Population structure promotes the evolution of costly sex in artificial gene networks

Alexander O. B. Whitlock*, **Ricardo B. R. Azevedo**† **and Christina L. Burch**‡,1

*Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, North Carolina, United States of America, †Department of Biology and Biochemistry, University of Houston, Houston, Texas, United States of America, ‡Biology Department, University of North Carolina, Chapel Hill, North Carolina, United States of America

ABSTRACT

Population structure is expected to promote the evolution of sex because it introduces two processes that have a disproportionate negative impact on asexual populations. First, population structure strengthens the influence of Hill-Robertson interference. Second, it increases the time to fixation of invading asexual mutants, allowing more time for the operation of Hill-Robertson interference and, in particular, Muller's Ratchet. Here, we evaluated the extent to which population structure promotes the origin and maintenance of costly sex using an artificial gene network model that explicitly incorporates interactions between genes. Our simulations include recombination and migration loads (costs of sex) and an evolving distribution of mutation effects (a benefit of sex) that were missing from previous studies of the evolution of costly sex. We found that population structure facilitated the origin and maintenance of costly sex in the expected ways. However, the maximum advantage of sex was realized in moderately structured populations. Highly structured populations experienced migration load in the form of Dobzhansky–Muller incompatibilities, decreasing the effective rate of migration between demes and, consequently, accelerating Muller's Ratchet in the *sexual* populations. Our work shows that population structure can facilitate the evolution of costly sex, but it also identifies an important limit on this mechanism—one that relied on the complex genetic architecture of the gene network model.

KEYWORDS Recombination; Hill-Robertson; Genetic architecture; Population structure; Cost of sex

Introduction

Sexual reproduction among eukaryotes is nearly ubiquitous (Bell 1982; Maynard Smith 1978; Vrijenhoek 1998) despite its substantial costs. Foremost among these is the risk that successful, coevolved genetic interactions will be destroyed by recombination. The production of males introduces a further cost, which may approach twofold in anisogamous species (Lehtonen *et al.* 2012; Gibson *et al.* 2017). Mating can also be a costly endeavor. In many species, an individual must expend time and resources to locate a mate, risking disease and predation in the process. The widespread prevalence of sex indicates it must convey extraordinary benefits, but the conditions that allow these benefits to counter its significant costs remain controversial.

One benefit of sex lies in its ability to increase the efficiency of selection by breaking up deleterious genetic associations (Burt 2000; Otto and Lenormand 2002). Such deleterious genetic associations can accumulate in finite populations through the combined activities of selection and drift, producing a phenomenon known as Hill-Robertson interference (Hill and Robertson 1966; Felsenstein 1974; Comeron *et al.* 2008). Hill-Robertson interference impairs evolvability and, in its strongest form, leads to the irreversible accumulation of deleterious mutations via a mechanism known as Muller's Ratchet (Muller 1964; Felsenstein 1974; Haigh 1978). Recombination counters Hill-Robertson interference, generating an advantage of sex. Prior theoretical work on panmictic populations demonstrated that Hill-Robertson interference, particularly in the form of Muller's Ratchet, facilitates the spread of modifiers that increase the recombination rate, that is, the *origin* 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). However, the advantage of sex in panmictic populations was insufficient to allow the evolution of *costly* sex, except when the modifier led to only a small increase in the probability that sex occurs (Keightley and Otto 2006; Hartfield *et al.* 2010). The advantage of sex was stronger in structured populations, where costly sex was able both to evolve (Martin and Lenormand 2006) and to resist invasion by asexual mutants (Peck *et al.* 1999; Salathé *et al.* 2006; Hartfield *et al.* 2012).

The extent to which these findings can be generalized to real organisms, however, is unclear, as most of these models employed a simple genetic architecture, with limited to no interaction between genes (but see Peck *et al.* 1999). This precluded an important cost of sex—recombination load, which results from the disruption of coadapted gene complexes. This distinction proved vital in our previous work that investigated the evolution of sex in an artificial gene network model that explicitly incorporated interactions between genes (Azevedo *et al.* 2006; Lohaus *et al.* 2010; Whitlock *et al.* 2016). In panmictic populations of artificial gene networks, the interactions between genes evolved to produce an advantage for sex, but recombination load constrained both its origin and its maintenance, and prevented costly sex entirely (Whitlock *et al.* 2016).

We now ask whether population structure can facilitate the evolution of costly sex in the artificial gene network model. Population structure is expected to promote the evolution of sex because it introduces two processes that have a disproportionate negative impact on asexual populations. First, population structure strengthens the influence of Hill-Robertson interference (Martin *et al.* 2006; Hartfield *et al.* 2012). Second, it increases the time to fixation of invading asexual mutants, allowing more time for the operation of Hill-Robertson interference and, in particular, Muller's Ratchet (Peck *et al.* 1999; Salathé *et al.* 2006). The benefit of sex arising from population structure is expected to operate broadly because all natural populations show at least some structure.

However, the complex genetic architecture of the gene network model, when combined with population structure, introduces another cost of sex: migration load. If we allow natural selection to operate in a spatially homogeneous manner, migrants will be equally well adapted to any deme, regardless of their sexual strategy. The offspring of sexual migrants will, however, experience a reduction in fitness (i.e., migration load) if they carry alleles that are incompatible with alleles in resident genotypes. Such Dobzhansky-Muller incompatilities (Dobzhansky 1937; Muller 1942) have been observed to evolve in a model similar to ours (Palmer and Feldman 2009). Migration load can therefore be understood as an extension of recombination load, in which recombination between residents of different demes disrupts coadapted gene complexes and produces low-fitness hybrids. Asexual populations experience no migration load. The impact of migration load on the benefits of sex imparted by population structure is unknown.

Here we evaluate the extent to which population structure supports the origin and maintenance of costly sex in a model with complex, evolving genetic architecture. We found that the relationship between population structure and the advantage of sex is non-monotonic. Small amounts of population structure promoted the origin and maintenance of costly sex by disproportionately increasing the strength of Muller's Ratchet in asexual populations, as expected. However, this advantage of sex was maximized in moderately structured populations. Highly structured sexual populations accumulated Dobzhansky–Muller incompatibilities that effectively prevented gene flow and, in some cases, disproportionately increased the strength of Muller's Ratchet in *sexual* populations.

Methods

Our gene network model is based on a model introduced by Wagner (1994, 1996).

Genotype

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.

Phenotype

The expression pattern of an individual 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

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

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

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

Starting from an initial 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*.

Fitness

The fitness of a viable genotype is given by

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

where $\delta(\mathbf{S}, \mathbf{S}') = \sum_{i=1}^{n} (s_i - s_i')^2/(4n)$ 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.

Offspring of asexual reproduction have fitness $W_{\text{asex}} = W$, whereas offspring of sexual reproduction have fitness $W_{\text{sex}} = W/C$, where $C \geq 1$ is the cost of sex ($C = 1$ and $C = 2$ represent no cost and a two-fold cost, respectively).

Random genotype

A random genotype is created by generating a random gene network and a random initial gene expression pattern. A random gene network is obtained by randomly filling an $\mathbf{R}$ matrix with $(1-\gamma)n^2$ zeros and γn^2 random numbers from a standard normal distribution, where γ is the connectivity density of the network. 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

Evolution is simulated using an individual-based, Wright-Fisher model with non-overlapping generations. Populations maintain a constant total size, N, and are subdivided into D demes of equal size, $N_d = N/D$, arranged in a ring. In each generation, individuals undergo a selection-reproduction-mutation-migration life cycle.

Initialization: Simulations are initiated by producing N clones of a single viable randomly generated genotype. The phenotype of this founding individual is set as the population's optimal phenotype. The optimum remains constant over the course of the simulation.

Selection: Every generation, in each deme, the individuals that will reproduce are chosen at random, with replacement, with probability proportional to their fitness (Equation 2).

Reproduction: If the parent reproduces asexually, it generates a clone of itself; if a parent reproduces sexually, it mates with another sexually reproducing parent and produces one haploid recombinant offspring. The recombinant $\mathbf{R}$ matrix is generated by copying rows from the $\mathbf{R}$ matrices of the parents with equal probability. This is equivalent to free recombination between regulatory regions and no recombination within regulatory regions.

Mutation: 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 γn^2 nonzero entries in $\mathbf{R}$ chosen at random; the mutated value is drawn randomly from a standard normal distribution.

Migration: Every generation, a number of individuals from each deme is randomly chosen (without replacement) to migrate to either neighboring deme, with no bias for fitness or reproductive mode. The number of migrants from one deme to one of its neighbors is Poisson distributed with parameter $mN_d/2$, where m is the migration rate. The numbers of migrants to and from individual demes are not guaranteed to be equal. Thus, following migration the population may transiently show variation in deme sizes. Selection and reproduction restore the size of every deme to N_d.

Reproductive mode

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 organisms 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 second individual may be the same as the first individual because sampling is done with replacement; the resulting selfing event is equivalent to asexual reproduction.

Invasion analyses

Populations were evolved for 10^4 generations under asexual (or sexual) reproduction to allow sufficient time for the population to approach mutation-recombination-selection-drift-migration equilibrium. We then mutated the allele at the modifier locus S (see "Reproductive mode," above) in a single randomly chosen individual, causing it to switch to the opposite reproductive mode, from asexual to sexual (or vice-versa). We measured the fixation probability of the novel modifier allele, u, relative to that of a neutral mutation ($u^* = 1/N$) in replicate invasion trials. During the invasion trials, individuals carrying the sexual modifier allele S experience a fixed cost of sex, C (see "Fitness"). For invasion trials in which the novel modifier allele fixed, we recorded the generation in which it fixed as T_{fix}.

Population metrics

Mean fitness: The mean fitness, $\overline{W}$, of all individuals present in the population at a given time (see Equation 2). The mean fitness of sexual and asexual individuals is denoted by $\overline{W}_{\text{sex}}$ and $\overline{W}_{\text{asex}}$, respectively.

Strength of Muller's Ratchet: Defined as

$$\Delta W/\Delta t = \frac{\ln W_2 - \ln W_1}{\ln t_2 - \ln t_1} \ ,$$

where W_i is mean fitness at generation t_i. To estimate the rate of change in mean fitness at the point in time when fitness declines due to the Ratchet were strongest, we calculated $\Delta W/\Delta t$ using the two points in our data series immediately prior to and after generation 10^3. Thus, $t_1 = 562$ and $t_2 = 1778$ generations in our calculations.

Deleterious mutation rate: Defined as $U_d = U(p_l + p_d)$, where U is the genomic mutation rate and p_l and p_d are the proportion of lethal and non-lethal deleterious mutations, respectively. U is constant throughout the course of a simulation but p_l and p_d can evolve. We estimated the quantity $p_l + p_d$ for a genotype by generating 100 copies of the genotype, each carrying one independently generated mutation, and evaluating the proportion of them that have lower fitness than the original genotype.

Mean fitness of recombinant offspring: Defined as $\overline{W}_R$, the mean fitness of offspring produced by recombination (without mutation) between individuals of the same deme, W_r, relative to the average fitness of its parents $\overline{W}_p$. We averaged $W_r/\overline{W}_p$ across N independently chosen pairs of individuals, where each parent was chosen with probability proportional to its fitness (i.e., in the same way the population reproduced in the evolutionary simulations).

Mean fitness of migrant offspring: Defined as $\overline{W}_M$, the mean fitness of offspring produced by recombination (without mutation) between individuals of neighboring demes, W_m, relative to the average fitness of its parents $\overline{W}_p$. We averaged $W_m/\overline{W}_p$ across N independent offspring, generated by recombination between N_d pairs of parents randomly chosen from each of the D pairs of neighboring demes. Both parents were chosen from among the individuals in their respective demes with probability proportional their fitness.

Population differentiation: Measured as $Q_{ST} = V_b/(V_w + V_b)$, where V_w and V_b are the variance within and among demes at a neutral locus, respectively. Each individual carries 20 neutral loci, which were neither linked to each other nor to any of the gene network loci. The founding genotype had a value of 0 at all 20 neutral loci. Every generation, each neutral locus in each individual acquired a random number of mutations drawn from a Poisson distribution with mean 1.0 (the neutral locus mutation rate). Each mutation changed the value of the neutral locus by adding a random value drawn from the standard normal distribution. V_w and V_b were calculated as variance components in a random-effects analysis of variance. We averaged Q_{ST} across the 20 neutral loci.

Maximum cost of sex: Defined as $C^O_{\max}$ and $C^M_{\max}$ to describe the maximum sustainable cost of sex during the origin and maintenance of sex, respectively. $C^O_{\max}$ is the maximum value of C for which $u_{\text{sex}} > u^*$ in sexual invasion trials. $C^M_{\max}$ is the maximum value of C for which $u_{\text{asex}} < u^*$ in asexual invasion trials (see "Invasion analyses"). For each set of equilibrium populations evolved at a particular m and N_d, we measured the modifier fixation probability, u, at each of at least 4 values of C and estimated $C_{\max}$ from the resulting data using logistic regression (Figures S2 and S3).

Equilibrium values: Defined as values of population metrics at generation 10^4 and indicated by a hat symbol (e.g., $\widehat{W}_{\text{sex}}$).

Parameter values

By default, we used the same parameters as in Whitlock *et al.* (2016): population size of $N = 10^3$ individuals, networks of $n = 100$ genes, with connectivity density $c = 0.05$, a genomic mutation rate of $U = 1$ per generation, and moderate stabilizing selection ($\sigma = 0.2$).

Statistical Analysis

All statistics were conducted using the R statistical package, version 3.4.1 (Ihaka and Gentleman 1996). Linear models that investigated the contributions of various population metrics to the long and short term advantages of sex were conducted using the function *lm* from the *stats* package.

All data were generated from 50 pairs of sexual and asexual populations. Each pair was founded by an independent, randomly generated genotype. Combined metrics from sexual and asexual populations, like $\widehat{W}_{\text{sex}}/\widehat{W}_{\text{asex}}$, were calculated separately for each pair, and the resulting 50 composite values were used for plotting and statistical tests.

Data Availability

Programs used to run all simulations were written in Python 2.7 and are available at https://bitbucket.org/aobwhitlock/whitlock-et-al-2018. Supplemental figures are available at FigShare.

Results

Population structure has a complex effect on the long-term advantage of sex

Previously, we showed that panmictic (i.e., unstructured) sexual populations of $N = 10^3$ individuals experiencing a genomic mutation rate of $U = 1$ and moderate stabilizing selection ($\sigma = 0.2$) evolved a mean fitness at equilibrium 5.7% ± 0.8% (mean and 95% confidence interval) higher than that of otherwise identical asexual populations (Whitlock *et al.* 2016). Here we investigate the extent to which population structure increases the long-term advantage of sex.

We manipulated population structure in two ways. First, we subdivided the total population into $D = 10$ to 100 demes of identical size $N_d = 10^3/D$ (note that D and N_d were not varied independently). Demes were arranged around a circle and migration occurred via a one-dimensional stepping-stone model (Kimura 1952). Second, we varied the migration rate (m) between neighboring demes from 2×10^{-4} to 2×10^{-1}. There was no migration (i.e., $m = 0$) between non-neighboring demes. Structured populations consisting entirely of either sexual or asexual individuals were allowed to evolve for 10^4 generations, by which time most populations had reached a mutation-selection-drift fitness equilibrium (Figure S1). The least structured populations (i.e., those subdivided into $D = 10$ demes of $N_d = 100$ individuals each, experiencing the highest migration rate, $m = 0.2$) evolved approximately like panmictic populations, achieving similar equilibrium mean fitness (Figure 1) and $Q_{ST} \approx 0$ (Figure 1). Decreasing either the deme size (N_d) or the migration rate (m) caused the level of genetic differentiation among demes, Q_{ST}, to increase, confirming that populations become more structured (Figure 1).

Relative to panmictic populations, small increases in population structure, imposed by either decreasing N_d or m, caused the magnitude of the long-term advantage of sex ($\widehat{W}_{\text{sex}}/\widehat{W}_{\text{asex}}$) to increase (Figure 1). This pattern was true provided the population consisted of relatively large demes ($N_d \gtrsim 50$). At $N_d = 50$ and $m = 2 \times 10^{-4}$, the average advantage of sex achieved a maximum that was 5.8-fold larger than it was in a panmictic population of the same total size.

Indeed, for each $m \leq 2 \times 10^{-2}$, there was an intermediate deme size, N^*_d, at which the advantage of sex was maximized. Further subdivision into demes that were smaller than N^*_d eroded the advantage of sex. Surprisingly, the effect of small deme size was most pronounced at the intermediate migration rate of $m = 2 \times 10^{-3}$, generating a strong *disadvantage* of sex in the smallest deme sizes ($N_d \leq 33$) at this migration rate.

The variance we observed in the long-term advantage of sex was almost entirely explained by the relative strength of Muller's Ratchet in sexual compared to asexual populations ($\Delta W_{\text{sex}}/\Delta t - \Delta W_{\text{asex}}/\Delta t$; $R^2 = 0.95, F_{1,34} = 669.3, p < 10^{-15}$; Figure 1). $\Delta W_{\text{sex}}/\Delta t - \Delta W_{\text{asex}}/\Delta t$ took on its highest values for combinations of m and N_d in which migration effectively countered the Ratchet in sexual ($\Delta W_{\text{sex}}/\Delta t > 0$) but not asexual ($\Delta W_{\text{asex}}/\Delta t < 0$) populations. Conditions in which $\Delta W_{\text{sex}}/\Delta t < \Delta W_{\text{asex}}/\Delta t < 0$, indicated by negative values, were entirely unexpected because they require that the Ratchet was accelerated in sexual populations compared to asexual populations. We next investigated the cause of this acceleration in Muller's Ratchet in some of the highly structured sexual populations.

Dobzhansky-Muller incompatibilities accelerate Muller's Ratchet in sexual structured populations

Evolving properties of the genetic architecture make substantial contributions to the long-term advantage of sex in panmictic populations (Whitlock *et al.* 2016). To investigate the extent to which an evolving genetic architecture contributes to the advantage of sex in structured populations, we monitored the mutation, recombination, and migration loads.

Both the mutation and recombination loads evolved in ways that contributed to the long-term advantage of sex (Figure 2), as we observed previously in panmictic populations (Whitlock *et al.* 2016). The lower equilibrium $\widehat{U}_d$ of sexual, compared to asexual, populations generated a fitness advantage for sexual populations of 6–9% ($1.06 < \exp(-\widehat{U}_{d,\text{sex}})/\exp(-\widehat{U}_{d,\text{asex}}) < 1.09$). Recombination load at equilibrium generated a fitness disadvantage for sexual populations of 5–8% ($0.92 < \widehat{W}_R < 0.95$). However, the differences among treatments in mutation and recombination loads (Figure 2) are not reflective of the differences among treatments in the overall advantage to sex (Figure 1; linear regression of $\widehat{W}_{\text{sex}}/\widehat{W}_{\text{asex}}$ against $\widehat{U}_d$: $R^2 = 0.0998$, $F_{1,34} = 3.769$, $p = 0.0606$; linear regression against $\widehat{W}_R$: $R^2 = 0.0153$, $F_{1,34} = 0.5275$, $p = 0.4726$).

A third property of the genetic architecture, the relative fitness of sexual migrant offspring, $\widehat{W}_M$, also evolved in our simulations (Figure 2). At equilibrium, $\widehat{W}_M$ depended predictably on both N_d and m. For large N_d and m, the fitness of migrant offspring was indistinguishable from that of resident offspring (i.e., $\overline{W}_M \approx \overline{W}_R$), as expected when there is relatively little differentiation between demes (low $\widehat{Q}_{ST}$). As N_d and m declined, so did $\widehat{W}_M$, until when $N_d \lesssim 50$ and $m \leq 2 \times 10^{-3}$, $\widehat{W}_M \approx 0$ and demes were strongly differentiated (high $\widehat{Q}_{ST}$). These reductions in $\widehat{W}_M$ must have resulted from the accumulation of alleles in some demes that show strong negative epistatic interactions with alleles present in other demes—i.e., Dobzhansky-Muller incompatilities. This extreme variation in $\widehat{W}_M$ had only a negligible direct effect on population mean fitness because $\widehat{W}_M$ was low only when migration rates were also low ($m \lesssim 2 \times 10^{-3}$). Instead, the main consequence of a low $\widehat{W}_M$ was to reduce the effective gene flow between demes, which in turn intensified Muller's Ratchet in sexual populations.

In highly structured populations with moderate migration rates (e.g., $N_d < 50$ and $m = 2 \times 10^{-3}$; hot pink line in Figure 2), the accumulation of Dobzhansky-Muller incompatibilities ($W_M \approx 0$) explains the faster fitness declines in sexual than in asexual populations ($\Delta W_{\text{sex}}/\Delta t < \Delta W_{\text{asex}}/\Delta t$; hot pink line in Figure 1). In this parameter range, the moderate migration experienced only by asexual populations more effectively countered Muller's Ratchet than the recombination experienced only by sexual populations. Outside this parameter range, the effective migration rate was similar in asexual and sexual populations, either because Dobzhansky-Muller incompatibilities didn't accumulate in sexual populations ($m > 10^{-3}$) or because the migration rate was near zero in asexual populations too ($m = 10^{-4}$).

The long-term advantage of sex is a good predictor of the evolutionary success of sex

Previously, we showed that sexual modifier mutations successfully invaded panmictic asexual populations of $N = 10^3$ and that panmictic sexual populations of the same size successfully resisted invasion by asexual modifier mutations. In other words, sexual reproduction evolved and was maintained in panmictic populations of $N = 10^3$ individuals. However, we observed this pattern in panmictic populations only if sex carried no direct costs (i.e., $C \leq 1$) (Whitlock *et al.* 2016). The addition of population structure is expected to promote the maintenance of costly sex by increasing the time (T_{fix}) that asexual modifier mutations spend in transit to fixation. In turn, increasing T_{fix} is expected to increase the accumulation of deleterious mutations in the invading asexual lineage, depressing its mean fitness and probability of fixation. This effect, of population structure on T_{fix}, is not expected to influence the *origin* of costly sex. Here we investigate the extent to which the predicted effect of population structure on T_{fix} combines with the complex effect of population structure on equilibrium mean fitness (Figure 1) to promote both the origin and maintenance of costly sex.

As expected, population structure promoted the maintenance of costly sex (Figures 3B and 3D). For each combination of m and N_d we used invasion assays to estimate the maximum cost of sex (C^M_{max}) under which the fixation probability of an asexual modifier mutation was lower than the neutral expectation ($u < u^*$, Figure S2). $C^M_{\text{max}} > 1$ for almost all the parameter combinations we examined and rose as high as 1.36 for the most favorable parameter combination. C^M_{max} significantly increased with both the mean T_{fix} among successful invaders ($F_{1,13} = 142.5$, $p < 10^{-7}$) and the equilibrium advantage to sex ($\widehat{W}_{\text{sex}}/\widehat{W}_{\text{asex}}$, $F_{1,13} = 343.7$, $p < 10^{-10}$). Moreover, T_{fix} and the equilibrium advantage of sex predicted C^M_{max} almost perfectly (multiple regression model containing only these two predictors, $R^2 = 0.98$).

Population structure also promoted the origin of costly sex (Figures 3A and 3C). To investigate the origin of sex we used invasion assays to estimate the maximum cost of sex (C^O_{max}) under which the fixation probability of a sexual modifier mutation was higher than the neutral expectation ($u > u^*$, Figure S3). Similar to C^M_{max}, $C^O_{\text{max}} > 1$ for almost all the parameter combinations we examined and increased significantly with the equilibrium advantage to sex ($F_{1,11} = 12.71$, $p = 0.0044$). However, C^O_{max} rose only as high as 1.1 for the most favorable parameter combination and showed no significant relationship with T_{fix} ($F_{1,11} = 3.62$, $p = 0.0835$). In addition, C^O_{max} was only somewhat predictable ($R^2 = 0.60$) from the quantities $\widehat{W}_{\text{sex}}/\widehat{W}_{\text{asex}}$ and T_{fix}.

We note that these results are for modifiers of sex that cause free recombination between rows of the genotype matrix, **R**. We also examined the behavior of modifiers of recombination that cause only small changes in recombination rate, using a model that allows recurrent mutation at the modifier locus. Modifiers of recombination behaved similarly to modifiers of sex. Using the parameter combination that was most favorable to the origin of sex, recombination rate increased over time when $C = 1.1$, but not when $C = 1.2$ (Figure 4). This similarity between the two types of modifiers was not observed in panmictic populations, where modifiers of recombination were more likely to evolve than modifiers of sex (Whitlock *et al.* 2016).

Discussion

Our work builds on prior observations that Hill-Robertson effects generate an advantage of sex that, in structured populations, can be large enough to explain the evolutionary maintenance of *costly* sex (Peck *et al.* 1999; Salathé *et al.* 2006; Martin and Lenormand 2006; Hartfield *et al.* 2012). Unlike the prior work, we employed an artificial gene network model that explicitly includes genetic interactions that produce recombination load.

We found that population structure facilitated the origin and maintenance of costly sex in the expected ways, increasing the

strength of Muller's Ratchet and slowing the fixation of invading asexual lineages. The maximum advantage of sex was realized in moderately structured populations. In some of the most structured populations, the advantage of sex was eroded by population differentiation among neighboring demes, that reduced the effective migration rate in sexual populations nearly to zero.

Both the short and the long-term advantages of sex in our structured populations depended primarily on the difference in the strength of Muller's Ratchet between sexual and asexual populations. The dependence of modifier fixation probabilities on the strength of Muller's Ratchet was observed in the work of Gordo and Campos (2008) and, therefore, did not depend on the complex genetic architecture of the gene network model. This dependence resulted simply because the Ratchet dynamics play out over the same timescale that determines whether modifier mutations are fixed or lost. The dependence of equilibrium mean fitness on the strength of Muller's Ratchet was not observed in prior work, because it required a complex genetic architecture. In the gene network model (Whitlock *et al.* 2016), as in biological systems (Poon and Otto 2000; Silander *et al.* 2007), compensatory mutations increase in frequency as fitness declines, stalling Muller's Ratchet at an equilibrium mean fitness that decreases as the strength of the Ratchet intensifies.

Differences in the strength of Muller's Ratchet between sexual and asexual populations depended, in turn, on the effective rate of migration between demes, $m_e \approx m\overline{W}_M$. Increasing structure decreased the efficacy of migration in sexual populations by promoting the accumulation of Dobzhansky-Muller incompatibilities (Dobzhansky 1937; Muller 1942; Palmer and Feldman 2009; Bank *et al.* 2012) that resulted in substantial increases in migration load. Consequently, in the most structured populations the mean fitness of hybrids between demes dropped to $\overline{W}_M \approx 0$ and the effective migration rate dropped to $m_e \approx 0$. Because asexual migrants reproduced without recombination and because migrant alleles were not maladapted to the local environment, asexual populations did not experience migration load (i.e., $\overline{W}_M = 1$ and $m_e = m$). Thus, asexual populations benefited from migration in some conditions (e.g., $m = 10^{-3}$ and $N_d < 33$) where sexual populations did not. In these conditions, Muller's Ratchet actually clicked more slowly in asexual than in sexual populations.

Earlier work had shown that migration load could generate a cost of sex in structured populations experiencing spatially *heterogeneous* selection, where migration can impede local adaptation by repeatedly introducing locally maladapted genotypes (García-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997; Hendry *et al.* 2001; Alleaume-Benharira *et al.* 2006; Bolnick and Nosil 2007). For example, the migration load experienced by sexual populations may explain the prevalence of asexual reproduction in marginal or peripheral habitats (Peck *et al.* 1998; Spitzer and Haygood 2007). Our results extend the potential role of migration load as a cost of sex to structured populations under spatially *homogeneous* selection. Our data also revealed a general mechanism for the cost of sex caused by migration load. Migration load, whether generated by the accumulation of Dobzhansky-Muller incompatibilities or by local maladaptation, reduces the effective migration rate, which intensifies Muller's Ratchet in sexual populations.

In addition to its effect on the strength of Muller's Ratchet, increasing population structure also promoted the evolution of sex through its effect on the invasion dynamics of new modifier mutations. Similar to prior investigations of the maintenance of sex (Peck *et al.* 1999; Hartfield *et al.* 2012), we showed that increasing population structure increased the time to fixation, T_{fix}, of invading asexual modifiers, promoting their mutational decay via Muller's Ratchet. In the gene network model, however, this decay is limited by the equilibrium advantage of sex. Indeed, T_{fix} and the equilibrium advantage to sex combine to predict, almost perfectly, the maximum cost at which sex was maintained in our simulations.

Structure also promoted the origin of sex. This effect of structure was not predictable from prior investigations of the evolution of sex, because it resulted from the complex genetic architecture in the gene network model. Sexual lineages experience a short-term fitness decline before they evolve robustness to ameliorate the accumulating recombination load and to acquire a long-term fitness advantage in the form of lower U_d. (Figures S1, 1, and 2). In panmictic populations, successful sexual invaders must arise by chance in a high fitness genetic background and quickly hitchhike with that genetic background to a frequency that prevents their loss during the short-term fitness decline (Whitlock *et al.* 2016). Population structure facilitated the origin of sex by slowing the loss of sexual invaders after this initial hitchhiking because the modifier mutation only needed to hitchhike to fixation within a single deme to be sheltered from the asexual population during the sexual lineage's short-term fitness decline.

Our results demonstrate that complex genetic architecture interacts with population structure to constrain the origin and maintenance of costly sex. The magnitude of both the long- and short-term advantages of sex are likely to be affected by many factors not considered here, including deviations from random mating other than those arising from population structure, ploidy, and environmental change (Shields 1982; Agrawal 2001; Siller 2001; Blachford and Agrawal 2006; Kirkpatrick and Jenkins 1989; Kondrashov and Crow 1991; Agrawal and Chasnov 2001; Otto 2003; Charlesworth 1993; Barton 1995; Otto and Nuismer 2004; Carja *et al.* 2014; Nowak *et al.* 2014). We posit that these factors may also interact with a complex genetic architecture to influence the evolution of sex, making them promising avenues of inquiry.

Acknowledgements

We thank Sonia Singhal for her comments on the manuscript. The University of North Carolina at Chapel Hill and the Research Computing group provided computational resources and support. This work was funded by grant DEB-1355084 awarded to C.L.B. and grant DEB-1354952 awarded to R.B.R.A. by the National Science Foundation and by a Dissertation Completion Fellowship awarded to A.O.B.W. by the University of North Carolina at Chapel Hill. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Figures

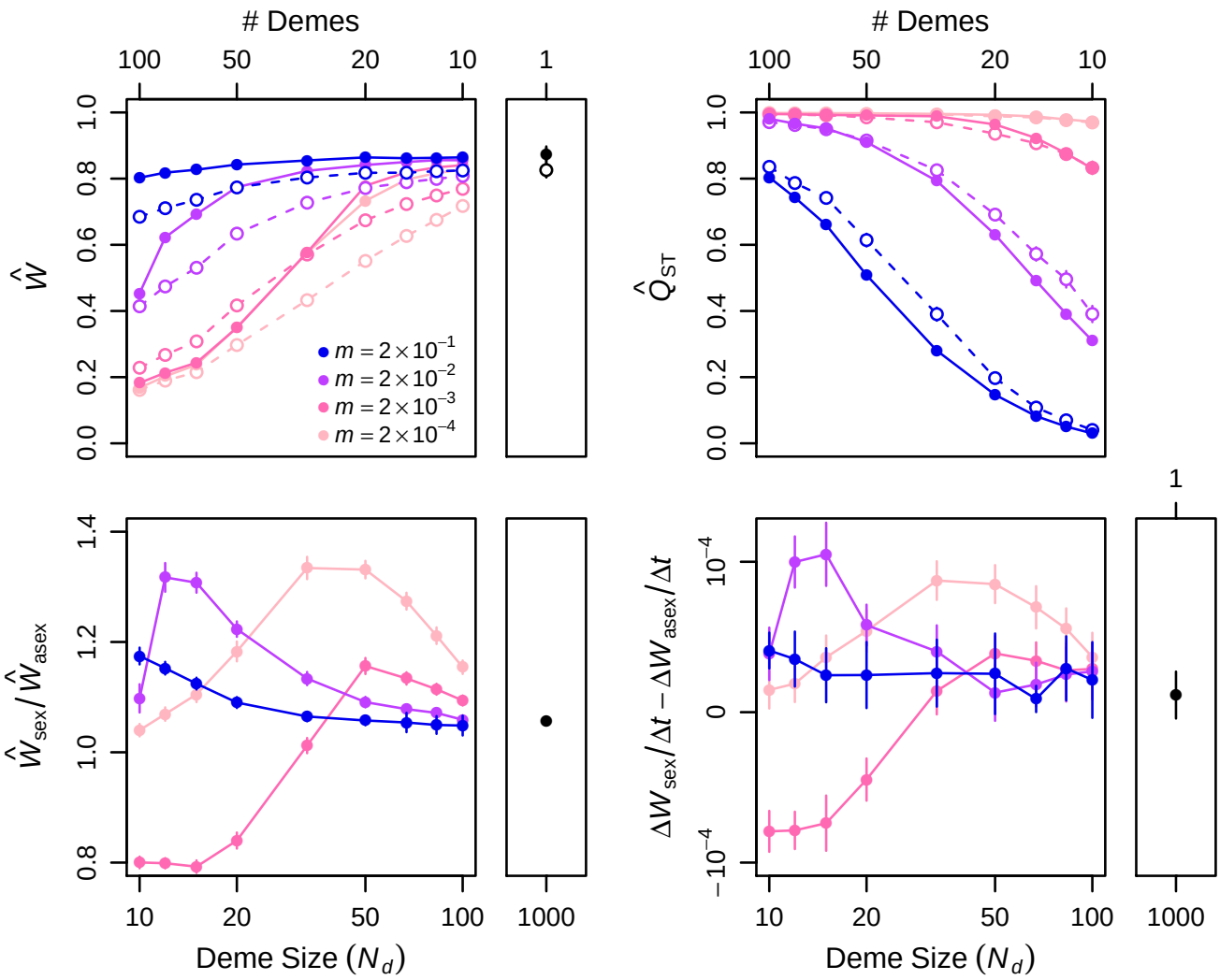

Figure 1 Migration rate and population subdivision interact in a complex way to determine the long-term advantage of sex. Mean fitness ($\hat{W}$), the fitness advantage of sexuals ($\hat{W}_{\text{sex}}/\hat{W}_{\text{asex}}$), and population differentiation ($\widehat{Q}_{ST}$) were calculated at generation 10^4, after populations of all migration rates and deme sizes had achieved fitness equilibria. The relative strength of Muller's Ratchet ($\Delta W_{\text{sex}}/\Delta t - \Delta W_{\text{asex}}/\Delta t$) was calculated at generation 10^3, when fitness declines due to the Ratchet were strongest. In the top panels, sexual and asexual populations are represented by closed and open circles, respectively. Large panels show the relationships between these equilibrium metrics and the deme number and size of subdivided populations. Small panels show the equilibrium metrics calculated from panmictic asexual and sexual populations of the same total size, $N = 10^3$ (obtained from Whitlock *et al.* 2016). Values are means and 95% confidence intervals based on 50 replicate populations initiated from different randomly chosen founders.

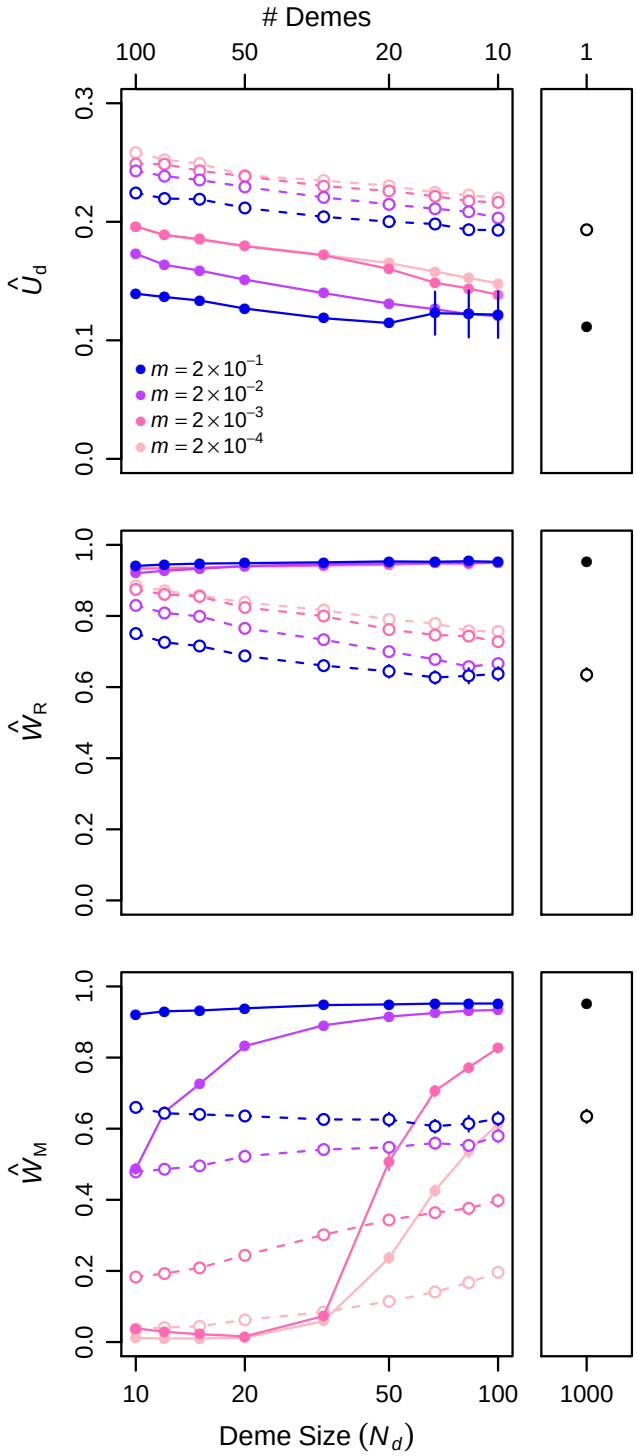

Figure 2 Migration rate and deme size affect three aspects of the equilibrium genetic architecture. Deleterious mutation rate ($\widehat{U}_d$), the mean fitness of recombinant offspring of parents from the same deme ($\widehat{W}_R$), and the mean fitness of recombinant offspring of parents from different demes ($\widehat{W}_M$) at generation 10^4, after populations of all migration rates and deme sizes achieved fitness equilibria. As in Figure 1, sexual and asexual populations are represented by closed and open circles, respectively, and values are means and 95% confidence intervals based on 50 replicate populations initiated from different randomly chosen founders. Large panels show the relationships between these equilibrium metrics and the deme number and size of subdivided populations. Small panels show the equilibrium metrics calculated from panmictic asexual and sexual populations of size $N = 10^3$ (obtained from Whitlock *et al.* 2016). Note that measures of $\widehat{W}_R$ and $\widehat{W}_M$ in asexual populations are provided for comparison only. During the evolution experiments, offspring produced by asexual reproduction experience $W_R = 1$ and $W_M = 1$, by definition.

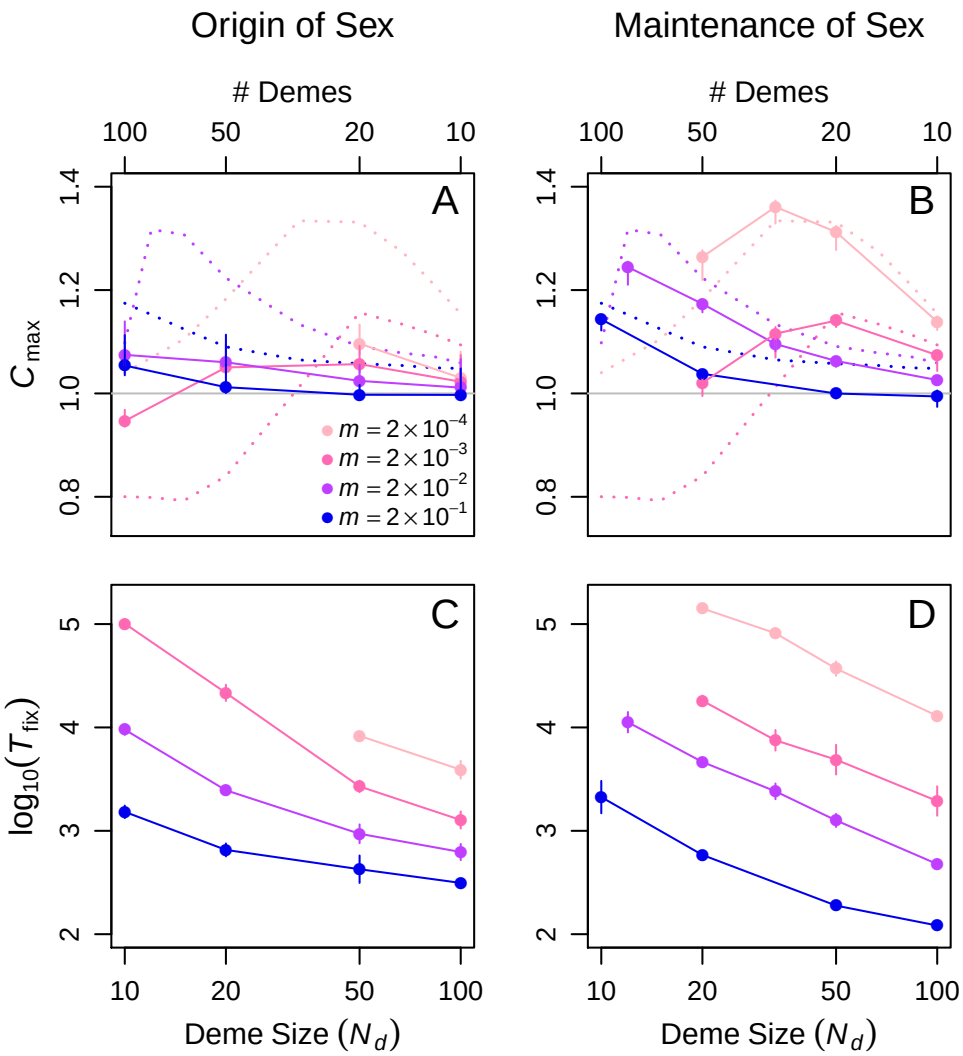

Figure 3 Population structure increases the transit time (T_{fix}) of invading sexual and asexual mutants, facilitating both the origin and maintenance of costly sex. (A) Points connected by solid lines show the maximum cost of sex (C_{max}) at which sexual mutants successfully invaded equilibrium asexual populations in each deme size and migration rate treatment. (B) Points connected by solid lines show the maximum cost of sex at which equilibrium sexual populations successfully resisted invasion by asexual mutants. Dotted lines in both (A) and (B) show the long-term advantage of sex, $\widehat{W}_{\text{sex}}/\widehat{W}_{\text{asex}}$, replotted from Figure 1, for comparison. (C and D) The average fixation time (T_{fix}) of sexual (C) and asexual (D) mutants that successfully invaded equilibrium asexual and sexual populations, respectively. Values are means and 95% confidence intervals based on all of the successful invasions in each condition. Missing points in the $m = 2 \times 10^{-3}$ and $m = 2 \times 10^{-4}$ series correspond to parameter combinations for which invasion trials were not run because they were not computationally feasible (i.e., T_{fix} was too long).

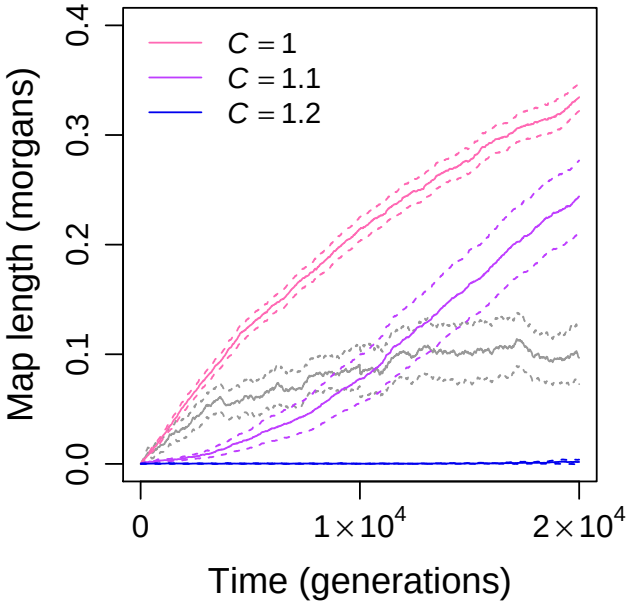

Figure 4 Evolution of the recombination rate under recurrent mutation at the recombination modifier locus. Color lines show the change in mean and 95% confidence interval, respectively, of the genome map length (i.e. mean crossover probability) over time in structured populations with $N_d = 50$ and $m = 2 \times 10^{-4}$ and costs of sex ranging from none ($C = 1$, pink) to substantial (purple and blue). Panmictic populations of the same total size ($N_{\text{total}} = 10^3$) experiencing no cost of sex ($C = 1$) are shown in gray for comparison. Data are from 50 replicates initiated with the equilibrium structured asexual populations shown in Figures 1 and S1 (pink, purple, and blue) or the equilibrium panmictic asexual populations of size $N = 10^3$ from Whitlock *et al.* (2016) (gray).

Literature Cited

Agrawal, A. F., 2001 Sexual selection and the maintenance of sexual reproduction. Nature **411**: 692–695.

Agrawal, A. F. and J. R. Chasnov, 2001 Recessive mutations and the maintenance of sex in structured populations. Genetics **158**: 913–917.

Alleaume-Benharira, M., I. R. Pen, and O. Ronce, 2006 Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. J. Evol. Biol. **19**: 203–215.

Azevedo, R. B. R., R. Lohaus, S. Srinivasan, K. K. Dang, and C. L. Burch, 2006 Sexual reproduction selects for robustness and negative epistasis in artificial gene networks. Nature **440**: 87–90.

Bank, C., R. Bürger, and J. Hermisson, 2012 The limits to parapatric speciation: Dobzhansky–Muller incompatibilities in a continent–island model. Genetics **191**: 845–863.

Barton, N. H., 1995 A general model for the evolution of recombination. Genet. Res. **65**: 123–145.

Barton, N. H. and S. P. Otto, 2005 Evolution of recombination due to random dirift. Genetics **169**: 2353–2370.

Bell, G., 1982 *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. University of California Press, Berkeley.

Blachford, A. and A. F. Agrawal, 2006 Assortative mating for fitness and the evolution of recombination. Evolution **60**: 1337–1343.

Bolnick, D. I. and P. Nosil, 2007 Natural selection in populations subject to a migration load. Evolution **61**: 2229–2243.

Burt, A., 2000 Perspective: Sex, recombination, and the efficacy of selection—Was Weismann right? Evolution **54**: 337–351.

Carja, O., U. Liberman, and M. W. Feldman, 2014 Evolution in changing environments: Modifiers of mutation, recombination, and migration. Proc. Natl. Acad. Sci. U. S. A. **111**: 17935–17940.

Charlesworth, B., 1993 Directional selection and the evolution of sex and recombination. Genet. Res. **61**: 205–224.

Comeron, J. M., A. Williford, and R. M. Kliman, 2008 The Hill-Robertson effect: evolutionary consequences of weak selection and linkage in finite populations. Heredity **100**: 19–31.

Dobzhansky, T., 1937 *Genetics and the origin of species*. Columbia Univ. Press, New York.

Felsenstein, J., 1974 The evolutionary advantage of recombination. Genetics **78**: 737–756.

García-Ramos, G. and M. Kirkpatrick, 1997 Genetic models of adaptation and gene flow in peripheral populations. Evolution **51**: 21–28.

Gibson, A. K., L. F. Delph, and C. M. Lively, 2017 The two-fold cost of sex: Experimental evidence from a natural system. Evolution Letters **1**: 6–15.

Gordo, I. and P. R. A. Campos, 2008 Sex and deleterious mutations. Genetics **179**: 621–626.

Haigh, J., 1978 The accumulation of deleterious genes in a population—Muller's ratchet. Theor. Popul. Biol. **14**: 251–267.

Hartfield, M., S. P. Otto, and P. D. Keightley, 2010 The role of advantageous mutations in enhancing the evolution of a recombination modifier. Genetics **184**: 1153 –1164.

Hartfield, M., S. P. Otto, and P. D. Keightley, 2012 The maintenance of obligate sex in finite, structured populations subject to recurrent beneficial and deleterious mutation. Evolution **66**: 3658–3669.

Hendry, A. P., T. Day, and E. B. Taylor, 2001 Population mixing and the adaptive divergence of quantitative traits in discrete populations: a theoretical framework for empirical tests. Evolution **55**: 459–466.

Hill, W. G. and A. Robertson, 1966 The effect of linkage on limits to artificial selection. Genet. Res. **8**: 269–294.

Ihaka, R. and R. Gentleman, 1996 R: a language for data analysis and graphics. J. Comp. Graph. Stat. **5**: 299–314.

Iles, M. M., K. Walters, and C. Cannings, 2003 Recombination can evolve in large finite populations given selection on sufficient loci. Genetics **165**: 2249–2258.

Keightley, P. D. and S. P. Otto, 2006 Interference among deleterious mutations favours sex and recombination in finite populations. Nature **443**: 89–92.

Kimura, M., 1952 "stepping-stone" model of population. Natl. Inst. Genet. Japan **3**: 62–63.

Kirkpatrick, M. and N. H. Barton, 1997 Evolution of a species' range. Am. Nat. **150**: 1–23.

Kirkpatrick, M. and C. D. Jenkins, 1989 Genetic segregation and the maintenance of sexual reproduction. Nature **339**: 300–301.

Kondrashov, A. S. and J. F. Crow, 1991 Haploidy or diploidy: which is better? Nature **351**: 314–315.

Lehtonen, J., M. D. Jennions, and H. Kokko, 2012 The many costs of sex. Tr. Ecol. Evol. **27**: 172–178.

Lohaus, R., C. L. Burch, and R. B. R. Azevedo, 2010 Genetic architecture and the evolution of sex. J. Hered. **101**: S142–157.

Martin, G. and T. Lenormand, 2006 The fitness effect of mutations across environments: a survey in light of fitness landscape models. Evolution **60**: 2413–2427.

Martin, G., S. P. Otto, and T. Lenormand, 2006 Selection for recombination in structured populations. Genetics **172**: 593–609.

Maynard Smith, J., 1978 *The Evolution of Sex*. Cambridge University Press, Cambridge.

Muller, H. J., 1942 Isolating mechanisms, evolution and temperature. Biol. Symp. **6**: 71–125.

Muller, H. J., 1964 The relation of recombination to mutational advance. Mutat. Res. **1**: 2–9.

Nowak, S., J. Neidhart, I. G. Szendro, and J. Krug, 2014 Multidimensional Epistasis and the Transitory Advantage of Sex. PLoS Comput. Biol. **10**: e1003836.

Otto, S. P., 2003 The advantages of segregation and the evolution of sex. Genetics **164**: 1099–1118.

Otto, S. P. and N. H. Barton, 2001 Selection for recombination in small populations. Evolution **55**: 1921–1931.

Otto, S. P. and T. Lenormand, 2002 Resolving the paradox of sex and recombination. Nat. Rev. Genet. **3**: 252–261.

Otto, S. P. and S. L. Nuismer, 2004 Species interactions and the evolution of sex. Science **304**: 1018–1020.

Palmer, M. E. and M. W. Feldman, 2009 Dynamics of hybrid incompatibility in gene networks in a constant environment. Evolution **63**: 418–431.

Peck, J. R., J. Yearsley, and G. Barreau, 1999 The maintenance of sexual reproduction in a structured population. Proc. R. Soc. B **266**: 1857–1863.

Peck, J. R., J. M. Yearsley, and D. Waxman, 1998 Explaining the geographic distributions of sexual and asexual populations. Nature **391**: 889–892.

Poon, A. and S. P. Otto, 2000 Compensating for our load of mutations: Freezing the meltdown of small populations. Evolution **54**: 1467–1479.

Salathé, M., R. Salathé, P. Schmid-Hempel, and S. Bonhoeffer, 2006 Mutation accumulation in space and the maintenance of sexual reproduction. Ecol. Lett. **9**: 941–946.

Shields, W. M., 1982 *Philopatry, inbreeding, and the evolution of sex*.

State University of New York Press, Albany.
Silander, O., O. Tenaillon, and L. Chao, 2007 Understanding the evolutionary fate of finite populations: The dynamics of mutational effects. PLoS Biol. **5**: e94.
Siller, S., 2001 Sexual selection and the maintenance of sex. Nature **411**: 689–692.
Spitzer, B. W. and R. Haygood, 2007 Migration Load and the Coexistence of Ecologically Similar Sexuals and Asexuals. The American Naturalist **170**: 567–572.
Vrijenhoek, R. C., 1998 Animal Clones and Diversity. BioScience **48**: 617–628.
Wagner, A., 1994 Evolution of gene networks by gene duplications: A mathematical model and its implications on genome organization. Proc. Natl. Acad. Sci. U. S. A. **91**: 4387–4391.
Wagner, A., 1996 Does evolutionary plasticity evolve? Evolution **50**: 1008–1023.
Whitlock, A. O. B., K. M. Peck, R. B. R. Azevedo, and C. L. Burch, 2016 An evolving genetic architecture interacts with Hill–Robertson interference to determine the benefit of sex. Genetics **203**: 923–936.