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Bloody-minded parasites and sex: the effects of fluctuating virulence

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Abstract

Asexual lineages can grow at a faster rate than sexual lineages. Why then is sexual reproduction so widespread? Much empirical evidence supports the Red Queen hypothesis. Under this hypothesis, coevolving parasites favour sexual reproduction by adapting to infect common asexual clones and driving them down in frequency. One limitation, however, seems to challenge the generality of the Red Queen: in theoretical models, parasites must be very virulent to maintain sex. Moreover, experiments show virulence to be unstable, readily shifting in response to environmental conditions. Does variation in virulence further limit the ability of coevolving parasites to maintain sex? To address this question, we simulated temporal variation in virulence and evaluated the outcome of competition between sexual and asexual females. We found that variation in virulence did not limit the ability of coevolving parasites to maintain sex. In fact, relatively high variation in virulence promoted parasite-mediated maintenance of sex. With sufficient variation, sexual females persisted even when mean virulence fell well below the threshold virulence required to maintain sex under constant conditions. We conclude that natural variation in virulence does not limit the relevance of the Red Queen hypothesis for natural populations; on the contrary, it could expand the range of conditions over which coevolving parasites can maintain sex.

Introduction

The predominance of sexual reproduction in nature continues to pose a problem for evolutionary theory. Asexual lineages have a higher per capita birth rate than sexual lineages, because asexual females invest all their resources in reproductive daughters, while sexual females must expend limited resources on sons. These sons cannot directly produce offspring, resulting in the 'twofold cost of males' (Maynard Smith, 1971a, 1978). Therefore, if an asexual mutant arises in a sexual population, the mutation is predicted to spread rapidly, driving sexual lineages extinct. Why then is sex such a common reproductive strategy?

Of the hypotheses proposed to resolve this paradox, the Red Queen hypothesis has garnered the most empirical support from natural and laboratory populations. This hypothesis argues that coevolving enemies impose negative frequency-dependent selection by adapting to infect common host genotypes and driving them down in frequency. Through segregation and recombination, sexual females can produce offspring with rare genotypes. This increases the probability that at least a fraction of their offspring escape attack from coevolving enemies (Hamilton, 1975, 1980; Levin, 1975; Jaenike, 1978). Thus, sex may have a fitness advantage over cloning that counterbalances the cost of males. The distribution of reproductive modes in nature is consistent with this model: asexual lineages dominate in parasite-free areas, while sexual lineages are more frequent in areas where parasite selection is strong (Levin, 1975; Bell, 1982; Lively, 1987; Schrag *et al.*,

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1994; Verhoeven & Biere, 2013). In addition, laboratory and field studies show that parasites rapidly adapt to infect common clonal genotypes (Lively & Dybdahl, 2000; Jokela *et al.*, 2009; Koskella & Lively, 2009), and sexual reproduction has been found to be associated with host–parasite coevolutionary hotspots (King *et al.*, 2009, 2011). Finally, in direct experimental support of the Red Queen hypothesis, Morran *et al.* (2011) showed that coevolution with a virulent bacterial parasite favoured the spread and maintenance of outcrossing males in populations of the androdioecious nematode *Caenorhabditis elegans*. In contrast, in populations of *C. elegans* faced with noncoevolving bacteria, outcrossing failed to spread and self-fertilization dominated (see also Slowinski *et al.*, 2016).

Despite this supporting evidence, theoretical results challenge the Red Queen hypothesis as a general explanation for sex. All hypotheses for the maintenance of sex require strong selection against asexual lineages. For the Red Queen hypothesis, this requirement means that parasites must be highly virulent. [Here, we define virulence as the reduction in host fitness due to infection (Read, 1994)]. For example, May & Anderson (1983) showed that a coevolving parasite needed to entirely erase the fitness of 90% or more of infected hosts to prevent the extinction of the sexual subpopulation. Subsequent models echoed this result (Howard & Lively, 1994; Parker, 1994; Otto & Nuismer, 2004). If the genetic diversity of the asexual subpopulation is limited relative to the sexual subpopulation, moderate virulence can allow for coexistence of sexual and asexual lineages (Lively, 2010b): Howard & Lively (1994) showed coexistence of a sexual subpopulation and a single invading clone when coevolving parasites reduced the fitness of their hosts by only 50%. Do parasites commonly exert even this moderate level of virulence in natural populations? Unfortunately, we lack a general sense of the selective effect of parasites in nature. In some natural host–parasite systems, parasites are known to kill or sterilize their hosts. Studies of high virulence cases show patterns consistent with the Red Queen hypothesis, including selection against common clones (Lively & Dybdahl, 2000; Decaestecker *et al.*, 2007; Koskella & Lively, 2009; Wolinska & Spaak, 2009) and high frequency of asexual individuals where the risk of parasitism is low (Lively, 1987; King *et al.*, 2009; Gibson *et al.*, 2016). In other systems, parasite virulence is more difficult to estimate. For example, virulence can be low under experimental removal of competitors but high under natural competitive interactions with conspecifics (Augspurger & Kelly, 1984; Lively *et al.*, 1995; Tseng, 2004; Bell *et al.*, 2006).

The Red Queen's virulence problem seems more intractable when we consider one of the few things we do know to be true about parasites in the wild: their distribution and selective effect vary with environmental conditions. For example, Mitchell *et al.* (2005) found that infection with the bacterial parasite *Pasteuria ramosa* substantially reduces reproduction of its host *Daphnia magna* at high temperatures (20–25 °C), but is relatively avirulent at low temperatures (10–15 °C). The authors suggested that this temperature effect may explain the absence of evidence for parasite-mediated selection in *Daphnia* populations in the wild, where temperatures are typically below 15 °C (Little & Ebert, 2001). Many additional studies found significant effects of the environment on parasite virulence (Bedhomme *et al.*, 2004; Vale *et al.*, 2008; Cornet *et al.*, 2014; Debes *et al.*, 2017), as well as infectivity (host susceptibility) (Fellowes *et al.*, 1999; Price *et al.*, 2004; Mitchell & Read, 2005; Cayetano & Vorburger, 2013b), genetic specificity (Ferguson & Read, 2002; Blanford *et al.*, 2003) and replication (Stacey *et al.*, 2003; Fels & Kaltz, 2006; Lambrechts *et al.*, 2006; Tseng, 2006; Laine, 2007; Vale & Little, 2009; Civitello *et al.*, 2015). Accordingly, experimental coevolution studies of phage with their bacterial hosts show that the rate, form and strength of coevolution are sensitive to resource availability (Forde *et al.*, 2004; Harrison *et al.*, 2013), temperature (Duncan *et al.*, 2017) and population mixing (Brockhurst *et al.*, 2003; Gómez *et al.*, 2015). Some authors have thus suggested that this variability in parasite selection severely limits the relevance of the Red Queen for natural populations (Blanford *et al.*, 2003).

High parasite virulence is essential to the maintenance of sex by coevolving parasites. Does environmental variation in parasite virulence then 'kill' the Red Queen hypothesis? To address this question, we used a simulation approach to test the effect of temporal variation in parasite virulence on the maintenance of a diverse sexual population in competition with an invading clone. Virulence was calculated as the reduction in the number of offspring produced by infected hosts relative to uninfected hosts. To simulate temporal environmental variation, we allowed the number of offspring produced by infected hosts to vary from generation to generation. Our key finding was that temporal variation in virulence did not limit the ability of parasites to maintain sex in their hosts. On the contrary, high variation in virulence promoted parasite-mediated selection for sex, maintaining sexual individuals when mean virulence was far less than that required to maintain sex under constant virulence. We accordingly predict that natural variation in virulence could increase, rather than restrict, the relevance of the Red Queen hypothesis for natural host–parasite populations.

Model

The scripts for all simulations used in this study can be found at the GitHub Repository Bloody-minded-parasites

Defining virulence

Our model follows the structure of Lively (2010a). Virulence is defined as the proportional reduction in fitness of infected (W_I) relative to uninfected (W_U) individuals:

$$V = \frac{W_U - W_I}{W_U} \quad 1$$

We treated all hosts as annual (i.e. no variation in death rates), so fitness values were defined as:

$$W_U = \frac{b_U}{1 + a_U N} \quad 2a$$

$$W_I = \frac{b_I}{1 + a_I N}, \quad 2b$$

where b_U and b_I give the number of offspring produced by uninfected and infected individuals, respectively, in the absence of competition. We simulate the effect of competition using the coefficients a_U and a_I, which scale the number of offspring of uninfected and infected hosts, respectively, by host density, given by total population size N. In both our model and that of Lively (2010a), offspring production of infected and uninfected hosts was equally sensitive to population density ($a_U = a_I$). The equation for virulence thus reduced to:

$$V = 1 - \frac{b_I}{b_U} \quad 3$$

We refer readers to Lively (2009) for a discussion of competition between sexuals and asexuals when parasite virulence is density dependent ($a_U \neq a_I$).

In Lively (2010a), virulence was held constant throughout a simulation. The key modification we made here was to allow b_I to vary between generations of a simulation. By holding b_U constant, this created temporal variation in parasite virulence. We generated a variety of distributions for b_I so that we could evaluate the outcome of competition between sexuals and asexuals at different mean virulence levels and different degrees of variation around those means. In the section 'Temporal variation in virulence,' we present the sampling procedure for b_I in full detail. We compared the outcomes of these stochastic virulence simulations with those of the Lively (2010a) model, in which virulence was held constant through time.

Model structure

As in Lively (2010a), hosts and parasites were haploid, and both had nine possible genotypes defined by three alleles at each of two loci. Hosts were either sexual or asexual, while all parasites were asexual. With the exception of genotype and reproductive mode, host individuals were identical. Likewise, with the exception of genotype, parasite individuals were identical. In each generation of the simulation, hosts were exposed to parasites. We used the standard matching-alleles model of infection genetics to determine the outcome of this exposure (Frank, 1993, 1996). The matching-alleles framework models self-nonself recognition in which hosts fail to recognize 'matching' parasites as nonself and accordingly do not mount an immune response (Grosberg & Hart, 2000). In our simulations, if a parasite carried the same allele as a host at both loci (a 'match'), the parasite successfully infected the host. If a parasite did not match a host at one or both loci, the parasite was killed by the host immune system and no infection resulted.

At generation $t + 1$, prior to recombination, the number of sexual individuals carrying allele i at locus 1 and allele j at locus 2 was given by eqn (1) in Lively (2010a):

$$S'_{ij} = (1 - s)S_{ij}(W_I P_{ij} - W_U(1 - P_{ij})), \quad 4$$

where P_{ij} is the probability of infection for the ij^{th} host genotype and s is the proportion of sons produced by sexual females. Hence, the term $(1-s)$ represents the costs of males, which is twofold when $s = 0.5$. To determine the number of asexual individuals of the ij^{th} genotype at generation t+1 (A'_{ij}), we use eqn (4), excluding the cost of males term (1-s). Following this selection step, the number of sexual individuals of the ijth genotype at generation $t + 1$ is then adjusted to reflect recombination:

$$S''_{ij} = N'[(1 - r)q_{ij} + rq_i q_j \quad 5$$

Here, N' is total host population size at generation t+1, r is the recombination rate (equivalent of ρ in Lively (2010a)), q_{ij} is the post-selection frequency of the ijth genotype in the host population, and q_i and q_j are the frequencies of the ith allele at locus one and the jth allele at locus two, respectively. There is no equivalent recombination step for the asexual subpopulation.

The probability of infection of a sexual or asexual individual of the ijth genotype is given as:

$$P_{ij} = 1 - e^{-z\left[\frac{S_{ijI} + A_{ijI}}{N'}\right]}, \quad 6$$

where z is the number of parasite propagules per infection that make contact with a subsequent host [z is the equivalent of β in Lively (2010a)]. S_{ijI} and A_{ijI} indicate the number of infected individuals of the ijth genotypes of the sexual and asexual subpopulations, respectively. Equation (6) gives the probability of a host encountering one or more parasite propagules of a matching genotype, where one propagule suffices to infect a host. Full details of this equation are presented in Lively (2010a); eqn 4). Each generation, one uninfected migrant and one infected migrant per genotype entered the population with probabilities m_U and m_I, respectively.

Temporal variation in virulence

To model temporal variation in virulence, we introduced stochastic variation in b_I, which was a constant in Lively

(2010a). To do so, we held b_U constant and sampled the ratio of the number of offspring produced by infected relative to uninfected hosts (b_I/b_U) from a beta distribution. The beta distribution is a flexible, continuous distribution bounded from 0 to 1. Therefore, the sampled ratio corresponded to a value of b_I that was less than or equal to b_U and ≥ 0. These beta-distributed ratios then became the per-trial probabilities for a beta-binomial distribution from which we sampled b_I.

For the beta-binomial distribution, the number of trials was equivalent to b_U. In other words, for each sample, a coin is flipped b_U times with the probability of success (e.g. heads) per flip (i.e. trial) equal to the ratio drawn for that sample. For our purposes, the probability of success was the probability of making one offspring. So, if b_U is 10 and the mean value of the ratio across generations (i.e. samples) is 0.4, then infected hosts will make on average four offspring, for a mean virulence of 0.6. The beta-binomial distribution was sampled each generation to define the value of b_I for all infected individuals, and thereby virulence, in that generation. The binomial distribution allowed us to generate discrete values for b_I. Compounding it with a beta distribution generated flexibility in the mean and variance of the distributions of b_I.

We used models from McKenzie (1985) to generate autocorrelated, beta-distributed values of the ratio b_I/b_U (Fig. S1). The variable ρ gives the degree of correlation between values of the ratio in generations t and $t + 1$, with the ratio in generation $t + 1$ equal to that in generation t when $\rho = 1$. For simulations with zero or positive autocorrelation ($\rho \geq 0$), we used the PBAR model (eqn 3 in McKenzie, 1985), which defines a first-order autoregressive process:

$$X_t = 1 - E_t(1 - G_t X_{t-1}) \quad 7$$

X has a beta-marginal distribution defined by the shape parameters α and β. E_t is a sequence of random values sampled from a beta (β, α-h) distribution. G_t is an independent sequence of random values sampled from a beta (h, α-h) distribution. h is a function of ρ:

$$h = \frac{\rho\alpha(\alpha + \beta)}{\beta + \rho\alpha} \quad 8$$

For simulations with negative autocorrelation ($\rho < 0$), we used the NBAR model (eqn 5 in McKenzie, 1985), which defines a similar first-order autoregressive process:

$$X_t = F_t(1 - G_t X_{t-1}) \quad 9$$

X again has a beta (α, β) marginal distribution. F_t is a sequence of random values sampled from a beta (α, β-h) distribution, and G_t is defined as above. h is now given as:

$$h = \frac{\rho(\alpha + \beta)}{\rho - 1} \quad 10$$

Simulations

All simulations were run in R v3.4.4 (R Core Team, 2013). We initiated each simulation with 8000 susceptible, sexual hosts, with approximately equal representation from each of the nine possible genotypes. After a burn-in period of 1000 generations, a single asexual mutant arose in the population. We modelled this by introducing a single individual of a genotype that was identical to one in the sexual population, but did not pay the cost of males. Following introduction of the clone, we ran the simulation for an additional 1100 generations. Parameter values are given in Table 1. We selected combinations of the shape parameters α and β that produced beta distributions with different means and variances for the ratio b_I/b_U. The distributions are shown in Fig. 1 with their corresponding α and β values. To obtain an accurate estimate of the performance of sex in these stochastic simulations, we ran each parameter set 250 times. As in Lively (2010a), we then the evaluated the average behaviour of the simulations in the final 100 generations.

Simulation Results

If b_I and, accordingly, virulence were held constant through time, parasite virulence had to be at least 0.5 to prevent extinction of the sexual subpopulation (Fig. 2: bottom row). Sexual individuals made up fewer than 1% of populations in the final generations of simulations in which virulence fell below this threshold. This is consistent with prior findings (Howard & Lively, 1994; Lively, 2009, 2010a, 2011). Though still

Table 1 Values for parameters used in simulations

Parameter	Value	Description
b_U	10	Number of offspring produced by healthy hosts
a_U, a_I	0.0001	Competition coefficients; scale b_U and b_I by population density
s	0.5	The proportion of sons produced by sexual females
r	0.2	Rate of recombination
z	10	Number of parasite propagules produced per infection that make contact with a host
m_U	0.1	Probability of introducing one healthy migrant per genotype per generation
m_I	0.02	Probability of introducing one infected migrant per genotype per generation
Variable		
α	see Fig. 1	Shape parameter for beta distribution for b_I/b_U
β	see Fig. 1	Shape parameter for beta distribution for b_I/b_U
ρ	−0.5, −0.1, 0, 0.1, 0.5	Degree of temporal autocorrelation for b_I

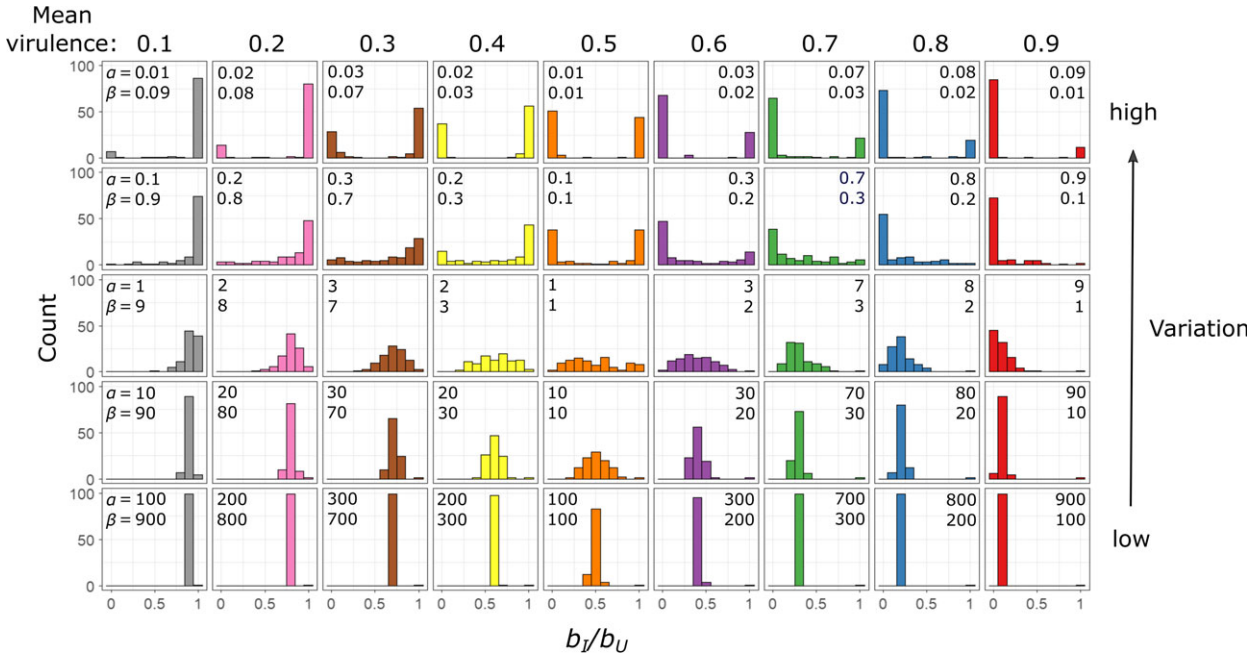

Fig. 1 Beta distributions for the ratio of the number of offspring produced by infected relative to uninfected hosts. Histograms show the distribution of 100 ratios sampled from the 45 different beta distributions used in this study. Specific values of the shape parameters α and β are shown in the upper corner of each panel (left corner for virulence ≤ 0.5, right corner for virulence > 0.5). Varying the ratio of α to β generated variation in the mean of the ratio b_I/b_U, which ranged from 0.9 (left) to 0.1 (right), corresponding to mean virulence of 0.1 to 0.9, respectively. Varying the magnitudes of α and β generated distributions with little (bottom) to substantial (top: u-shaped) variation in virulence. We then generated temporal variation in b_I by sampling b_I from a binomial distribution with these beta-distributed ratios as the per-trial probabilities. Mean virulence was then calculated using eqn. 3.

dominant, sexual individuals declined slightly in mean frequency in simulations in which virulence was highest (Fig. 2: bottom row, $V = 0.8$, 0.9). This likely reflected increased fluctuations in asexual and sexual population size as virulence increased, which lowered the mean representation of the dominant sexual subpopulation.

We then evaluated the maintenance of sex in simulations in which virulence varied from generation to generation. If temporal variation in virulence reduces the ability of parasite-mediated selection to maintain sex, we predicted that the proportional representation of sexual individuals at the end of a simulation would decline as variation increased around a given mean

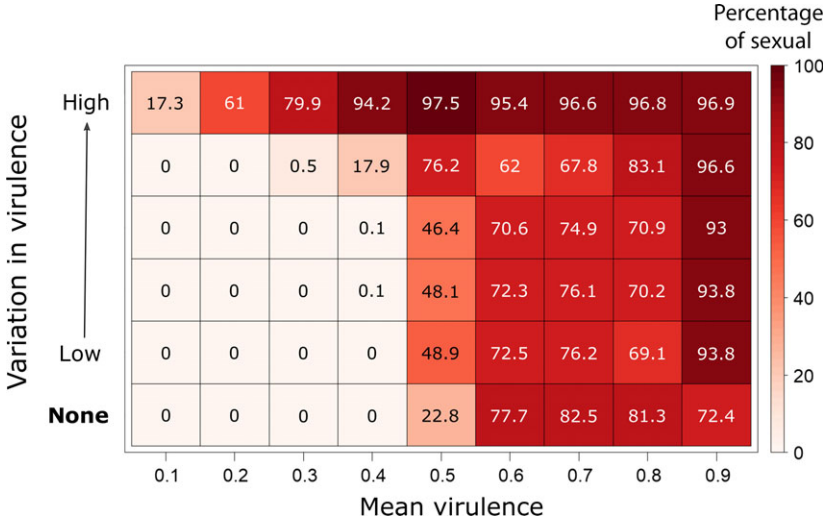

Fig. 2 The maintenance of sex according to mean virulence and the degree of temporal variation in virulence. Shading and numeric values indicate the mean percentage of individuals that were sexual in the final 100 generations of a simulation. Mean percentages were estimated from 250 simulations per parameter set. The bottom row gives the results of simulations in which virulence was held constant. Above this bottom row, variation in virulence increases from low to high, with sampling distributions corresponding to those shown in Fig. 1.

virulence. We first tested this prediction in the absence of temporal autocorrelation ($\rho = 0$).

We did not see support for this prediction at low levels of variation. Low-to-moderate temporal variation in virulence did not qualitatively change the pattern observed at constant virulence (Fig. 2). At these low levels of variation, we continued to see coexistence of sexual and asexual individuals when mean virulence exceeded 0.5, and extinction of sex at lower mean virulence. Low variation in virulence did alter the mean frequencies at which sexual individuals were maintained in the population. The most striking change was evident at a mean virulence of 0.5, where even minor variation in virulence more than doubled the prevalence of sexual individuals (Fig. 2: 22.8% sexual under no variation to 48.9% under low variation). Low variation corresponded to a slight reduction in the mean frequency of sexual individuals at higher mean virulence ($V = 0.6$–0.8), but an increase in their mean frequency at the highest mean virulence ($V = 0.9$). In the context of our model, we conclude that low-to-moderate temporal variation in virulence does not reduce the ability of parasite-mediated selection to maintain sex.

At high levels of variation, we saw the opposite of the predicted result. High temporal variation in virulence favoured sex, decreasing the mean virulence required to prevent extinction of sex (Fig. 2: upper two rows). Given sufficient variation, sexual individuals were able to coexist with asexual competitors when mean virulence was as low as 0.1 (Fig. 2: top left). High variation in virulence also tended to increase the mean frequencies at which sexual individuals were maintained. High variation around a mean virulence of 0.5 or greater (Fig. 2, top row) reduced the fraction of the population that was asexual at the ends of simulations to < 5%. Thus, in the context of our model, we concluded that high temporal variation in virulence enhanced the ability of parasite-mediated selection to maintain sex.

Variation in virulence induced larger fluctuations in population size and infection rate for the asexual relative to the sexual subpopulation. Trajectories of simulated populations show the asexual subpopulation declining sharply in number, and frequency, when parasite virulence shifted upward (Fig. 3b,c). This appears to arise from the fact that, when common, the asexual subpopulation was substantially more infected than the sexual subpopulation (Fig. 3, bottom row). The fluctuations were largest under high variation in virulence: windows of low virulence (Fig. 3c, top) were followed by sharp increases in asexual frequency (Fig. 3c, middle) and infection rate (Fig. 3c, bottom). The heavily infected asexual subpopulation then crashed when virulence increased.

Environmental variables may be weakly temporally autocorrelated (Halley, 1996; Inchausti & Halley, 2002). We examined the impact on sex of temporal autocorrelation in virulence for moderate levels of mean virulence ($V = 0.4$–0.6). Temporal autocorrelation did not change the effect of variation in virulence on the maintenance of sex (Fig. S1, S2). Notably, the most realistic estimate for temporal autocorrelation ($\rho = 0.1$, weak

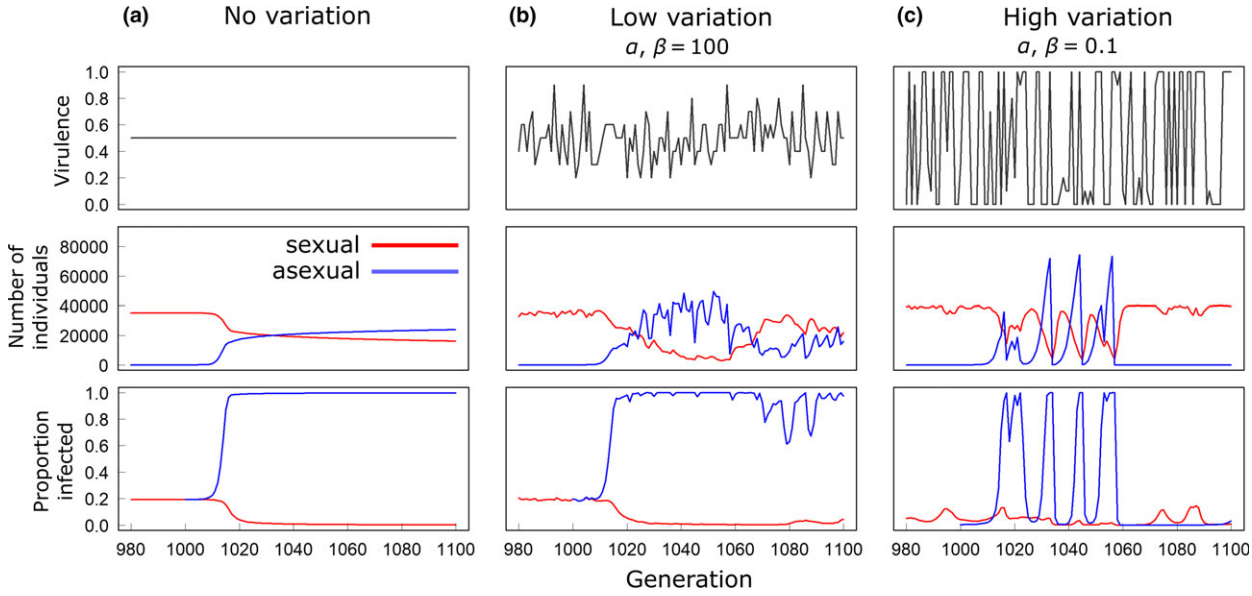

Fig. 3 Representative trajectories of simulated populations. Panels show virulence (upper row), the number of sexual (red) and asexual (blue) individuals (middle row), and the proportion of individuals infected (bottom row) through time, from generations 980–1100. Mean virulence was set to 0.5 for all simulations, and variation increases from (a) none to (b) low to (c) high. Each simulation was initiated with 8000 sexual individuals, and a single asexual individual was introduced at generation 1000.

positive autocorrelation) had little impact on the mean percentage of sexual individuals maintained at the end of simulations (Fig. S2a).

Discussion

The maintenance of sexual reproduction requires that a strong selective force counterbalance the per capita birth rate advantage of asexual competitors. Coevolving parasites can, in theory, confer a sufficient selective advantage on sex, but only if parasite virulence is high. This requirement of high virulence poses a major obstacle for the Red Queen hypothesis: very little data exist on virulence in natural populations, but sufficiently high virulence is thought to be rare in the wild. Additionally, ample empirical data show virulence to be unstable, shifting with a myriad of environmental conditions. Here, we used a simulation approach to ask: Does temporal variation in virulence further limit the ability of coevolving parasites to maintain sex? Based upon our simulation results, the answer is no. Low-to-moderate variation in virulence did not impact the outcome of competition between sexuals and asexuals, while high variation strengthened parasite-mediated selection for sex (Fig. 2).

This latter result shows that temporal variation in virulence could in fact relax the strict virulence requirements suggested by prior Red Queen models (May & Anderson, 1983; Howard & Lively, 1994; Parker, 1994; Otto & Nuismer, 2004). Under constant virulence, the maintenance of sex requires virulence of, at minimum, 0.5. In other words, parasites must reduce the fitness of infected hosts by 50% or more to prevent the extinction of sex. Here, we found that, with temporal variation in virulence, sex was maintained when mean virulence fell as low as 0.1. This means that parasite-mediated selection can maintain sex even when parasites reduce host fitness by only 10% on average, if in rare generations fitness is reduced substantially more (as much as 90%) (Fig. 2).

Why would variation in virulence favour sex? The trajectories of our simulated populations suggest possible explanations for this result. The asexual subpopulation suffered disproportionately when virulence increased between generations. Consistent with the Red Queen hypothesis, we see that in our simulations coevolving parasites preferentially infected the clone when it was common (Fig. 3, bottom rows). Accordingly, increases in virulence (Fig. 3b,c, top) were followed by sharp declines in the number of asexual individuals (Fig. 3b,c, middle). This process was accentuated by temporary declines in virulence, which released asexuals from parasite selection. Asexuals could then realize their per capita birth rate advantage, increasing to very high frequencies. This very common clone became the primary target of parasite selection as the coevolving parasite population rapidly adapted to infect it. When parasite virulence increased in subsequent generations, asexual numbers dropped substantially. This is most evident at high variation in virulence, as in Fig. 3c. Variation in virulence thus appears to strengthen negative frequency-dependent selection by amplifying parasite-mediated oscillations in asexual frequency.

In our model, parasites must maintain their ability to infect hosts in order for temporal variation in virulence to promote parasite-mediated selection for sex. In natural settings, virulence may not vary independently of other traits, such as parasite reproduction and transmission (Anderson & May, 1982; Ewald, 1983; Bedhomme *et al.*, 2004; Alizon *et al.*, 2009). The positive effect of variation on sex might accordingly be diminished if virulence were to covary with parasite reproduction or transmission. Adding even more complexity, experimental studies commonly reveal a genetic basis to the response to environmental variation (e.g. Ferguson & Read, 2002; Blanford *et al.*, 2003; Fels & Kaltz, 2006; Vale *et al.*, 2008; Lazzaro & Little, 2009): for example, Laine (2007) showed that, for the fungal parasite *Podosphaera plantaginis* infecting ribwort plantain, a 2 °C decrease in temperature reduces the fitness of some parasite strains by nearly half, while other strains are unaffected. Simulating a genetic basis for temporal variation in virulence requires that the shape of the distribution for parasite virulence vary across genotypes. This extension lies outside the scope of the current study, but we predict that the effects on sex would resemble those observed here.

Most prior studies of environmental variation and coevolution have focused upon another condition of parasite-mediated selection for sex: tight genetic specificity of host and parasite. Many Red Queen models, including ours, use a matching-alleles interaction matrix, which assumes that a parasite genotype is the best at infecting one host genotype and relatively poor at infecting all other genotypes. The idea here is that parasite adaptation to infect a common host genotype comes with a loss of infectivity on rare host genotypes. This trade-off exerts negative frequency-dependent selection, maintaining polymorphism in the host population. What if the specificity of this genetic interaction were unstable, shifting in response to the environment? Such a GxGxE interaction might prevent a parasite population from applying consistent selection against the most common host genotypes. To address this prediction, Mostowy & Engelstädter (2011) modelled temporal variation in both the strength and specificity of coevolutionary selection. They found that temporal variation in the specificity of a matching-alleles interaction can weaken negative frequency-dependent selection by periodically halting parasite-mediated oscillations in allele frequencies. In their model, this temporal variation in specificity seemed to have a greater impact on coevolutionary dynamics than did variation in the strength of selection, which was the

focus of our study. To simulate temporal variation in the strength of selection, they varied the host's fitness cost of infection and the parasite's fitness cost of failing to infect. This variation changed the speed at which allele frequencies oscillated but did not qualitatively change the form of those oscillations. Mostowy & Engelstädter (2011) did not directly address the maintenance of sex. It would be valuable to extend our study to include variation in specificity, as in Mostowy & Engelstädter (2011), and contrast the relative significance of variation in virulence vs. variation in specificity for the maintenance of sex.

The empirical data at hand indicate that variation in virulence, and other main effects that contribute to the strength of parasite selection, may be more relevant to natural coevolutionary interactions than variation in specificity. A few laboratory studies support a shift in the specificity of infection with environmental change (Tétard-Jones *et al*., 2007; Bryner & Rigling, 2011; Sadd, 2011), while other studies have employed sufficient replication to reject meaningful GxGxE effects (Vale & Little, 2009; Cayetano & Vorburger, 2013a,b). Their relevance in nature is unknown. The evidence for variation in virulence is stronger: laboratory studies clearly show that virulence can vary with environmental conditions (Ferguson & Read, 2002; Bedhomme *et al*., 2004; Mitchell *et al*., 2005; Cornet *et al*., 2014; Mursinoff & Tack, 2017). We do not know how these laboratory-based results play out in natural populations, but virulence consistently varies with minor environmental change in the laboratory, implying that temporal variation in virulence may be common in nature. Based upon our theoretical results, estimates of the mean and range of virulence in a diversity of natural host–parasite interactions would serve as valuable data for evaluating the generality of the Red Queen hypothesis.

To conclude, our simulation results show that variation in virulence does not check the Red Queen. Minor variation has little effect on parasite-mediated selection for sex, while substantial variation in virulence promotes negative frequency-dependent selection, favouring sex. As in several other theoretical studies (Salathé *et al*., 2008; Engelstädter & Bonhoeffer, 2009; Lively, 2009, 2010a, 2011), the inclusion of biological reality expands, rather than restricts, the conditions under which coevolving parasites maintain sex.

Maynard Smith (1985) pointed out that, to maintain sex, the natural environment must be 'bloody-minded,' such that favoured combinations of alleles become disfavoured in subsequent generations (Maynard Smith, 1971b, 1978):

> There is, at least at first sight, a serious difficulty with such models. ...they require the environment to behave in a very odd way.... Not only do environmental features change: correlations between features changes. It is hard to believe that God is as bloody-minded as that.
>
> (p. 168: Maynard Smith, 1985)

Jaenike (1978) and Hamilton (1980) subsequently suggested to Maynard Smith that this bloody-mindedness could come from parasites, rather than the environment *per se*.

> Suppose that alleles at two linked loci are concerned with resistance to a parasite, which has a corresponding pair of virulence loci. This can give rise to cyclical changes in genotype frequency, and to changing linkage disequilibria. It is not God, but a parasite, that is being bloody-minded.
>
> (p. 169: Maynard Smith, 1985)

Here, we find that temporal variation in the environment may offer hosts a brief reprieve, but parasites remain as bloody-minded as ever.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Figure S1 Temporal autocorrelation.
Figure S2 The maintenance of sex under temporally autocorrelated variation in virulence.

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