s operate by increasing RS and increasing

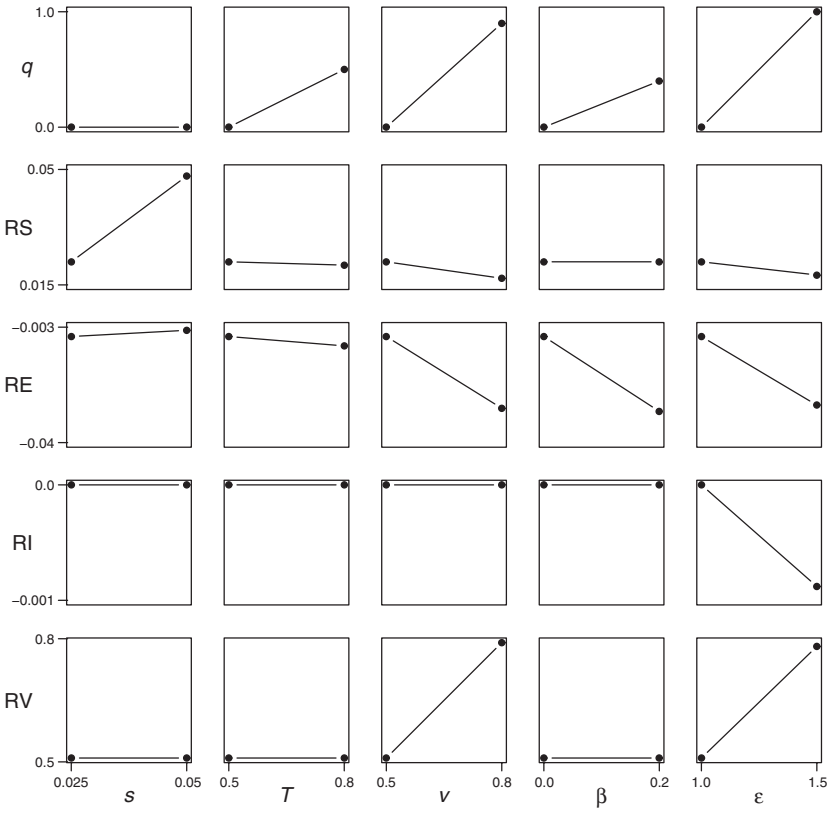

Fig. 6 Effect of model parameters s, T, v, β, ε on the realized parameters RS, RE, RI, RV when varied independently. Also shown is the resulting effect on model predictions for the probability (Q) that the sexual population goes to fixation following invasion by an asexual population.

the magnitude of RI, and the latter is more important both in terms of the probability of increasing Q and the amount by which Q is increased. Finally, while β is not correlated with RI, ε is correlated with RE, reinforcing the fact that the interaction is converted into epistasis, whereas the opposite is not true. The transmission probability, T, has an appreciable effect on the probability of the fixation of sex (Q) without affecting any of the realized quantities (Fig. 6).

To sum up, the probability of fixation of sexual reproduction is mostly driven by RV, RI and RE. The parameters most strongly associated with increases of Q are infection parameters (v and T), epistasis (β), and the strength of interactions between infection and deleterious mutations (ε). When the latter exist ($\varepsilon > 1$), deleterious mutations are converted into additional virulence (increase in RV) and to a lesser extent virulence is converted into deleterious mutations (increase in RS); interestingly, they are both converted into epistasis (increase in RE) even in the absence of 'real' epistasis (i.e. even when $\beta = 0$); naturally, interactions also lead to increases in the magnitude of RI.

Discussion

In isolation, ecological and genetic theories struggle to explain the evolutionary maintenance of sex. Therefore, models incorporating joint action of ecological and genetic processes have gained prominence (West *et al.*, 1999). Although it is highly plausible that most sexually reproducing organisms have to contend with coevolving parasites and deleterious mutations, theoretical studies of their joint effects are limited and do not consider the potential interaction between the two. One of our main results is that such interactions have a profound effect on the evolutionary maintenance of sex. Synergistic interactions (whereby the fitness costs of carrying deleterious mutations and parasite infection are greater than would be expected if they acted independently) promote the maintenance of sexual reproduction and indeed can reverse predictions concerning the ability of an asexual mutant to invade a sexual population.

In the absence of parasites and epistasis, and for the parameter values of the mutation rate to and selective effect of deleterious mutations we used, our model always predicts that asexual mutants will replace sexually reproducing organisms. Alternatively, without deleterious mutations, sexual reproduction is maintained only rarely and then only for extremely virulent and transmissible parasites. The combination of both parasites and mutation increases the range of parameter values for which sex is maintained. Qualitatively similar results were also found by Howard & Lively (1994), although their model had both viability selection and fertility selection (depending separately on infection and deleterious mutations), whereas our model uses only viability

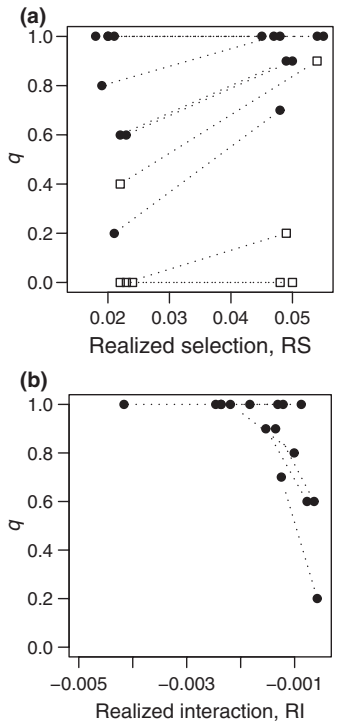

Fig. 7 Effect of increases of the strength of selection against deleterious mutations, *s*, on the realized strength of selection, RS, and the realized strength of interaction, RI, and through them, on the probability that a sexual population fully resists invasion by an asexual mutant (Q). Points connected by a dotted line have the same parameter values except for s; *s* increases from left to right in (a) and from right to left in (b). In the presence of interactions ($\varepsilon > 1$) increasing *s* results in (a) an increase in the realized strength of selection, RS ($\varepsilon > 1$ filled circles vs. $\varepsilon = 1$ open squares) and (b) an increase in the magnitude of the realized strength of interaction, RI. Both of these effects result in an increase in the probability that a sexual population goes to fixation following an invasion by an asexual mutant (Q).

selection. This advantage to sex is only predicted when the invading asexuals are monoclonal; a small multiclonal asexual population can only be repelled in the presence of parasites with extremely high transmissibility and virulence. However, there are many invasion scenarios where we would not expect such clonal diversity. For example, asexuals derived from sexuals via a meiosis-suppressing mutation is a process that is unlikely to occur frequently in a given population. Even in cases where multiclonal invasion is plausible (e.g. egg-hatching from a diapausing egg bank), we must ultimately ask how the asexual population first established, at which point it is more plausible to assume invasion by one clone.

It is worth noting that because of the finite population size framework used here another mechanism (different from Muller's Ratchet) involved in the evolution of sex is at play, the 'finite population hypothesis' (Keightley & Otto, 2006; Otto & Gerstein, 2006). Under this hypothesis, sex is favoured in finite populations because, by rendering evolutionary processes at different loci independent from each other, it enables sexual populations to better respond to selection than their asexual counterparts. However, for the parameter values used here, and particularly for a two-fold cost of sex, this mechanism alone would not lead to the fixation of sexual reproduction (see Fig. 2, Keightley & Otto, 2006).

We extended the Howard & Lively (1994) results by introducing epistasis (parameter β) and an interaction between infection and mutation (parameter ε) in a simple modelling framework. Both of these interactions are important for the evolutionary maintenance of sex. A synergistic interaction between infection and mutations ($\varepsilon > 1$) is capable of changing the evolutionary outcome from fixation of the asexual population to maintenance of the sexual. Synergistic epistasis also has an effect in this model, because it can alter host fitness and have some influence on the outcome of the fixation of reproductive modes for certain synergistic levels of interaction ($\varepsilon > 1$) (Fig. 4a and b). Intuitively, we can understand why synergistic processes would favour sex in our model. We assume that there are two components to host fitness (w_m and w_p, introduced in the Methods section). For certain parasite and genetic parameter values, these fitness components will be appreciably lower in asexual individuals (because they are unable to either escape parasites or purge deleterious mutations). If these components combine in a synergistic way then they can easily overcome the cost of sex and, further, can render sex viable even in situations where there is antagonistic epistasis. Our results add to existing theory which states that the precise form of epistasis may not be important in selecting for sex provided that it is synergistic (Howard & Lively, 1998). These results are qualitatively robust to changes in resident and invading population sizes and to the genetic composition of the invading asexuals (i.e. monoclonal or biclonal). The robustness of results to increases in population size is intuitively expected because in large populations, the probability of fixation of an allele at a given locus is low and consequently, the advantage to sex, conferred by genetic recombination, is maintained.

Motivated by the mutational deterministic hypothesis (Kondrashov, 1988, 1993), there have been several empirical tests for synergistic epistasis among deleterious mutations (deVisser *et al.*, 1996; Elena & Lenski, 1997; Elena, 1999; Wloch *et al.*, 2001; Rivero *et al.*, 2003; Cooper *et al.*, 2005; Kouyos *et al.*, 2007; Martin *et al.*, 2007). These experiments have produced very mixed results for the average level of epistasis with either no epistasis or antagonistic interactions among deleterious mutations being a fairly common occurrence. Importantly, some of these analyses (e.g. Elena & Lenski, 1997; Kouyos *et al.*, 2007) revealed high variability of the sign of epistasis, which runs contrary to the mutational

deterministic hypothesis. We have found that sex can be maintained even when there is antagonistic epistasis among deleterious mutations. This requires a sufficiently strong compensatory synergistic interaction between infection and deleterious mutations. This result extends the conditions for the maintenance of sex to situations that have, until now, been problematic. Moreover, not testing for such interactions in experimental or natural systems could lead to entirely misleading results.

We also find an influence of synergistic epistasis on promoting the fixation of sexual reproduction (e.g. Fig. 4b). The implication is that the role of epistasis can be modulated by the nature of the interaction between infection and deleterious mutations. In fact, we note that increasing the strength of interaction between mutations and infection (ε) results in an increase in 'realized' epistasis (RE), and once RE is driven to sufficient values, it begins to influence the maintenance of sex (Figs 5b and 6). As well as potentially helping to reconcile the empirical observations of variable epistasis and obligate sexual reproduction in several species, our work is broadly in agreement with existing theoretical work which has found that epistasis on its own is not central in the question of the maintenance of sex, e.g. (Howard & Lively, 2002; Keightley & Otto, 2006).

The modelling work has identified novel mechanisms that can play a pivotal role in the evolutionary maintenance of sex. We have shown that parasite virulence (v) and the strength of interaction between infection and deleterious mutations (ε) can enhance realized epistasis (RE), which is associated with an increased ability of a sexual population to resist invasion by an asexual mutant. Further, we have demonstrated that mutation load is converted into realized virulence (RV) through the interaction between the fitness effects of deleterious mutations and infection (ε). This increased virulence strongly selects for sex. An interaction between infection and deleterious mutations allows the core parameter s (strength of selection against deleterious mutations) to act on the realized strength of selection and the realized strength of interaction and both pathways promote the evolutionary maintenance of sex. An increased parasite transmission probability (T) also has an appreciable effect on the probability that the sexual population fixes when challenged with an asexual mutant invasion. Previous theoretical research has shown this (Howard & Lively, 1994), and here we illustrate that it does not act through increasing the realized quantities that we calculate (Fig. 6). Increasing the transmission rate simply increases the prevalence of infection.

Taken together, this variety of mechanisms underlines the importance of considering interactions as part of the 'pluralist' theory of sex. We assert that computational models can be very useful to develop theory on the evolutionary maintenance of sex, particularly the emerging ideas that aim to unite the ecological and genetic schools of thought.

There are many ways in which this work may be developed. The first is to analyse the dependence of our results on the specific model of infection genetics. Here, we have used a MA model. In general, the specific types of infection genetics exhibited in natural host–parasite interactions are not well known, but include self/nonself recognition systems (e.g. MA), antigen/antibody type interactions (e.g. inverse MA) and gene-for-gene (GFG) systems (Agrawal & Lively, 2002; Nuismer & Otto, 2004; Otto & Nuismer, 2004). A somewhat polarised debate has ensued between advocates of the GFG system and the MA system. Agrawal & Lively (2002) argue that these are in fact two compatible extremes of a continuum, and that the highly dynamical frequency-dependent aspects of MA models are observed across most of the MA-GFG continuum. This suggests that the MA model is somewhat more general than the GFG model. In our study, we are interested in cases where polymorphisms at loci implicated in infection genetics are maintained (otherwise the advantage of recombining alleles via sexual reproduction is moot). For this to occur in GFG systems, there have to be costs for hosts to resist infection and parasites to be able to overcome host resistance. Given that polymorphism is maintained, then we expect advantages to sexually reproducing individuals (negative frequency-dependent selection and purging deleterious mutations) to be present. Consequently, we predict that results from our model with matching allele infection genetics (which avoids having to balance costs of resistance/virulence) would be qualitatively unaffected by switching to a GFG system with suitable costs to ensure polymorphism is maintained. Although some previous work (Parker, 1994) comparing matching allele infection genetics and GFG infection genetics did find that GFG models were less conducive to sex, this was simply through the loss of polymorphism through absent or insufficient costs.

Another important development will be the consideration of higher ploidy levels (particularly in the host population) for two reasons. First, it is important to consider the role of dominance of deleterious mutations because it has been shown that it may affect the evolution of sex (Otto, 2003). Second, transitions from sexual to asexual reproduction are often accompanied by an elevation in ploidy level (Otto & Whitton, 2000). This will not only change the host-parasite infection genetics (Nuismer & Otto, 2004), but will also impact on the consequences of deleterious mutations, i.e. by allowing the masking of recessive deleterious mutations (Agrawal & Chasnov, 2001). The modelling work introduced here suggests that interactions will be of importance, potentially with more complex processes introduced via dominance and infection genetics.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Tabulated model results for the effect of core parameters (s, U, T, ν, β, ε) on realized parameters (RS, RE, RV, RI) in a sexual population at mutation-selection balance and in the presence of parasites.

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