Sexual reproduction and Muller's ratchet in digital organisms

Dusan Misevic[1], Richard E. Lenski[1,2] and Charles Ofria[3]

[1]Department of Zoology, [2]Department of Microbiology & Molecular Genetics, [3]Department of Computer Science & Engineering, Michigan State University, East Lansing, MI 48824
Corresponding author: Dusan Misevic (dule@alife.org)

Abstract

The evolution of sexual reproduction has long been a major problem in biology. According to one theory, sex opposes the fitness-destroying process of Muller's ratchet, which occurs by the stochastic loss of high-fitness genotypes in small populations. Sex opposes the ratchet by allowing genotypes with different deleterious mutations to produce mutation-free offspring. We used the Avida digital-evolution software to investigate sex in relation to Muller's ratchet. Populations of digital organisms mutated, competed, and evolved in a complex environment. Populations were either asexual or sexual; in the latter case, parental genomes recombined to produce offspring. We also varied genomic mutation rates and population sizes, which at extreme values often caused mutational meltdowns and population extinctions. Our results demonstrate that sex is advantageous for population survival under some conditions. However, differences in extinction probabilities were usually small, occurred over a narrow range of mutation rates and population sizes, and the advantage of sex for population survival required many generations. Also, the mean fitness of surviving asexual populations was often greater than in sexual populations. This last result indicates the need for work that compares the statistical distribution of mutational effects and epistatic interactions in asexual and sexual populations.

Introduction

The mixing of genomes via sexual reproduction and the resulting recombination of genetic variants are widespread and important to life on Earth (Maynard Smith; 1978 Bell 1982). For over a century, biologists have sought to explain the evolutionary origin and maintenance of sex (Weismann 1889; Ghiselin 1988; West et al. 1999). While there have been important advances, there are still more questions than answers, making this research area both interesting and active (Rice 2002).

There are many potential costs associated with sexual reproduction, including time and energy spent in searching for a suitable mate and in the act of mating (Lewis 1983). Moreover, at the genetic level there often exists a two-fold cost of sex, which is variously called the cost of meiosis or the cost of males. This cost alone implies that asexual populations should grow twice as fast as sexual ones, all else being equal (Maynard Smith 1971). This two-fold cost presents a major hurdle that must be overcome if the evolution of sex is to be understood. Many theories have been proposed to explain why sex arose and how it persists despite these costs, but most have not been adequately tested (Kondrashov 1993) and none are broadly supported by those tests that have been performed to date (West et al. 1999). One of the main theories is attributed to the famous geneticist H. J. Muller, and it proposes that sex is beneficial in opposing what is now referred to as Muller's ratchet.

Muller's ratchet is a stochastic process that leads to the loss of genetic information from asexual populations via the loss of those high-fitness genotypes that are free of any deleterious mutations (Muller 1964; Felsenstein 1974). The ratchet depends on both mutation and drift, where drift refers to changes in gene frequencies caused by the random sampling of a finite number of genes from the previous generation. Drift is especially important, and selection is relatively weak, in small populations that are most sensitive to sampling effects. By chance, the most fit genotypic class can be lost from a small population, especially if the genomic mutation rate is high. Once this class is lost from an asexual population, it cannot be recovered owing to the low probability of beneficial mutations in a small population. The metaphorical ratchet has thus advanced one notch and its action is irreversible; each subsequent loss of the most fit genotypic class advances the ratchet another notch. By contrast, in a sexual population the most fit class can potentially be reconstructed, even after it has been lost, via recombination between two genotypes that carry different mutations. The harmful effects of Muller's ratchet are thus opposed by sex.

Small populations subject to the ratchet may even go extinct if the resulting fitness decay causes the birth rate to drop below the death rate. This feedback can produce a vicious cycle, in which declining fitness leads to a drop in population size, which speeds up the ratchet causing further fitness loss. This cycle has been described as a mutational meltdown (Lynch et al. 1993, 1995). In this study, we use population survival as one metric to compare the effect of Muller's ratchet in sexual and asexual populations.

Several experiments have shown the fitness-destroying effect of Muller's ratchet in diverse microorganisms including viruses (Chao 1990; Duarte et al. 1992), bacteria (Andersson and Hughes 1996), and protozoa (Bell 1989). At least one study with viruses further showed that genetic recombination could oppose the ratchet (Chao et al. 1997). Research in evolutionary computation has also examined the ratchet, with an emphasis on designing strategies to eliminate its adverse effects from applied optimization

algorithms (Nowak and Schuster 1989; Prügel-Bennett 1996; Zitzler et al. 2000; Laumanns et al. 2001). However, questions about the operation of Muller's ratchet and its interaction with reproductive mode are difficult to answer using biological systems (Kondrashov 1982; Maynard Smith 1988). In this paper, we therefore use digital organisms to examine the effects of Muller's ratchet over a range of population sizes and mutation rates, and we test whether sexual reproduction can substantially impede the ratchet's harmful effects.

Methods

We use digital evolution software called Avida to study Muller's ratchet and its interaction with reproductive mode. Avida maintains and monitors experimental populations of digital organisms, which are self-replicating computer programs written in a customized assembler-like language (Adami 1998; Ofria and Wilke 2004). Avida has been used for several other studies of evolutionary dynamics and outcomes (Lenski et al. 1999; Wilke et al. 2001; Lenski et al. 2003). Digital organisms in Avida evolve on rugged fitness landscapes characterized by diverse epistatic interactions, they have intricate genotype-phenotype maps that emerge from a complex developmental program, and they can exhibit quasi-species dynamics (Lenski et al. 1999; Adami et al. 2000; Wilke et al. 2001; Lenski et al. 2003). They approach the level of complexity of organic viruses, making results obtained with Avida biologically relevant and of general interest (Adami 2002; Wilke and Adami 2002). In Avida, genomes have 26 possible instructions at each position. All organisms descend from an ancestral program used to seed a population. Organisms execute the programs encoded by their genomes, including commands that enable them to copy and divide their genomes. The `copy` instruction duplicates a single instruction. During this duplication process, the instruction has a probability of being miscopied and changed to a different instruction in the offspring's genome; mutations from one instruction to any other are equally likely. In this study, we held the genome length constant by setting rates of insertion and deletion mutations to zero. The genomic mutation rate, U, equals the mutation probability per instruction copied multiplied by the genome length. The value of U is controlled by the investigator and was varied in our experiments.

Each digital organism occupies a cell in a rectangular lattice. The size of the lattice sets the maximum population size, which was also varied in our experiments. After a `divide` instruction is executed, the genome is split into two; the duplicated genome (the offspring), is placed into a random cell in the lattice, which kills the organism that previously occupied that position. Although death is random, the danger of being overwritten provides a selective advantage to organisms that replicate faster. Also, if any organism has not reproduced after executing its instructions an average of 15 times each, it dies and is removed from the population. If all the individuals in a population fail to reproduce within this allotted time, then the population has become extinct. In this study, organisms could accelerate the execution of their genomic instructions, and thus their reproduction, by performing certain logic functions (Lenski et al. 2003). If an organism performs one of these functions, then it receives some corresponding resource that provides energy and accelerates execution of its genomic program. Aside from differences in their ability to perform logic functions, all organisms would execute their genomes at the same rate. Even in that case, fitness can vary among organisms depending on their relative gestation time (number of executed instructions necessary to produce an offspring). An organism's expected fitness equals the product of the baseline energy available to all organisms (made proportional to genome length to eliminate selection on genome size per se) and bonuses received for performing logic operations, divided by the gestation time. Organisms do not have access to, and cannot manipulate, their expected fitness. Realized fitness is affected, however, by population structure and interactions among organisms.

In this study, we introduce a new command to Avida that causes the digital organisms to reproduce sexually. We use this variant command to compare evolution in asexual and sexual populations. When executed, the `divide-sex` command separates a copied genome from its parent, but it does not immediately place that new genome into the population. Instead, the new genome goes into a separate location called the *birth chamber*. If the chamber is empty, the new genome remains there until a second genome arrives. When two genomes are present, they recombine and then both resulting offspring are placed at random into the population. [Notice that this mechanism for sexual reproduction does not involve the two-fold cost of sex, although we could have introduced such a cost by placing only one of the two recombinant offspring in the population. It is likely that the most primitive biological forms of sex did not have to overcome this two-fold cost (Maynard Smith 1978), and so we began this research by placing both recombinants in the population. Even so, as we show below, the conditions favoring sexual reproduction with respect to Muller's ratchet are fairly narrow.] Recombination occurs by taking a single continuous region (with two random endpoints) from one genome and swapping it with the corresponding region from the other genome. Genomes are circular and fixed in length; genomic positions are defined by distance from the first command executed and direction of execution. The initial speed of execution of an offspring's genome is set to the weighted average of its two parents, with weights based on the proportion that each parent contributed to the offspring's genome. Under asexual reproduction, the initial speed is inherited from the sole parent.

We performed the evolution experiments with Avida in two stages. Briefly, the *first stage* used large populations in order to evolve digital organisms that were well adapted to their environment. For the *second stage*, these organisms were moved into much smaller populations to examine the

effects of Muller's ratchet. Maximum population size was identical in all cases during the first stage, but this size was varied in the second stage. Mutation rates were also varied across runs, but the rate was held constant in both stages of a given lineage. Similarly, reproductive mode (asexual or sexual) varied across runs, but this mode was held constant during both stages of any lineage. Further details on the two evolutionary stages are provided below.

First evolutionary stage: All runs started with a hand-written ancestor, which had a genome of 100 instructions. The ancestor was capable of self-replication, but it could not perform any logic functions. Ten replicate experiments were run with each of five genomic mutation rates (U = 0.1, 0.3, 1, 3, 10) and with reproduction being either strictly asexual or sexual for the population. Thus, there were 100 runs of the first stage. Replicates differ only in the random number seed, which then affects all the stochastic events during the run, such as mutations and offspring placement. The maximum population size (N) was 3600 organisms for all runs in the first stage. Genome length was held constant in all runs. Nine different resources could be obtained by digital organisms that evolved the ability to perform logic functions; these resources were available in infinite supply. Experiments ran for 100,000 updates, where an update is an arbitrary unit of time in Avida corresponding to the execution of 30 instructions, on average, per individual organism. In these first-stage runs, one generation required roughly 10 updates; the exact value depends on the number of instructions needed to produce an offspring, which often changes during evolution. At U = 0.1 and 0.3, the experiments ran for an additional 500,000 updates in order to compensate for the slower adaptation at these lower mutation rates; this extension ensured there were genotypes that could use all nine resources in each first-stage treatment. During each run, we recorded the numbers of organisms using each resource as well as the mean and highest fitness in the population. At the end of each run, we saved the most fit genotype (provided it was able to use all nine resources) for use in the second evolutionary stage.

Second evolutionary stage: These runs used the pool of well-adapted genotypes from the first evolutionary stage as starting material to investigate the effect of Muller's ratchet on small populations. Each small population had the same mutation rate and same reproductive mode as its first-stage progenitor. For each of the five mutation rates, one sexual and one asexual organism were randomly chosen from the pool of genotypes saved at the end of the first-stage runs. Each of these ten genotypes (also referred to as proximate ancestors) was then used to start 100 replicate experiments

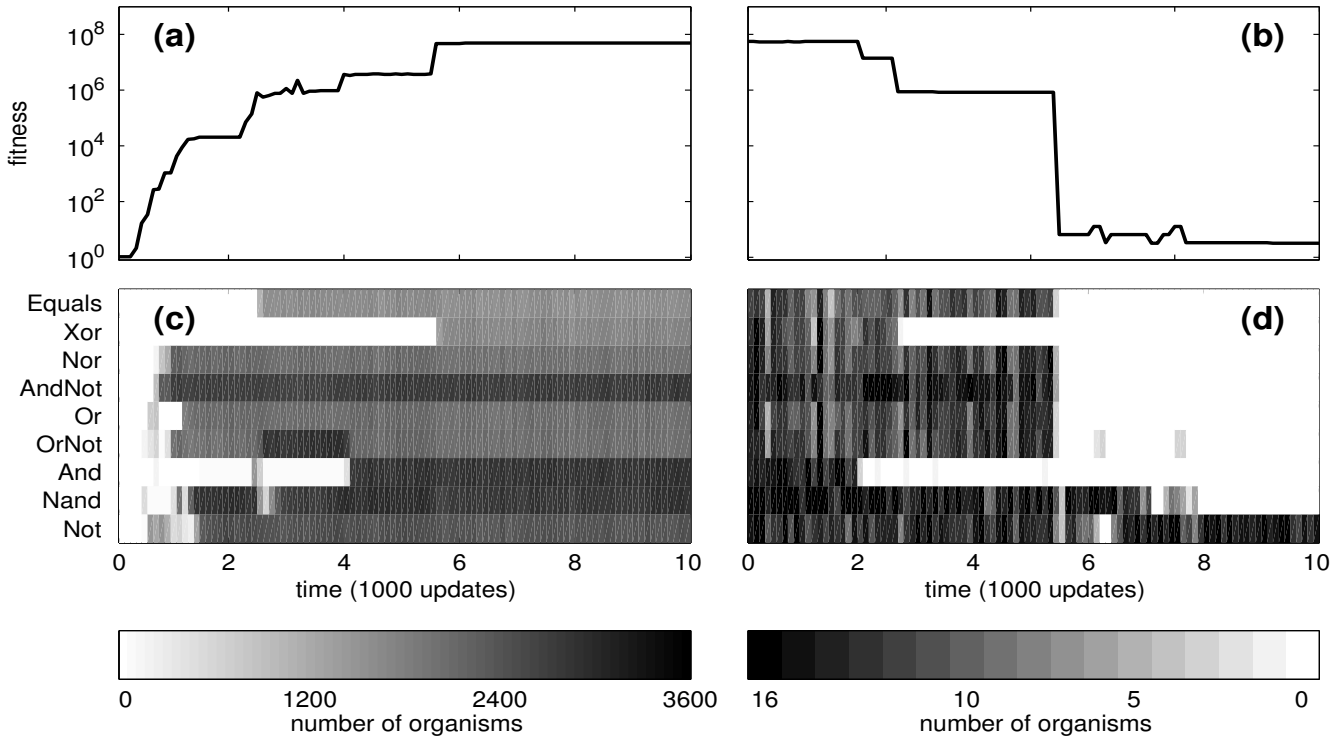

Figure 1. Trajectories for maximum fitness and resource use during evolution in large and small asexual populations. (a) Maximum fitness in a large population (N = 3600) during the initial 10,000 updates with genomic mutation rate U = 0.3; **(b)** Maximum fitness in a small population (N=16) that began with the most fit genotype from (a), and which continued at the same mutation rate; **(c)** Number of organisms performing each of the nine rewarded logic functions, indicated by shading intensity (scale below), in the same large population as in (a); **(d)** Number of organisms performing these logic functions in the same small population as in (b). Note the different scales in (c) and (d).

Reproduction: asexual, sexual		Genomic mutation rate, U				
		0.1	0.3	1	3	10
Population size, N	4	75, 80	**8, 41**	0, 1	0, 0	0, 0
	8	98, 100	92, 98	**60, 87**	0, 3	0, 0
	16	100, 100	100, 100	98, 99	**67, 95**	0, 0
	32	100, 100	100, 100	100, 100	97, 99	**18, 70**
	64	100, 100	100, 100	100, 100	99, 100	98, 99
	128	100, 100	100, 100	100, 100	100, 100	100, 100

Table 1. Survival of asexual and sexual populations when population size is small and Muller's ratchet operates. The two numbers in each cell show the number (out of 100 runs) of surviving asexual and sexual populations, respectively, for each combination of genomic mutation rate and maximum population size. Paired values are shown in **bold** when they were significantly different (see text for details).

at each of six small population sizes (N = 4, 8, 16, 32, 64, or 128), for a total of 6000 second-stage runs. All parameters other than population size were identical to those in the first-stage experiments. The second-stage runs lasted for 500,000 updates, during which we again recorded the number of organisms using each resource, as well as the mean and highest fitness. We also recorded whether the population had gone extinct by the end of the run.

Results and Discussion

Adaptation and decay: In the first stage of the evolution experiments, several populations at each mutation rate produced highly adapted genotypes that could perform all nine logic functions and thereby obtain the corresponding resources. Figure 1a shows the maximum-fitness trajectory over the first 10,000 updates for a representative first-stage population that evolved with U = 0.3 while reproducing asexually. Figure 1b shows the corresponding trajectory for a second-stage population founded by the most fit genotype from the first stage, but with the maximum population size now reduced from 3,600 to 16 organisms. The step-like changes in maximum fitness are typical of the experiments. The steps reflect, in large measure, the adaptive gains and maladaptive losses of logic functions that occurred in the large and small populations, respectively (Figs. 1c and 1d).

Population survival and extinction: In order to test if sexual reproduction could substantially impede Muller's ratchet, we compared the number of sexual and asexual populations that survived to the end of the second stage. Recall that mutation accumulation by Muller's ratchet can cause individuals to fail to reproduce and die, leading to a decline in population size which, if severe, might cause a mutational meltdown and eventual extinction. In total, we compared the fate of asexual and sexual populations under 30 different combinations of mutation rate and population size (Table 1). For 13 combinations with relatively large population sizes, low mutation rates, or both, all 100 asexual and all 100 sexual populations survived to the end of the experiment. In 4 combinations subject to both high mutation rates and small population sizes, all 200 populations went extinct, regardless of their reproductive mode. In the remaining 13 combinations of mutation rate and population size, the number of surviving sexual populations was greater than the number of surviving asexual populations. For 4 of these combinations, the difference was significant based on Fisher's exact test (two-tailed $p < 0.05$) with a Bonferroni correction to adjust for performing 30 tests (Sokal and Rohlf 1995). Also, the likelihood that all 13 cases in which there was a difference would, by chance, trend in the same direction is very small (binomial test, $p < 0.001$). The data on population survival and extinction therefore support the hypothesis that sexual reproduction can slow the advance of Muller's ratchet and prevent mutational meltdown. On the other hand, most of the differences in extinction probabilities are fairly small, they depend on the particular parameter values for mutation rate and population size, and the survival advantage to sex requires thousands of generations to be manifest.

Mean fitness of surviving organisms: While the data on population survival are consistent with the hypothesis that sex is beneficial in opposing Muller's ratchet, the mean fitness values of survivors suggest a more complicated picture. Owing to the large number of experiments (100 populations for each of 60 combinations of population size, mutation rate, and reproductive mode), we cannot present all of the fitness data. However, Figure 2 shows the most important patterns. All of the populations in this figure evolved with genomic mutation rates set to 0.3; the three panels show data obtained for population sizes of 4, 16, and 64. Fitness values are expressed relative to the proximate ancestor, and were transformed owing to their tremendous range. At the lowest population size (Fig. 2a), surviving sexual populations had slightly higher mean fitness values than did their surviving asexual counterparts, although this difference was not significant (two-tailed t-test, $p > 0.5$). For both reproductive modes, the final mean fitness values were very low relative to the ancestors. The situation was more complicated, however, at somewhat larger population sizes (Figs. 2b and 2c). As expected, the

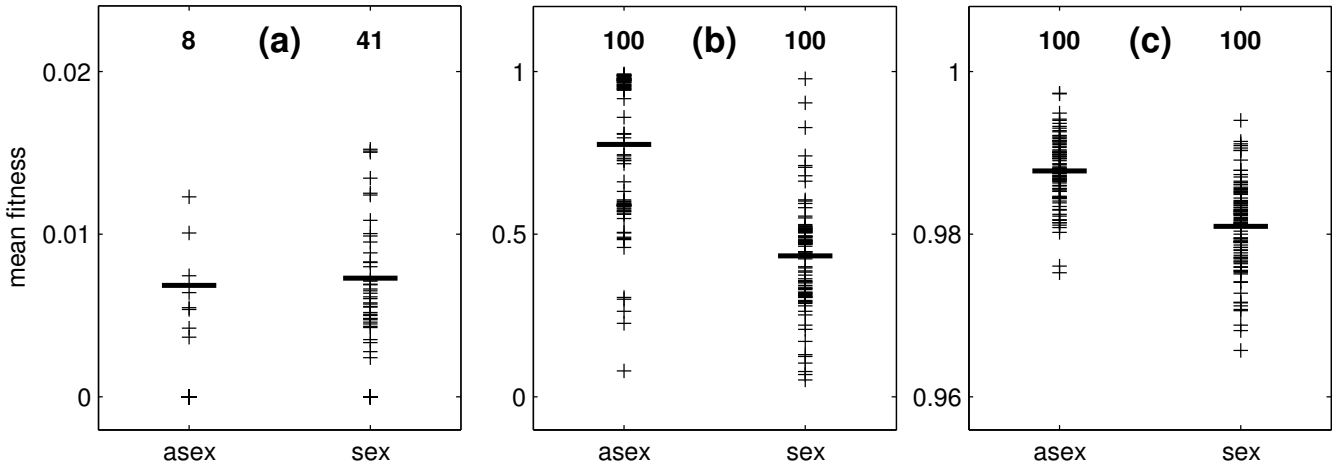

Figure 2. Distributions of mean fitness in asexual and sexual populations that survived Muller's ratchet. Panels **(a)**, **(b)**, and **(c)** correspond to maximum population sizes of 4, 16, and 64, respectively. All populations shown here evolved with a 0.3 genomic mutation rate. Asexual and sexual populations are shown at the left and right, respectively, in each panel. Each + symbol shows the mean fitness of one surviving population; the horizontal bar shows the mean value across the surviving populations for each treatment. The numbers of surviving populations in each treatment are shown along the top (see Table 1). Owing to the tremendous range of fitness values within and between treatments, mean fitness, W, is transformed as $\log(W+1)/\log(W_0)$, where W_0 is the fitness of the proximate ancestor. Note the changes in fitness scale between panels.

mean fitness under both reproductive modes was much higher at N = 16 than at N = 4, and mean fitness was higher still with N = 64. Unexpectedly, however, the asexual populations had higher mean fitness than did the sexual populations at these larger population sizes (two-tailed t-test, both $p < 0.0001$). It appears that sexually reproducing populations, while better able to survive Muller's ratchet in very small populations, may accumulate more harmful mutations than asexual populations at somewhat larger population sizes. Data obtained from other combinations of population size and mutation rate gave similar results.

Distribution of mutational effects: As a first effort to understand one factor that might have contributed to lower mean fitness of sexual than asexual populations, we subjected the proximate ancestors to an in-depth mutational analysis. We constructed all 2500 one-step mutants (25 alterative instructions at each of 100 genomic sites) for the first-stage sexual and asexual genotypes that were ancestral to the populations shown in Figure 2. Table 2 shows the fraction of one-step mutations that were lethal, deleterious (non-lethal), neutral, and beneficial. The asexual genotype had a substantially higher proportion of mutations that were lethal, while the sexual type had a correspondingly higher proportion of deleterious but non-lethal mutations. Those populations derived from this asexual genotype would have had a higher risk of extinction, especially in the smallest populations, as a consequence of the higher fraction of lethal mutations. But surviving asexual populations might also have been purged of their most deleterious mutations, leaving these survivors with higher fitness than those from the sexual populations. The generality of these differences as a function of reproductive mode remains to be seen, as do such other factors as the extent and form of epistatic interactions between mutations (Lenski et al. 1999). But these preliminary data do suggest that prior evolution under the different reproductive modes can influence subsequent evolution. In other words, there are multiple interacting and dynamical feedbacks that shape evolving genomes, and they will complicate efforts to discern the various forces responsible for the origin and maintenance of sexual reproduction (Lenski 1999).

	Lethal	Deleterious	Neutral	Beneficial
Sexual	0.2420	0.6392	0.1088	0.0100
Asexual	0.4236	0.5052	0.0708	0.0004

Table 2. Distribution of one-step mutational effects on fitness in sexual and asexual ancestors of some second-stage populations. The proportion of the 2500 different mutations are shown for one sexual genotype and one asexual genotype that evolved during the first stage with U = 0.3, and which then served as the ancestors for the second-stage evolution shown in Figure 2.

Summary and future directions: Sexual reproduction has several disadvantages relative to asexual reproduction, which begs the question of why sex is common in nature. Many potential advantages of sex have been hypothesized, including that sexual reproduction opposes the maladaptive effect of Muller's ratchet in small populations. We used the Avida software to perform evolution experiments with digital organisms that would test this hypothesis. Our results demonstrate the effect of Muller's ratchet in small populations. At high mutation rates and in very small populations, the ratchet often led to mutational meltdowns

caused by the vicious cycle of mutation accumulation and population decline. Sexual populations survived this effect significantly better than asexual populations, but only over a fairly narrow range of parameter values. Opposing this advantage, surviving organisms in asexual populations unexpectedly had higher mean fitness than those in sexual populations at some other parameter values. This last result points toward the need for systematic analyses of the effect of reproductive mode on genetic architecture, including the distribution of mutational effects on fitness as well as the extent and form of epistatic interactions among mutations. Avida is well-suited for such analyses, which we intend to pursue in our future work on the evolution of sex.

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