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REVIEW

Current hypotheses for the evolution of sex and recombination

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Abstract

The evolution of sex is one of the most important and controversial problems in evolutionary biology. Although sex is almost universal in higher animals and plants, its inherent costs have made its maintenance difficult to explain. The most famous of these is the twofold cost of males, which can greatly reduce the fecundity of a sexual population, compared to a population of asexual females. Over the past century, multiple hypotheses, along with experimental evidence to support these, have been put forward to explain widespread costly sex. In this review, we outline some of the most prominent theories, along with the experimental and observational evidence supporting these. Historically, there have been 4 classes of theories: the ability of sex to fix multiple novel advantageous mutants (Fisher–Muller hypothesis); sex as a mechanism to stop the build-up of deleterious mutations in finite populations (Muller's ratchet); recombination creating novel genotypes that can resist infection by parasites (Red Queen hypothesis); and the ability of sex to purge bad genomes if deleterious mutations act synergistically (mutational deterministic hypothesis). Current theoretical and experimental evidence seems to favor the hypothesis that sex breaks down selection interference between new mutants, or it acts as a mechanism to shuffle genotypes in order to repel parasitic invasion. However, there is still a need to collect more data from natural populations and experimental studies, which can be used to test different hypotheses.

Key words: evolution of sex, fitness-associated sex, Hill–Robertson interference, mutational deterministic hypothesis, Red Queen hypothesis

INTRODUCTION

What is sex?

Sexual reproduction is usually defined as a means of propagation that requires 2 parents to combine genetic material, usually by uniting 2 cells (gametes) containing chromosomes from the parents, in order to form a zygote. Before gametes are produced, the parents' genomes first undergo recombination during meiosis (Kleckner 1996). Therefore, researchers concerned with the evolution of sex are also interested in determining what conditions favor the evolution of recombination, as it is seen as a precursor to the appearance of obligate sex.

This contrasts with asexuality, where a parent clones its genotype to reproduce. Asexuality is very rare in nature, with only approximately 0.1% of animal species reproducing asexually (Vrijenhoek 1998). Most asexu-

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als' lineages have recently evolved from sexual predecessors (Vrijenhoek 1998; Simon *et al.* 2003), although there may exist a few 'ancient' asexuals, the best-known candidate being the bdelloid rotifers (Vrijenhoek 1998).

The prevalence of sexual reproduction indicates that there should be a clear and obvious reason as to why it is advantageous. However, this is far from the case – the origin and maintenance of sexual reproduction has remained one of the most elusive questions in evolutionary biology. The reason for this is that sex incurs major costs in comparison to asexual reproduction (Maynard Smith 1978), and to this day, no universally accepted explanation exists as to how sex evolved and is maintained in the face of these disadvantages. This review will describe some of the major costs associated with sex, and the most prominent hypotheses that have been put forward to explain its evolution and maintenance.

Sex is a costly endeavor

The most famous of the major costs has been labelled as the 'twofold cost' of sex. This manifests itself through 2 outcomes, due to the fact that sexual females invest resources into the production of males, or male gametes in the case of hermaphrodites, which in themselves do not themselves provide any resources to the next generation (see the recent review by Lehtonen *et al.* 2012 for more information). The first and probably most common usage refers to a 'cost of males' (Maynard Smith 1978), illustrated in Fig. 1. With biparental sexual reproduction, a male and a female have to meet in order to reproduce.

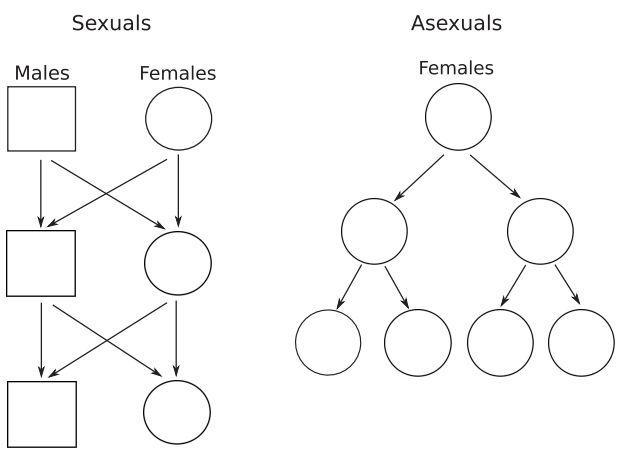

Figure 1 A schematic illustrating the twofold cost of males. Males are represented by squares and females by circles.

If this results in the birth of 1 son and 1 daughter, on average, then population will be maintained at a constant size. However, in a population of asexuals, energy is invested only in the female function, rather than in both female and male functions. Therefore, parthenogenetic females simply need to clone themselves, and males become irrelevant. As a result, if each parent produces two female offspring, an asexual population can quickly double in size and easily displace existing sexuals. The twofold cost also refers to a 'cost of meiosis' in anisogamous organisms (Williams 1975; Lively & Lloyd 1990), where each sexual parent only contributes half its genes to its offspring, decreasing its genetic contribution and thus the relatedness between parent and offspring.

There are additional costs that can affect the possible emergence of sexual reproduction. Recombination can destroy positive associations between selected clusters of alleles, reducing an individual's fitness, so selection will act against maintaining recombination (Nei 1967). Sexuals also have to expend energy to find mates, and there is the risk of sexual reproduction spreading diseases between parents, or to their offspring (Lockhart *et al.* 1996; Otto 2009). However, it has been argued that the cost of males can be decreased through sexual selection (Agrawal 2001; Siller 2001), increased intraspecific competition amongst asexuals (Doncaster *et al.* 2000) or sexuals increasing their variance in fecundity (Blachford & Doebeli 2009). Costs to sex have been observed in field studies of *Antennaria parlinii* (Michaels & Bazzaz 1986), *Potamopyrgus antipodarum* snails (Jokela *et al.* 1997) and psychid moths (Kumpulainen *et al.* 2004). A 'recombination load', caused by breaking apart selected combinations of genes, has also been observed in *Drosophila melanogaster* (Charlesworth & Charlesworth 1975).

Direct advantages to sex and recombination

Several physiological explanations have been offered to suggest why sex may be advantageous. These 'direct' hypotheses account for the evolution of sex and recombination due to an immediate effect they confer on a host's fitness. This is in contrast to 'indirect' hypotheses, which explain the evolutionary advantages of sex and recombination through mixing genetic material from 2 parents (Otto & Lenormand 2002).

One direct hypothesis is that sexual reproduction repairs damaged DNA and, therefore, 'regenerates' the genome (Bernstein *et al.* 1988). However, there is little evidence that recombination is essential for DNA repair, even though it could offer an inexpensive way of

doing so (Maynard Smith 1988). Experiments with *Bacillus subtilis* and *Haemophilus influenzae* also failed to find evidence for transformation (and, therefore, recombination) evolving in order to repair damaged DNA (Redfield 1993). Subsequent experiments also show that competence and transformation protect *Streptococcus pneumoniae* against non-DNA-damaging processes, indicating that transformation may not necessarily have evolved solely as a mechanism to repair damaged DNA (Engelmoer & Rozen 2011). The repair hypothesis also struggles to explain the maintenance of sexual reproduction, because double-strand breaks are induced during meiosis in sexuals (Kleckner 1996). It also does not consider the evolution of asexual diploids, which can utilize the second copy of a specific gene as a template for DNA repair (Otto & Lenormand 2002).

Another proximate explanation for sex is that it can improve the transmission of 'selfish' genes (Goddard *et al*. 2001). In sexual individuals, transposable elements can be passed on to every offspring produced, even if present as a heterozygote; hence, their rate of spread will be faster in comparison to non-selfish genes. Sex could then evolve as a byproduct of ensuring rapid transmission of such elements, even if they cause a substantial fitness reduction to the host (Hickey 1982). However, although this could explain the initial emergence of sex, it cannot easily explain how sex is maintained. Once selfish genes invade a population and reach a high frequency, asexual individuals should be able to propagate selfish elements just as quickly as sexuals (Otto & Lenormand 2002).

Population genetics advantages to sex and recombination

Because such direct, short-term hypotheses have limited power in explaining the evolution of sexual reproduction, much more attention has focused on indirect population genetics-based hypotheses instead. The concept underpinning these explanations is that by combining genomes from different backgrounds, sex and recombination create better genotypes that would not be formed asexually. Such fitter sexual individuals are more likely to reproduce and persist in the long term. This idea was memorably summarized by Williams (1975) who compared the different mating systems acting in a fluctuating environment to buying lottery tickets. Asexuality was akin to buying lots of tickets that all had the same number. Sex, however, was similar to buying greatly fewer tickets, but with each one having different numbers, therefore more likely to produce a 'winner'. The main population-genetics hypotheses can be placed into 1 of 3 categories:

1. *Breaking apart selection interference.* Also known as Hill–Robertson interference (Hill & Robertson 1966), this is where selection acting on 1 locus interferes with selection acting at a second, linked locus in a finite population. Recombination breaks apart such interference and improves the response to selection. The classic theories related to this broad idea are the Fisher–Muller hypothesis (Fisher 1930; Muller 1932), where sexuals can combine beneficial alleles into the same genome, and Muller's ratchet (Muller 1964) caused by the irreversible build-up of deleterious mutations in finite asexual populations.
2. *Parasitic resistance (also known as the Red Queen hypothesis)*. By recombining genomes, sexuals are more likely to create new genotypes that are able to adapt to environments that fluctuate deterministically. The best-known application of this hypothesis concerns sex as a means to resist parasitic infection (Jaenike 1978; Hamilton *et al.* 1990).
3. *Mutational deterministic.* This hypothesis is based on a deterministic model of an infinite population. If the deleterious mutation rate is high enough and deleterious mutants act synergistically (i.e. a collection of deleterious mutants cause a greater reduction in log fitness than expected if acting independently), recombination can restore fitness variance that would otherwise decrease due to deleterious mutation accumulation (Kondrashov 1982, 1993).

This review will give an overview of each of these theories and their ability to explain the prevalence of sex. Although there exist realistic scenarios under which each hypothesis could explain the evolution of costly sex, none have managed to provide a sufficient explanation as to why costly sex is ubiquitous in nature, especially since there has been little empirical testing of some of these hypotheses. This is because it has been hard (until recently) to obtain accurate data against which to test these theories. The difficulty of explaining sex has led to the suggestion that a 'pluralist' approach might provide the best answer, where several processes work in tandem to overcome the twofold cost of sex (West *et al.* 1999). Although West *et al.* (1999) considered Red Queen and mutational deterministic processes working together, subsequent research has also considered how other mechanisms interact to potentially maintain sex (e.g. Howard & Lively 2002; Hartfield *et al.* 2010). Here, we shall separate the discussion of the different hypotheses, due to different mechanisms affecting

the evolution of sex in each case (e.g. finite population size, fluctuating selection or the effects of epistasis).

BREAKING APART SELECTION INTERFERENCE

Early theories: Fisher–Muller hypothesis and Muller's ratchet

In finite asexual populations, sampling and drift can impede the efficacy of selection acting at linked loci. By recombining genomes, the response to selection at individual loci is increased, leading to a higher mean fitness. It has been argued (Burt 2000) that a similar idea was first put forward by Weismann (1887), who contended that sexual reproduction "may be regarded as a source of individual variability, furnishing material for the operation of natural selection" (Weismann 1887, p. 609). This increase in genome-wide variability thereby causes sex to bring together favorable alleles, while less-fit genomes will be 'weeded out'.

Fisher (1930) and Muller (1932) formalize this argument as applied to the case of 2 beneficial alleles arising at separate, linked loci. Both argue that sexuals can recombine the 2 alleles into a new genome, quickly creating a fitter individual. Asexuals, in contrast, would have to wait for both mutants to arize and fix in sequence in the same lineage, which would take a greatly increased amount of time. This claim is verified by Christiansen *et al.* (1998), who determines that in asexual populations, the production of a genome containing both mutants would take on the order of

$$1/\sqrt{N\mu^2} \text{ generations, but only } 1/\sqrt[3]{\frac{1}{3}RN\mu^2}$$

in sexual populations, where μ is the mutation rate at each locus, R the recombination rate between the 2 loci, and N is the haploid population size. Figure 2 outlines a schematic of this process.

Another classical hypothesis to explain the evolution of sex and recombination in finite populations was put forward by Muller (1964). In nature, he notes that mutations are mainly deleterious (which has been confirmed by subsequent studies, such as Eyre-Walker & Keightley 2007), and back mutation to restore the wildtype allele is rare. Both conditions will lead to the buildup of deleterious mutations, but initially there will exist a proportion of mutation-free individuals. In finite populations, such a class of individuals will eventually be lost

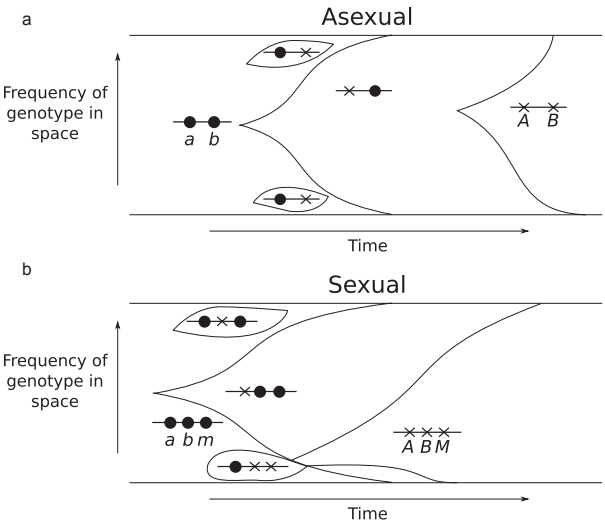

Figure 2 How the Fisher–Muller hypothesis works. (a) shows how an asexual lineage will sequentially fix advantageous alleles *A* and *B* from initial allelels *a* and *b*; and (b) shows how a modifier allele for increased recombination *M* can combine the 2 alleles to create the fitter genome much more quickly. As such, the modifier allele *M* spreads through hitchhiking (Otto & Barton 1997; Roze & Barton 2006). The figure is modified from Muller (1932).

and cannot be recreated. This loss constitutes 1 'click' of the ratchet. Over subsequent generations, the loss of the least-loaded class will continue, which would also lead to deleterious alleles fixing in the population (Charlesworth & Charlesworth 1997). This can cause an irreversible degradation of the genome that can drive a population to extinction (Lynch *et al.* 1993). Therefore, sex and recombination are beneficial through the recreation of genomes having smaller numbers of deleterious mutations, preventing degradation of the genome over time. Subsequent work has shown that only a small amount of recombination is needed to stop the ratchet (Charlesworth *et al.* 1993). Furthermore, deleterious mutations are unlikely to build up if compensatory mutations arise, except in very small populations (Poon & Otto 2000).

General selection interference and the Hill–Robertson effect

In the years following Muller (1964), there was renewed controversy around whether the Fisher–Muller mechanism or Muller's ratchet could explain the ubiquity of sex, especially in comparisons of finite-popula-

tion models against infinite-population models (reviewed in Felsenstein 1974). The Fisher–Muller hypotheses and Muller's ratchet are explored and united in a seminal paper by Felsenstein (1974), which explains how the Fisher–Muller hypothesis and Muller's ratchet are conceptually the same as what he describes as the Hill–Robertson effect, named after the paper by Hill and Robertson (1966). In it, a mixture of diffusion equations and computer simulations are used to demonstrate that in finite populations, selection and drift creates chance associations between alleles, with negative associations persisting for longer. Genetically, this reflects the reduced effectiveness of selection at a specific allele through chance associations with a selected linked locus. This process leads to an increase in fitness variance at the focal site, due to the effect of selection acting on linked sites, but also a reduction in total fitness variance in the population, usually (but not always) leading to a subsequent decrease in the effective population size, N_e. Felsenstein (1974) used simulations to investigate how recombination increases the fixation rate of beneficial mutations (Fisher–Muller hypothesis), and stops the build-up of deleterious mutations (Muller's ratchet), if different selection coefficients and mutation rates are used.

Further research has investigated various types of selection interference that can be described as a Hill–Robertson effect. Such processes generally lie in 1 of 4 main categories, as summarized by Charlesworth *et al.* (2009):

1. *Genetic hitchhiking*. A selective sweep arising in the genome can drag alleles at linked loci with it to fixation, reducing levels of genetic variability around it (Maynard Smith & Haigh 1974). Neutral alleles are generally affected, although weakly deleterious alleles could also hitchhike in regions of low recombination (Hadany & Feldman 2005; Chun & Fay 2011; Hartfield & Otto 2011), or polymorphism could be lost at linked loci under balancing selection (Peck 1993). Sweeps can also interfere with fixation of new advantageous alleles if they arise at linked sites, as described in the Fisher–Muller hypothesis (Barton 1995b). This is also known as 'clonal interference' if acting in clonal organisms (Gerrish & Lenski 1998).
2. *Background selection*. Deleterious mutations enter the population via mutation and are generally removed quickly by selection. This also removes neutral variation around the site of deleterious alleles (Charlesworth *et al.* 1993, 1995; Hudson & Kaplan 1995). Background selection could also impede the spread of advantageous alleles (Johnson & Barton 2002; Bachtrog & Gordo 2004) and allow other deleterious alleles to persist in the population for a longer time (Barton 1995b).
3. *Muller's ratchet*, as described above.
4. *Weak Hill–Robertson effects*. If a large number of linked sites are subject to reversible mutation between advantageous and deleterious states, then linkage can cause deleterious alleles to persist at frequencies above that expected by mutation-selection equilibrium (McVean & Charlesworth 2000).

Because of all these different classifications, most of the classical theories explaining the evolution of sex in finite populations are usually described as special cases of a single theory, in which recombination is beneficial by breaking apart interfering mutations and increasing the response to selection.

Hill–Robertson effects selecting for sex and recombination

In a general analysis of the Hill–Robertson effect, Barton (1995b) shows how recombination between 2 selected alleles could increase the efficacy of selection acting on both. Otto and Barton (1997) demonstrate that this mechanism can select for increased levels of recombination at a modifier locus, but the increase in frequency of a recombination modifier is only significant if linkage between loci is initially tight, because even a small amount of recombination could greatly increase the efficacy of selection. Further analyses demonstrate how negative linkage disequilibrium arises by chance in finite populations, even if the population started in linkage equilibrium, which leads to selection for increased recombination (Barton & Otto 2005). Recombination also helps to accelerate the spread of advantageous alleles over time, after interference is broken down (Roze & Barton 2006). However, Barton and Otto (2005) and Roze and Barton (2006) conclude that although breaking down interference selects for higher levels of recombination, the effect is only strong in small populations and cannot explain the evolution of costly sex in large populations.

This view has changed over the past 5 years, with the finding that breaking down selection interference can select for increased levels of recombination in larger populations, if it acts over multiple linked loci. Otto and Barton (2001) show that if acting over 3 loci experiencing directional selection, a modifier is not favored in

populations of $N > 10\ 000$ chromosomes unless synergistic epistasis is present between loci (which also creates negative associations that selects for recombination; see section on mutational deterministic). However, in 11-locus simulations, a modifier is selected for in populations larger than 10 000 individuals. Extending this, Iles *et al.* (2003) demonstrate that stronger selection for recombination modifiers arises in very large, finite populations (the largest population investigated consisted of 100 000 haploid individuals), if individuals contain more loci experiencing directional selection. Keightley and Otto (2006) show that recombination could be very strongly selected for in large populations consisting of individuals subject to recurrent deleterious mutation across multiple, linked loci. It has also been shown that these large advantages to recombination could potentially overcome a twofold cost of sex, but only if modifier genes increase the frequency of sex to low levels.

The studies mentioned above mainly consider selection for recombination, if populations are subject to deleterious mutation (except Iles *et al.* 2003), where mutations are solely advantageous). However, such work does not consider the effects of both advantageous and deleterious mutations acting together. Peck (1994) and Peck *et al.* (1997) demonstrate how sex can move novel advantageous alleles away from deleterious genetic backgrounds, increasing their fixation probability and leading to a higher population mean fitness. Therefore, the presence of both advantageous and deleterious mutation can offer additional selection for recombination. This is demonstrated by Hartfield *et al.* (2010), whose simulations show greater selection acting on a recombination modifier in populations subject to recurrent advantageous and deleterious mutation at multiple loci compared to populations exposed to deleterious mutation only.

Effect of spatial structure

Peck *et al.* (1999) and Salathé *et al.* (2006) demonstrate that costly sex can be maintained against asexual invasion, if there is sufficient population subdivision. This is a consequence of asexuals accumulating excessive deleterious mutations, because population subdivision increases the time needed for an invading asexual to fix in the entire population. Figure 3 shows a schematic of this process. Salathé *et al.* (2006) also note that population subdivision disfavors asexuals because the smaller within-deme population sizes increase the rate of Muller's ratchet (Gessler 1995; Higgins & Lynch 2001). The benefits of sex also increase with population size, because this further slows down the spread of an asexual (Salathé *et al.* 2006). Martin *et al.* (2006) demonstrate that subpopulations can maintain polymorphism, increasing selection on a recombination modifier even in very large overall population sizes. Hartfield *et al.* (2012) explore asexual invasion into a structured

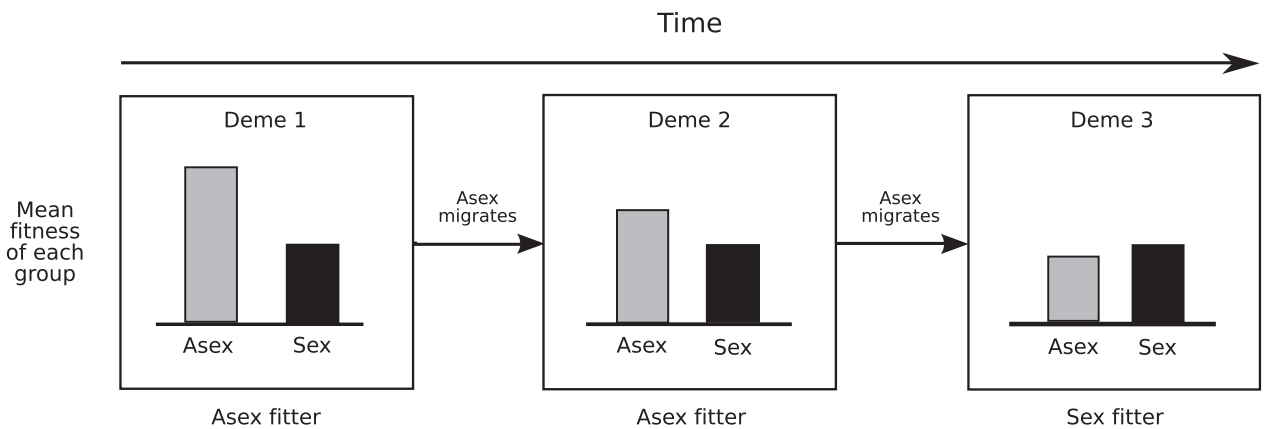

Figure 3 The maintenance of sex in a subdivided population. Initially, an asexual has a twofold advantage and fixes in the first deme. However, when it migrates to a second deme, its advantage would be lower due to deleterious mutation accumulation. Given enough time and demes to transfer to, asexuals would eventually have a lower mean fitness than sexuals, so sexuals eventually outcompete asexuals. Only 3 demes are shown here for brevity, but the argument can be applied to any number of demes, given a low enough migration rate.

population subject to deleterious and advantageous mutation and various levels of population subdivision, and demonstrate that large populations (N = 40 000 overall) with modest levels of F_{st} (approximately 0.15 was the lowest level found) maintained sex with a twofold cost. The greatest advantage to sex arose if demes were arranged in a stepping-stone formation, with individuals subject to both advantageous and deleterious mutation.

Effect of diploidy

All the above studies considered haploid individuals, but most species in nature are diploid. Not only do sexual diploids undergo crossing-over, which alters the arrangement of alleles on different loci at the same chromosome, but also segregation, which alters the associations within a specific locus. In infinite populations, segregation can cause sexuals to obtain a higher mean fitness through restoring the fittest homozygous genotype (Chasnov 2000; Otto 2003), and aid the fixation of advantageous alleles in diploid populations with intermediate dominance (h = 1/2) (Kirkpatrick & Jenkins 1989). Furthermore, sex is selected for under a wider range of deleterious selective strengths and dominance values in inbred populations, as inbreeding is more likely to create unfit homozygotes that are subsequently purged by selection (Agrawal & Chasnov 2001; Otto 2003).

Recent models have incorporated finite population sizes, where selection interference also arises between loci. Haag and Roze (2007) show that at a single locus subject to deleterious mutation, with recessive heterozygote mutants (h < 1/2), small sexual populations (generally less than N = 10 000) have a lower fitness load compared to asexuals, where the fitness load is defined as the difference between the population's mean fitness and its maximum possible value. The load is reduced in sexuals because segregation breaks down negative associations that arise due to drift within loci. These negative associations are more prevalent in subdivided populations, generating higher loads in asexuals relative to sexual populations.

However, Roze (2009) and Roze and Michod (2010) show that recombination and sex modifiers in finite populations are selected *against* if deleterious mutants are partially recessive (h < 0.2), because recombination tends to break correlations in heterozygosity across multiple, linked loci, and thus reduce the frequency of genotypes that are heterozygous at multiple loci, which have the highest fitness. This result makes it harder to explain whether breaking apart selection interference could explain the ubiquity of sex, because there is evidence that most deleterious alleles are partially recessive in nature (Simmons & Crow 1977).

Experimental evidence for selection for sex by breaking down selection interference

Several experimental studies have demonstrated that sex can increase the efficacy of selection by breaking down interference between loci. This paper will only focus on those demonstrating that breaking apart selection interference creates a benefit for sexuals populations compared to asexuals. There are many other studies demonstrating that recombination increases the efficacy of selection at the genetic level; these are discussed further in Charlesworth *et al.* (2009) and references therein.

Malmberg (1977) verifies the Fisher–Muller hypothesis using the bacteriophage T4, by observing that cells that interchange strands with others have a higher rate of adaptation compared to those with no recombination. A similar finding is observed in *D. melanogaster* by Rice and Chippindale (2001) and in *Chlamydomonas reinhardtii* where sexuals were better able to overcome the effects of clonal interference, leading to an accelerated adaptation (Colegrave 2002). However, while those studies show that sex is advantageous through accelerating adaptation, Zeyl and Bell (1997) find that sexual strains of yeast only increased mean fitness in environments to which populations were already adapted, as opposed to strains placed in novel environments. This suggests that sex is maintained predominantly through preventing the buildup of deleterious mutations via Muller's ratchet. Poon and Chao (2004) use population bottlenecking in a system of the RNA bacteriophage Φ6 in order to mimic the effects of genetic drift, and observe that asexuals have a lower response to selection compared to sexuals. Similarly, sexuals strains of yeast in a stressed environment had a higher fitness compared to asexuals, and a higher variance in fitness, again increasing the response to selection, in line with Weismann's hypothesis (Goddard *et al.* 2005). Morran *et al.* (2009) show that *Caenorhabditis elegans* evolved outcrossing not only to increase the rate of adaptation, but also to prevent deleterious mutational meltdown. Finally, asexual lineages of *P. antipodarum* snails were found to accumulate deleterious mutations at an accelerated rate in mitochondrial genomes, as measured using polymorphism to divergence ratios at noncoding and synonymous sites (Neiman *et al.* 2010).

SEX TO RESIST PARASITES: ESCAPING THE RED QUEEN

Coronation of the Red Queen

During the 1970s, the hypothesis that sex evolved to break down selection interference was subject to much criticism (reviewed in Jaenike 1978 and Maynard Smith 1978). At the time, it was seen as a 'group selectionist' argument: that is, sex evolves due to the benefit it offers to a group of individuals, as opposed to the benefits offered to modifier genes that increase levels of sex. It was also unclear at the time whether there was a sufficiently long-term benefit to breaking down selection interference that could explain the maintenance of sex. Because of this, Jaenike (1978) proposed, using a verbal model, that sex could be beneficial through creating rare genotypes, which can resist parasites that have adapted to infect individuals with a specific genotype.

This hypothesis gained prominence after Hamilton (1980) formalized Jaenike's argument and demonstrated that, with fluctuating selection, sexuals could gain a twofold fitness advantage over asexuals, if the recombination rate and fecundity of sexuals was high enough. This enabled the creation of a higher frequency of rare genotypes that are resistant to parasites. However, by more explicitly considering the epidemiological dynamics of parasitic infection (such as pathogen reproductive and infection rates), May and Anderson (1983) show that sex only has a twofold fitness advantage if infection is nearly lethal to the host. Hamilton *et al.* (1990) demonstrate that, given a high enough number of loci that determine the host's infection susceptibility, and if individuals are subject to rank-order truncation selection (i.e. the least fit are automatically killed off), then costly sex could fix in a population. Note, however, that this model assumes individuals consisting of multiple link loci subject to selection, and truncation selection that creates synergistic epistasis between deleterious loci. Therefore, sex could confer additional benefits through breaking apart selection interference, or restoring fitness variance according to the mutational deterministic model (see next section).

This basic model suffered from limitations, specifically with regards to whether the creation of rare genotypes could select for modifier genes for increased recombination. Ladle *et al.* (1993) extend Hamilton's model to a subdivided population, and demonstrate that if there are large discrepancies between host and parasite migration rates then sex would be selected against. This is because migration restores genotypes from other regions that would otherwise be rare in that deme, removing the benefits to sex. With regards to sex in fluctuating environments, Barton (1995a) shows that linkage disequilibrium needs to change sign rapidly (every 2 to 5 generations) for recombination to be favored in infinite populations. Furthermore, Otto and Nuismer (2004) show that under a variety of species interactions, increased levels of recombination would be disfavored because adapted gene combinations would be broken apart, unless the fitness reduction due to parasitic infection was strong.

Expansion of Red Queen models

Subsequent investigations of the Red Queen hypothesis aimed to answer either 1 of 2 main questions. First, what processes select for increased sex and recombination under this mechanism? Second, are there any ways in which the basic model can be extended so that host–parasite interactions select for sex under a wider range of biologically realistic conditions? Both these issues have been rigorously investigated using theoretical studies.

Concerning the first question, Peters and Lively (1999) determine that antagonistic coevolution leads to fluctuating linkage disequilibrium and epistasis in parasites and hosts. This leads to selection for recombination modifiers through the creation of rare genotypes that confer higher fitness. Conversely, there is little advantage to recombination through increasing additive genetic variance in fitness, which improves an individual's response to directional selection. This result is formalized by Gandon and Otto (2007), who also find that increased parasitic virulence causes more rapid fluctuations in host and parasite genotypes, which are the conditions that favor higher rates of recombination. This finding could be used to determine whether advantages to sex observed in field and experimental studies arise due to Red Queen dynamics, or instead through breaking apart selection interference (Barton 2010). This point is especially of interest because Barton and Otto (2005) demonstrate that selection interference is present at loci subject to fluctuating selection in finite populations, and recombination can be beneficial through breaking it down. Peters and Lively (2007) and Salathé *et al.* (2009) determine that recombination modifiers spread mainly due to a 'delayed short-term benefit', which is the advantage of recombination through creating rare genotypes that have maximum possible fitness in the generations immediately after their creation.

The basic Red Queen models have been extended in numerous ways. Lythgoe (2000) demonstrate that if parasites attack a vertebrate immune system, sex is selected for within parasites; however, such advantages could not overcome a twofold cost. Similarly, Lively (2010a) modifies May and Anderson's (1983) model, so that the infection rate is proportional to the number of infected hosts, leading to sexual and asexual hosts coexisting over time. Other studies find scenarios where sex can be maintained under a broader range of conditions, such as if 'similarity selection' is assumed (i.e. the offspring that a certain parasite is likely to infect is genetically similar to the host's parent) (Agrawal 2006); strong selection against noninfecting parasites relaxes the need for parasitic infection to be strongly deleterious to hosts (Salathé *et al.* 2008); similarly, if virulence is density-dependent and the population death rate is low, then parasitic infection can maintain sex, even if it is weakly virulent (Lively 2009, 2010b). However, Agrawal (2009a) finds that conditions favoring evolution of recombination in haploid Red Queen models do not generally cause equally strong selection for sex in diploids. Mostowy *et al.* (2010) also find different dynamics if multiple parasites can infect an individual; sex is generally selected against if simultaneous infection of a host is common, because this breaks down fluctuating linkage disequilibrium.

Empirical evidence for the Red Queen hypothesis

Although existing theoretical work suggests that the Red Queen hypothesis only selects for sex under specific circumstances, a wide body of empirical studies exist, based on field studies and recent experimental work, showing that parasite interactions select for increased levels of sex in nature.

The fundamental prediction of the Red Queen hypothesis – that exposure to parasitic infections maintains sexual reproduction – has been directly tested and observed in both field and laboratory studies. Jokela *et al.* (2009) clearly show that previously common asexual clones of *P. antipodarum* snails were driven to extinction within a few years, while sexuals remained at a high frequency. Common clones were also more susceptible to sympatric *Microphallus* sp. parasites (those that arise in the same region as the asexual population under investigation), leading to negative frequency-dependent selection. King *et al.* (2009) provide the first experimental evidence of Red Queen dynamics maintaining sex in *P. antipodarum*. In a direct experimental test of the Red Queen hypothesis, Morran *et al.* (2009) show that *C. elegans* evolved higher levels of outcrossing when exposed to the bacterial pathogen *Serratia marcescens*. Morran *et al.* (2011) demonstrate that 'sexual' *C. elegans*, which were genetically manipulated to be obligately outcrossers, become fixed very quickly if populations are constantly exposed to coevolving strains of the pathogen, indicating the presence of coevolving parasites directly selecting for sex. Other studies by Lively (1987) and Kumpulainen *et al.* (2004) demonstrate the existence of a positive correlation between parasitic infection and the frequency of sexuals. In laboratory experiments, Fischer and Schmid-Hempel (2005) observe significantly increased levels of recombination in *Tribolium castaneum* beetles subject to infection by the *Nosema whitei* parasite.

Field studies and laboratory experiments have also verified specific assumptions and predictions of the Red Queen hypothesis. These assumptions include whether parasitic infection is frequency-dependent and infection disproportionally affects asexuals; whether asexuals were derived from sexual ancestors (as assumed in the model of Hamilton *et al.* 1990); and also determining the selective disadvantage of noninfected parasites. Dybdahl and Lively (1995) demonstrate that asexual and sexual species of *P. antipodarum* coexist in the same area, with asexuals evolving from sexual species, verifying the assumption that asexual clones are derived from and compete with local sexual populations. Such asexuals mostly diverged 20 000–70 000 years ago, with a few ancient asexuals also present, with divergence times of 500 000 years (Neiman *et al.* 2005). King *et al.* (2011a) show that increased rates of infection also promote clonal diversity, and trematode parasites die if they fail to infect the host, offering evidence for strong selection against parasites (King *et al.* 2011b), which is needed to maintain sex under the Red Queen hypothesis (Salathé *et al.* 2008). Koskella and Lively (2009) verified the prediction of the Red Queen hypothesis that common host genotypes should reduce in frequency over time, due to parasitic infection.

Mutational deterministic hypothesis

In the mutational deterministic hypothesis, negative disequilibrium persists in infinite populations subject to deleterious mutation, if deleterious mutants act synergistically. That is, a collection of deleterious mutants will cause a larger detriment to an individual's fitness than expected if they acted independently. This leads to the evolution of increased levels of recombination,

which can overcome a twofold cost of sex if the deleterious mutation rate is high enough. However, it will be seen that the clear yet strict conditions needed to maintain obligate sex under this model are not found to be widespread in nature. As a consequence, the hypothesis is losing favor as an explanation of the evolution of sex and recombination.

The mutational deterministic hypothesis attracted attention when several theoretical papers appeared demonstrating that recombination can be selected for in infinite populations. Previous models had shown that recombination is selected against because it breaks apart the fittest genotype (Nei 1967). The necessary condition needed for recombination to be advantageous is the presence of negative linkage disequilibrium between loci, which can be generated by synergistic epistasis (Feldman *et al.* 1980). Recombination places more deleterious mutants together in the same genome, which causes a larger fitness reduction than if each mutant acted individually, due to the presence of epistasis. These individuals are then more likely to die, purging more deleterious alleles from the population (Kondrashov 1982). This leads to a highly reduced fitness load in sexuals, while the load in asexuals is always equal to $1 - e^{-U}$ at mutation-selection equilibrium, for genome-wide deleterious mutation rate U, irrespective of the distribution of fitness effects (Kimura & Maruyama 1966). Furthermore, if the genomic mutation rate U is greater then 1, then the benefit is large enough to overcome the twofold cost of sex (Kondrashov 1993).

Charlesworth (1990) investigates in detail the role played by epistasis in selecting for sex, and shows that the benefits to recombination increase with more chromosomes, and longer map length per chromosome. Barton (1995a) quantifies what range of epistasis would select for increased recombination. It is shown that for epistasis to create negative associations between loci that persist over time, it has to be weak and negative. Specifically, under a quasi linkage equilibrium (QLE) scheme (where selection and epistasis are weak relative to the recombination rate [Kirkpatrick *et al*. 2002]), where the recombination modifier has a small effect on overall recombination rate ($\delta p << 1$), then the rate of increase of a recombination modifier is equal to:

$$\frac{\delta p}{\rho_{MAB}} D(\lambda - \varepsilon) \qquad (1)$$

where δp is the increase in recombination caused by the modifier; ρ_{MAB} is the recombination rate across the set of 3 loci (modifier locus M and selected loci A, B); D is the linkage disequilibrium between selected loci; ε the degree of epistasis; and λ is a compound parameter that takes the form:

$$\lambda = -s_a s_b \left[\frac{1}{\rho_{MA}} + \frac{1}{\rho_{MB}} - 1 \right] \qquad (2)$$

Here, s_a and s_b are the selection coefficients of the deleterious alleles at selected loci A and B. Eqn 1 encapsulates both the short-term effect of recombination through breaking apart favoured combinations of alleles, and creating a long-term increase in the population mean fitness. Because $D < 0$ is needed for recombination to be selected for (otherwise recombination is disadvantageous through breaking apart adapted collections of alleles), Eqn 1 shows that epistasis should lie in the range $\lambda < \varepsilon < 0$. Figure 4 outlines a schematic of the parameter space needed to select for recombination. Otto and Feldman (1997) verify this analysis, and also show that initial levels of linkage have to be tight for increased recombination to evolve.

Testing the mutational deterministic hypothesis

The above analysis makes clear that in order for sex to be widespread, the genomic mutation rate should be high (generally greater than or equal to 1), and synergistic epistasis needs to exist between deleterious mutants. Because of these simple predictions, and its focus on deleterious mutants (which are prevalent in nature), it was, until recently, an appealing explanation for widespread costly sex. More is now known about the deleterious mutation rate for several species. Some do seem to have genomic deleterious mutations rates greater than 1, such as *Drosophila* (Haag-Liautard *et al.* 2007; Keightley *et al.* 2009), *C. elegans* (Denver *et al.* 2004) and hominids (Eöry *et al.* 2010). Studies generally show equivocal evidence as to whether a deleterious mutation rate greater than 1 is common in coding regions (Keightley & Eyre-Walker 2000; Baer *et al.* 2007). However, because non-coding regions are also subject to deleterious mutation (Eöry *et al.* 2010), it is reasonable to assume that U can easily exceed 1 in higher eukaryotes. More importantly, widespread net epistasis has not been found in nature; a large variance in epistasis is generally found in organisms, which disfavours the evolution of increased recombination (Otto & Feldman 1997). (See Kouyos *et al.* [2007] for a review of data, as well as a discussion on the limitations of these studies.)

It should be noted, however, that a high mutation rate and synergistic epistasis is only needed for sex to overcome a twofold cost. Deleterious mutations that act synergistically, but arise at lower rates, can still cause higher rates of recombination to evolve (Kouyos *et al.* 2007). Sex could have a twofold advantage if mutational deterministic processes are combined with other mechanisms, such as fluctuating selection (West *et al.* 1999), or finite-population effects such as Muller's ratchet (Howard 1994; Howard & Lively 1998, 2002). However, the current general consensus is that the conditions required to fulfill the mutational deterministic hypothesis are not widespread in nature, and other mechanisms can more easily explain the production of negative associations between loci that are needed to select for sex and recombination (Kouyos *et al.* 2007).

Other hypothesis on the evolution of sex and recombination

The 3 hypotheses discussed so far (i.e. selection interference, Red Queen dynamics and the mutational deterministic model) are the most prominent mechanisms proposed to explain the maintenance of costly sex. Nevertheless, alternative, less prominent ideas have been proposed that can aid selection for sex.

An idea that has been recently explored extensively is the idea of fitness-associated recombination (FAR), and the related mechanism of fitness-associated sex (FAS). This is the idea that if populations are subject to environmental stress, the level of recombination and sex increases in individuals with lower fitnesses, so as to restore the fittest genotype (Hadany & Otto 2007). Recombination and sex would then be associated with the fittest individuals, causing modifiers coding for fitness-associated recombination and sex to spread. Research into this mechanism was motivated by numerous experimental studies showing that organisms are more likely to evolve higher levels of recombination if subject to fitness stresses (Hadany & Beker 2003).

Redfield (1988) first demonstrated that FAR is beneficial in a model consisting of less-fit bacteria that undergo transformation, while the fittest individuals reproduce asexually. This leads to an increase in the mean

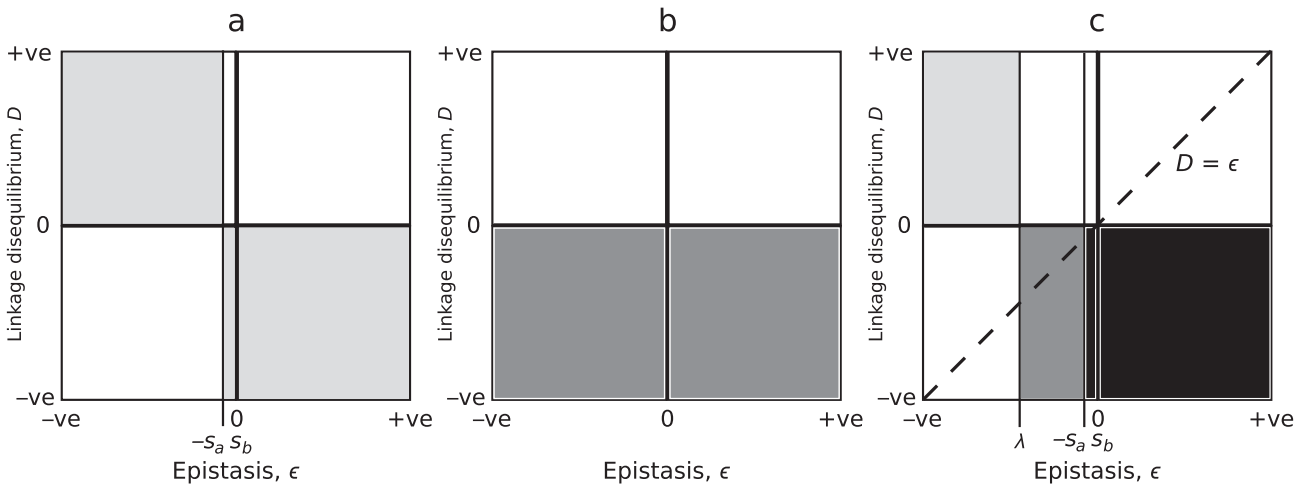

Figure 4 Explaining the parameter space under which recombination is favored according to the mutational deterministic model. The '+ve' and '–ve' terms on the axis indicate where the epistasis and linkage disequilibrium parameters are positive and negative, respectively. (a) There is a short-term advantage if the mean fitness of 'extreme' genotypes (*AA* and *aa*) is greater than that of 'intermediate' genotypes (*Aa* and *aA*); recombination is beneficial if it breaks up intermediate genotypes in this case, which arises (approximately) if D and ε have opposite signs (light grey sections). (b) Recombination offers a long-term advantage through increasing the genetic variance in the population, which arises if intermediate types are underrepresented; that is, if $D < 0$ (dark grey sections). (c) A modifier is therefore selected for if the short-term advantage outweighs the long-term disadvantage (light grey), if the long-term advantage outweighs short-term disadvantage (dark grey) or if both short-term and long-term effects are beneficial (black). In an infinite model in a homogeneous environment, disequilibrium is only formed through epistasis; therefore, recombination can only evolve when the $D = \varepsilon$ line overlaps the key areas, which arises if $\lambda < \varepsilon < 0$. The figure is adapted from Lenormand and Otto (2000) and Otto and Lenormand (2002).

fitness of the population. Gessler and Xu (2000) show, using computer simulation, that FAR is selected for in populations at linkage equilibrium, where recombination modifiers that are not fitness-dependent would be selectively neutral. Hadany and Beker (2003) demonstrate analytically that populations subject to FAR have a higher mean fitness compared to those subject to uniform recombination rates (UR). This result holds either in infinite populations with 1 or 2 loci, if selection at each locus is stronger than the deleterious mutation rate, because FAR is more able to create positive associations between alleles in lower-fitness individuals, compared to UR. This scenario results in FAR populations having a higher mean fitness when compared with equivalent UR populations. Agrawal *et al.* (2005) find that while FAR is advantageous in haploids, the benefits do not extend to diploids (except if *cis-trans* effects are present), because recombination is likely to break apart advantageous associations between the recombination modifier and a beneficial allele in heterozygotes.

However, FAS could be beneficial in diploids due to segregation. Hadany and Otto (2007) find that FAS could evolve if the costs of sex were not too high. Although the dynamics of such a modifier are complex, 1 steady-state found is the 'extreme' case where the fittest class of individuals are purely asexual, with others undergoing some degree of FAS. Hadany and Otto (2009) show that FAS accelerated adaptation in haploids and diploids, if there were some cost of sex (so as to activate the fitness-dependent sex mechanism), and if heterozygotes engaged in sex more often than expected, compared to the multiplicative rates of sex in homozygotes.

Another recently-discussed hypothesis is that sex and recombination are favored in populations spread over heterogeneous environments, where different alleles at the same locus have different environmental-dependent selective effects. In such a situation, recombination can be favored in an infinite population of haploids, if there is a wider range of epistasis values between the selected loci compared to homogeneous populations, depending on the covariance in selection coefficients between loci across environments (Lenormand & Otto 2000). Similar to the FAS mechanism, Agrawal (2009b) finds an advantage to sex in spatially heterogeneous environments, if the fitness of heterozygotes is greater than the mean fitness of the 2 homozygotes at a locus (Fig. 5). This is because migration causes an excess of homozygotes to be introduced into each population, so sex is advantageous through creating a fitter heterozygote state.

Unfortunately, there is limited experimental evidence as to whether fitness-associated mechanisms or heterogeneous environments select for increased sex in nature. Becks and Agrawal (2010) find that increased levels of sex emerge in the monogonont rotifer *Brachionus*

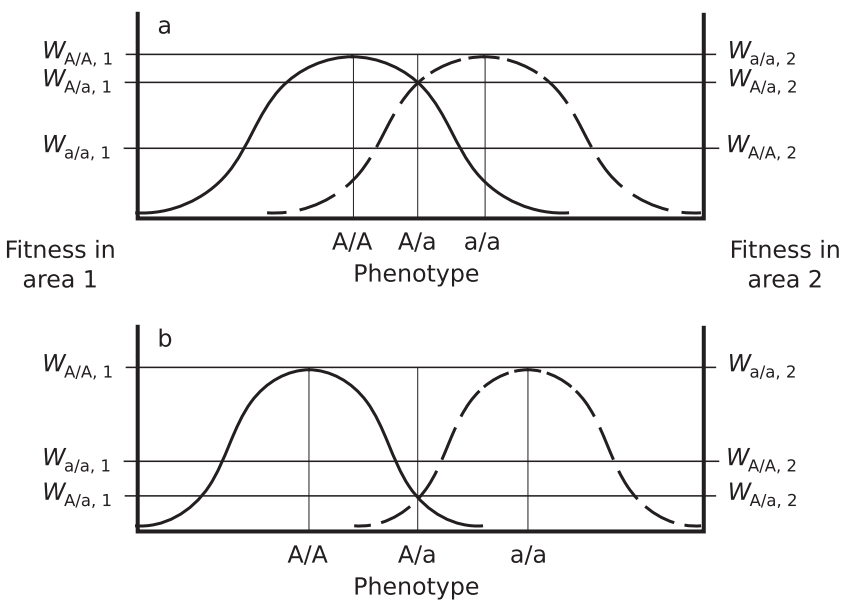

Figure 5 An example of where sex would be favored in diploids in spatially heterogeneous environments. (a) The diploid locus is under stabilizing selection; because the fitness of the heterozygote is greater than the mean of the homozygotes, sex would be advantageous as it would form more heterozygotes from the excess homozygotes created by migration. (b) If the optima lie too far apart then heterozygotes would be disadvantageous, so sex would be selected against. The figure is adapted from Agrawal (2009b).

calyciflorus if it is transferred between 2 heterogeneous environments. No evidence is found that such sex is fitness-dependent. It is also asserted that drift effects do not select for higher levels of sex in this experimental system, because there are no differences in the frequency of sexuals if the population size increases, and sexuals do not have a higher variance in fitness across the entire population (Becks & Agrawal 2011).

CONCLUSION

Although there still does not exist a clear explanation as to why sex is ubiquitous, most of the fundamental processes that sex utilizes are well understood. The 2 most currently promising hypotheses regarding the evolution of sex revolve around recombination breaking apart selection interference, and as a mechanism to produce rare genotypes that are less susceptible to co-evolving parasites. The mutational deterministic model has lost favor as a strong explanation, due to the lack of widespread synergistic epistasis found in nature. Other hypotheses, such as FAS or the effect of heterogeneous environments, are intriguing, but require more thorough investigation to determine whether they can actually explain the widespread appearance of sex.

Despite the major theoretical and empirical advances made over the past 20 years, this question is far from resolved. Both selection interference and Red Queen hypotheses provide cases where costly sex can be maintained (when acting over a subdivided population in the former case, and if generally strong parasitic selection is present in the latter case). However, neither hypothesis has yet to produce theoretical models that confidently account for the appearance of sex over a wide range of biological scenarios. This is especially true with regards to the evolution and maintenance of sex in diploids (Agrawal 2009a; Roze 2009; Roze & Michod 2010), despite the existence of empirical studies showing benefits to diploid sexuals (e.g. see Morran *et al.* 2009, 2011). One of the major tasks facing theoreticians is in determining what scenarios lead to the maintenance of sex in diploids. Another major theoretical direction is investigating how multiple processes aid selection for sex, such as the presence of both advantageous and deleterious mutation (Hartfield *et al.* 2010, 2012), or if hosts are subject both to parasitic infection and deleterious mutation accumulation in Red Queen models (Howard & Lively 1998, 2002). Usually, such models show larger advantages to sex than if just 1 mechanism operates.

However, of greater importance is the need to collect more experimental and empirical data, to test the large number of theoretical predictions. This situation has improved over the past 10 years, with numerous studies showing that sex can be advantageous through breaking apart selection interference, by co-evolving with parasites and by switching between heterogeneous environments. However, more experimental evidence is needed to discriminate between the major hypotheses, as well as more field evidence to determine what mechanism selects for sex in nature. Therefore, future experiments should try not just to determine what environments might select for sex, but also attempt to separate the effects of directional selection from fluctuating environments. This information can then be used to inform whether sex is selected for in fluctuating conditions due to the presence of homogeneous environments or parasite interactions, or whether it instead evolves as a means to break apart selection interference and increase the response to selection.

More data are also needed to test and verify specific predictions made by theoretical work, in light of recent theoretical predictions. Specifically, more studies similar to that of Zeyl and Bell (1997) are needed to determine whether the presence of advantageous mutations increase selection for sex and recombination, or whether the main advantages lie in purging deleterious alleles. It also needs to be verified whether homogeneous spatial populations cause a sufficient mutation meltdown in asexuals that could lead to the maintenance of sex. More empirical studies should also determine whether the evolution of sex and recombination leads to an increase in fitness variance, as this observation can inform on the underlying mechanism (Becks & Agrawal 2011).

Although there has been considerable success in verifying predictions made by the Red Queen hypothesis, recent theoretical advances have yet to be fully investigated. These include whether weakly virulent parasites select for sex or the effect of multiple parasites on the maintenance of sexuals. The outcome of such studies could provide key insights into which genetic processes are the main determinants in the evolution of sex, and might even provide an answer to this notorious scientific enigma.

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