WHY BOTHER WITH SEX? THE EVOLUTION OF RECOMBINATION IN AN ARTIFICIAL GENE NETWORK MODEL

Alexander O. B. Whitlock

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Approved by:

Christina Burch

Shawn Gomez

Ronald Swanstrom

Todd Vision

Christopher Willett

ABSTRACT

Alexander O. B. Whitlock: Why bother with sex? The evolution of recombination in an artificial gene network
(Under the direction of Christina Burch)

Sex is ubiquitous in the natural world, but its costs are high and the nature of its benefits remains controversial. Previous studies have suggested that a major advantage of sex is its ability to eliminate interference between selection on linked mutations, a phenomenon known as Hill-Robertson interference. However, those studies may have missed both important advantages and disadvantages of sexual reproduction because they did not allow the distributions of mutational effects and interactions (i.e., the genetic architecture) to evolve. Using an artificial gene network model that incorporates evolution of genetic interactions, we allowed populations of a range of sizes and structures of sexual or asexual individuals to evolve to a mutation-selection-drift equilibrium. Sexual reproduction had a long-term advantage in the form of an equilibrium fitness advantage. This was due to a combination of the evolution of more robust genetic architecture and the elimination of Hill-Robertson interference, with the size of the fitness advantage increasing with genetic drift. To investigate the origin and maintenance of costly sex, we introduced a mutation which switched reproductive mode into equilibrium populations at a range of costs of sex. The principle determinant of the success of sex was the transit time of a new mutation to fixation, but the determinant of the maximum cost sex could support and resist invasion was determined by its equilibrium fitness advantage, and costly sex never successfully originated. This contradicts previous studies which demonstrated that the cost supported by sex increased with time to fixation. The key to this difference is that the evolution of genetic interactions curbed the deleterious impact of Hill-Robertson interference on the asexual population, decreasing the potential benefit of

sex. The finite maximum sexual fitness advantage capped the maximum sustainable cost to a similar value. Therefore, while we demonstrated conditions which promote the maintenance of costly sex, the sustainable cost was still relatively modest and unlikely to be substantially increased through further manipulations of population structure in absence of other factors that increase the sexual fitness advantage.

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C	Cost of sex
C_{max}	Maximum cost at which sex is advantageous
F_{ST}	Wright's F statistic of population differentiation
LD	Linkage disequilibrium
L_R	Recombination load
m	Migration rate
N	Population size
N_d	Deme size
N_e	Effective population size
T_{fix}	Transit time from mutation to fixation
U_d	Deleterious mutation rate
$\hat{V}$	Neutral variance at equilibrium
$\bar{W}$	Population mean fitness
$\hat{W}$	Population equilibrium mean fitness
$\hat{W}_{sex}/\hat{W}_{asex}$	Equilibrium fitness advantage of sex

CHAPTER 1: INTRODUCTION

> "We do not even in the least know the final cause of sexuality; why new beings should be produced by the union of the two sexual elements, instead of by a process of parthenogenesis." -Charles Darwin, 1862

Sex is nearly ubiquitous among eukaryotes, with over 99.9% of animals and 99% of plants reproducing sexually at least some of the time (Weismann, 1887; Maynard Smith, 1978; Bell, 1982; Vrijenhoek, 1998; Otto and Lenormand, 2002). Obligate asexual lineages are rare, sparsely speciated, and appear to be short-lived (Bell, 1982), implying bleak odds for long-term success without sex (Judson and Normark, 1996; Little and Hebert, 1996; Birky-Jr., 1996; Normark et al., 2003; Simon et al., 2003). Even bdelloid rotifers, the most notorious exception, share suspiciously similar alleles between otherwise divergent species (Mark Welch et al., 2004; Hillis, 2007), raising the possibility of some form of rare genetic exchange. The prevalence of sexual reproduction would seem to suggest clear benefits and few disadvantages, yet just the opposite is true. A century and a half after Darwin's observation, the explanation for the origin and maintenance of sex remains one of the most puzzling questions in evolutionary biology.

Sexual reproduction carries the potential for enormous costs (Smith, 1978), including the cost of recombination itself. Because the fitness of a phenotype is the product of selection for combinations of genes that work favorably together, it is not clear how random reshuffling of genes could be advantageous. Given that the immediate effect of recombination is the destruction of beneficial genetic interactions, the resulting fitness decrease should cause recombination to be selected against (Nei, 1967).

Obligate sexual eukaryotes suffer an additional infamous burden in the form the so-called "two-fold cost of sex" (Maynard Smith, 1978; Bell, 1982). Every member of an asexual

population can produce progeny, but sexual population must invest resources in production of two sexes, even if one sex contributes little to the offspring. In organisms which do not produce males, this can instead be thought of as the "two-fold cost of meiosis", or genome dilution, in which each sexual parent contributes only half of its genes to its offspring (Williams, 1975; Lively and Lloyd, 1990). A parent reproducing sexually only propagates 50% of its genome, instead of passing along the entire thing as under clonal reproduction. Therefore, all else (such as fecundity and niche size) being equal, the benefit of sex in organisms paying these costs must be at least twofold.

Finally, in order to reproduce sexually, individuals must invest time and resources towards finding a mate, which may be especially problematic in a low-density species, making themselves vulnerable to disease and predation in the process.

The population-wide impact of these costs could be alleviated if organisms only engaged in sexual reproduction occasionally and under optimal conditions. *Saccharomyces cerevisiae*, for example, is facultatively sexual, switching from mitosis to meiosis under low fitness conditions (Hadany and Otto, 2009). However, this facultative sexual reproduction has an additional cost: while mitotic reproduction takes approximately 90 minutes, meiosis lasts on the order of days.

This apparent contradiction, in which a costly strategy is also extraordinarily common, is the paradox of sex. Indeed, the Reduction Principle (Altenberg and Feldman, 1987) states that in a randomly mating population at equilibrium, without mutation, modifiers of recombination can only decrease in a population. One of the first explanations proposed is that sex produces the genetic variation required for adaptation (Weismann, 1887). Though this concept is intuitively appealing, it is not a sufficient explanation because sex does not necessarily increase variation, and when it does, this variation may be maladaptive.

First, sex may act to decrease variability. Consider a gene with two alleles, A and a, found in a population at Hardy-Weinberg proportions. If the recessive a is favorable, the frequency of aa homozygotes will increase following selection, removing the population

from Hardy-Weinberg proportions. Asexual reproduction will maintain the distribution built by selection, but sexual reproduction restores Hardy-Weinberg proportions, producing intermediate genotypes at the expense of extreme genotypes.

Second, sex may generate genetic variation that is maladaptive. For example, the sickle cell trait is strongly advantageous when heterozygous. Were reproduction to occur asexually, heterozygotes would be expected to go to fixation in the population, resulting in a population free of both sickle cell anemia and malaria. However, sexual reproduction restores the genotypes to Hardy-Weinberg proportions. Though this does increase variance, it does so at a cost to mean fitness. While these examples are obviously not universally applicable, they do demonstrate a need for a more sophisticated explanation.

As may be expected for such a long-lived problem, over 20 hypotheses have been proposed to explain the prevalence of sex. While explanations predicting a direct physiological benefit to sex have been proposed, the most accepted hypotheses predict the benefit is indirect, in which the benefit of sex is its ability to break up linkage disequilibrium (LD). When LD is predominantly negative, that is, when genotypes of intermediate fitness are overrepresented in the population relative to expectations based on allele frequency, natural selection is less efficient. Recombination regenerates extreme phenotypes, restoring the additive genetic variation required for efficient selection (Feldman et al., 1980). Expectations regarding the source of LD and the specific benefit of destroying it can be placed in three categories: The Mutational Deterministic Hypothesis, Red Queen, and Hill-Robertson interference, each of which will be be discussed further.

The Mutational Deterministic Hypothesis (MDH) predicts that recombination allows populations to purge deleterious mutations. According to the MDH, negative LD accumulates in an infinite population due to a combination of selection and mutation, with synergistic epistasis between deleterious mutations. When recombination breaks up LD and regenerates extreme phenotypes, some of these genotypes will contain multiple deleterious mutations. Due to the synergistic effects of these mutations, the fitness of the genome will be low. If that

genome dies or fails to reproduce, those mutations will be purged from circulation (Kimura and Maruyama, 1966; Kondrashov, 1982, 1984). Because sexual reproduction facilitates the removal of deleterious alleles, sexual populations have a lower mutation load than asexual populations.

This prediction requires two things: first, a high genomic mutation rate. A genomic mutation rate of $U >= 1$ is predicted to have a high enough deleterious mutation supply for the benefits to surpass the twofold cost (Kondrashov, 1993). Second, epistasis must be weak and negative in order to create persistent negative associations between loci (Barton, 1995). However, the conditions that support MDH's role in selection for recombination may not be widespread in nature. Genomic mutation rates have been quantified for a variety of organisms and, while some do seem to be above 1, including *Drosophila* (Haag-Liautard et al., 2007) and *C. elegans* (Denver et al., 2004), many are much lower (Drake et al., 1998). Additionally, empirical evidence for the sign of epistasis is mixed and evidence exists for negative positive, and no epistasis. Indeed, studies find variation in how genes interact, both between organisms and within genomes (Rice, 2002; Kouyos et al., 2007; Whitlock et al., 1995; Elena and Lenski, 1997; de Visser and Elena, 2007). Neither is there evidence that synergistic epistasis evolves as a result of recombination (Desai et al., 2007; MacCarthy and Bergman, 2007). As the parameter requirements seem to be rarely met, MDH is not likely a major contributor to the success of sex.

Another prediction, the Red Queen hypothesis, predicts that sex allows escape from antagonistic coevolution. In an environment with a fluctuating optimum, genetic combinations in linkage disequilibrium that were built by selection become disadvantageous over time. Recombination is advantageous both because it can destroy this now-deleterious LD, and because it can rapidly generate new phenotypes, one of which may be a closer match to the new optimum (Sturtevant, 1938; Charlesworth, 1976; Hamilton, 1980; Bell, 1982; Salathé et al., 2009). As such, modifiers of recombination are expected to be able to spread in the time immediately following an optimum shift (Peters and Lively, 2007). Under conditions of

strong selection, frequent optimum shifts, and high recombination rates, a model implemented by (Hamilton, 1980) demonstrated that sex was able to overcome a two-fold cost.

The negative frequency-dependent selection that results from antagonistic coevolution between species, most notably between hosts and parasites, can produce optimum shifts of this nature (Jaenike, 1978; Hamilton, 1980). Antagonistic coevolution causes fluctuation in linkage disequilibrium and epistasis in both parasites and their hosts (Peters and Lively, 1999) leading to a predicament similar to Lewis Carroll's Red Queen, in which both species must evolve as quickly as they can just to stay in the same place relative to one another (Bell, 1982).

Both experimental and empirical evidence support a role for parasitic interactions in the frequency of sex. Frequency of sexual reproduction in *P. antipodarum* correlates with the density of trematode parasites, with asexual reproduction predominating in regions with low parasite load (Lively, 1987; Jokela et al., 2009), with additional evidence of negative frequency-dependent selection, in which the asexual clones which reached high frequency were driven to low frequency or extinction, while sexual reproduction resulted in population stability (Jokela et al., 2009). Experimental coevolution between hosts and parasites has demonstrated time-lagged, frequency-dependent selection (Britt Koskella, 2007), and hosts in populations with high rates of pathogen or parasite infection evolved increased recombination rates (Morran et al., 2009, 2011) both of which support a role for antagonistic coevolution in the maintenance of sex. Generally, there is a pattern of increased prevalence of sexual reproduction in regions with high density of parasites (Neiman et al., 2009), and it has been proposed that the strong positive correlation between longevity, small brood size, and recombination rate demonstrates that recombination is favored in long-lived organisms as a way to create offspring that are relatively resistant to rapidly evolving parasites (Burt and Bell, 1987).

However, despite the empirical patterns, theoretical models have only found a benefit to costly sex within a narrow parameter range (Roze, 2009). Selection must be strong-to-lethal

(May and Anderson, 1983; Howard and Lively, 1994; Otto and Nuismer, 2004) and linkage disequilibrium must cycle rapidly, on the order of every two to five generations (Barton, 1995). This is especially problematic because in many organisms, the part of the genome under selection for host-parasite interaction is confined to a single region, yet the entire genome pays the cost of recombination. Simulations with diploid populations are even less likely to find a benefit to sex (Agrawal and Otto, 2006). As a result, the extent to which antagonistic coevolution contributes to the broad maintenance of sex is not clear.

Sex can also act to destroy selection interference among loci. Though the previous hypotheses have been purely deterministic, real populations are finite, and genetic drift also influences the evolution of the population. In a finite population without recombination, linkage disequilibrium accumulates due to a combination of mutation, selection, and drift. This results in Hill-Robertson interference, in which selection at one locus interferes with selection at another linked locus (Hill and Robertson, 1966; Felsenstein, 1974; Comeron et al., 2008). By destroying linkage disequilibrium, sex exposes the loci to selection. Hill-Robertson interference can take at least four forms.

The Fisher-Muller effect (Fisher, 1930; Muller, 1932; Gerrish and Lenski, 1998) describes interference between beneficial mutations in the population. In an asexual population, beneficial mutations arising in different individuals will interfere with one another's fixation. Unless the mutations occur sequentially in the same lineage–a potentially lengthy affair–one of the beneficial mutations will be wasted, slowing the rate of adaptation. In a sexual population, however, recombination can easily bring together and fix mutations that arose in different individuals. Fisher-Muller effects are likely to be prominent in populations with an ample supply of beneficial mutations (Crow and Kimura, 1969), such as large populations, populations that are far from the optimum, or those that are undergoing directional selection. Once a modifier of recombination has brought together beneficial alleles, these gene combinations flourish and proliferate, bringing with them the modifier that brought them together (Felsenstein and Yokoyama, 1976; Otto and Barton, 2001; Barton and Otto,

2005).

In a linked genome, the fate of a new mutation is inextricably tied to the genetic background in which it arises, regardless of its individual selection coefficient. In a linked genome containing both a beneficial and a deleterious mutations, the apparent fitness consequence of each is decreased by its counterpart, forestalling efficient selection for or against either mutation. As a result, a beneficial mutation that arises in an otherwise low-fitness linked genome may be lost due to background selection (Charlesworth, 1993b; Peck, 1994; Charlesworth, 1994). Similarly, a deleterious mutation in an otherwise high fitness linked genotype may hitchhike to fixation (Maynard Smith and Haigh, 1974; Peck, 1994; Bachtrog and Gordo, 2004; McDonald et al., 2016). Both hitchhiking and background selection decrease the effective population size. By freeing mutations from the genetic background in which they arose, recombination increases additive genetic variance and allows efficient selection.

Muller's ratchet is predicted to have the strongest effect in small populations, where genetic drift has the most influence. Over time, genetic drift stochastically causes fixation of deleterious mutations. With each fixation event, the number of accumulated mutations in the least-loaded genotype increases by one, and barring a rare beneficial mutation or back-mutation, the population's fitness has been irrevocably decreased (Muller, 1964; Felsenstein, 1974; Haigh, 1978; Gordo and Charlesworth, 2000). To use Muller's analogy, the ratchet has been tightened a click. As mutations accumulate, the ratchet continues to tighten, with the potential for a mutational meltdown and the loss of the asexual population (Haigh, 1978; Lynch and Gabriel, 1990; Gabriel et al., 1993). Sexual recombination, however, can halt the ratchet. Recombination between two unfit genotypes can restore the most fit ancestral genotype and salvage the population. Though evolution of compensatory interactions can avert Muller's Ratchet in some instances (Poon and Otto, 2000; Silander et al., 2007; Neher and Shraiman, 2012), other populations have been observed to undergo mutational meltdowns and extinction (Higgins and Lynch, 2001).

Hill-Robertson interference has substantial empirical and experimental support and, as

all populations are finite and subject to some level of drift, is expected to function broadly (Comeron et al., 2008). It is less dependent on epistasis than other models, and has been demonstrated to support the evolution of sex given positive, negative or absent epistasis (Felsenstein and Yokoyama, 1976; Otto and Barton, 2001; Barton and Otto, 2005). Even though it is drift-based, it does not require an especially small population size. In a large population size, increasing the number of loci under selection increases the potential for linkage to affect genetic variation (Iles et al., 2003). The addition of structure to large populations introduces the potential for substantial genetic drift within demes (Martin et al., 2006). As a result, Hill-Robertson interference is of particular interest as an explanation for the prevalence of sexual reproduction due to its apparent ability to promote a strong advantage of sex across a wide parameter range (Otto and Barton, 2001; Iles et al., 2003; Barton and Otto, 2005; Keightley and Otto, 2006; Gordo and Campos, 2008; Hartfield et al., 2010, 2012).

MODELS OF THE EVOLUTION OF SEX

> "So far, we have been able to study only one evolving system and we cannot wait for interstellar flight to provide us with a second. If we want to discover generalizations about evolving systems, we will have to look at artificial ones."
> -John Maynard Smith, 1992

Computational models both complement and extend results from biological organisms. Not only are results from simulations more repeatable and analyzable, thousands of generations of evolution can be simulated in a afternoon. The compromise is that such scale necessitates simplifications. As it is said, "All models are wrong, but some are useful" (Box, 1979). A model falls into the latter category only to the extent that its simplifications do not affect its results.

Many models used to investigate the evolution of sex have made the assumption that properties of genetic architecture–e.g. the interactions between genes which include epistatic interactions, the distribution of mutational effects, and modularity (Hansen, 2006; Rice,

2008)–do not evolve. While this assumption may be appropriate if genetic architecture did not interact with sex, there is substantial evidence that it is otherwise. For example, many models are based on a simplified two or three locus model (McVean and Charlesworth, 2000; Gardner and Kalinka, 2006; Livnat et al., 2008, 2010; Hodgson and Otto, 2012; Park and Krug, 2013), even though real genomes are larger and more complex, a property which has been shown to increase the benefit of sex (Lenski et al., 1999; Iles et al., 2003; Sanjuán and Elena, 2006; Charlesworth et al., 2009; Barbuti et al., 2012). Other models have used only deleterious mutations (Keightley and Otto, 2006; Gordo and Campos, 2008), or assumed that the distribution of mutational effects is constant (McVean and Charlesworth, 2000; Wilke, 2004; Charlesworth et al., 2009; Barbuti et al., 2012; Park and Krug, 2013). Yet, mutations can be deleterious, neutral or beneficial (Sanjuán et al., 2004), and the distribution of mutational effects can evolve in just a few generations (Burch and Chao, 2004; Montville et al., 2005; Sanjuán et al., 2007; Silander et al., 2007).

Most significantly, recombination itself influences the evolved properties of genetic architecture (Azevedo et al., 2006; Gardner and Kalinka, 2006; Misevic et al., 2006; MacCarthy and Bergman, 2007; Livnat et al., 2008; Martin and Wagner, 2009; Livnat et al., 2010; Lohaus et al., 2010), and the genetic architecture that evolves under sexual reproduction has the potential to shape the benefits of sex (Kondrashov, 1982, 1988; Charlesworth, 1990; Otto and Feldman, 1997). As a consequence, omitting evolution of genetic interactions has the potential to change both the mechanism and the impact of sex.

Here, we simulate evolution using an artificial gene network model (Wagner, 1996; Siegal and Bergman, 2002) which explicitly encompasses evolution of genetic architecture, including recombination load, compensatory interactions, distribution of mutational effects, robustness and epistasis. The consequences of the interaction between Hill-Robertson interference and an evolving genetic architecture for the evolution of costly sex is the focus of this work.

CHAPTER 2: AN EVOLVING GENETIC ARCHITECTURE INTERACTS WITH HILL-ROBERTSON INTERFERENCE TO DETERMINE THE BENEFIT OF SEX

The vast majority of organisms alive today have experienced some form of genetic exchange, or sex, in their recent evolutionary history, despite substantial costs (Weismann, 1887; Maynard Smith, 1978; Bell, 1982; Otto and Lenormand, 2002). Sex breaks up favorable genetic combinations and increases the risk of transmission of pathogens and selfish genetic elements. Sexual reproduction is often slower than asexual reproduction. In many sexually reproducing eukaryotes, sex involves costs of finding and attracting a mate, and of mating in itself; in anisogamous species, if one sex contributes little to progeny production, sexual reproduction carries a two-fold cost of producing that sex. The ubiquity of sex implies that it must confer considerable benefits to overcome these costs. However, the nature of these benefits is not well understood. In fact, over twenty hypotheses have been proposed to explain the benefits of sex (Bell, 1982; Kondrashov, 1993; Hurst and Peck, 1996; Otto and Lenormand, 2002). While hypotheses predicting direct benefits exist (e.g., improved DNA repair, Bernstein et al., 1985), the main benefits of sex are believed to be indirect, such as increased evolvability (Weismann, 1887; Maynard Smith, 1978; Bell, 1982; Kondrashov, 1993; Burt, 2000; Otto and Lenormand, 2002).

Indirect benefits of sex result from the ability of recombination to break down the linkage disequilibrium (LD) generated by mutation, genetic drift, and natural selection. If LD is predominantly negative, that is, if genotypes with the highest and lowest fitness are

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underrepresented in the population, then recombination can generate these extreme genotypes and increase the efficiency of natural selection (Otto and Lenormand, 2002). In the absence of recombination, negative LD can accumulate in a population either through the action of natural selection alone (Red Queen hypothesis: Hamilton 1980; Mutational Deterministic hypothesis: Kimura and Maruyama 1966; Kondrashov 1982, 1988; Charlesworth 1990; Barton 1995; Otto and Feldman 1997), or through the combined actions of selection and genetic drift (Hill-Robertson interference: Hill and Robertson 1966; Felsenstein 1974; Comeron et al. 2008). Although there is evidence supporting the existence of Red Queen and Mutational Deterministic benefits of sex in specific populations, the conditions required to generate these benefits are thought to be too restrictive to provide a general explanation for the ubiquity of sex (Charlesworth, 1993a; Barton, 1995; Otto and Feldman, 1997; Otto and Nuismer, 2004). In contrast, Hill-Robertson interference is thought to operate broadly (Comeron et al., 2008), and to provide strong benefits of sex (Otto and Barton, 2001; Iles et al., 2003; Barton and Otto, 2005; Keightley and Otto, 2006; Gordo and Campos, 2008; Hartfield et al., 2010).

Hill-Robertson interference is a phenomenon whereby, in a finite population, selection acting at one locus reduces the efficiency of selection at linked loci (Hill and Robertson, 1966; Felsenstein, 1974; Comeron et al., 2008). It takes at least four forms. First, deleterious alleles fix stochastically—a process known as Muller's ratchet (Muller, 1964; Haigh, 1978; Gordo and Charlesworth, 2000). Second, selection against deleterious alleles removes linked beneficial alleles from the population as a side effect—a process known as background selection (Charlesworth et al., 1993; Charlesworth, 1994; Peck, 1994). Third, competition between individuals carrying independent beneficial alleles slows down the spread of these beneficial alleles—a process known as the Fisher-Muller effect (Fisher, 1930; Muller, 1932; Gerrish and Lenski, 1998). Fourth, selection for beneficial alleles causes linked deleterious alleles to rise in frequency in the population as a side effect—a process known as hitchhiking (Maynard Smith and Haigh, 1974; Peck, 1994). All forms of Hill-Robertson interference are strongest in asexuals, whose entire genomes are completely linked, and are weakened by sex.

The relative strength of the benefits of sex arising from these different forms of Hill-Robertson interference are known to depend critically on population size and on the distribution of mutational effects on fitness. For example, Muller's ratchet is strongest in small populations that often experience deleterious mutations, whereas the Fisher-Muller effect is strongest in large populations that often experience beneficial mutations. The increase in the strength of the Fisher-Muller effect between *beneficial* mutations with population size is intuitive because population size (N) affects the beneficial mutation supply rate (NU_b, where U_b is the beneficial mutation rate). More surprising is the recent finding from evolutionary simulations that interference between *deleterious* mutations can, on its own, also generate a large benefit of sex that increases with population size (Otto and Barton, 2001; Iles et al., 2003; Barton and Otto, 2005; Keightley and Otto, 2006; Gordo and Campos, 2008; Hartfield et al., 2010). This finding is surprising because neither Muller's Ratchet (Muller, 1964; Haigh, 1978; Gordo and Charlesworth, 2000) nor background selection (Hudson and Kaplan, 1994, 1995) is expected to increase in strength with population size. The mechanism underlying the dependency of the benefit of sex on population size was little explored in this previous body of work and will be our focus here.

Our intuition is that assumptions made in previous work on the evolution of sex—that one or more of the deleterious mutation rate, distribution of mutation effects, and the epistatic interactions between mutations do not evolve—affected the magnitude and nature of the advantages of sex generated by Hill-Robertson interference in that work (e.g., Fisher, 1930; Muller, 1932, 1964; Hill and Robertson, 1966; Felsenstein, 1974; Haigh, 1978; Peck, 1994; Charlesworth, 1994; Barton, 1995; Otto and Feldman, 1997; Gordo and Charlesworth, 2000; Otto and Barton, 2001; Iles et al., 2003; Barton and Otto, 2005; Keightley and Otto, 2006; Gordo and Campos, 2008; Hartfield et al., 2010). The deleterious mutation rate, the distribution of mutational effects, and the epistatic interactions between mutations are all properties of the genetic architecture (Box 1) that are known to play an important role in the indirect benefits of sex (Kondrashov, 1982, 1988; Charlesworth, 1990; Otto and Feldman,

1997). These properties can evolve in just a few generations (Burch and Chao, 2004; Montville et al., 2005; Sanjuán et al., 2007; Silander et al., 2007). We know, from both theoretical (Poon and Otto, 2000) and empirical (Silander et al., 2007) studies, that an evolving genetic architecture impacts the benefits of sex in populations that are sufficiently small to be subject to Muller's ratchet. As the fitness of asexual populations declines through operation of the ratchet, the rate of compensatory mutations increases, eventually halting the ratchet and limiting this cost of asexuality. We know less about how an evolving genetic architecture impacts the benefit of sex in large populations.

In previous work, we studied a gene network model that explicitly incorporates an evolving genetic architecture (Wagner, 1996; Siegal and Bergman, 2002) and found that sex selects for a lower deleterious mutation rate, lower recombination load, and negative epistasis (Azevedo et al., 2006; Lohaus et al., 2010), three changes in the genetic architecture predicted to favor the maintenance of sex. We noticed that population size affected the extent to which sexual reproduction led to an increase in mean fitness at equilibrium (the long-term advantage) and successfully invaded asexual populations (the short-term advantage), but we did not systematically explore these effects (Lohaus et al., 2010).

Here we build on our earlier work on the gene network model to investigate the extent to which Hill-Robertson interference interacts with the evolving genetic architecture to determine the advantage of sex. We compare sexual and asexual reproduction, manipulating the contribution of Hill-Robertson interference by altering population size. We look for a long-term advantage of sex by monitoring the evolution of fitness and the genetic architecture as sexual and asexual populations approach equilibrium. We also look for a short-term advantage of sex by monitoring the invasion of equilibrium sexual populations by asexual mutants, and vice versa. We show that both genetic architecture evolution and Hill-Robertson interference contribute to the advantages of sex in the gene network model and that the magnitudes of their relative contributions depend on population size, as expected. As population size increased, the contribution of Hill-Robertson interference to both the short- and long-term

advantages of sex increased.

MATERIALS AND METHODS

Our gene network model is based on a model introduced by Wagner (1994, 1996). A haploid genotype is modeled as a network of n genes, each encoding a transcription factor that can, potentially, regulate its own expression or the expression of other genes. The gene network is represented by an $n \times n$ matrix, $\mathbf{R}$, where $r_{ij} \in \mathbf{R}$ is the regulatory effect of the product of gene j on gene i. Thus, row i of $\mathbf{R}$ represents gene i, including its cis-regulatory elements. Genes are evenly distributed on a linear chromosome in the same order as the rows in the $\mathbf{R}$ matrix ($i = 1, 2, \ldots, n$). Genes 1 and n are at a map distance λ from each other. Adjacent pairs of genes are at a map distance $\lambda/(n-1)$ from each other.

The expression pattern of an individual, the *Phenotype*, is represented by the vector $\mathbf{S}$, where $s_i \in \mathbf{S}$ is the expression state of gene $i = 1, 2, \ldots, n$. Expression states are discrete: a gene is either on ($s_i = +1$) or off ($s_i = -1$).

The expression pattern of an individual at time t is given by the system of difference equations:

$$s_i(t+1) = f\left[\sum_{j=1}^{n} r_{ij} s_j(t)\right] \tag{1}$$

where f is a step function that determines how the input from the gene network controls the expression of the target gene:

$$f(x) = \begin{cases} +1 & \text{if } x \geq 0 \\ -1 & \text{if } x < 0 \end{cases}$$

Starting from an initial gene expression pattern $\mathbf{S}(0)$ at time $t = 0$, gene expression changes according to Equation 1 and is judged to reach a steady state if the following criterion is met: $\mathbf{S}(t) = \mathbf{S}(t-1)$. If a genotype does not achieve a gene expression steady state within $t \leq 100$ time steps, it is considered inviable ($W = 0$, see next section). If a genotype achieves a gene expression steady state within $t \leq 100$ time steps, it is considered viable ($W > 0$), and the steady state gene expression pattern $\hat{\mathbf{S}}$ is its *phenotype*.

Most random genotypes (see below) fail to produce a gene expression steady state (Pinho et al., 2014).

The fitness of a viable genotype is given by:

$$W = \exp\left[-\frac{D(\hat{\mathbf{S}}, \dot{\mathbf{S}})}{\sigma}\right] \tag{2}$$

where $D(\mathbf{S}, \mathbf{S}') = \sum_{i=1}^{n}(s_i - s_i')^2/(4n)$ measures the difference between expression patterns $\mathbf{S}$ and $\mathbf{S}'$, $\hat{\mathbf{S}}$ is the phenotype corresponding to the genotype, $\dot{\mathbf{S}}$ is the optimal phenotype, and $\sigma > 0$ is inversely related to the strength of stabilizing selection.

A random genotype is created by generating a random gene network, $\mathbf{R}$, and a random initial gene expression pattern, $\mathbf{S}(0)$. A random gene network is generated by randomly assigning to its r_{ij} regulatory elements $(1-c)n^2$ zeros and cn^2 non-zero random variates drawn from a standard normal distribution (i.e., with zero mean and unit variance), where c is the connectivity density of the network. Networks with more than one weakly connected component (Newman, 2010) are discarded. A random initial gene expression pattern is generated by filling the n entries of $\mathbf{S}(0)$ with either -1 or $+1$ with equal probability.

Evolution is simulated using an individual-based, Wright-Fisher model with constant population size, N, and non-overlapping generations. Individuals undergo a selection-reproduction-mutation life cycle. At the beginning of a simulation, a viable random genotype is cloned N times to found a population. The optimal phenotype is defined as the phenotype of the founder. This aspect of the environment remains constant throughout the simulation.

Parents for the next generation are chosen at random, with replacement, with probability proportional to their fitness (Equation 2). If the parent reproduces asexually, it generates a clone of itself. If two parents reproduce sexually they form a transient diploid, and produce one haploid recombinant offspring. The recombinant $\mathbf{R}$ matrix is generated by choosing one parent at random and copying the first row of its $\mathbf{R}$ matrix; the next row of $\mathbf{R}$ is copied from the same parent except if a crossover occurs, in which case the corresponding row of the $\mathbf{R}$

matrix of the other parent is copied instead. The process repeats for each row, switching between parents each time a crossover location is encountered. For each offspring, the number of crossovers is drawn randomly from a Poisson distribution with mean 2λ, where λ is the genetic map length in M (morgans). Crossover locations are chosen randomly and occur between genes. No crossovers occur within the regulatory regions of a gene, maintaining each row as a completely linked locus. Unless otherwise stated, sexual reproduction occurs with free recombination (i.e $\lambda/(n-1) = 0.5$ M).

Both asexually and sexually produced offspring inherit the initial pattern of gene expression, $\mathbf{S}(0)$, of the parents. This aspect of the environment also remains constant throughout the simulation.

Each individual offspring acquires a random number of mutations drawn from a Poisson distribution with mean U, the genomic mutation rate. A mutation is represented by a change to the value of one of the dn^2 nonzero regulatory elements r_{ij}, chosen at random; the mutated value is drawn randomly from a standard normal distribution. Mutation cannot create new regulatory interactions (i.e., a zero entry cannot become nonzero), but can transiently remove regulatory interactions (i.e., a nonzero entry may become approximately zero).

The reproductive mode of an individual is determined by its genotype at a modifier locus M. Unless otherwise stated, the M locus is unlinked to the genes involved in the gene network. There are two alleles at the modifier locus: m and M. We have implemented three different genetic bases for these reproductive modes, described below. Under all implementations, if a population is fixed for the m allele every individual reproduces asexually, and if it is fixed for the M allele every individual reproduces sexually. Thus, we refer to the m and M alleles as being *for* asexual and sexual reproduction, respectively. In all simulations where the M locus modifies reproductive mode, the m allele specifies no recombination (i.e., $\lambda = 0$ M), and the M allele specifies free recombination (i.e., $\lambda/(n-1) = 0.5$ M).

In the Separate Sex method of reproduction, the sexual and asexual subpopulations are reproductively isolated from each other. Sexuals do not experience a cost of finding mates.

One individual is chosen for every reproductive event with probability proportional to its fitness. If it carries the m allele, it reproduces asexually. If it carries the M allele, a second individual carrying an M allele is chosen with probability proportional to its fitness, and the two individuals reproduce sexually and produce one recombinant offspring.

In the Recessive Sex method of reproduction, asexual individuals always reproduce asexually; sexual individuals sometimes reproduce asexually. Two individuals are chosen for every reproductive event with probability proportional to their fitness. If one or both of the individuals carries the m allele, one of them reproduces asexually, regardless of its genotype at the M locus. If both individuals carry the M allele, they reproduce sexually and produce one recombinant offspring.

In the Dominant Sex method of reproduction, sexual individuals always reproduce sexually, but they may recombine with asexual individuals. Two individuals are chosen for every reproductive event with probability proportional to their fitness. If both individuals carry the m allele, one of them reproduces asexually. If one or both of the individuals carry the M allele, they reproduce sexually and produce one recombinant offspring. The offspring inherits one of the parental alleles at the M locus, chosen randomly.

Fitness variation is given as the mean fitness ($\overline{W}$) and mean log fitness ($\overline{\ln W}$) of all individuals present in the population at a given time (see Equation 2). Genetic variance in log fitness is the total variance in $\ln W$ among all individuals present in the population at a given time.

Mean and variance in log fitness under linkage equilibrium is calculated as mean and variance in $\ln W$ for a population with the same allele frequencies but no LD (Barton, 1995). We estimated these parameters from a sample of 100 "chimeras." A chimeric $\mathbf{R}$ matrix was constructed by picking each row from the $\mathbf{R}$ matrices of any individual in the population with equal probability.

To estimate effective population size, N_e, a neutral locus was incorporated in the simulation. In sexuals, the neutral locus was not linked to the gene network loci. In each generation, the

neutral locus acquired a random number of mutations drawn from a Poisson distribution with mean 1. Each mutation added to the neutral locus value a random draw from the standard normal distribution. The equilibrium variance ($\hat{V}$) at an unlinked neutral locus is expected to be N, the census population size. We calculated N_e as $\hat{V}$ at the neutral locus. N_e estimates were obtained at generation 10^4, at which time all but the largest populations ($N = 10^4$) had achieved equilibrium variance at the neutral locus.

The deleterious mutation rate is defined as $U_d = U(p_d + p_l)$, where U is the genomic mutation rate, and p_d and p_l are the proportion of non-lethal deleterious and lethal mutations, respectively (see Box 1). U is constant throughout the course of a simulation but p_d and p_l can evolve. We estimate the quantity $p_d + p_l$ for a genotype by generating 100 copies of the genotype carrying a single mutation and evaluating the proportion of them that have lower fitness than the original genotype.

Recombination load is defined as $L_R = \overline{\omega - \rho}$, where ω is the mean fitness of a pair of parental genotypes and and ρ is the fitness of a single recombinant offspring from them, without mutation (see Box 1). L_R was calculated by averaging this measure across N independently chosen pairs of individuals, where each parent was chosen with probability proportional to their fitness (i.e., in the same way the population reproduced in the evolutionary simulations).

We define multiplicative epistasis between two mutations, i and j, as $\varepsilon = W_{i,j}/W - W_iW_j/W^2$, where W is the fitness of the unmutated (test) genotype, W_i and W_j are the fitnesses of the single mutants, and $W_{i,j}$ is the fitness of the double mutant. Otto and Feldman (1997) introduced the standardized epistasis coefficient $\varepsilon^* = \varepsilon/\bar{s}^2$, where $\bar{s} = (W_i+W_j)/(2W)-1$ is the mean effect of a single mutation. We calculated means and variances of ε^* across pairs of random non-lethal deleterious mutations, introduced individually and in combination into a random sample of 100 viable individuals without replacement (for $N < 100$, we sampled 100 viable individuals with replacement).

For invasion analysis, populations were evolved for 10^4 generations under either asexual or sexual reproduction (i.e., the population was fixed for either the m or M allele, respectively),

to allow sufficient time for the population to approach mutation-recombination-selection-drift equilibrium. We then mutated the allele at the modifier locus M (see Reproductive mode, above) in a single randomly chosen individual. In most population size $\times$ reproductive mode treatments, we measured the fixation probability of the novel modifier allele, u, relative to that of a neutral mutation ($u^* = 1/N$) in N replicate invasion trials per independently evolved population, for a total of $50N$ replicates. For the largest $N = 10^4$ populations, computational time limited us to $5N$ replicate asexual invasion trials and $10N$ replicate sexual invasion trials.

To measure evolution of recombination rate, populations were evolved for 10^4 generations with a modifier locus that was linked to a randomly chosen row of the **R** matrix and fixed for an allele that specified a map length of $\lambda = 0$ M. After generation 10^4, the modifier locus experienced mutations at a rate of 10^{-3} per generation. Mutational effects on λ were ± 0.05 M, with equal probability. Alleles conferring $\lambda < 0$ were discarded. When two individuals with map lengths λ_1 and λ_2 reproduced, the expected number of crossovers in the offspring was $\lambda_1 + \lambda_2$.

The parameter values used here differed from those used in previous work on the evolution of genetic architecture (Siegal and Bergman, 2002; Azevedo et al., 2006; MacCarthy and Bergman, 2007; Lohaus et al., 2010): the random gene networks were larger ($n = 100$ genes) and sparser ($c = 0.05$), and the genomic mutation rate was higher ($U = 1$). These modifications have three advantages. First, the greater number of genes allows mutations to have a broad range of potential fitness effects, including beneficial, neutral, slightly deleterious and lethal. Second, the higher U allows populations to show considerable mutation load at equilibrium (Martin and Wagner, 2009). Third, real gene networks are relatively sparse (Leclerc, 2008).

All statistics were conducted using the R statistical package, version 3.2.1 (Ihaka and Gentleman, 1996). Comparisons of evolutionary trajectories were conducted using the function *lme* of the *nlme* package to generate linear mixed-effects models (Pinheiro and Bates, 2000).

In these models, ln(Time) in generations, Sex (i.e., reproductive mode: sexual or asexual), and ln(N) were modeled as fixed effects. Statistical tests using linear models with only one fixed effect or with multiple fixed effects are described inline or in a detailed table, respectively. In all linear models, population founder was modeled as a random effect.

RESULTS

Sex has a long-term advantage. We simulated the evolution of haploid gene networks in populations ranging in size from $N = 10$ to 10^4 individuals. We set the genomic mutation rate to be high ($U = 1$) and stabilizing selection to be moderate ($\sigma = 0.2$) to ensure the operation of all components of Hill-Robertson interference: Muller's ratchet in smaller populations, the Fisher-Muller effect in larger populations, and hitchhiking and background selection at all population sizes. Mutants differing from the target expression state at i and $i + 1$ genes differed in fitness by no more than 5% (i.e., $W_i - W_{i+1} < 0.05$, for all i). Populations were evolved for 10^4 generations, allowing sufficient time for populations of all sizes to approach mutation-recombination-selection-drift equilibrium. To examine the evolutionary contributions of changes in the genetic architecture in these populations, we monitored mean fitness ($\overline{W}$), deleterious mutation rate (U_d), epistasis (ε^*), and recombination load (L_R) over the course of the simulations (Figure 1, note that time is plotted on a log scale).

Over the short term (generations 1 through 10), the most striking difference between sexual and asexual populations is that mean fitness declines significantly more quickly in large sexual populations than in large asexual populations (statistical analysis in Appendix table 1). This pattern characterizes populations of at least 100 individuals (ln(Time) $\times$ Sex interaction estimated separately for each $N \geq 100$: $|t| \geq 3.989$, d.f. = 447, $p < 0.0001$, all tests) and appears to be largely the result of the recombination load increasing in sexual populations through generation 10 (effect of ln(Time) on L_R: $|t| = 3.975$, d.f. = 1699, $p < 0.0001$). Smaller populations did not show a significant change in mean fitness in the first 10 generations (main effect of ln(Time) and ln(Time) $\times$ Sex interaction estimated separately for each $N < 100$: $|t| \geq 1.946$, d.f. = 447, $p > 0.05$, all tests).

Over the longer term (at 10^4 generations; Figure 1B), sexual populations evolved significantly higher mean fitness at equilibrium than asexual populations ($\widehat{W}_{\text{sex}} > \widehat{W}_{\text{asex}}$), and the magnitude of the difference depended on population size (Appendix table 2). In populations of 100 individuals or fewer, the difference appears primarily attributable to Muller's ratchet, as all asexual populations in this size range exhibited a fitness decline between generations 100 and 10^4 (Figure 1A; effect of ln(Time) on mean fitness estimated separately for each $N \leq 100$: $|t| \geq 8.469$, d.f. = 399, $p < 0.0001$, all tests). Only the smallest sexual and asexual populations ($N = 10$) evolved to indistinguishable equilibrium mean fitnesses, suggesting that the costs of recombination load in sexual populations and of Muller's ratchet in asexual populations were of similar magnitude at this population size.

In populations of more than 100 individuals, the equilibrium mean fitness was determined by the evolving genetic architecture (Figure 1B). Both the deleterious mutation rate, U_d (Appendix table 3; $p < 0.0001$), and the recombination load, L_R (in sexuals: $|t| = 7.251$, d.f. = 299, $p < 0.0001$), decreased significantly with population size. The proportions of all types of mutations—beneficial, neutral, deleterious, and lethal—evolved, but reductions in the proportion of lethal mutations (p_l) and parallel increases in the proportion of neutral mutations (p_n) made the strongest contributions to the decreases in U_d (Figure 8). The equilibrium mean fitness of large populations was well predicted by the mutation-selection balance equation ($\widehat{W} \approx e^{-\overline{U}_d}$; Figure 2), with large asexual populations closely matching the prediction (all $N \geq 333$ differing by $< 1\%$) and sexual populations falling slightly below the prediction due to recombination load (all $N \geq 100$ differing by $> 2.5\%$).

Sexual populations evolved negative epistasis between deleterious mutations (Figure 1), consistent with earlier results using a similar model with fewer genes (n=10; Azevedo et al., 2006; MacCarthy and Bergman, 2007; Lohaus et al., 2010). However, the negative epistasis cannot account for the long-term advantage of sex in our model. If the negative epistasis we observed had produced a long-term advantage of sex, as expected (Kimura and Maruyama, 1966; Kondrashov, 1988; Charlesworth, 1990), then sexual populations would have evolved a

higher fitness at equilibrium than that predicted by the mutation-selection balance equation. We found the opposite pattern (Figure 2).

Although the operation of Muller's ratchet (Kimura et al., 1963) was apparent only in populations of 100 individuals or fewer ($\widehat{W} \ll e^{-\overline{U}_d}$; Figure 2), Hill-Robertson interference was also operating in larger asexual populations. Background selection reduced neutral genetic variation, a metric of N_e, significantly more in large asexual populations than in small asexual populations (Figure 3B and Appendix table 4). Thus, Hill-Robertson interference had an indirect effect on the mean fitness of larger populations via its effect on the efficiency with which selection acted to reduce U_d (Figure 1B). In further support of this conclusion, when sexual and asexual populations were subjected to a mutation rate ($U = 0.1$) that was too low for changes in U_d to have an appreciable effect on mean fitness, but sufficiently high to drive background selection, we observed no difference in mean fitness between sexual and asexual populations even at $N = 10^4$ (Figure 9). In addition, when network connectivity (c) was too low to drive differences among sexual and asexual populations in equilibrium U_d, we again observed no difference in mean fitness between sexual and asexual populations (Figure 11).

Sex has a short-term advantage in large populations. The data in Figure 1 document a long-term advantage to sexual reproduction at all population sizes. As a result, equilibrium sexual populations are expected to outcompete equilibrium asexual populations in head-to-head competition. However, the data in Figure 1 also indicate a short-term disadvantage associated with recombination load that is expected to impede both the origin and maintenance of sexual reproduction; a sexual mutant arising in an asexual population has an immediate disadvantage because it *starts* experiencing recombination load, whereas an asexual mutant arising in a sexual population has an immediate advantage because it *stops* experiencing recombination load.

We next investigated whether the short-term advantages of sex were sufficient to enable sexual mutants to invade equilibrium asexual populations, despite this short-term disadvantage.

Following the approach of Keightley and Otto (2006), we investigated the origin of sex by introducing a sexual mutant into equilibrium asexual populations. We similarly investigated the maintenance of sex by introducing an asexual mutant into equilibrium sexual populations. We then monitored the fate of the mutations until they were either fixed or lost from the population. We measured the fixation probability of the invading allele (u) relative to that of a neutral mutation ($u^* = 1/N$) in at least $5N$ replicate invasion trials at each population size.

In Figure 4, we show the effect of population size on these relative fixation probabilities, u/u^*. At small population sizes, asexual modifiers invaded successfully more often than sexual modifiers, and this difference increased with population size until it achieved a maximum near $N = 100$. As population size increased further, the trend reversed so that sexual modifiers invaded successfully more often than asexual modifiers in large populations ($N > 10^3$; Figure 4). In the largest populations we tested ($N = 10^4$), sexual mutants invaded asexual populations significantly more often than the neutral expectation ($u/u^* = 1.987$, $n = 1.56 \times 10^5$, $p = 0.0005$ by an exact binomial test). Although we report only the results of our Separate Sex implementation of sexual reproduction (see Materials and Methods, Reproductive mode) in Figure 4, we obtained qualitatively identical results using Recessive Sex (Figure 12A). Dominant Sex was neither able to invade nor to resist invasion by asexual modifiers (Figure 12B) for reasons we discuss in the Appendix.

Examining only the largest populations ($N = 10^4$), we explored the sensitivity of the modifier fixation probabilities to the genome-wide mutation rate (U). Like the long-term advantage described above, this short-term advantage of sex disappeared when we reduced the mutation rate to $U = 0.1$ (Figure 9). Although this mutation rate was sufficiently high to cause evolution of genetic architecture in the form of a decreased U_d, it did not translate to a fitness advantage because mutations, deleterious or otherwise, are rare. Despite the substantial background selection at this mutation rate (Keightley and Otto, 2006), asexual modifiers readily invaded equilibrium sexual populations and sexual modifiers were unable to invade equilibrium asexual populations (Figure 9).

The short-term advantage of sex is caused by Hill-Robertson interference, not epistasis. In our invasion simulations, the immediate population genetic consequence of introducing sex into an asexual population is the break up of linkage disequilibrium (LD). Breaking up LD is expected to have two consequences. First, mean fitness will decline as beneficial combinations of alleles (positive LD) built up by selection are broken up; this selects against sex. Second, additive genetic variance in fitness will rise as negative LD built up by a combination of selection and genetic drift is broken up; this selects for sex. Figure 3 shows that both of these predictions are met for log fitness ($\ln W$) for populations of 100 or more individuals.

If these immediate consequences of sex determined the invasion success of sexual modifiers, then we expect the increase in additive genetic variance to outweigh the decrease in mean fitness only in the largest populations ($N = 10^4$; Figures 4 and 12A). More precisely, higher recombination is expected to evolve if the net advantage of eliminating LD is positive, i.e., if $\Delta\overline{\ln W} + \Delta\mathrm{var}(\ln W) > 0$, where Δ indicates the difference between a statistic in the real population and in a hypothetical population with the same allele frequencies but in linkage equilibrium (Barton, 1995). Figure 3B shows that at generation 10^4 the net advantage of eliminating LD increases with population size and that $\Delta\overline{\ln W} + \Delta\mathrm{var}(\ln W) > 0$ for all asexual populations of 100 or more individuals (paired t-test: $t \geq 3.417$, $df = 49$, $p \leq 0.0013$).

These results agree qualitatively, but not quantitatively, with the data in Figures 4 and 12A, where sex invades successfully only in much larger populations. One possible reason for the discrepancy is that the Barton (1995) prediction is for weak modifiers of recombination, whereas our results are for a maximal increase in the rate of recombination (increasing the genetic map length from $\lambda = 0$ to 49.5 M). To test this possibility, we ran additional invasion simulations where we introduced into equilibrium asexual populations a weaker modifier of recombination that increased map length from $\lambda = 0$ to only 0.05 M. The weaker modifier mutations exhibited higher fixation probabilities, exceeding the neutral expectation in populations of at least 10^3 individuals (Figure 13).

In our model, the determinant of the short-term advantage of sex, negative LD, appears to have arisen from Hill-Robertson interference rather than from the negative epistasis that evolved in our simulations (Figure 1). Otto and Feldman (1997) predict the evolution of higher recombination rate only if the epistatic effects of mutations satisfy the following condition:

$$3\overline{\varepsilon^*} + (\overline{\varepsilon^*})^2 + \mathrm{var}(\overline{\varepsilon^*}) < 0$$

where ε^* is a standardized epistasis coefficient (see Materials and Methods, Genetic architecture). None of the 50 populations summarized in Figure 1 (sexual or asexual) satisfied that condition at generation 10^4. Thus, epistasis cannot explain the accumulation of negative LD in large asexual populations. Instead, it must have been caused by Hill-Robertson interference.

Changes in the genetic architecture influence both the origin and maintenance of sex. Changes in the genetic architecture played a decisive role in generating a long-term advantage of sex (Figure 1). Here we investigate the role of changes in the genetic architecture in the short-term advantage of sex. To understand why the origin and maintenance of sex was favored only when population size was large, we investigated the mean fitness dynamics and fixation times of the sexual and asexual genotypes that successfully invaded (Figure 5). The immediate and short-term fitness consequences of mutations that alter reproductive mode were predictable from the dynamics of genetic architecture evolution. Asexual modifiers arising in sexual populations experienced an immediate fitness benefit due to the disappearance of recombination load and the advantageous genetic architecture (low U_d) they inherit from their sexual predecessors. The latter advantage decayed over time as asexual invaders evolved toward the asexual equilibrium. Most successful asexual invasions occurred quickly (Figure 5A, black points and boxplots), before the mean fitness of the invaders (Figure 5A, black lines) decayed below that of the resident sexual population (Figure 5A, dashed gray lines).

In contrast, sexual modifiers that successfully invaded asexual populations experienced a short-term fitness decline during the time when recombination load was accumulating, but evolved back toward the sexual equilibrium after sufficient time had passed to evolve

an advantageous genetic architecture (compare fitness trajectories in Figure 5B to U_d and L_R trajectories in Figure 1A). Successful sexual modifiers arose by chance in high fitness genomes, retained a higher fitness than the asexual mean for around 100 generations (Figure 5B, red lines) and hitchhiked to a relatively high frequency as a result (Figure 13). In populations of size $N \leq 100$, the only sexual modifiers that fix appear to do so by hitchhiking quickly to fixation. In larger populations ($N \geq 10^3$), the initial hitchhiking of sexual modifier mutations was critical to their invasion success because it enabled their persistence over the long timescale needed for the sexual invaders to evolve a higher mean fitness (Figure 5B, red lines) than that of the resident asexual population (Figure 5B, dashed gray lines). Similarly, population size (N) critically affected invasion probabilities because increasing N increased the transit time (t_{fix}) of new mutations to fixation (Figure 5, red and black points and boxplots). Because the evolution of asexual disadvantages and sexual advantages is time-dependent, sexual resident populations and sexual invaders can be successful only if they persist long enough for these differences to evolve. Thus, our data reveal that the evolutionary success of sex at only the largest population sizes resulted from an interaction between the increase in t_{fix} and the differences in fitness dynamics between sexual and asexual invaders (Figure 5).

Selection favors moderate recombination rates. Thus far, we have compared asexual reproduction to sexual reproduction with free recombination. However, we found that a small increase in recombination rate is favored even when sex is not ($N = 10^3$, compare Figures 4 and 12A with 13), suggesting that "a little sex may go a long way" (Hurst and Peck, 1996) in our model. To investigate this phenomenon further, we allowed recombination rate to evolve in populations of $N = 10^3$ individuals. Like our investigations of the evolution of sexual reproduction, we began these simulations with populations that had evolved to a mutation-selection-drift equilibrium in the absence of recombination. Unlike the earlier simulations, mutations at the modifier locus were recurrent and had only small effects on the length of the genetic map λ ($\pm 0.05M$) and, therefore, the recombination rate. Mean map

length among 50 replicate simulations increased to $\overline{\lambda} \approx 0.1M$ within 1.5×10^4 generations (Figure 6). Thus, selection in the gene network model readily promoted the evolution of moderate, but not high, recombination rates in populations of $N = 10^3$ individuals.

DISCUSSION

We simulated evolution in a computational model of gene networks in order to determine how Hill-Robertson interference interacts with an evolving genetic architecture to impact the evolutionary origin and maintenance of sex. We found that the benefit of sex increased with population size, in agreement with earlier studies (Iles et al., 2003; Keightley and Otto, 2006; Gordo and Campos, 2008; Hartfield et al., 2010). Those studies identified Hill-Robertson interference as the principal cause of this pattern. We found that Hill-Robertson interference also played a role in our model in creating both a long-term and a short-term advantage of sex. But we also showed that the long- and short-term advantages of sex were determined by differences between sexual and asexual populations in the evolutionary dynamics of two properties of the genetic architecture, U_d and L_R. We next sought to quantify the contribution of Hill-Robertson interference to these dynamics.

We documented two differences between sexual and asexual populations that likely impacted the evolution of U_d. First, sexual populations uniquely experienced recombination load, L_R. We know from previous work that selection to minimize L_R, alone, results in increasing robustness to both recombination and mutation, lowering U_d (Azevedo et al., 2006; Misevic et al., 2006; Gardner and Kalinka, 2006; Martin and Wagner, 2009; Lohaus et al., 2010). Second, asexual populations uniquely experienced Hill-Robertson interference that reduced N_e (Figure 3B). As in Keightley and Otto (2006), the reduction in N_e increased with population size, N. At $N = 100$, N_e was reduced by 36% (from 72 to 46 individuals); at $N = 10^4$, N_e was reduced by 91% (from 5957 to 510 individuals). The reduced N_e in asexuals is expected to reduce the efficiency with which selection lowers U_d. In sum, sexuals may have evolved lower U_d than asexuals both because sexuals experienced stronger selection to do so and because Hill-Robertson interference reduced the efficiency of selection on U_d in asexuals.

We quantified how these differences in the strength and efficiency of selection contributed to the equilibrium U_d in sexual and asexual populations in Figure 7. In that figure, we compare the equilibrium U_d between sexual and asexual populations of the same census size, N, and of the same effective size, N_e. Differences in U_d between populations of the same census size resulted from differences in both the strength and efficiency of selection, whereas differences in U_d between populations of the same effective size resulted from differences only in the strength of selection. In Figure 7 we see that the effect on U_d of differences in the strength of selection (line **b**) decreased as population size increased, whereas the combined effect of differences in the strength and efficiency of selection (line **a**) was constant across the population sizes we examined. Thus, although differences in the strength of selection played a larger role than differences in the efficiency of selection at all the population sizes we examined, the relative contribution of selection efficiency grew with population size. In populations larger than 10^4 individuals, the reduced selection efficiency caused by Hill-Robertson interference may have eventually come to play the dominant role in determining U_d and, consequently, mean fitness in asexual populations.

Our finding that Hill-Robertson interference contributed to the advantages of sex in part through its indirect effect on the evolution of genetic architecture differs from the findings of previous models in which the genetic architecture was static and Hill-Robertson interference directly determined the advantages of sex (Iles et al., 2003; Keightley and Otto, 2006; Gordo and Campos, 2008; Hartfield et al., 2010). The evolvable genetic architecture in the gene network model likely impacted the contribution of Hill-Robertson interference in a number of ways. We focused on the evolving U_d because it was the primary determinant of equilibrium mean fitness in large populations, but we think that the evolving compensatory mutation rate 8 also played an important role. Compensatory mutations increased in frequency as fitness declined, ensuring that our populations achieved a fitness equilibrium. The fitness equilibrium was an important aspect of our model. The absence of compensatory mutations in the previous models (Muller, 1964; Haigh, 1978; Keightley and Otto, 2006; Gordo and

Campos, 2008) ensured a perpetual decline in population mean fitness via Muller's ratchet, regardless of population size, that would have been accelerated by Hill-Robertson interference in asexual populations. In our gene network model, Muller's ratchet was eventually halted by an increasing frequency of compensatory mutations even at the smallest population sizes (Figure 1). As a result, direct effects of Hill-Robertson interference on advantages of sex in the gene network model were limited to populations that were small enough for Muller's ratchet to operate over a wide fitness range.

One major difference between our results and those of earlier studies of Hill-Robertson interference, is that we observed only moderate advantages of sex. The long-term advantage of sex observed here ($\widehat{W}_{\text{sex}}/\widehat{W}_{\text{asex}} = 1.04$ for $N = 10^4$) was substantial but may be considered weak compared to the 2-fold cost experienced by many sexual species in nature. The short-term advantage was even weaker: it disappeared when we imposed as little as a 1% cost of sex (14). We note, however, that modifiers of sex generated smaller short-term advantages than modifiers of recombination (compare the $N = 10^3$ populations in Figures 4, 13 and 6), as has been observed in other models (Keightley and Otto, 2006).

We identified two additional factors that contribute to the advantages of sex in our model. First, a higher mutation rate U increases the advantage of sex. This has been found in other models (Keightley and Otto, 2006; Gordo and Campos, 2008). Note that the deleterious mutation rate evolved in our simulations ($U_d \approx 0.2$) is high, but not unrealistically so. For example, *Drosophila melanogaster* shows $U_d \approx 1$ (Haag-Liautard et al., 2007). Second, a higher gene network connectivity increases the advantage of sex. We improved biological realism (Leclerc, 2008) by using much sparser networks ($c = 0.05$) than earlier studies of this model (typically, $c = 0.75$; Siegal and Bergman, 2002; Azevedo et al., 2006; MacCarthy and Bergman, 2007; Lohaus et al., 2010) A connected transcriptional regulatory network of 75 transcription factors in yeast estimated $c = 0.024$ (MacIsaac et al., 2006). Networks with similar connectivity in our model were not able to generate an advantage of sex ($c = 0.02$, 11). However, the yeast estimate increases as new data becomes available (compare Harbison

et al., 2004; MacIsaac et al., 2006). In addition, we do not know the extent to which this yeast network is representative of other networks in nature. A strict comparison of connectivities between our networks and real biological networks is likely misleading because we only considered random gene networks, a pattern of connectivity that is probably unrealistic (Milo et al., 2002; Shen-Orr et al., 2002).

The magnitude of both the long- and short-term advantages of sex are likely to be affected additionally by many factors we have not considered here, such as, deviations from random mating (Shields, 1982; Jaffe, 2000; Agrawal, 2001; Siller, un 7; Blachford and Agrawal, 2006), population structure (Peck et al., 1999; Agrawal and Chasnov, 2001; Salathé et al., 2006; Roze, 2009; Hartfield et al., 2012), ploidy (Kirkpatrick and Jenkins, 1989; Kondrashov and Crow, 1991; Agrawal and Chasnov, 2001; Otto, 2003; Haag and Roze, 2007; Roze, 2009), number of loci (Iles et al., 2003), and environmental change (Barton, 1995; Otto and Nuismer, 2004; Carvalho et al., 2014), leaving many questions yet to be answered.

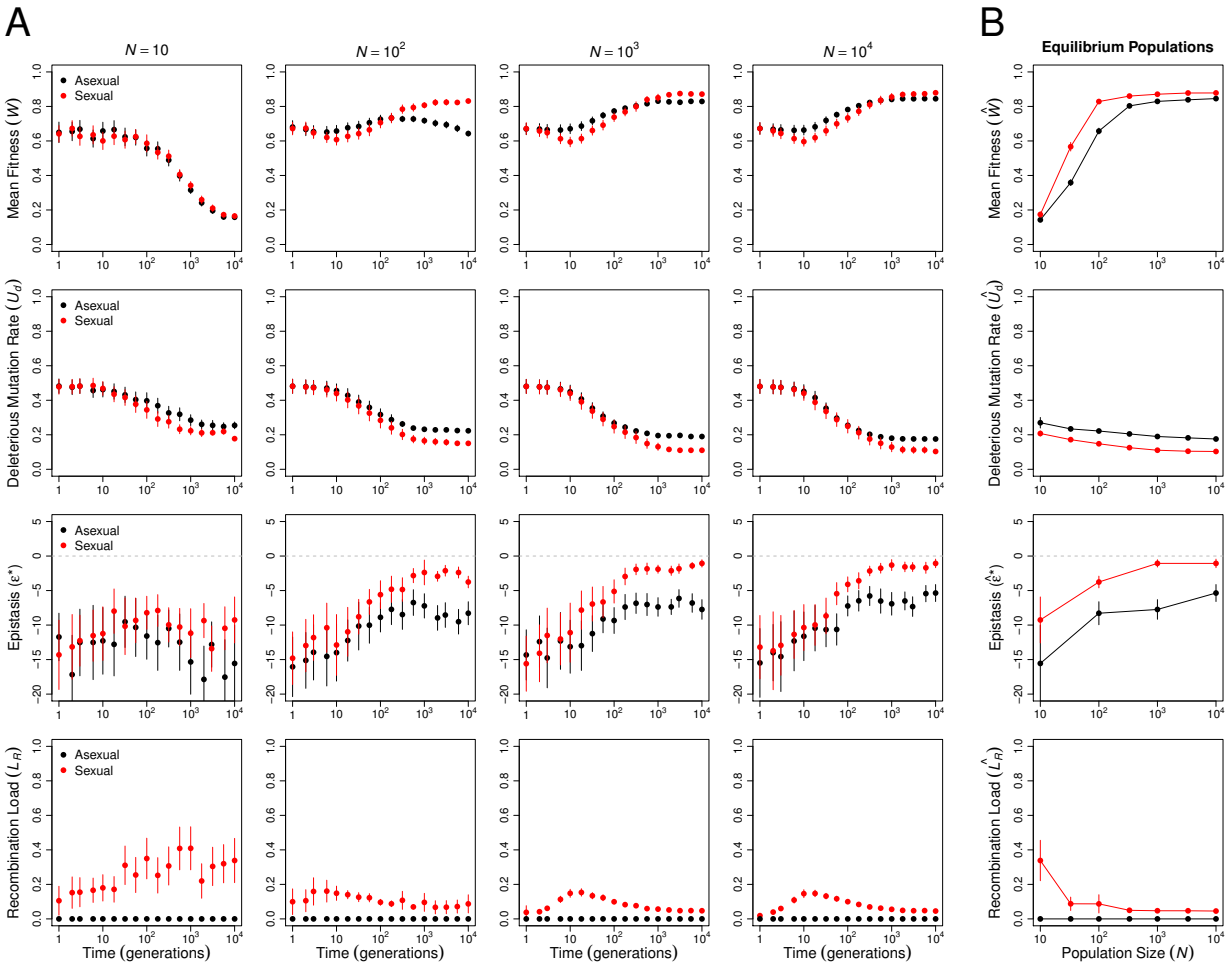

Figure 1: Sex has a long-term advantage. (A) Changes in mean fitness (W), deleterious mutation rate (U_d), epistasis (ε^*), and recombination load (L_R) over time in asexual (black) and sexual (red) populations of various sizes (N). (B) Means at generation 10^4, after populations of all sizes had achieved equilibrium in all properties. Values are means and 95% confidence intervals based on 50 replicate populations initiated from different randomly chosen founders.

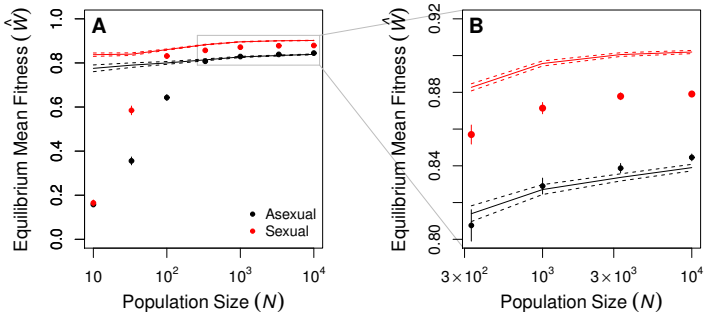

Figure 2: Equilibrium mean fitness shows the effects of Muller's ratchet, mutation load and recombination loadThe equilibrium mean fitness of large populations differed only slightly from the expectation at mutation-selection balance (Box 1). Values are means and 95% confidence intervals of the observed fitness in asexual (black) and sexual (red) populations after 10^4 generations of evolution (replotted from Figure 1B). Solid lines show the expectation under the mutation load equation in Box 1 and dashed lines show 95% confidence intervals calculated from the observed U_d in each population. Panels show data from all populations (A) or from only the largest populations (B).

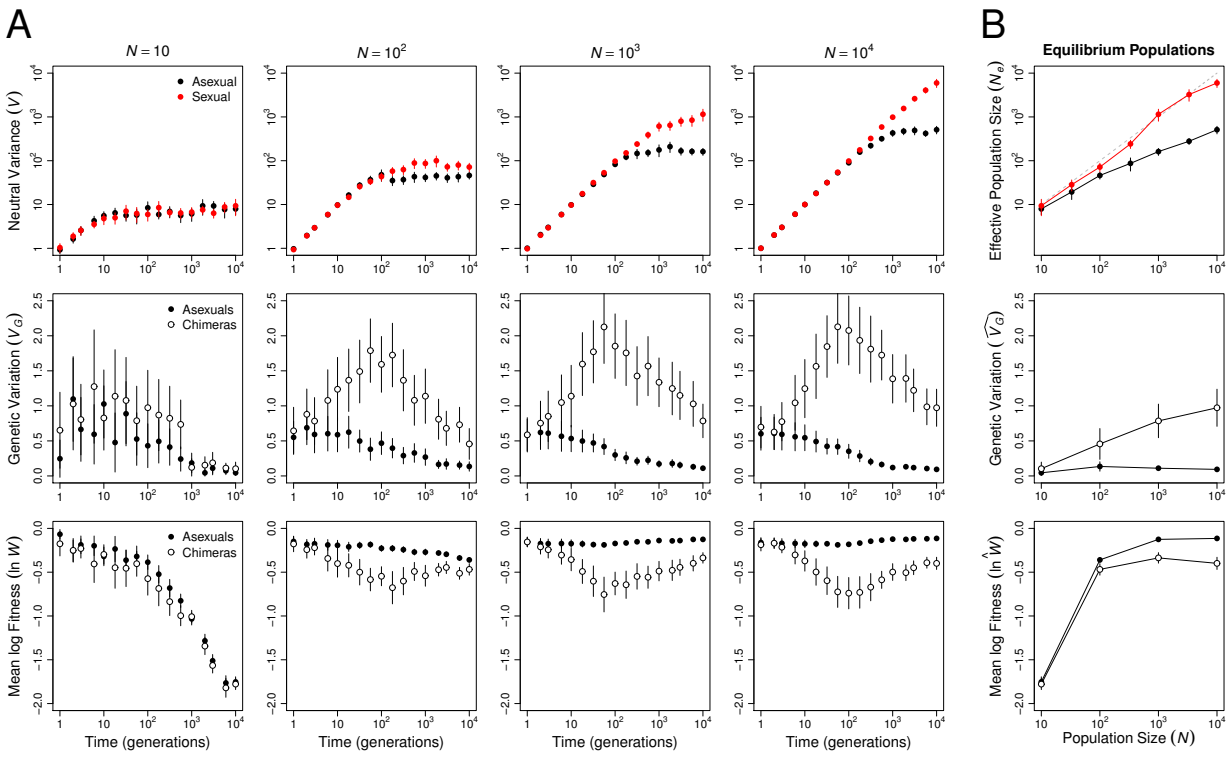

Figure 3: Hill-Robertson interference affected asexual populations of all sizes. (A) Hill-Robertson interference depressed variance at a neutral locus (V) in asexual (black) compared to sexual (red) populations (top row). The LD that accumulated in asexual populations also decreased genetic variance in log fitness, var($\ln W$), and increased mean log fitness, $\overline{\ln W}$. Data in the middle and bottom rows compare these metrics in the real asexual populations (closed circles) and populations of chimeras with the same allele frequencies but no LD (open circles). (B) Means of each metric at generation 10^4. Effective population size (N_e) was estimated from $\hat{V}$ (see Materials and Methods, Population metrics). Values are means and 95% confidence intervals based on 50 replicate populations initiated from different randomly chosen founders.

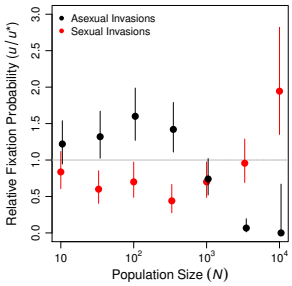

Figure 4: Sex has a short-term advantage in large populations. Asexual (black) or sexual (red) mutants were introduced into equilibrium sexual or asexual populations, respectively, at an initial frequency of $1/N$. Frequencies of the modifier mutations were monitored until the modifiers were either fixed or lost. Values are the proportion of fixations (u) divided by the neutral expectation ($u^* = 1/N$) and 95% confidence intervals based on $\geq 5N$ replicate invasion trials for each population size N. The data shown here are from the Separate Sex implementation of reproductive mode (see Materials and Methods, Reproductive mode). Analogous data for Recessive Sex and Dominant Sex are shown in Figure 12.

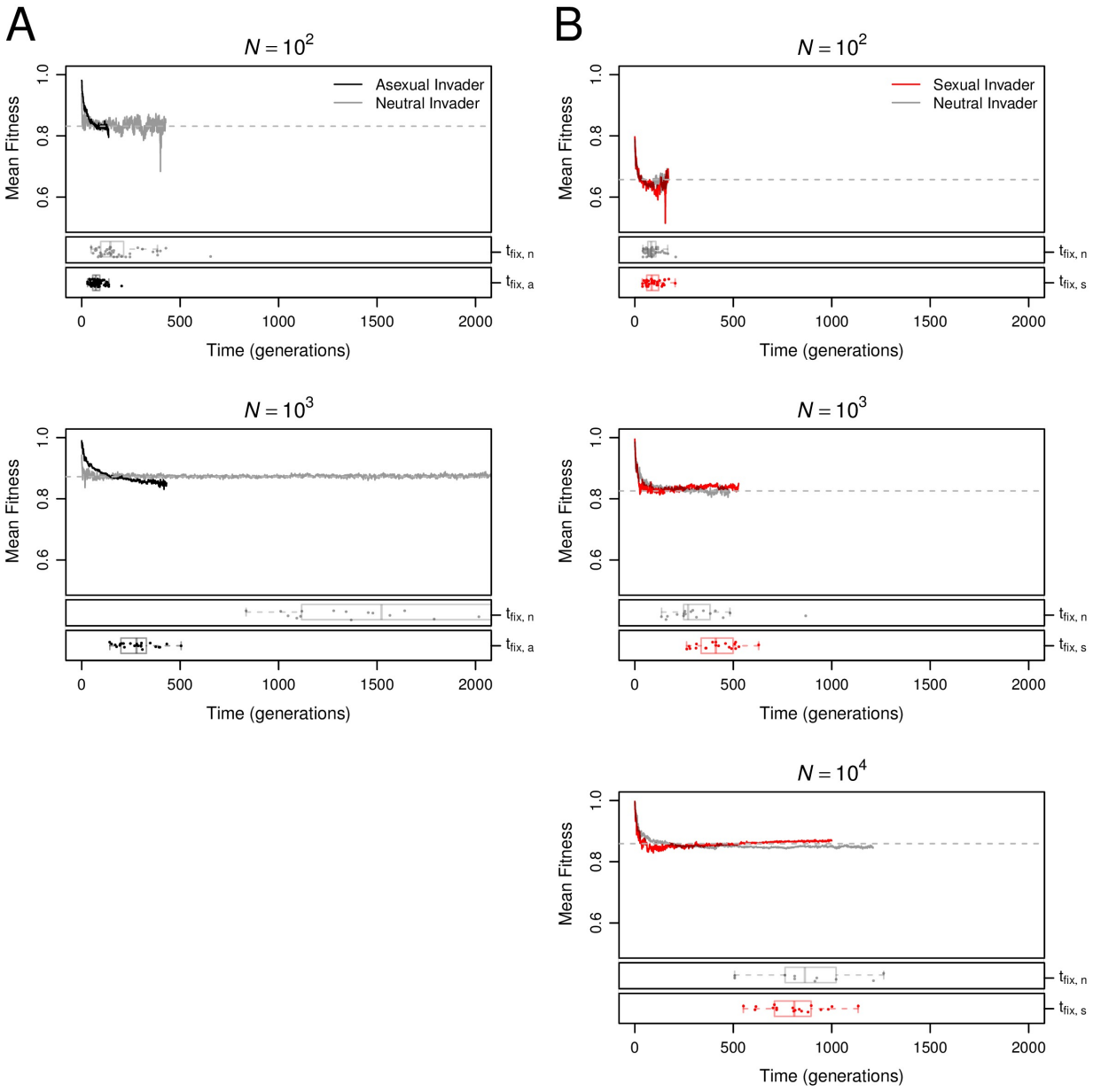

Figure 5: Changes in the genetic architecture influence both the origin and maintenance of sex. We monitored the fixation and loss of asexual mutants introduced into equilibrium sexual populations (black lines, panel A), of sexual mutants introduced into equilibrium asexual populations (red lines, panel B), and of neutral mutants introduced into both sexual and asexual populations (solid gray lines, panels A and B, respectively). Lines show the evolution of mean fitness among invading mutants, averaged over at least 10 successful invasions. The equilibrium mean fitness of the populations being invaded is represented by a gray dashed line across each plot. Points and corresponding boxplots shown at the bottom of each plot indicate the time of fixation for individual neutral ($t_{\mathrm{fix,n}}$), sexual ($t_{\mathrm{fix,s}}$), or asexual ($t_{\mathrm{fix,a}}$) mutations.

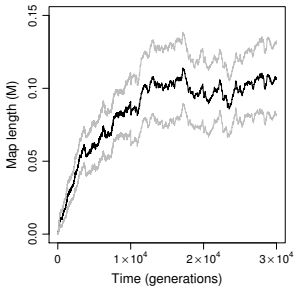

Figure 6: Evolution of the recombination rate under recurrent mutation at the recombination modifier locus. Black and gray lines show the change in mean and 95% confidence interval, respectively, of the genome map length (i.e. mean crossover probability) over time. Data are from 50 replicates initiated with the equilibrium asexual populations of size $N = 10^3$ shown in Figure 1.

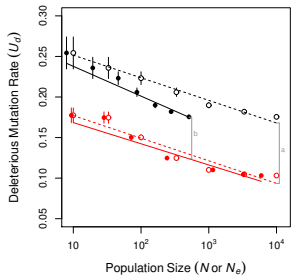

Figure 7: Hill-Robertson interference explains part of the difference in equilibrium U_d between sexual (red) and asexual (black) populations. Equilibrium values of the genome-wide deleterious mutation rate U_d versus census population size N (open circles, replotted from Figure 1) and versus effective population size N_e (closed circles). Lines are best fit linear models obtained separately using N (dashed lines) or N_e (solid lines) as a dependent variable together with reproductive mode. The total difference in U_d exhibited by sexual and asexual populations of census size $N = 10^4$ (gray line **a**) is attributable to both differences in the strength and efficiency of selection acting on genetic architecture. The difference in U_d exhibited by sexual and asexual populations of effective size $N_e = 510$ (gray line **b**) is the proportion of the total difference that remained after controlling for differences in the efficiency of selection that arise through Hill-Robertson interference.

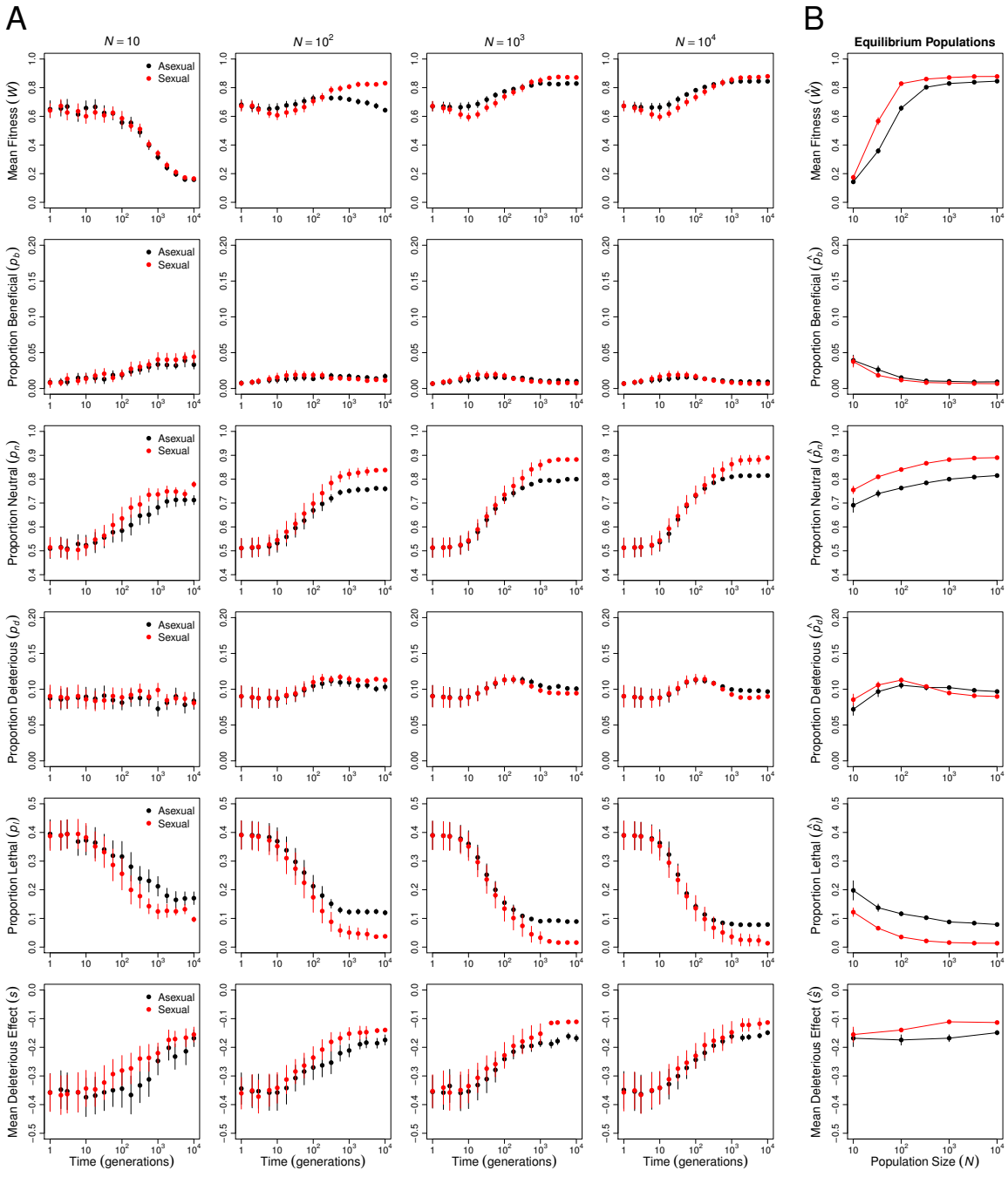

Figure 8: Evolution of the distribution of mutation effects. (A) Change in mean fitness (replotted from Figure 1), proportions of mutations (p_b, p_n, p_d, p_l) that are beneficial ($s > 0$), neutral ($s = 0$), deleterious ($-1 < s < 0$), and lethal ($s = -1$), and the mean effect of deleterious mutations (s) over time in asexual (black) and sexual (red) populations of various sizes (N). Note that the deleterious mutation rate U_d plotted in Figure 1 is a composite of the deleterious and lethal mutations displayed here: $U_d = U(p_d + p_l)$. (B) Mean of each property at generation 10^4 (equilibrium). Values are means and 95% confidence intervals based on 50 replicate populations.

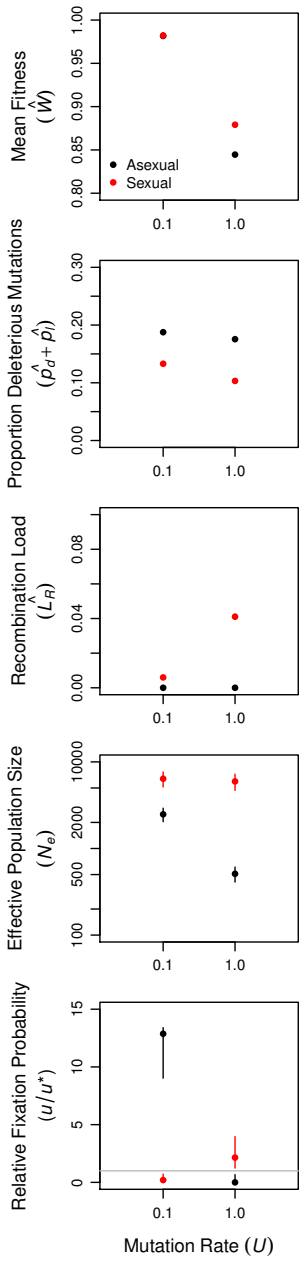

Figure 9: Equilibria and invasion probabilities for large populations ($N = 10^4$) at low ($U = 0.1$) and high ($U = 1$) genome-wide mutation rates. The top 4 panels show means and 95% confidence intervals for asexual (black) and sexual (red) populations at generation 10^4. Note that the proportion of deleterious mutations ($p_d + p_l$) shown here includes both deleterious ($-1 < s < 0$) and lethal ($s = -1$) mutations. The bottom panel shows relative fixation probabilities (u/u^*) of sexual (red) and asexual (black) modifier mutations introduced into these equilibrium asexual and sexual populations, respectively. Data shown here were generated using the Separate Sex mode of reproduction.

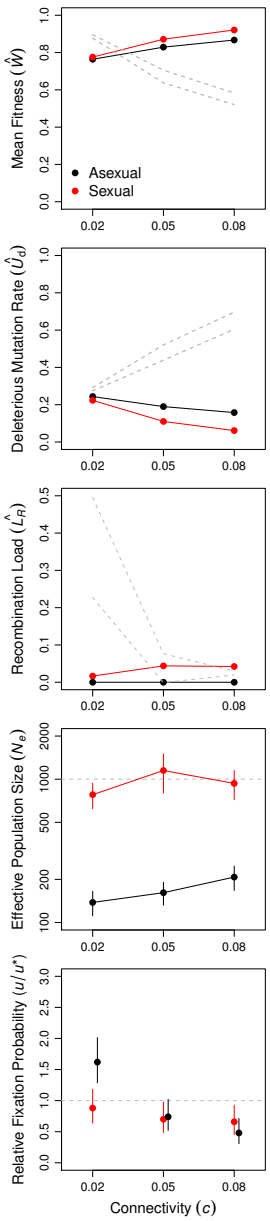

Figure 10: Network connectivity (c) impacts the long- and short-term advantages of sex. The top 4 panels show means and 95% confidence intervals for asexual (black) and sexual (red) populations at generation 10^4, after populations at each connectivity had achieved an equilibrium in all metrics. Dashed gray lines indicate the 95% confidence intervals after the first generation in panels 1–3 and the census size of all populations ($N = 10^3$) in panel 4. Note that asexual and sexual populations do not differ after the first generation because there has been no opportunity for selection at this time point. The bottom panel shows relative fixation probabilities (u/u^*) of sexual (red) and asexual (black) modifier mutations introduced into these equilibrium asexual and sexual populations, respectively, with the dashed gray line indicating the expectation for neutral mutations. Data shown here were generated using the Separate Sex mode of reproduction.

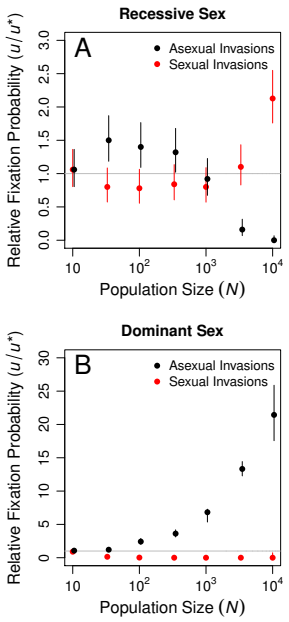

Figure 11: Relative fixation probabilities (u/u^*) of recessive and dominant modifiers of sex. Individual asexual (black) or sexual (red) modifier mutations were introduced into equilibrium sexual or asexual populations, respectively, at an initial frequency of $1/N$. Populations were then allowed to evolve using either the Recessive Sex (A) or Dominant Sex (B) reproductive mode (see Materials and Methods, Reproductive mode). In both cases, frequencies of the modifier mutations were monitored until the mutations were either fixed or lost. Data are the proportion of fixations (u) divided by the neutral expectation ($u^* = 1/N$) and 95% confidence intervals based on $\geq 5N$ replicate invasion trials for each population size (N).

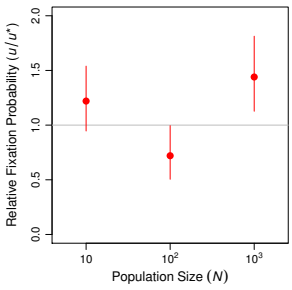

Figure 12: Relative fixation probabilities (u/u^*) of a modifier of recombination. The modifier mutation increased the genetic map length from $\lambda = 0$ to 0.05 M and acted additively. Individual modifier mutations were introduced into equilibrium asexual populations, at an initial frequency of $1/N$. Populations were then allowed to evolve. Frequency of the modifier mutation was monitored until it was either fixed or lost. Data are the proportion of fixations (u) divided by the neutral expectation ($u^* = 1/N$) and 95% confidence intervals based on $50N$ replicate invasion trials for each population size (N).

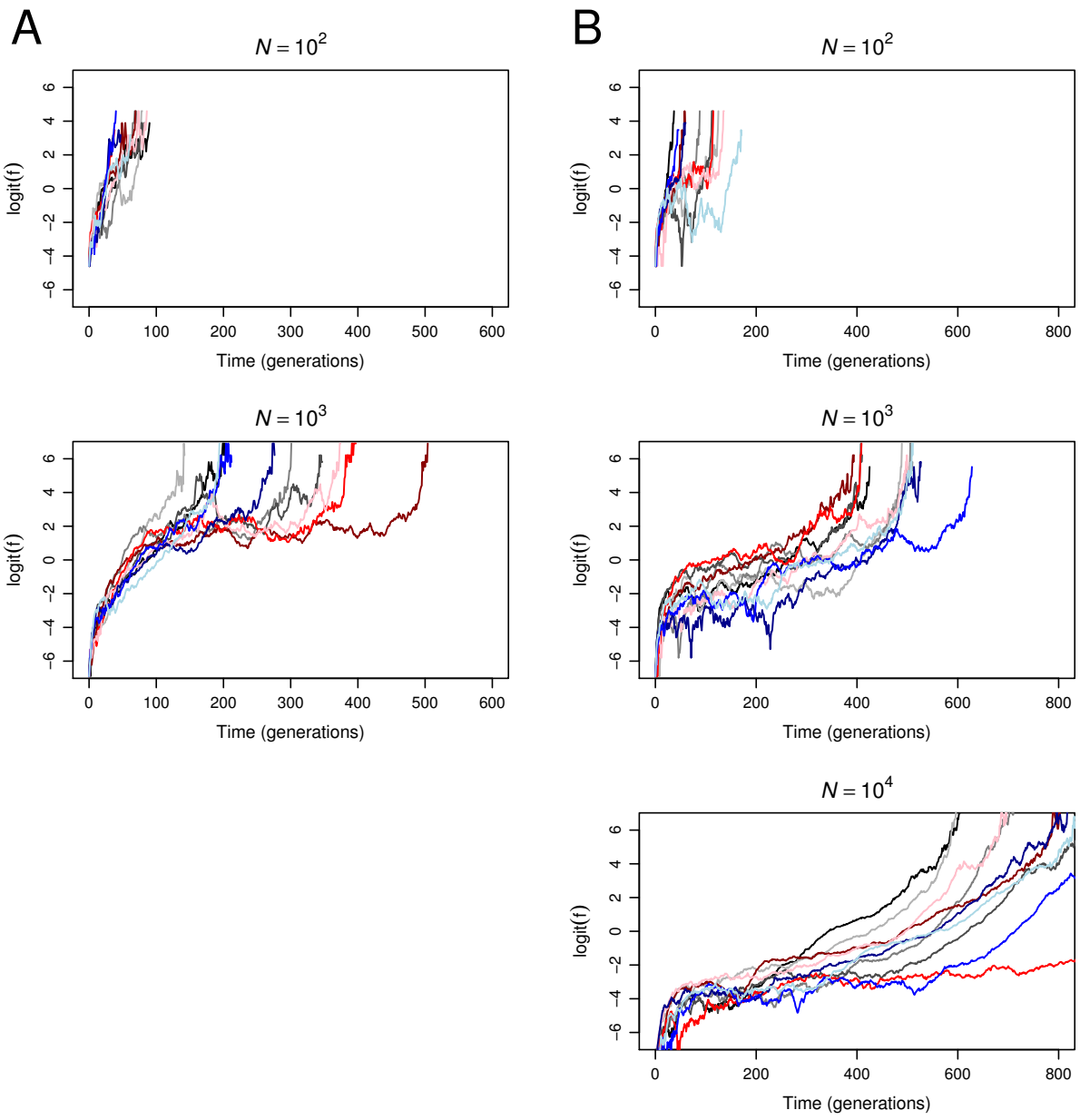

Figure 13: Frequency dynamics during successful invasions by modifiers of sex. Individual asexual (A) or sexual (B) modifier mutations were introduced into equilibrium sexual or asexual populations, respectively, at an initial frequency of $1/N$. Populations were then allowed to evolve using the Separate Sex reproductive mode. Lines show the logit transformed of the modifier mutation frequency over time for each of a random sample of 10 successful invasions.

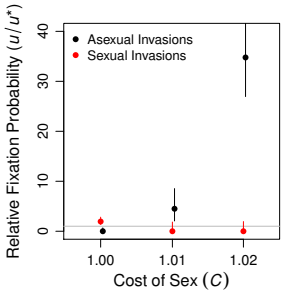

Figure 14: Costly sex does not evolve. Individual asexual (black) or sexual (red) modifier mutations were introduced, respectively, into equilibrium sexual or asexual populations of $N = 10^4$ individuals at an initial frequency of $1/N$. Asexually produced offspring have fitness $W_{\text{asex}} = W$ (Equation 2). Sexually produced offspring have fitness $W_{\text{sex}} = W/C$, where $C \geq 1$ is the cost of sex (e.g., $C = 2$ models a two-fold cost). Populations were then allowed to evolve with either no cost of sex ($C = 1$) or very small costs of sex ($C = 1.01$ or 1.02). Frequencies of the modifier mutations were monitored until the mutations were either fixed or lost. Data are the proportion of fixations (u) divided by the neutral expectation ($u^* = 1/N$) and 95% confidence intervals based on $\geq 2N$ replicate invasion trials at each cost of sex.

CHAPTER 3: POPULATION SUBDIVISION MAXIMIZES THE SUSTAINABLE COST OF SEX

Sexual reproduction among eukaryotes is both nearly ubiquitous (Bell (1982); Maynard Smith (1978); Vrijenhoek (1998)) and extraordinarily costly. Sexual reproduction destroys successful genetic combinations built by selection. In many eukaryotes, an individual reproducing sexually must expend time and resources to locate a mate, risking disease and predation in the process. Anisogamous species which must invest in males are further disadvantaged. In the absence of mitigating factors such as parental contribution or increased fecundity, the production of males alone carries a twofold cost. The prevalence of sex despite these costs implies it must convey extraordinary benefits, yet the nature of these benefits is controversial.

While there are over twenty hypotheses seeking to reconcile this disparity, (Bell, 1982; Kondrashov, 1993; Hurst and Peck, 1996; Otto and Lenormand, 2002), the primary benefits of sex are thought to be indirect (Weismann, 1887; Maynard Smith, 1978; Bell, 1982; Kondrashov, 1993; Burt, 2000; Otto and Lenormand, 2002), resulting from the ability of recombination to break up linkage disequilibrium (LD). When LD in a population is predominantly negative, that is, when genomes with intermediate fitness are overrepresented at the expense of high and low fitness genomes, selection is inefficient. Recombination between intermediate genotypes can generate extreme genotypes, restoring the efficiency of selection.

One hypothesis of particular interest, due to its broad applicability (Comeron et al., 2008), predicts that without recombination, negative LD accumulates in finite populations through a combination of selection and drift(Hill and Robertson, 1966; Felsenstein, 1974; Comeron et al., 2008). Essentially, selection at one locus reduces the efficiency of selection at other linked loci. This phenomenon, known as Hill-Robertson interference, takes several forms: background

selection (Charlesworth et al., 1993; Charlesworth, 1994), hitchhiking (Maynard Smith and Haigh, 1974; Peck, 1994), the Fisher-Muller effect (Fisher, 1930; Muller, 1932; Gerrish and Lenski, 1998), and Muller's ratchet (Muller, 1964; Haigh, 1978; Gordo and Charlesworth, 2000). Hill-Robertson interference has the potential to constrain the evolvability of populations or cause a fitness decline in small populations. Previous investigations have demonstrated that recombination decreases the impact of Hill-Robertson interference and provides a strong advantage to sex. This advantage allowed sexual mutants to invade asexual populations as well as to resist invasion by asexual mutants (Otto and Barton, 2001; Iles et al., 2003; Barton and Otto, 2005; Keightley and Otto, 2006; Hartfield et al., 2010, 2012).

Although Hill-Robertson interference is increasingly accepted as an important contributor to the evolution of sex, its effects have rarely been examined in the context of the evolution of interactions between genes, i.e. genetic architecture. This has the potential to obscure both the advantages and the disadvantages of sex; recombination selects for beneficial genetic architecture (Azevedo et al., 2006; Gardner and Kalinka, 2006; Martin and Wagner, 2009; Livnat et al., 2010; Lohaus et al., 2010), but the destruction of successful genetic interactions is one of the chief costs of sex. To address this knowledge gap, we previously used an artificial gene network model that explicitly incorporates evolving genetic architecture (Wagner, 1996; Siegal and Bergman, 2002) to investigate the origin and maintenance of sex. We demonstrated that sexual populations evolved more robust genetic architectures that exhibited lower deleterious mutation rates and lower recombination loads, resulting in an equilibrium fitness advantage to sexual populations.

Sexual equilibrium fitness ($\hat{W}_{sex}$) increased with population size, but the equilibrium advantage of sex ($\hat{W}_{sex}/\hat{W}_{asex}$) was maximized in intermediate-size populations ($10 > N > 100$) that were both small enough to experience Muller's ratchet and large enough for selection to produce a robust genetic architecture. By contrast, we found that sexual populations resisted invasion by asexual mutants only in the largest populations ($N > 10^3$). The key to this result was that asexual invaders initially have a fitness advantage, but the advantage

degrades over time. For the invaders to be repelled, transit time of a new mutation to fixation (T_{fix}) must be greater than the amount of time it takes for asexuals to lose the fitness advantage. This condition was met only with a large N, demonstrating that T_{fix}, not $\hat{W}_{sex}/\hat{W}_{asex}$, was the primary determinant of the maintenance of sex. However, when we imposed even a 1% cost to sex, even the largest populations were unable to resist invasion. Thus, our results could not explain the maintenance of costly sex in the face of invading asexual mutants.

In that work, we manipulated T_{fix} through population size, but an increase in T_{fix} can also be effected through addition of population structure (Peck et al. (1999); Salathé et al. (2006); Martin et al. (2006); Hartfield et al. (2012)). Population structure is a particularly promising line of investigation because it has the additional impact of decreasing the effective population size (N_e), raising potential for substantial genetic drift and a broad impact of Hill-Robertson interference even within large populations. The addition of population structure has the potential to neatly sidestep the limitation to the maintenance of sex we observed in panmictic populations, in which the two conditions expected to promote the maintenance of sex, a high $\hat{W}_{sex}/\hat{W}_{asex}$ and a high T_{fix}, were found at different population sizes. Indeed, previous investigations found that increasing T_{fix} through a combination of population structure and large population size supported the maintenance of sex with a cost approaching twofold (Hartfield et al., 2012). However, because these previous investigations did not incorporate compensatory interactions, there was no fitness equilibrium or limitation on Muller's ratchet, a consequence with the potential to exaggerate the benefits of sex.

Here we build on our previous work in the artificial gene network model by investigating the maintenance of costly sex in a structured population. Specifically, we simulate evolution in structured populations which feature different deme sizes and different migration rates between demes. We examine the contribution of population structure to the impact of Hill-Robertson interference, the equilibrium mean fitness and maintenance of sex during asexual invasions into equilibrium sexual populations. Using these results, we determine

how the interactions between population structure, T_{fix}, and the evolution of compensatory interactions affect the cost that can be sustained by sexual reproduction.

MATERIALS AND METHODS

Our gene network model is based on a model introduced by Wagner (1994, 1996). Methods used uniquely in this investigation are described below, with the full explanation of the model found in Chapter 2.

The reproductive mode of an individual is determined by its genotype at a modifier locus S, unlinked to the genes involved in the gene network. There are two alleles at the modifier locus: s and S. If a population is fixed for the s allele every individual reproduces asexually, and if it is fixed for the S allele every individual reproduces sexually. Thus, we refer to the s and S alleles as being *for* asexual and sexual reproduction, respectively.

The sexual and asexual subpopulations are reproductively isolated from each other. Sexual individuals do not experience a frequency-dependent cost of finding mates. One individual is chosen for every reproductive event with probability proportional to its fitness. If it carries the s allele, it reproduces asexually. If it carries the S allele, a second individual carrying an S allele is chosen with probability proportional to its fitness, and the two individuals reproduce sexually and produce one recombinant offspring. The offspring inherits the S allele from one of the parents.

Structured populations were created by subdividing populations of size $N = 1000$ into a ring of 10 or 20 equal-sized demes of N_d organisms. Demes were assumed to have a carrying capacity of N_d, and maintained a constant population size, with N_d offspring produced in each generation. Demes were not allowed to go extinct. In each generation, randomly-chosen individuals from each deme, with no bias for fitness of reproductive mode, migrate to either neighboring deme with a poisson-distributed probability set at a migration rate of m. Because the numbers of migrants to and from individual demes are independent, migration results in a transient state in which actual N_d may not equal carrying capacity, but is restored by

selection and reproduction, which produce N_d offspring. Population isolation was calculated as Wright's F statistic, F_{ST} (Wright, 1931).

Statistical analysis: All statistics were conducted using the R statistical package, version 3.2.1 (Ihaka and Gentleman, 1996). Comparisons of evolutionary trajectories were conducted using the function *lme* of the *nlme* package to generate linear mixed-effects models (Pinheiro and Bates, 2000). In these models, ln(Time) in generations, Sex (i.e., reproductive mode: sexual or asexual), N_d, and ln(m) were modeled as fixed effects. In all linear models, population founder was modeled as a random effect.

RESULTS

We simulated evolution of haploid artificial gene networks in structured populations of $N = 1000$ individuals. Structure was imposed by subdividing populations into demes of either $N_d = 50$ or $N_d = 100$ individuals and imposing migration rates (m) between neighboring demes ranging from 2×10^{-2} to 2×10^{-4}, parameters predicted to maximize based on results from panmictic populations (Figure 16). We set a high genomic mutation rate ($U = 1.0$) and moderate stabilizing selection ($\sigma = 0.2$) to ensure operation of Hill-Robertson interference. Mutants differing from the target expression state at i and $i+1$ genes differed in fitness by no more than 5% (i.e., $W_i - W_{i+1} < 0.05$, for all i). Structured populations consisting entirely of either sexuals or asexuals were allowed to evolve for 10^4 generations, by which time all populations had reached a mutation-selection-drift equilibrium. To examine the impact of population structure on the evolutionary contributions of Hill-Robertson interference and of changes in the genetic architecture to the mean fitness of sexual and asexual populations, we monitored mean fitness ($\overline{W}$), deleterious mutation rate (U_d), recombination load (L_R), within-deme genetic variance at a neutral locus (V), and genetic differentiation among demes (F_{ST}) over the course of the simulation (Fig 15).

As we observed previously in panmictic populations, after a brief decline as mutations begin accumulating, the fitness of both asexual and sexual populations rose. This was coincident with a decrease in U_d in both populations, as well as a decrease in L_R in the sexual

populations, both of which indicate evolution of robust genetic architecture. All populations reached a mutation-selection-drift equilibrium within 10,000 generations. (Figure 15).

At equilibrium, sexual populations evolved higher mean fitness than asexuals under all conditions. Increases in population structure were deleterious to the fitness of all populations, but had a disproportionately deleterious impact on asexual populations (log(Migration rate) $\times$ Sex interaction $\times$ Deme Size: $|t| = 4.5$, d.f. $= 543$, $p < 0.0001$), resulting in a $\hat{W}_{sex}/\hat{W}_{asex}$ that was maximized at the highest level of structure (Figure 17)

The equilibrium mean fitness was determined by the evolved properties of the genetic architecture, especially the equilibrium U_d in both populations, as well as L_R in the sexual population. Though U_d declines in all populations, it declines further in sexual populations ($U_d \times$ Sex interaction: $|t| = 7.41$, d.f. $= 543$, $p < 0.0001$). This difference comes in part due to the impact of recombination on evolution of genetic architecture–as previously demonstrated, selection to minimize L_R is sufficient to increase robustness to both recombination and mutation (Azevedo et al., 2006; Lohaus et al., 2010). and genetics paper–but the disproportionately deleterious impact of population structure demonstrates the contribution of Hill-Robertson interference as well.

Hill-Robertson interference decreased the fitness of all asexual populations, both directly and indirectly, depending level of structure. In all asexual populations, within-deme neutral genetic variance was lower than their sexual counterparts, indicating operation of Hill-Robertson interference in the form of background selection. The resulting impaired efficiency of selection for robust genetic architecture indirectly decreased the fitness of asexual populations by preventing a decrease in U_d. At low levels of structure, Hill-Robertson interference manifests solely through impaired evolution of mutational robustness, as demonstrated by an equilibrium mean fitness that meets the expectation under mutation load ($\widehat{W} = e^{-\overline{U}_d}$). However, at intermediate and high levels of structure, equilibrium mean fitness falls below that expectation ($\widehat{W} \ll e^{-\overline{U}_d}$), demonstrating a direct impact of Hill-Robertson interference in the form of Muller's ratchet (Figure 18).

Although sexual populations exhibited an equilibrium fitness advantage, this long-term fitness advantage may not ensure the maintenance of sex against invasion by a mutant asexual lineage if asexual reproduction has a large short-term benefit. Given a short-term benefit to asexuality, sex can be maintained only if T_{fix} is large enough for asexual invaders to lose the short-term advantage before they go to fixation. To determine the combined impact of the cost of sex and of population structure on the maintenance of sex, we conducted invasion experiments in which a single individual within an equilibrium sexual population received a mutation that caused it to begin reproducing asexually, after Hartfield et al. (2010, 2012). We monitored the fate of the asexual mutant until it either reached fixation or was lost from the population. We recorded the fixation probability of asexual mutants (u) relative to that of a neutral mutation ($u* = 1/N$). We monitored invasions of populations subdivided into demes of size $N_d = 100$ and all combinations of the following migration rates ($m = 2e-4, 2e-3, 2e-2$) and costs of sex $C = 1, 1.05, 1.1, 1.15, 1.2$).

In Fig. 19, we show the effect of population structure on these relative fixation probabilities (u/u^*). When sex has no cost, any level of structure allowed populations to resist asexual invasion. Within an intermediate range of costs, increased structure supported the maintenance of sex, but the benefit of structure was lost at costs exceeding that range. The maximum cost at which sexual populations resisted asexual invasion at greater than neutral expectation (C_{max}) rose with population structure. At the highest level of structure, C_{max} was $\hat{W}_{sex}/\hat{W}_{asex}$ were similar (Figure 20).

Our results so far match the prediction that the genetic drift induced by population structure increases $\hat{W}_{sex}/\hat{W}_{asex}$ and aids the maintenance of costly sex. In previous investigations of panmictic populations, we found T_{fix} to be the primary determinant of a sexual population's ability to resist invasion. In order to understand how the impact of population structure on $\hat{W}_{sex}/\hat{W}_{asex}$ and T_{fix} interacts with the evolutionary dynamics of the asexual invaders, we investigated the timecourse and fitness dynamics of simulations in which asexual mutants successfully invaded (Figures 21 and 22). Asexual modifiers arising in sexual

backgrounds experienced an immediate fitness benefit because they inherited the beneficial genetic architecture of their sexual predecessor, but no longer experienced L_R. The genetic architecture decayed over the course of the invasion, until the fitness of the asexual lineage reached the asexual equilibrium. As expected, the conditions that favor the maintenance of sex, low costs of sex (C; Figure 21) and low migration rate (m; Figure 22), both produced higher T_{fix}. Asexual invasions were more likely at high C and m, conditions in which fixations occurred more quickly than the decay of the initial advantage (Figure 22).

By increasing both T_{fix} and $\hat{W}_{sex}/\hat{W}_{asex}$, structure increased the probability of the maintenance of costly sex in two ways. The increase in $\hat{W}_{sex}/\hat{W}_{asex}$ increased the maximum maintainable cost that could be imposed on the sexual population without entirely negating the sexual equilibrium advantage. Second, given $\hat{W}_{sex} + C > \hat{W}_{asex}$, the increase in T_{fix} decreased the probability of invasion by allowing sufficient time for the genetic architecture of the asexual lineage to degrade to its equilibrium state. With increasing structure, C_{max} approaches $\hat{W}_{sex}/\hat{W}_{asex}$ (Figure 20).

DISCUSSION

Here we conducted evolutionary simulations in an artificial gene network in order to examine the contribution of population structure to the maintenance of sexual reproduction in the context of an evolvable genetic architecture. Consistent with prior investigations, we found that incorporation of structure to a population increased the probability of the maintenance of costly sex (Peck et al. (1999); Salathé (2006); Hartfield et al. (2012)). These studies identified structure's contribution to T_{fix} to be the principle determinant of the success of sex and of the cost that could be sustained. While we also demonstrate that T_{fix} increased the likelihood of the maintenance of sex, we found the maximum supported cost of sex to be limited by $\hat{W}_{sex}/\hat{W}_{asex}$.

At equilibrium, sexual populations maintained a fitness advantage over asexual populations, resulting in a stable $\hat{W}_{sex}/\hat{W}_{asex} > 1$ for each parameter set. Though Hill-Robertson interference decreased the fitness of all asexual populations, with intermediate and highly

structured populations suffering an additional penalty from Muller's ratchet, the impact was ultimately limited by the evolving genetic architecture. As fitness declined, the frequency of compensatory mutations increased, halting Muller's ratchet in favor of a long-term fitness equilibrium which is consistent with previous investigations, both theoretical (Poon and Otto, 2000) and empirical (Silander et al., 2007).

The genetic interactions that limited the operation of Muller's ratchet were responsible for our finding that even the most structured sexual populations were able to support only a modest cost of sex (C). When Muller's ratchet is allowed to run unchecked, asexual populations undergo mutational meltdown and W_{sex}/W_{asex} continuously increase over time. Extending T_{fix} indefinitely has the potential to extensively increase the cost that can be tolerated and the apparent benefit of sex. However, because $\hat{W}_{sex}/\hat{W}_{asex}$ was stable at equilibrium in the gene network model, extending T_{fix} increased the success of sex only while T_{fix} was less than the time it takes for asexual genetic architecture to degrade to its equilibrium state. Because sex will never have a fitness advantage exceeding its equilibrium advantage, adding a cost which fully negates the equilibrium advantage (that is, if $\hat{W}_{sex}/\hat{W}_{asex} < C$) guarantees a perpetual fitness advantage to asexual reproduction. As a result, the maximum cost at which sex can maintain an advantage in a population is similar to the $\hat{W}_{sex}/\hat{W}_{asex}$ in that population.

Though the limits of computational tractability prevented exploration of the parameter space in which previous investigations found the greatest benefit to sex, we predict that incorporating those conditions, namely a larger N subdivided into more demes, each with a larger N_d, would have had little qualitative effect on our results. Consider two populations of size N but with different structures, the first of which has few demes with moderate N_d but a very low m, and the second having many demes with a very low N_d but a moderate m. Though they may have the same F_{ST}, the latter could be predicted to support a higher cost because pronounced Hill-Robertson interference in small demes will maximize $\hat{W}_{sex}/\hat{W}_{asex}$, and T_{fix} must only be sufficient for the invading lineage's genetic architecture to be degraded

to its equilibrium state. Though the T_{fix} of the former may be substantial, the moderate N_d and low genetic drift will result in a smaller $\hat{W}_{sex}/\hat{W}_{asex}$. Because T_{fix} and $\hat{W}_{sex}/\hat{W}_{asex}$ contribute differently towards the maintenance of sex, C_{max} is not a straightforward function of F_{ST}. While further exploration of structure that results in the combination of low N_d and low m is likely to identify a range that supports a quantitatively higher costs of sex, this comes at the expense of an increasingly unrealistic, narrow parameter space. Therefore, though Hill-Robertson interference results in an advantage to sex, it cannot explain the success of costly, frequent sex.

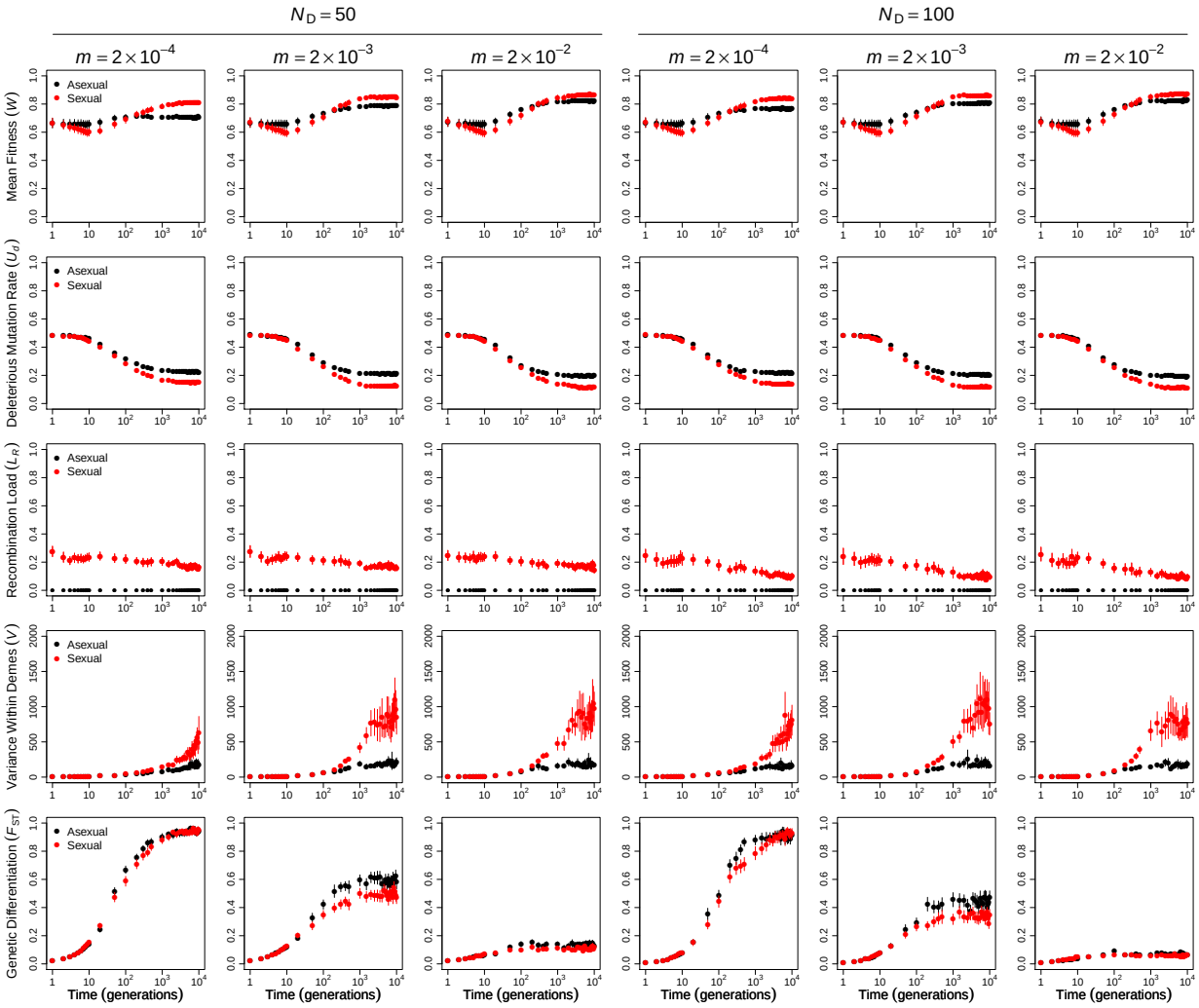

Figure 15: Evolution of genetic architecture gives sex an equilibrium advantage. Changes in mean fitness ($\overline{W}$), deleterious mutation rate (U_d), recombination load (L_R), within-deme neutral variance (V), and F_{ST} over time in asexual (black) and sexual (red) populations of migration rates (m) and deme sizes (N_d). Values are means and 95% confidence intervals based on 50 replicate populations initiated from different randomly chosen founders.

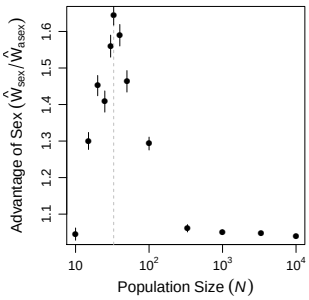

Figure 16: Operation of Muller's ratchet maximizes the equilibrium advantage of sex in intermediate N. Equilibrium sexual advantage is the ratio of mean equilibrium sexual fitness over mean equilibrium asexual fitness at generation 10^4, after populations of all sizes had achieved equilibrium in all properties. Values are means of 50 replicate populations initiated from different randomly chosen founders.

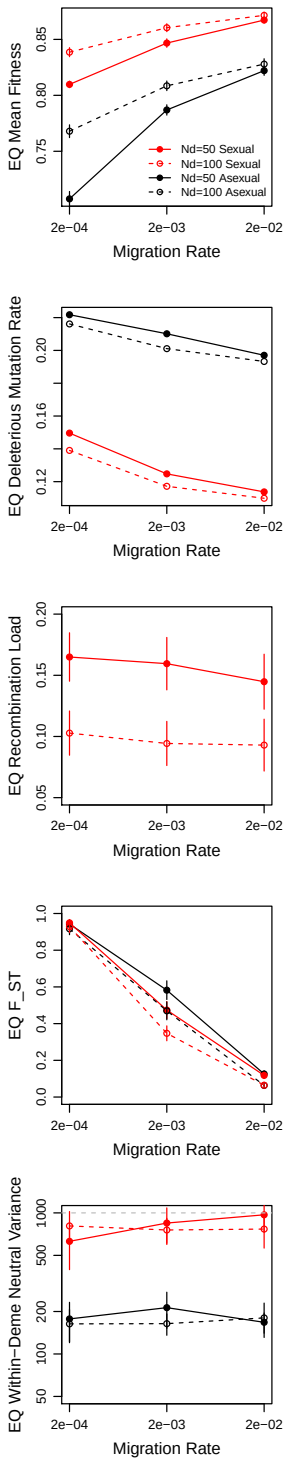

Figure 17: Sex has an equilibrium advantage. Means at generation 10^4, after populations of all sizes had achieved equilibrium in all properties. Equilibrium values of within-deme neutral variance are also shown. Values are means and 95% confidence intervals based on 50 replicate populations initiated from different randomly chosen founders.

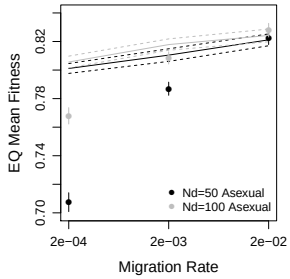

Figure 18: Equilibrium mean fitness of asexual populations shows the effects of Muller's ratchet and mutation load. The population equilibrium mean fitness matched the expectation at mutation-selection balance only at the highest migration rate. Values are means and 95% confidence intervals of the observed fitness in asexual (populations after 10^4 generations of evolution. Solid lines show the expectation under the mutation load equation and dashed lines show 95% confidence intervals calculated from the observed U_d in each population.

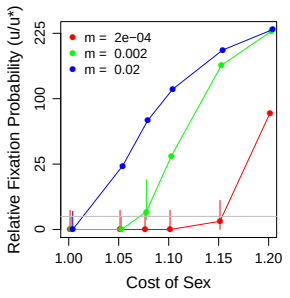

Figure 19: Population structure supports maintenance of costly sex. Asexual mutants were introduced into equilibrium sexual at an initial frequency of $1/N$. Frequencies of the modifier mutations were monitored until the modifiers were either fixed or lost. Values are the relative fixation probability of the modifier mutation, given as proportion of fixations (u) divided by the neutral expectation ($u^* = 1/N$) and 95% confidence intervals.

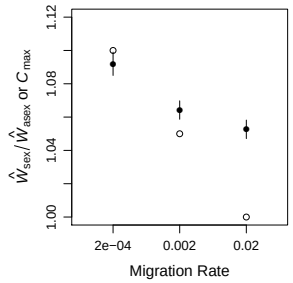

Figure 20: Sexual fitness advantage and maximum sustained cost increase with structure. Asexual mutants were introduced into equilibrium sexual at an initial frequency of $1/N$. Frequencies of the modifier mutations were monitored until the modifiers were either fixed or lost. Values are equilibrium sexual fitness advantage ($\hat{W}_{sex}/\hat{W}_{asex}$, closed circles) and the maximum cost of sex at which the fixation rate of an asexual mutation is lower than the neutral expectation (C_{max}, open circles) and 95% confidence intervals based on invasion trials for each population size N.

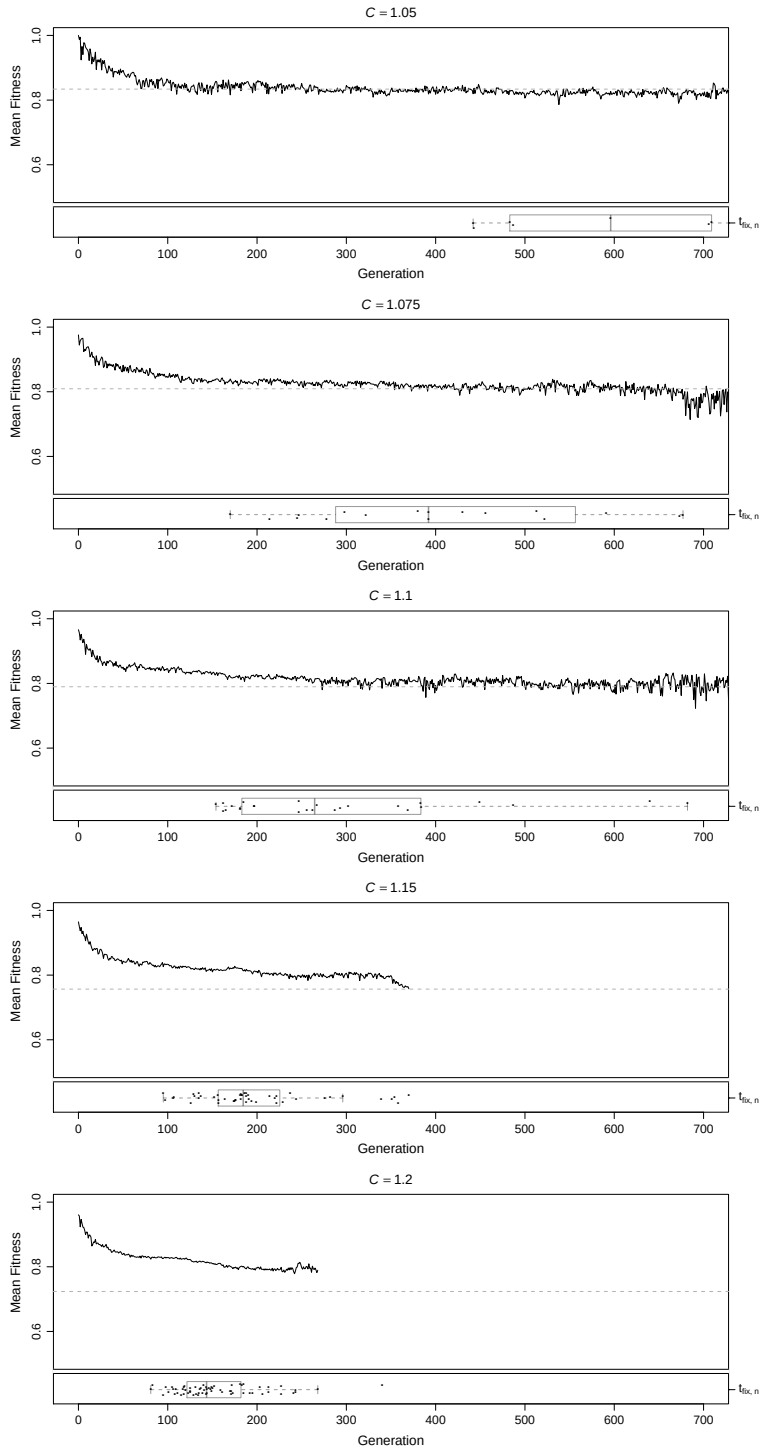

Figure 21: Evolving genetic architecture influences the success of costly sex. We monitored the fixation and loss of asexual mutants introduced into equilibrium sexual populations with $m = 0.02$ between demes at several costs of sex. Lines show the evolution of mean fitness among invading mutants. The equilibrium mean fitness of the populations being invaded is represented by a gray dashed line across each plot. Points and corresponding boxplots shown at the bottom of each plot indicate the time of fixation for individual mutations.

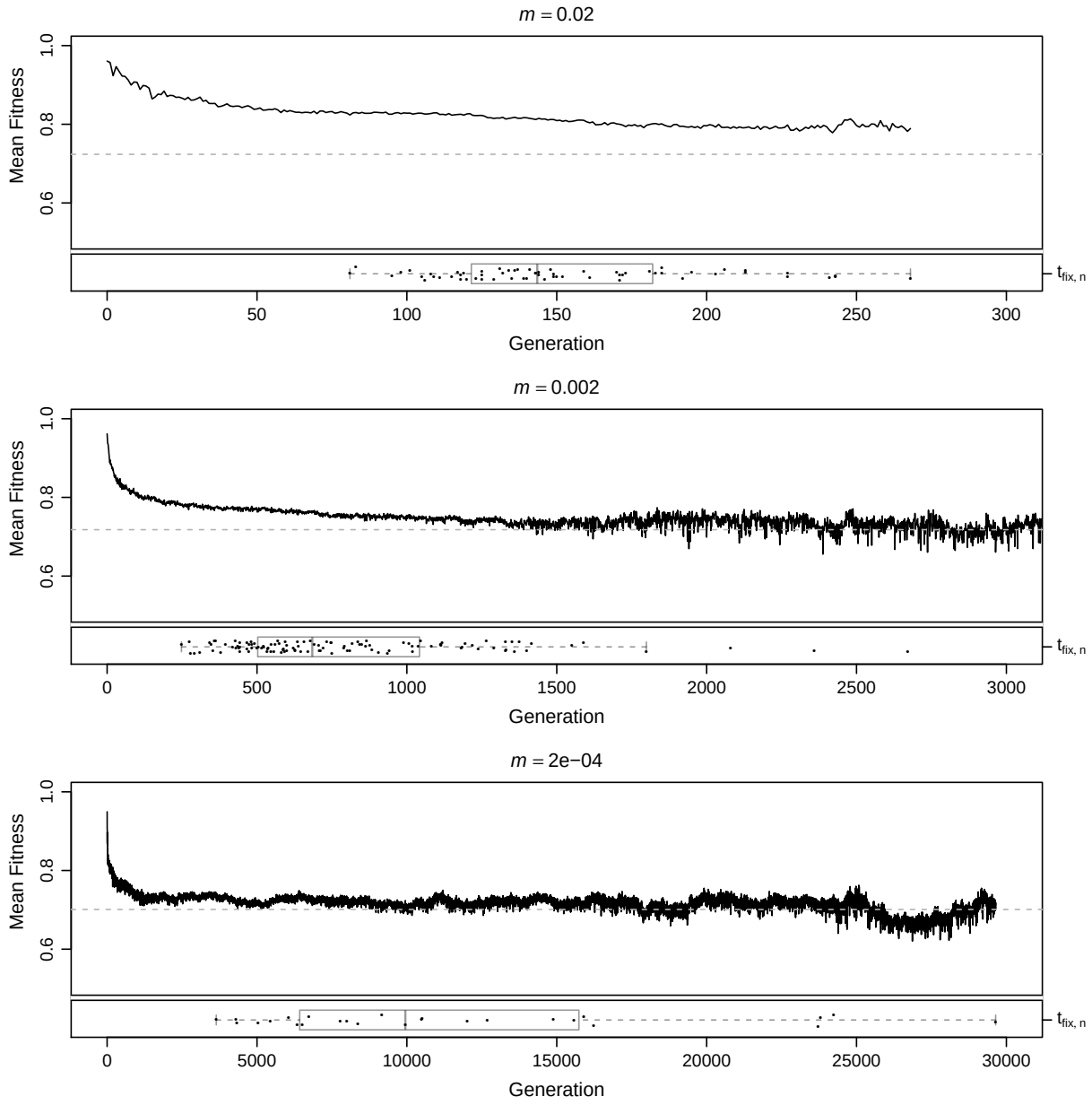

Figure 22: Population structure slows transit time and increases the maintenance of costly sex. We monitored the fixation and loss of asexual mutants introduced into equilibrium sexual populations that carried a cost of sex of 1.2 at each of three migration rates. Lines show the evolution of mean fitness among invading mutants. The equilibrium mean fitness of the populations being invaded is represented by a gray dashed line across each plot. Points and corresponding boxplots shown at the bottom of each plot indicate the time of fixation for individual mutations. Note different scales of time axis.

CHAPTER 4: CONCLUSIONS

Though there is no universally accepted explanation for the ubiquity or benefits of sexual reproduction, Hill-Robertson interference, a drift-based hypothesis in which efficiency of selection at one locus is impeded by selection at linked loci (Hill and Robertson, 1966; Felsenstein, 1974; Comeron et al., 2008), is especially promising because it is thought to both operate broadly and provide strong benefits to sex (Otto and Barton, 2001; Iles et al., 2003; Barton and Otto, 2005; Keightley and Otto, 2006; Comeron et al., 2008; Gordo and Campos, 2008; Hartfield et al., 2010, 2012). Without recombination, the negative linkage disequilibrium that accumulates through a combination of drift, mutation and selection impairs selection (Maynard Smith and Haigh, 1974; Charlesworth et al., 1993; Charlesworth, 1994; Peck, 1994), potentially limiting the evolvability of large populations (Fisher, 1930; Muller, 1932; Gerrish and Lenski, 1998) or decreasing the fitness of small populations (Muller, 1964; Haigh, 1978; Gordo and Charlesworth, 2000). The additive genetic variance revealed by recombination restores selection's ability to purge deleterious mutations and fix beneficial mutations. Computational simulations and theoretical investigations of Hill-Robertson interference have had promising results, showing a clear benefit to sex that increased with population size and structure (Otto and Barton, 2001; Iles et al., 2003; Barton and Otto, 2005; Keightley and Otto, 2006; Gordo and Campos, 2008; Hartfield et al., 2010, 2012), with some finding benefits substantial enough to account for the elusive twofold cost of sex. However, these models did not incorporate genetic interactions or evolution of genetic architecture, a choice with potential for both disadvantages and advantages to sex. While sex may select for genetic architecture that supports its own maintenance (Azevedo et al., 2006; Misevic et al., 2006; Livnat et al., 2008; Lohaus et al., 2010), omission of genetic interactions also allows sex to avoid the profound cost of recombination load (Bell, 1982). Furthermore,

because evolution of genetic architecture can limit the deleterious impact of Hill-Robertson interference in asexuals (Poon and Otto, 2000; Silander et al., 2007), simulations without genetic interactions have the potential to overstate the benefit of sex. Therefore, the impact of evolving genetic architecture on the effects of sex is difficult to predict.

To address how interactions between Hill-Robertson interference and evolving genetic architecture contribute to the evolution of sex, both its long-term benefit as well as its origin and maintenance, we simulated evolution of gene networks with parameters that ensured the operation of all forms of Hill-Robertson interference. Consistent with (Iles et al., 2003; Keightley and Otto, 2006; Gordo and Campos, 2008; Hartfield et al., 2010, 2012), we found that the destruction of Hill-Robertson interference generated an advantage to sex that increased with population size, a surprising finding, as neither Muller's ratchet (Muller, 1964; Haigh, 1978; Gordo and Charlesworth, 2000) nor background selection (Hudson and Kaplan, 1994, 1995) are expected to be stronger in large populations. However, we found that even the largest populations ($N = 10^4$) were unable to support even a 1% cost of sex. From there, we quantified the contributions of Hill-Robertson interference and evolution of genetic architecture to the evolution of sex.

All populations reached mutation-selection-drift equilibrium, resulting in a stable equilibrium advantage to sex ($\hat{W}_{sex}/\hat{W}_{asex} > 1$). We identified three mechanisms that contributed to the advantage of sex, two of which manifested as mutational robustness, an evolved property of genetic architecture. Recombination itself selected for robust genetic architecture (Azevedo et al., 2006; Misevic et al., 2006; Gardner and Kalinka, 2006; Martin and Wagner, 2009; Lohaus et al., 2010), while background selection in asexual populations interfered with selection for robust genetic architecture. As a result, selection for robust genetic architecture was both stronger and more efficient in the sexual population. The lower mutation load caused by robust genetic architecture contributed to the sexual equilibrium fitness advantage under all conditions and was entirely responsible for the equilibrium advantage in intermediate-to-large ($10^3 \leq N \leq 10^4$) panmictic populations.

The third mechanism contributing to the advantage of sex was the direct impact of Muller's ratchet on asexual populations, observed only in populations small enough for substantial genetic drift. As a result, small asexual populations ($N \leq 10^2$) suffered fixation of directly deleterious mutations in addition to the genetic architecture-mediated fitness impact of an elevated mutation load. However, despite Muller's ratchet, even the smallest asexual populations ($N = 10$) reached a fitness equilibrium because evolution of the distribution of mutational effects resulted in a rate of compensatory mutations that was inversely related to population fitness. Fitness-determined rates of compensatory interactions limited the impact of Muller's Ratchet, an observation which is consistent with other investigations, both theoretical (Poon and Otto, 2000) and empirical (Silander et al., 2007).

These genetic architecture dynamics are the key difference between our findings and previous work in models lacking compensatory genetic interactions (Keightley and Otto, 2006; Hartfield et al., 2010, 2012). Without compensatory genetic interactions, populations do not reach a fitness equilibrium. Instead, the continual operation of Muller's ratchet, regardless of N, ensured a perpetual fitness decline that was accelerated by asexual reproduction. The result was a dynamic, perpetually rising $\bar{W}_{sex}/\bar{W}_{asex}$. In the absence of evolving genetic interactions, the only mechanism generating the long-term benefit to sex was the operation of Muller's ratchet, and the magnitude of the long-term benefit of sexual reproduction was determined by length of invasion alone.

We also found the principle determinant of the success of the origin or maintenance of sex to be transit time of new mutation to fixation (T_{fix}); because all sexual populations had an equilibrium advantage, to be successful, sexual lineages had only to persist in the population long enough for the equilibrium fitness relationship to be restored. This feat was only possible with $N = 10^4$ but the minimal $\hat{W}_{sex}/\hat{W}_{asex}$ was insufficient for sex to originate or be maintained with any cost. Though populations of intermediate size ($10 < N < 100$) generated the maximum equilibrium sexual fitness advantage, sex was not able to originate or be maintained in this size range because the time required to restore the condition of

$\bar{W}_{sex} > \bar{W}_{asex}$ was longer than T_{fix}.

The potential to optimize fitness and time in the same population provided motivation for the addition of population structure. In addition to increasing T_{fix}, the substantial drift induced by population structure predicts activity of Muller's ratchet even in a large population ($N = 10^3$). Manipulating the level of population structure allowed us to distinguish the individual impacts of $\hat{W}_{sex}/\hat{W}_{asex}$ and T_{fix} to the maintenance of costly sex. As in panmictic populations, the interaction of T_{fix} with the evolutionary dynamics of the invaders was the primary determinant of the *probability* of fixation. However, we found that the maximum permissible *cost* of sex was determined by $\hat{W}_{sex}/\hat{W}_{asex}$. The impact of T_{fix} can be further demonstrated through comparison of asexual invasions into sexual populations (without cost) of $N = 10^3$, one of which was panmictic and the other subdivided into 10 demes with $m = 0.02$. They had similar $\hat{W}_{sex}/\hat{W}_{asex}$. Yet, asexuals readily invaded sexual panmictic populations, but never invaded sexual structured populations.

Ultimately, $\hat{W}_{sex}/\hat{W}_{asex}$ set an upper limit for the maintainable cost of sex (C_{max}). Though addition of cost decreased $\hat{W}_{sex}$, the rising T_{fix} induced by population structure allowed time for $\bar{W}_{asex}$ to approach $\hat{W}_{sex}$ until the condition of $\bar{W}_{asex} < \hat{W}_{sex}$ was met. $\hat{W}_{sex}/\hat{W}_{asex}$ declined as the cost of sex rose. When cost caused $\hat{W}_{sex}/\hat{W}_{asex} < 1$, no amount of population structure could prevent asexual invasion. Therefore, high levels of structure increased the maintenance of costly sex, but the static equilibrium fitness values limited the maximum cost to a value indistinguishable from the $\hat{W}_{sex}/\hat{W}_{asex}$.

Manipulation of population structure that increases genetic drift, such as smaller N_d, might be expected to increase $\hat{W}_{sex}/\hat{W}_{asex}$ and support a higher C_{max} than observed here. However, we observed that a little migration goes a long way towards decreasing the impact of drift on the equilibrium mean fitness of asexual populations. The greatest $\hat{W}_{sex}/\hat{W}_{asex}$ we observed was 1.1, in a population with $N_d = 50$ and $m = 2 \times 10^{-4}$, but panmictic populations of $N = 50$ (equivalent to a population with $N_d = 50$ and $m = 0$) had $\hat{W}_{sex}/\hat{W}_{asex} = 1.6$. In addition, results from panmictic populations indicate a floor to the drift-induced fitness

benefit. At $N = 10$, asexual and sexual populations suffered alike, evolving to the same $\hat{W}$. Additionally, attempting to increase C_{max} through manipulation of population structure requires further departure from biologically plausible conditions. We found $C_{max} = 1.1$ at $F_{ST} = 0.9$, a value exceeding comparisons of species divided by continents (reviewed in Hartfield et al., 2012). Because C_{max} is not a straightforward function of F_{ST}, population parameters could likely be found that maintain a substantial $\hat{W}_{sex}/\hat{W}_{asex}$ at a lower F_{ST} while maintaining a substantial $\hat{W}_{sex}/\hat{W}_{asex}$, but it comes at the cost of an increasingly stringent parameter space and is unlikely to change the qualitative outcome.

FUTURE DIRECTIONS

While these results confirm that the interaction of Hill-Robertson effects and an evolving genetic architecture results in an equilibrium advantage for sex, the maximum sustainable cost we found was modest compared to costs predicted in nature, and the most obvious route to a quantitative increase in supported cost comes with the detriment of an increasingly stringent parameter space. Therefore, I propose that future work introduce directional selection for a shifting optimum, replacing the static $\hat{W}_{sex}/\hat{W}_{asex}$ with a dynamic $\bar{W}_{sex}/\bar{W}_{asex}$.

Ample experimental evidence has demonstrated that sexual populations are able to adapt more quickly than asexual populations following an environmental shift (de Visser et al., 1999; Colegrave et al., 2002; Kaltz and Bell, 2002; Bachtrog and Gordo, 2004; Poon, 2004; Goddard et al., 2005; Misevic et al., 2010; Becks and Agrawal, 2012; Barbuti et al., 2012; Park and Krug, 2013; McDonald et al., 2016), and current research has demonstrated that both Hill-Robertson interference and evolution of genetic architecture drive this observation. However, computational investigations of the benefits of sex in adapting populations have been lacking; investigations which have incorporated Hill-Robertson interference have not incorporated evolution of genetic architecture (Otto and Barton, 1997; Martin et al., 2006; Charlesworth et al., 2009; Hodgson and Otto, 2012), and vice versa (Misevic et al., 2006; Draghi and Wagner, Feb; Livnat et al., 2008; Draghi et al., 2010; Livnat et al., 2010). Our previous

results demonstrated that neither of these factors can be overlooked as both contribute the benefit of sex.

In our work under stabilizing selection, only small populations experienced direct effects of Hill-Robertson interference, but a regime of periodic optimum shifts is expected to increase Hill-Robertson interference at all population sizes. Following an optimum shift, while $\bar{W}$ is approaching the theoretical new optimum, i.e. the lag load phase (Maynard Smith, 1976), the adapting populations enjoy a higher rate of beneficial mutations, most of which will occur in different individuals within the population. Sexual populations are poised to incorporate the new mutations through recombination, but in an asexual population, lineages containing beneficial mutations will compete against one another and some beneficial mutations will inevitably be lost due to Fisher-Muller interference (Fisher, 1930; Muller, 1932; Gerrish and Lenski, 1998; Cooper, 2007; Green and Mason, 2013). As a result, asexual populations must fix beneficial mutations sequentially, causing them to adapt more slowly than would be expected from their beneficial mutation supply (de Visser et al., 1999; Colegrave et al., 2002). Fisher-Muller interference is the primary manifestation of Hill-Robertson interference in adapting asexual populations, but fixation of beneficial mutations also offers an opportunity for fixation of linked deleterious mutations and loss of V (Maynard Smith and Haigh, 1974; Maynard Smith, 1978; Peck, 1994), especially if selective sweeps are frequent (Kaplan et al., 1989).

Under stabilizing selection, the effect of Hill-Robertson interference on the evolution of genetic architecture manifested as lower mutational robustness and higher U_d at equilibrium (Figures 1 and 17). Under directional selection for a shifting optimum, deleterious effects of Hill-Robertson interference experienced during adaptation may linger in the evolved properties of the genetic architecture even after the population has achieved its theoretical target. Following an optimum shift, both sexual and asexual populations will experience selection for mutations that produce the new optimum phenotype, but selection to maintain beneficial genetic architecture is also ongoing. Fisher-Muller interference in asexual populations may

force a compromise between the short-term benefit of a mutation that produces the new fitness optimum versus the long-term benefit of a mutation that promotes beneficial genetic architecture. While the former will shorten the time until the new optimum is achieved, the latter may dampen fitness and evolvability long-term. Because robustness promotes adaptability (Wagner and Altenberg, 1996; Schroeder et al., 2004; Wagner, 2008; Draghi et al., 2010), suboptimal genetic architecture may further impede future adaptation.

For genetic architecture in an unstable environment to be beneficial, it must be evolvable. Evolvability itself is an evolved property of genetic architecture (Lipson et al., 2002; Meyers et al., 2005; Draghi and Wagner, Feb; Steiner, 2012). In our experiments to date, selection was primarily for mutational robustness: fitness in large populations was entirely attributable to U_d (and L_R in sexual populations). As a result, we have so far only defined robustness in terms of the genome, as defined by the size of the neutral network of each genome, and indirectly measured by the distribution of mutational effects (Wagner et al., 2008). Robustness may additionally be defined as a property of the phenotype, given as the number of genotypes that produce each phenotype. While the former predicts low evolvabilty, the latter predicts high evolvability (Meyers et al., 2005; Wagner et al., 2008). Because stabilizing selection selects only for genetic robustness while directional selection selects for both, only directional selection causes evolution of evolvability (Lipson et al., 2002; Meyers et al., 2005; Draghi and Wagner, Feb; Steiner, 2012; Melo and Marroig, 2015).

Because asexual reproduction is expected to slow adaptation to a shifting optimum as well as impede selection for beneficial genetic architecture, we predict directional selection will promote both long-term and short-term benefits of sex. In a major departure from evolution under stabilizing selection, the direct impact of Hill-Robertson interference may rise with N as the rising NU_b increases the potential for Fisher-Muller interference. Because Hill-Robertson interference is also expected to impair the evolution of beneficial genetic architecture, optimum shifts that occur with a period that is shorter than the time required for populations to reach equilibrium may impact the asexual population in two ways. First,

direct effects of Hill-Robertson interference will cause the asexual population to spend a greater proportion of time in the lag load phase. Second, the indirect effects may decrease the evolvability of the genetic architecture, resulting in lag load phases that increase in length over time.

In order to differentiate the impact of Hill-Robertson interference from selection to minimize L_R on evolved properties of genetic architecture, as well as to understand how and to what degree mutational robustness affects adaptability, it is necessary to further characterize the evolved properties of genetic architecture. We have not yet investigated the mechanism of the evolution of robustness, but sex has been previously demonstrated to select for a modular genome (Misevic et al., 2006; Livnat et al., 2008; Draghi and Wagner, Feb; Martin and Wagner, 2009; Livnat et al., 2010). In intriguing work in the gene network model, Martin and Wagner demonstrated that mutational robustness evolved in both sexual and asexual populations, but genome modularity only evolved in sexual populations 2009. Modularity was characterized by regulatory values biased towards extreme values. In the absence of sex, no such pattern was seen; the distribution of regulatory effects was similar and centered at zero in asexual evolved networks, unevolved networks, and unstable networks. The strength and distribution of regulatory values is a promising metric to test in our work because it has the potential to explain differences in the mechanism of robustness between sexual and asexual populations. Namely, selection for recombinational robustness in sexual populations also increases mutational robustness, but selection for mutational robustness in asexual populations does not confer recombinational robustness, and is additionally less robust to mutation. Both observations could be explained if recombination selects for networks in which fewer "correct" regulatory values are required to maintain a gene expression state.

Evolution in an unstable environment is expected to have broad qualitative differences from evolution under stabilizing selection. In what may be the largest departure from our previous work, sex has the potential for a substantial short-term fitness benefit in addition to an equilibrium benefit. If asexual adaptation is significantly impaired, lag load may

result in substantial, transient values of $\bar{W}_{sex}/\bar{W}_{asex}$, which have the potential to exceed $\hat{W}_{sex}/\hat{W}_{asex}$. In a second major departure, we expect Hill-Robertson interference to have a direct fitness impact at all population sizes and structures. Finally, sexual populations may have a long-term benefit during periods of quasi-equilibrium between optimum shifts, and, if asexual populations are less evolvable, this benefit may increase over repeated rounds of adaptation.

These differences are likely to change the evolutionary dynamics of the interaction between cost of sex and time. The predicted short-term benefit of sex may permit the origin of costly sex; while the immediate assumption of L_R prevented the origin of costly sex in a population at equilibrium, destruction of previously-fit linkage disequilibrium will be beneficial, or at least less deleterious, during adaptation. Additionally, as we demonstrated in Chapter 3, T_{fix} and $\hat{W}_{sex}/\hat{W}_{asex}$ determined different aspects of the maintenance of costly sex, with the minimum feasible T_{fix} increasing with cost. This relationship may be relaxed by a dynamic $\bar{W}_{sex}/\bar{W}_{asex}$; if the short-term benefit of sex periodically exceeds its long-term benefit, a short T_{fix} may periodically be permissible. What remains to be seen is if the combined impact of evolution of genetic architecture and Hill-Robertson interference can generate an advantage to sex sufficient to sustain its costs, and if they can do so within a realistic parameter range.

APPENDIX 1: CHAPTER 2 SUPPLEMENT

Reproductive mode

We explored the sensitivity of our results to the particular implementation of sex by investigating the origin and maintenance of sex using three different genetic bases for reproductive mode|Separate Sex, Recessive Sex, and Dominant Sex (see Materials and Methods, Reproductive mode). Under all implementations, if a population is fixed for the m allele, every individual reproduces asexually, and if it is fixed for the M allele every individual reproduces sexually. Thus, the genetic basis of reproductive mode can impact evolutionary dynamics only during the time when populations are polymorphic for the modifier allele, i.e. during invasions by sexual or asexual mutants.

In Figure 11, we show the effect of population size on the relative fixation probabilities (u/u^*) for the Recessive Sex and Dominant Sex implementations of reproductive mode. The **Recessive Sex** results were qualitatively identical to the Separate Sex results reported in the main text (Figure 4). At small population sizes, asexual modifiers invaded more often than sexual modifiers, but at the largest population sizes we saw the opposite pattern. Sexual modifiers invaded more often than asexual modifiers only in populations larger than 10^3 individuals (Figure 11A). In the largest populations we tested ($N = 10^4$) sexual mutants fixed in asexual populations more often than the neutral expectation ($u/u^* = 2.13$, $n = 5.5 \times 10^5$, $p < 0.0001$ by an exact binomial test). The reasons for the success of Recessive Sex in large populations were identical to those for Separate Sex discussed in the main text. In particular, successful sexual modifier mutations arose in high fitness genetic backgrounds, remained linked to the background on which they arose, and quickly hitchhiked to a relatively high frequency as a result. This early high frequency was critical to the ability of these modifier mutations to persist for the long time required for sexuals to evolve an advantageous genetic architecture (i.e., reductions in U_d and L_R).

Results using the **Dominant Sex** implementation (Figure 11B) differed dramatically from the Separate and Recessive Sex implementations of reproductive mode. In populations of more

than 100 individuals, asexual mutants readily invaded sexual populations, whereas sexual mutants rarely if ever invaded asexual populations. Several phenomena likely contributed to this outcome. First, under Dominant Sex, sexual modifier mutations do not remain linked to the genetic background on which they arise. As a result, sexual modifiers that arose on high fitness backgrounds did not quickly hitchhike to a high frequency, unlike the Separate and Recessive Sex modifiers. Second, a dominant sexual modifier is initially rare and, consequently, sexual M genotypes reproduce by recombining with asexual m genotypes in the generations immediately after they arise. Recombination load remains high in this case because gene flow from the asexuals counters the action of selection to minimize it.

Tables

Table 1: Analysis of population mean fitness data from first 10 generations[a].

Parameter	Estimate	s.e.	t-value	p-value[e]
(Intercept)	0.665	0.0177	37.6	< 0.001
log(time)[b]	-0.0111	0.0054	-2.05	0.0409
Sex[c]	-0.0010	0.0110	-0.094	0.925
$\log(N)$[d]	0.0009	0.0012	0.685	0.493
log(time) $\times$ Sex	-0.0094	0.0077	-1.22	0.223
log(time) $\times \log(N)$	0.0010	0.0009	1.09	0.277
Sex $\times \log(N)$	0.0011	0.0018	0.604	0.546
log(time) $\times$ Sex $\times \log(N)$	-0.0028	0.0012	-2.28	0.0224

[a] Linear mixed-effect model: $\overline{W} \sim$ log(time) * sex * $\log(N)$ + random(founder)

[b] Time in generations was modeled as a continuous variable.

[c] Sex = reproductive mode, sexual or asexual.

[d] Population size, N, was modeled as a continuous variable.

[e] p-values based on 3443 degrees of freedom for all parameters.

Table 2: Analysis of population mean fitness at generation 10,000[a].

Parameter	Estimate	s.e.	t-value	p-value[b]	
(Intercept)	0.0634	0.0198	3.19	0.0015	
Sex	0.164	0.0281	5.83	< 0.001	
$\log(N)$	0.0997	0.0032	31.2551	< 0.001	
Sex $\times \log(N)$	-0.0137	0.0045	647	-3.037	0.0025

[a] linear mixed-effect model: $\overline{W} \sim$ sex * $\log(N)$ + random(founder) [b] p-values based on 647 degrees of freedom for all parameters.

Table 3: Analysis of the deleterious mutation rate U_d at generation 10,000[a].

Parameter	Estimate	s.e.	df	t-value	p-value[b]
(Intercept)	0.277	0.0041	68.0	< 0.001	
$\log(N)$	-0.0117	0.0006	-18.1	< 0.001	
Sex	-0.0700	0.0057	-12.3	< 0.001	
$\log(N) \times$ Sex	-0.0008	0.0009	-0.840	0.401	

[a] linear mixed-effect model: $U_d \sim \log(N)$ * sex + random(founder) *b* p-values based on 647 degrees of freedom for all parameters.

Table 4: Analysis of neutral variation at generation 10,000[a].

Parameter	Estimate	s.e.	df	t-value	p-value[b]
(Intercept)	-5.34	1.78	-3.01	0.002can8	
$\log(N)$	2.69	0.286	9.42	< 0.001	
Sex	-26.6	2.52	-10.6	< 0.001	
$\log(N) \times$ Sex	7.42	0.404	18.4	< 0.001	

[a] linear mixed-effect model: $V \sim \log(N)$ * sex + random(founder), where V is the equilibrium between-individual variance at the neutral locus for each replicate population.
b p-values based on 647 degrees of freedom for all parameters.

Table 5: Analysis of U_d at generation 10,000[a]

	Value	Std.Error	t-value	p-value[b]
(Intercept)	0.1800	0.0096	18.8396	0.0000
log(Migration_Rate)	-0.0058	0.0015	-3.9281	0.0001
Sex_Locus	-0.1006	0.0135	-7.4605	0.0000
Deme_Size	-0.0001	0.0001	-0.6076	0.5437
log(Migration_Rate):Sex_Locus	-0.0035	0.0021	-1.6674	0.0960
log(Migration_Rate):Deme_Size	0.0000	0.0000	0.4313	0.6664
Sex_Locus:Deme_Size	0.0001	0.0002	0.6242	0.5327
log(Migration_Rate):Sex_Locus:Deme_Size	0.0000	0.0000	0.7984	0.4250

[a] Linear mixed-effect model: $U_d \sim$ log(time) * sex * N_d * m + random(founder)

[b] p-values based on 543 degrees of freedom for all parameters.

Table 6: Analysis of population mean fitness at generation 10,000[a]

	Value	Std.Error	t-value	p-value[b]
(Intercept)	0.9713	0.0105	92.2493	0.0000
log(Migration_Rate)	0.0368	0.0016	22.6664	0.0000
Sex_Locus	-0.0348	0.0149	-2.3401	0.0196
Deme_Size	-0.0009	0.0001	-6.6746	0.0000
log(Migration_Rate):Sex_Locus	-0.0189	0.0023	-8.2548	0.0000
log(Migration_Rate):Deme_Size	-0.0002	0.0000	-11.5697	0.0000
Sex_Locus:Deme_Size	0.0005	0.0002	2.8523	0.0045
log(Migration_Rate):Sex_Locus:Deme_Size	0.0001	0.0000	4.5008	0.0000

[a] Linear mixed-effect model: $\overline{W} \sim$ log(time) * sex * N_d * m + random(founder)

[b] p-values based on 543 degrees of freedom for all parameters.

Table 7: Analysis of recombination load at generation 10,000[a]

	Value	Std.Error	t-value	p-value[e]
(Intercept)	0.1749	0.0432	4.0507	0.0001
log(Migration_Rate)	-0.0066	0.0066	-1.0016	0.3175
Deme_Size	-0.0009	0.0005	-1.6808	0.0941
log(Migration_Rate):Deme_Size	0.0000	0.0001	0.5376	0.5913

[a] Linear mixed-effect model: $L_R \sim$ log(time) * N_d * m + random(founder)

[b] p-values based on 247 degrees of freedom for all parameters.

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