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Can sexual selection drive extinction and speciation?
A case study in yeast

A thesis submitted in partial fulfillment of the requirement
for the degree of Bachelor of Science in Biology from
The College of William and Mary

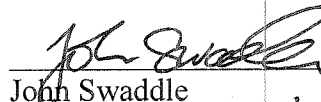
by

Luke Philip Reding

Accepted for Honors



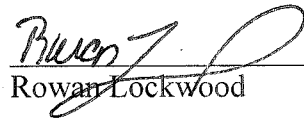
Helen Murphy



John Swaddle



Matthias Leu



Rowan Lockwood

Williamsburg, VA
April 24, 2012

INTRODUCTION

Natural selection is a mechanism of evolution whereby the heritable characteristics of organisms that contribute to increased survival and reproduction are better represented in future generations. Individuals in a population that carry phenotypes (i.e. the physical expression of the genes) that enable them to reproduce and survive better leave more offspring relative to individuals with other phenotypes; hence phenotypes that are linked to increased reproduction will increase in frequency over time in the population.

Sexual selection is the subset of natural selection that describes the collection of evolutionary processes that lead to differential mating success among individuals (Andersson 1994). Sexual selection is usually partitioned from the rest of natural selection for several reasons. First, sexual selection acts on phenotypes involved in mating or obtaining mates, whereas natural selection encompasses a broader suite of traits that also influence the probability of survival. Hence, for a given phenotype, natural and sexual selection can act in opposite directions (see below). Second, the mating interests of males and females are often not aligned with one another. Classically, males invest little in a single reproductive encounter and so compete to get access to more and more females, whereas females invest much more time, energy, and resources in a mating attempt and hence tend to be more choosy about who they mate with (Trivers 1972). This conflict in interests often sets up an evolutionary arms between the sexes over mating strategies, sometimes leading to the evolution of traits that promote mating opportunities for one sex while directly harming the opposite sex (Hotzy and Arnqvist 2009; Johnstone and Keller 2000; Rice 1996). Overall, sexual selection can lead to a reduction in individual and population fitness not predicted by natural selection alone.

In any population of sexually reproducing organisms there is potential for sexual selection. The greater the differences in mating success between individuals in a population, the stronger the potential action of sexual selection. Sexual selective strength is therefore strongly tied to the mating skew in the population (i.e. the skew in distribution of mating success). When all individuals in the population obtain equal mates or mating opportunities, there is little to no potential for sexual selection to act. I will refer to this as weak sexual selection. Weak sexual selection characterizes many monogamous bird species that mate once and rarely seek extra-pair matings (e.g. *Diomedea exulans*, Weimerskirch et al. 2005; *Branta leucopsis*, Black 2001; for monogamous mammal species, see Kleiman 1977). Alternatively, if a few individuals in a population obtain all the mating opportunities, while the majority of individuals consequently obtain no mates, there is great potential for sexual selection acting on the heritable traits of those few who obtained all the matings. I will refer to this as strong sexual selection. Túngara frogs (*Physalaemus pustulosus*) exhibit such a strong mating skew where many males obtain no or few matings, while a few males obtain more than ten (Fig. 1) (Ryan 1983).

Importantly, sexually selected traits are costly. The existence of these costs (e.g. time looking for mates, increased predation risk due to increased conspicuousness, mate-mate contests, production and maintenance of elaborate traits, sexual conflict; Andersson 1994; Andersson and Iwassa 1996; Chapman et al. 1995) makes it likely that strong sexual selective pressures will cause traits to vary from their ecological optimum, thus adversely affecting the survival of individuals while maximizing mating success for each sex.

While empirical examples of the costs of sexually selected traits abound (e.g. Chapman et al. 1995; Gerber et al. 2010; Hamilton 1979; Holland and Rice 1999; Promislow et al. 1992), a particularly illustrative example can be seen in the aforementioned túngara frogs (*P. pustulosus*).

Females of this species prefer to mate with males that advertise with more complex calls (Akre and Ryan 2010), yet frog-eating bats (*Trachops cirrhosus*) preferentially cue into these more elaborate calls (Ryan et al. 1982). Thus males advertising with complex calls are simultaneously experiencing a greater predation risk and greater mating success. This idea is depicted graphically in Fig. 2, where the difference between θ and y represents the difference in selective pressures between natural and sexual selection, respectively. The resulting phenotypic distribution is a compromise between the natural and sexually selected optima (Panel e in Fig. 2).

Though sexual selection has been extensively studied on the individual level, relatively little attention has been paid to the macroevolutionary consequences of sexual selection. One primary prediction is that because sexual selection is associated with reduced survival for the individual, strong sexual selection pressures could lead to extinction (Dawkins and Krebs 1979; Kirkpatrick 1982; McLain 1993), especially when environmental conditions change (Kokko and Brooks 2003; Tanaka 1996).

Despite this prediction, the relationship between sexual selection and extinction has remained largely experimentally untested. Though several studies have attempted to characterize this relationship, all have relied on correlational evidence and proxies for both extinction risk and strength of sexual selection. For example, several studies have investigated whether dichromatic bird species, assumed to experience more intense sexual selection, suffer from lower establishment rates when introduced to islands. While there is some evidence for this (Cassey 2002; McLain et al. 1995; McLain et al. 1999; Sorci et al 1998), others have failed to identify such a relationship (Donze et al. 2004). Ultimately, such studies 1) cannot rule-out overlooked factors that actually explain the patterns in the data; 2) rely on correlational evidence and thus cannot show that sexual selection is really the force driving the observed patterns; and 3) are

often subject to bias depending on the type of sexually selected trait used as a surrogate for overall sexual selective pressure (Morrow and Pitcher 2003), as well as trait(s) used for extinction risk.

Though previous experiments have investigated how sexual selection influences individual-level components of fitness which may be related to population viability (Holland and Rice 1999; Promislow et al. 1998; Radwan et al. 2004), only one to my knowledge has explicitly investigated the relationship between sexual selection and extinction. Jarzebowska and Radwan (2010) examined prevalence of population extinction in fifty lines of the bulb mite *Rhizoglyphus robini* that were either allowed to mate normally or underwent enforced monogamy. The biological relevance of this study is unclear, however, as each population consisted of a scant five individuals.

Interestingly, the second primary prediction concerning the effect of sexual selection over evolutionary time involves speciation, in some sense the opposite of extinction. Strong sexual selection pressures coupled with a change in some mate preference or phenotype within a population can cause prezygotic isolation between populations well before other genetic or ecological incompatibilities arise. This ultimately leads to reproductive isolation between populations, creating new species (Andersson 1994, Panhuis et al. 2001). Thus, it is thought that strong sexual selection can be a significant factor contributing to speciation processes (Coyne and Orr 2004; Higashi et al. 1999; Kirkpatrick and Ravigné 2002; Lande 1981; reviewed by Panhuis et al. 2001). Both these predictions contribute to the idea of sexual selection acting as a ‘double-edged sword,’ both promoting speciation and extinction (Morrow and Pitcher 2003; Owens et al. 1999).

As with extinction risk, the relationship between sexual selection and speciation has remained elusive. Studies examining this relationship suffer from the same inherent flaws of those describing sexual selection and extinction. Many studies, examining widespread taxonomic groups, have found some support that some measure of sexual selection (e.g. plumage dimorphism, testis size, number of mating partners) is associated with more specious genera (Arnquist et al. 2000; Barraclough et al. 1995; Mank 2007; Moller and Cuervo 1998; Owens et al. 1999). Other studies have failed to find such relationship (Gage et al. 2002; Morrow et al. 2003). Whether such a pattern is observed is apparently dependent on the traits analyzed, how speciation is measured, and the extent to which non-independence among species phenotypes due to ancestry is controlled for. Again, these comparative studies are correlational and cannot show that sexual selection does indeed promote species divergence if such a pattern is observed.

What is missing is a study that experimentally manipulates sexual selection strength and examines the resulting changes in risk of extinction and potential for speciation. Such a study could show causation of sexual selection instead of presenting correlational evidence of sexual selection's effects over macroevolutionary time. It could also potentially help explain patterns of evolution in the fossil record, explain patterns of biodiversity and phylogenetic relatedness, identify species of conservation concern, as well as shed light on the mechanisms that lead to the origin of species. The difficulties in tracking populations and species over a timescale necessary to test the effects of sexual selection on extinction and speciation have made such a controlled experiment logistically impractical to date.

Here, I use replicate populations of baker's yeast (*Saccharomyces cerevisiae*) founded from a single individual colony to investigate the macroevolutionary consequences of sexual selection. Yeast reproduce both sexually and asexually (Replansky et al. 2008), have a short

generation time, the strength of sexual selection can be experimentally controlled (Rogers and Greig 2009), and some measure of extinction (Lenski et al. 1991) and speciation (Dettman et al. 2007) can be ascertained.

Yeast are emerging as model organisms for studies of evolution and sexual selection (Replansky et al. 2008). Diploid yeast undergo sporulation (meiosis) when starved of nutrients, creating four spores: two of mating type MAT_a and two of mating type MAT_α (see Fig. 3 for yeast lifecycle). Mating in yeast occurs when haploids of opposite mating types fuse to return to the diploid stage. Yeast mating is pheromone-dependent. Both MAT_a and MAT_α haploid cells produce mating pheromones: MAT_a cells produce ‘a factor’ encoded by the MFA1 gene, while MAT_α cells produce ‘α factor’ encoded by the MFA2 gene (Rogers and Greig 2009). This pheromone has been shown to be costly, with phenotypically and genetically inferior individuals paying a higher cost of pheromone production (Smith and Greig 2010). Haploid yeast cells respond across a concentration gradient to the haploid of the opposite mating type that secretes the most pheromone (Jackson and Hartwell 1990). A ‘high α-signaling’ allele experimentally introduced into a mixed population of pheromone-deficient MAT_a and ‘low α-signaling’ MAT_α haploids was shown to increase almost to fixation in five of six replicate populations experiencing strong sexual selection (Rogers and Greig 2009), suggesting that mate choice in yeast favors strong pheromonal signalers (Jackson and Hartwell 1990).

In this experiment, yeast strains were first genetically altered with specific antibiotic resistances and auxotrophic markers to allow for selection during different parts of the yeast life cycle (diploids, MAT_a, and MAT_α cell types in isolation). This allowed us to obtain pure cultures of MAT_a and MAT_α cells. I then created four replicate populations for each of five treatment groups: asexual (to control for the effect of sex), weak sexual selection, strong sexual

selection, and two intermediate treatments. (These intermediate treatments are not reported below due to time limitations.) The populations were subjected to different intensities of sexual selection by manipulating the ratio of MAT α to MAT α cells, creating situations of more extreme reproductive skew in each successive treatment group (Figs. 4 and 5). All 20 populations were evolved for a total of 180 generations, or six experimental cycles.

Extinction risk was assayed two different ways. First, we estimated rate of adaption to the new experimental environment. If sexual selection increases extinction risk, we would expect that populations experiencing strong sexual selection adapt at a slower rate than those experiencing weak sexual selection (Fig. 6). Next, we assayed extinction risk by challenging the populations to a new environment by altering the temperature. Similarly, we expect that the populations experiencing strong sexual selection to fair poorly in a new environment compared to a populations experiencing weak sexual selection. Speciation potential was assayed by spore viability resulting from crosses between population within treatment groups. If sexual selection increases genetic divergence between populations, crosses between populations experiencing strong sexual selection should yield fewer viable spores than those crosses between populations experiencing weak sexual selection (Fig. 7).

MATERIALS AND METHODS:

Strains:

Our goal was to arrange antibiotic and auxotrophic markers such that a unique combination of each was expressed in each cell type (diploid, MATa, and MAT α cells). Such a system allows selection of a specific cell type from media containing one or more undesirable types of cells. This is an important aspect of our experimental design when 1) selecting for diploids and 2) obtaining pure cultures of MATa and MAT α cells. We therefore constructed a MATa strain that alone could grow in the presence of the antibiotic hygromycin (hyg) but without the amino acid histidine, a MAT α strain that could alone grow without the addition of the amino acid leucine. Finally, we placed two different antibiotic markers at the same locus, one in each haploid strain, to ensure that diploids would be able to grow in the presence of both kanamycin (kan) and cloNAT (nat).

MATa construct: YPS3340 (see Table 1 for all strain genotypes) was crossed with YJN27 to produce a MATa yeast isolate termed Isolate H. *Ste2p* is the promoter to the gene that codes for the receptor for MAT α mating pheromone, and is only activated in MATa cells. Coupled to the *hyg* marker, which confers resistance to the antibiotic (hyg), this construct allows selection for MATa cells in our experimental cycle.

MAT α construct: YJN20 was crossed with Y8205 to obtain MAT α Isolate C. Importantly, this isolate contained the construct *lyp1 Δ ::Ste3p-leu2*, a deletion of the *lyp1* gene, which is one of three amino acid permeases and exhibits functional redundancy. This gene is replaced by the *Ste3p-leu2* construct. *Ste3p* is the promoter of the gene that codes for the receptor for MATa

mating pheromone and is only expressed in MAT α cells. This promoter was coupled to *leu2*, which is deleted in the Y8205 genetic background. This construct allows for selection of MAT α cells even when present in both MAT α and MATa cell types.

Isolates C and H were then crossed to obtain Isolate J. This Isolate J was then crossed to YPS3060 to obtain YLR13, the ancestral MAT α . A second cross of Isolates C and H produced Isolate K. Isolate K was crossed with Isolate J to produce YLR12, the ancestral MATa. YLR13 and YLR12 were mated to produce YLR11, the ancestral diploid.

In all ancestral strains, the mating type switching gene, *ho*, was deleted and replaced by an antibiotic marker (*nat* in MATa and *kan* in MAT α). This allowed selection of diploids (which have two copies of the *ho* gene and thus double antibiotic resistance) from cultures that contained unmated haploid cells. Deleting the *ho* gene was important for another reason: an individual haploid yeast cell with an intact *ho* gene can divide by mitosis, undergo a switch to the opposite mating type, then mate with newly cloned self. Because we wanted to specify the ratio of MATa to MAT α cells in each of our experimental groups, this gene was deleted to prevent mating type switching.

Marked Strains. Fitness assays (see below) were used to determine the relative fitness of the evolved strains relative to the ancestral condition. To use this assay, it was necessary to visually distinguish the evolved from the ancestral strain when plated on agar. A ‘marked’ (pink-colored) ancestral strain (YLR14) was therefore created, which is similar to the ancestral diploid (YLR13) except that it contains an adenine auxotrophy (*ade2*^{-101^{ochre}}), which inactivates the gene SUP4

and makes the colonies of these yeast appear pink (Fig 8). This way, we could easily distinguish between our evolved (white) and ancestral (pink) strain colonies growing on an agar plate.

To create YLR 14, YMZ336, a diploid homozygous for the adenine autotrophy described above, was sporulated and dissected into MAT α and MAT α cells. One of these MAT α cells was crossed with YLR12 and the resulting diploid dissected. The resulting haploids were plated to selective media and YLR15 was identified as having the same phenotype of the ancestral type YLR12, with the addition of the pink-inducing adenine auxotrophy. Similar crosses were done with YLR13 to obtain YLR16, the our marked ancestral MAT α . Finally, YLR15 and YLR16 were mated to obtain YLR14, the marked ancestral diploid.

Media:

A novel media type termed Evolving Medium (hereafter EM; 0.1% dextrose, 0.17% yeast nitrogen base, 0.1% glutamic acid, 250 mg/L histidine, 250 mg/L leucine, 0.2M NaCl), was used to evolve the yeast throughout the experiment. 0.1% dextrose represents one twentieth the dextrose amount of standard growth media (see YPD below). This limited dextrose concentration, along with the addition 0.2M salt, was used to create a harsh environment to which the yeast could adapt during the course of the experiment. This allowed examination of how adaptation proceeds under the treatments outlined below. In a medium where the yeast are well-provisioned, there is little differential survival and thus little room for selection to act (see blue and yellow dots in Fig. 1 of Goddard et al. 2005). Glutamic acid was substituted for ammonium sulfate (used in standard recipes) because it was more amenable to the use of antibiotics in media (The McCusker Lab: Resources). 250 mg/L represents an abundance of

histidine and leucine; typically less than 100 mg/L is used in standard preparations. We used an abundance of these amino acids to exclude the possibility of generating selection pressures that favored mutants able to grow on reduced histidine or leucine, which would compromise our experimental design.

Diploid growth medium. Diploid populations were evolved in EM supplemented with the antibiotics Kanamycin (kan) and cloNat (nat) at 375 mg/L and 250 mg/L, respectively. These were high concentrations, but high concentrations are needed when using double antibiotics medium (The McCusker Lab: Resources), and we wanted to ensure nothing except our ancestral diploid was able to grow in the EM.

MATa selection medium. EM without histidine was used to select for MATa cells (MATa cells can produce histidine endogenously; MAT α cells contain a fatal histidine auxotrophy). This medium was supplemented with 400mg/L Hygromycin (hyg), another antibiotic for which MATa cells express resistance.

MAT α selection media. EM without leucine was used to select for MAT α cells as MAT α but not MATa cells produce leucine endogenously.

Competition assay medium: Competitions were performed in EM to which 100 mg/L adenine was added (the marked strains contain an adenine auxotrophy).

Sporulation media (1% potassium acetate, 250 mg/L histidine, 250 mg/L leucine, and 1.5% agar) was autoclaved and pipetted into the wells of a 24-well plate. YPD plates were 1% yeast extract, 2% peptone, 2% dextrose, and 1.5% agar.

The zymolyase stock solution was prepared by combining 3.6g sorbitol, 20 mg zymolyase 100T, and 1 ml 2-mercaptoethanol with 19 ml sterile water (J. Jasmin, personal correspondence), filter sterilizing, and stored at -80°C until use.

Ancestral strains were suspended in 15% glycerol and frozen at -80°C until use. All twenty populations of the evolved strains were frozen every three experimental cycles.

Treatment Groups:

We evolved four replicates of five treatment groups under various strengths of sexual selection by varying the number of MAT α cells relative to the number of MATa cells. The greater the disparity between the two, the greater the potential for sexual selection to occur, as there is greater variation in mating success when several MAT α cells go unmated. This is common way of manipulating the strength of sexual selection (Holland and Rice 1999; Promislow et al. 1998; Radwan et al. 2004; Rogers and Greig 2009). Four of the five treatment groups involved direct manipulation of the mating type ratio at the mating stage of the experimental cycle (in order of increasing sexual selection; all are MATa:MAT α): 1:1, 1:2, 1:5, and 1:10 (Fig. 5). 1:10 was chosen as the most intense sexual selection treatment because this should be approximately the number of MAT α cells that can surround a MATa cell on the two-dimensional surface of the

mating plate. The two intermediate treatment groups are not reported here due to time limitations. The 1:1 treatment group is termed the weak sexual selection group, as all haploid yeast had equal mating opportunities. The 1:10 group is termed the strong sexual selection group, as each MAT α cell could choose between ten MAT α cells, resulting in differential mating success among the MAT α cells. A third treatment, termed the asexual group, was not allowed to undergo meiosis in order control for any effects of sex regardless of sexual selection strength.

Experimental Cycle:

Each experimental cycle lasted one week and provided four days for pure diploid cultures to adapt to the novel media. Once per cycle, the diploids in each population were sporulated and placed in selective media to obtain pure cultures of MAT α and MAT α cells. These were recombined in various mating type ratios (depending on the treatment group), given ample time to mate, and the cycle began anew.

Serial transfers. A single diploid colony was used to found all populations to begin the experiment. This colony was grown in EM for 24 hours at 30°C, then transferred to 2ml EM in 20 wells of a 24-well plate. After 24 hours of asexual growth, 20 μ L of each population was transferred to a new 24-well plate containing fresh media and replaced in the 30°C incubator. This was done a total of three times during each cycle, representing four 24-hour periods of diploid growth.

Meiosis. 24 hours after the final serial transfer, each population (with the exception of the four asexual populations that were placed at 4°C until the serial transfer phase) was induced to undergo meiosis. Each population was transferred to a 15ml Falcon tube and centrifuged at 3,000 rpm for 3 minutes at room temperature. The supernatant was discarded and each population was washed once with 2 ml sterile water and was resuspended in 50µL water. Each population was then transferred to a sporulation plate (which allows for a good sporulation rate (>90%, personal observation) to be achieved over a short period of time) and incubated at 30°C.

Roughly 36 hours after placing the populations on sporulation plates, sporulation was verified for a subsample of all population through visual inspection via microscopy. The sporulated cells were resuspended in 1ml water and placed in a 15ml Falcon tube. This was placed at 55°C for 45 minutes to kill all diploid cells (Rogers and Greig 2009). Elimination of diploids was necessary to ensure only cells undergoing meiosis and subsequent sex were passed through to the next cycle.

Isolation of pure haploid cultures. 100 µL zymolyase stock solution was added to each population to dissolve the ascii protecting the haploid spores. These were transferred to a rotating wheel for 4 - 4.5 hours at 30°C. 2 µL Triton X was then added to each tube and each population was vortexed on high for at least five minutes to separate spore clumps that can form during the zymolyase digestion. Each population was then centrifuged and the supernatant discarded. Each population was resuspended in 500 µL distilled water, and half of each population was transferred to MAT α or MAT α selective medium (see media above).

Mating and sexual selection. After 24 hours, pure MAT α and MAT α cultures were obtained. MAT α and MAT α cells from the same population were then recombined in various ratios by volume (the following are MAT α : MAT α): 1:1, 1:2, 1:5, 1:10. The number of MAT α cells was constant across treatment groups to equalize effective population size across treatments. The appropriate volumes of cells were centrifuged at 13,300 rpm at room temperature for two minutes and resuspended in a small amount of water (~20 μ L). The MAT α and MAT α cells from the same population were placed on solid EM plate and mixed thoroughly by pipetting up and down to ensure mixing of the two cells types. The populations were then left overnight to mate at 30°C.

Diploid selection. The following morning, ~12 hours after mating, EM (supplemented with kan and nat, the two antibiotics at the *ho* locus) was placed on top of the mated cultures to select for diploids. The serial transfer process began again after 24 hours.

Generations per cycle. The yeast populations grew to confluence after our 1:100 serial dilution in a 24-hour period, yielding 6.6 generations (assuming discrete doubling-- 2^x generations--yeast reach carrying capacity after 100-fold dilution; therefore: $\log(100)/\log(2) = 6.64$ generations). After four days of diploid growth, ~26 generations were obtained. The remaining portion of the experimental cycle was spend either on sporulation media (where no cellular divisions should occur) or in the selection media. Therefore, approximately 30 generations were obtained per cycle.

Population Assays

Relative Fitness Assays. Fitness relative to the ancestral condition was used as a measure of general adaptation to a static environment and thus resistance to extinction. We expect that because sexual selection carries costs for individuals, these costs may be manifested at the population level and thus populations experiencing strong sexual selection may adapt to the harsh EM environment at a slower rate than populations experiencing weak sexual selection (Fig. 6).

Cultures of the marked ancestor and evolved strain to be competed were allowed to grow for 48 hours in EM + adenine (24 hours followed by a 1:100 serial transfer, then 24 hours of additional growth). After 24 hours, the optical density of each strain was taken and the strains were mixed together in appropriate volumes to ensure the number of each type of cell was roughly equal. Ten samples of this master mix were plated to YPD using a 1:5000 dilution with distilled water to determine the initial relative abundance of each strain. 20 μ L of this mix were then added to 10 wells of a 24-well plate containing 2ml EM + Adenine. After 24 hours at 30°C, the mix was diluted 1:100 and the cultures were allowed to grow for an additional 24 hour period. Two samples from each of the ten wells were then plated 1:5000 to YPD. The number of white (evolved) and pink (marked ancestral) cells were counted on each of the total 30 plates per fitness assay using a hand counter while marking individual colonies with a marker (see Fig. 8 for a representative plate).

Calculations of relative fitness were based on Lenski (1991). A Malthusian parameter (m) was determined for each of the ten samples for each population. This was calculated as:

$$m(A) = \ln \frac{\# A \text{ colonies after 48 hr competition} \times 100}{\# \text{ of initial } A \text{ colonies before competition period}}$$

The $\times 100$ term in the numerator refers to the 1:100 dilution from the master mix to the sample wells. The number of colonies after the 48 hour competition period was taken as the average of the two samples for a given well, while the initial number of colonies was taken as the average number of colonies from the ten samples of the original master mix. Relative fitness (W) was calculated as the ratio of Malthusian parameters, i.e.,

$$W = \frac{m(\text{evolved})}{m(\text{marked ancestor})}$$

Biologically, this term represents that the evolved strain increased at a rate W times that of the ancestral strain. Importantly, the marked ancestor and actual ancestor (YLR13 and YLR14) are identical in terms of fitness. Competition between these two strains yielded $W = 1.000$.

Adaptation to a novel environment. A key theoretical prediction from the literature is that sexual selection could increase the susceptibility of a population to extinction during environmental change (Kokko and Brooks 2003, Tanaka 1996). We sought to test this with our populations of yeast by competing the evolved populations with the ancestor in different environmental contexts. These competitions were carried out exactly as above, except that some aspect of the yeast's environment was altered.

In one assay, we competed the yeast in EM + adenine as above, but placed the yeast at 37°C, a non-optimal temperature.

Spore viability. Because sexual selection is thought to be related to speciation potential, we expect that sexual selection should increase the genetic incompatibilities between the yeast populations (Fig. 7). To test this, the viability of haploid cells resulting from crosses between replicate populations within each of the treatment groups was assessed.

At the sixth experimental cycle, each population was sporulated and placed in selective media, resulting in pure populations of either MAT α or MAT α cells. Within each of the three treatment groups, the resulting MAT α cultures were crossed with MAT α cultures from a different replicate population, for a total of (4 choose 2) six crosses per treatment group. The resulting diploid cells were sporulated and the viability of the resulting spores was recorded. These results are not yet reported here but are forthcoming.

Statistical analyses

All statistical analyses were performed in R (R Core Development Team 2012). To test for statistical differences in fitness between the treatment groups, mixed-model analysis of variance (ANOVA) was applied, using population number as a nested, random factor within a fixed treatment factor (weak sexual selection, strong sexual selection, or asexual) (Goddard et al. 2005). This accounts for the non-independence between fitness estimates from the same population. Including population as a random factor within each treatment group precluded the

use of orthogonal contrasts and Tukey's honest significant differences test to compare two treatment groups. Instead, additional ANOVAs between the two treatment groups of interest were performed.

RESULTS:

Susceptibility to extinction was assayed by calculating fitness relative to the marked ancestral diploid in various environmental conditions as a proxy for rate of adaptation to a new environment. First, relative fitness was calculated for each population in the asexual, strong sexual selection, and low sexual selection treatment groups in EM at 30°C after three experimental cycles (~90 generations). Overall, there were no significant differences between the three treatment groups tested (ANOVA, $F_{2,9} = 1.399$, $p = 0.298$) (Fig. 9). Interestingly, the strong sexual selection group as a whole appeared to suffer from little increase in relative fitness compared (treatment average $W = 1.006$) compared to the asexual (average $W = 1.044$) and weak sexual selection groups (average $W = 1.038$). There was much variation in fitness within each treatment group, especially the strong sexually selected group (green populations in Fig. 10). Interestingly, the fourth strong sexual selection population actually decreased in fitness over these first three cycles ($t = 2.89$, $d.f. = 15.04$, $p = 0.011$).

At the same time point, relative fitness at 37°C was also assayed. The distributions of fitnesses was different between treatment groups (ANOVA, $F_{2,9} = 10.68$, $p = 0.004$). Subsequent ANOVAs between each of the three pairs of treatments indicated that the asexual treatment had lower fitness than both the strong or weak sexual selection population (Fig. 11). There were no differences between the strong and weak sexually selected treatments (ANOVA, $F_{1,6} = 1.321$, $p = 0.294$). The asexual treatment showed relatively little between-population variation in comparison with the two other treatment groups, notably, again the strong sexual selection group (Fig. 12). The overall range of fitness values was much higher in the 37°C assay, as expected if the evolved populations have undergone significant adaptation to surviving in the EM.

DISCUSSION:

If sexual selection is related to extinction risk, we would predict a greater propensity towards extinction (slower rate of adaptation) in populations experiencing strong sexual selection compared to populations experiencing weak sexual selection. After three experimental cycles, we were unable to detect a statistical difference between the strong and weak sexual selection groups. Despite this, overall fitness of the strong sexual selection group was the lowest of the three treatments, near the ancestral fitness of 1.0 (Fig. 9). The fitness values for one of these populations was statistically lower than that of the ancestor. This may be indicative that sexual selection hampers adaptation thus reducing population viability and perhaps increasing extinction risk.

This reduction in fitness may be a consequence of increased pheromone production by MAT α cells, which could shift advantageous phenotypes away from their naturally-selected optimum to maximize mating success. Such an effect would be important, as it would show the natural evolution of a phenotype that increases mating success at the expense of naturally selected fitness over many generations in a large population. If this were true, we would expect those populations showing a decrease in fitness to produce more α -pheromone than those populations showing an increase in fitness. Rogers and Greig (2009), however, failed to detect fitness differences between two yeast lines expressing different amounts of pheromone, though in a more complete later analysis, pheromone production was shown to decrease fitness by over 30% (Smith and Greig 2010). This prediction could be tested using halo assays, which allow some measurement of pheromone strength (Rogers and Greig 2009).

If sexual selection reduces fitness in the wild as it may in the lab, what role does sexual selection play in conservation biology? Should we include metrics of sexual selection when determining those species that most deserve protection? Doherty et al. (2003) provide an interesting insight: birds experiencing greater sexual selection experience more frequent local extinctions, but also higher turnover rates via colonization events. These frequent colonizations mask the deleterious effects of sexual selection on a large scale, which would explain why large-scale studies that look at population dynamics with respect to sexual selection strength often fail to find any pattern. Dispersal ability may therefore be an important factor buffering populations under strong sexual selection from collapse. It would be interesting to experimentally test how dispersal factors play a role in maintenance of population viability in populations under strong sexual selection, a question that could presumably be addressed using an experimental system similar to the one developed here. An experiment that occasionally transferred yeast *between* populations and recorded the resulting fitnesses before and after these transfers could presumably illuminate how dispersal affects extinction probability in populations experiencing strong sexual selection. This may be an important research goal, especially as increased habitat fragmentation may impede the natural dispersal tendencies of organisms in the wild.

When competed at a higher temperature, the asexual populations suffered a severe fitness disadvantage compared to the two sexual treatments, which were roughly equivalent in terms of fitness. Populations experiencing strong and weak sexual selection appear to weather environmental changes the same, at least with respect to the one aspect of the environment altered here (temperature). Temperature is one of many aspects of a yeast's environment, and a more informative assessment of how sexual selection is related to extinction susceptibility in a

novel environment would encompass a broader suite of environmental factors, such as altering the carbon source used for metabolism or salt concentration of the media.

Importantly, relative fitness was significantly reduced in the asexual treatment compared to either of the two other treatment groups. This may be a consequence of the benefits of sex during adaptation. Sex can break up deleterious alleles from nearby advantageous ones, increasing the rate at which advantageous alleles become fixed and deleterious alleles are purged from the population (Goddard et al. 2005). This does not mean that fitness should not increase in the absence of sex, but rather that it will increase at a slower rate. This is consistent with the findings in this study (Fig. 11), and has been previously been documented in yeast (e.g. Goddard et al. 2005).

Overall, there was much between-population variation in fitness. Variation within treatments is expected, however, as the serial transfers (i.e. bottlenecking) of the populations during the experimental cycle represents a strong stochastic force, and the yeast cells that colonized the populations initially were a constraint on the subsequent evolution of the population. Populations founded by yeast cells that, by chance, harbored beneficial genetic mutations or alleles that allowed them to grow and reproduce faster in the EM could increase in fitness faster than those populations that weren't as fortunate. This between-treatment variation was probably aided by our use of yeast strains whose genome was roughly 75% from wild yeast isolates, which have greater standing genetic diversity on which selection can act than most laboratory yeast strains.

Even without the stochasticity induced by the serial transfers, the differences between populations of the same treatment group is consistent with how evolution has proceeded in similar experimental evolution experiments. For example, Rogers and Greig (2009) found that

evolution with respect to the frequency of an introduced high-signalling allele differed between populations experiencing strong sexual selection. The allele frequency was somewhat variable throughout the experiment in all populations, but reached fixation (or near fixation) in five of the six replicate populations. One population, however, saw a significant decrease in allele frequency to under twenty percent, demonstrating that evolution is often unpredictable. Even when evolving yeast in a very controlled environment without the use of serial transfers, fitness estimates can vary substantially between populations receiving the same treatments (see Fig. 1 of Goddard et al. 2005).

Interestingly, it seemed that range of fitnesses for the strong sexual selection treatment was much larger than any of the other two treatments. This is analogous to what we find in nature: sexually selected traits are much more variable than non-sexually selected traits.

Strong sexual selection pressures are also thought to increase speciation potential. We would therefore predict that the progeny (i.e. spores) of individuals from two populations experiencing strong sexual selection might suffer from reduced viability if sexual selection accelerates genetic divergence. Though data are still forthcoming, it is likely that there are few differences in spore viability between treatments. Genetic incompatibilities between populations accumulate very slowly over time and our ability to detect such differences are hindered by the length of the experiment. Furthermore, speciation in this context is largely dependent on prezygotic isolation. In our system, this would mean that a given haploid individual from a population under strong sexual selection would preferentially mate with an individual of the same population, and not of another (i.e. assortative mating). Perhaps the MATa individuals become more tolerant to α -pheromone, and thus more of it is required to initiate mating with a

MAT α individual. This is pure speculation, however, and potential for prezygotic isolation between populations of yeast has yet to be recognized.

Yeast have proved an effective system for studying how sexual selection effects populations in over several generations. In this study, the ability to estimate population-level fitness with relative ease is an especially powerful technique. Few experimental systems exist in which an evolved strain can be competed directly with its ancestor. Furthermore, speciation potential and rate of fitness change (as an estimate of likelihood of extinction) can be quantified without having to worry about potentially confounding extrinsic factors (e.g. climate change). Finally, using yeast also allows the use of various genetic tools. If, in a future experiment, divergence between yeast populations is observed, in theory the genes responsible for these incompatibilities could be identified. This is a main goal of speciation research (The Marie Curie SPECIATION Network 2012).

FIGURES

Figure 1

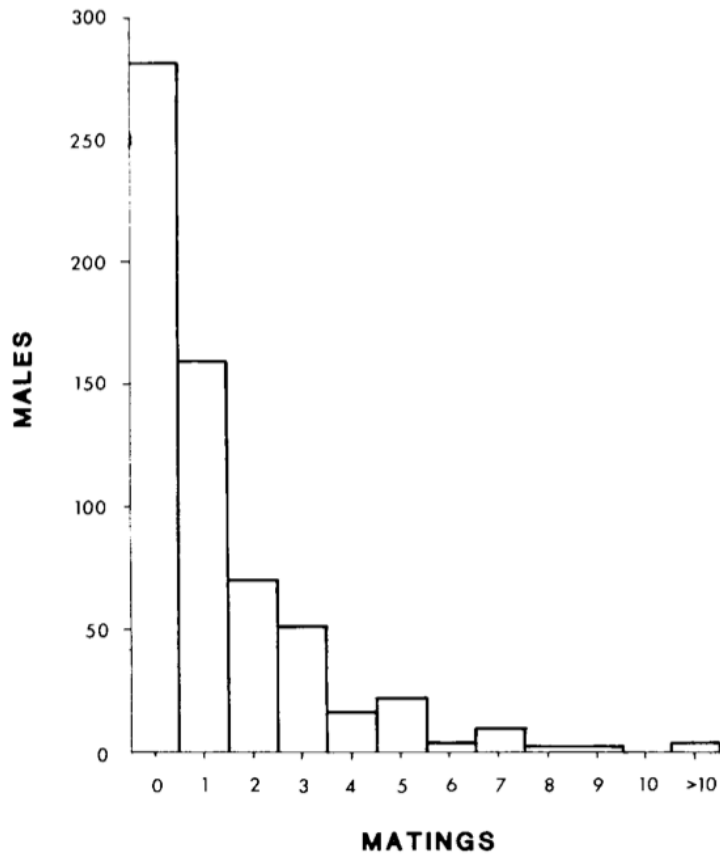
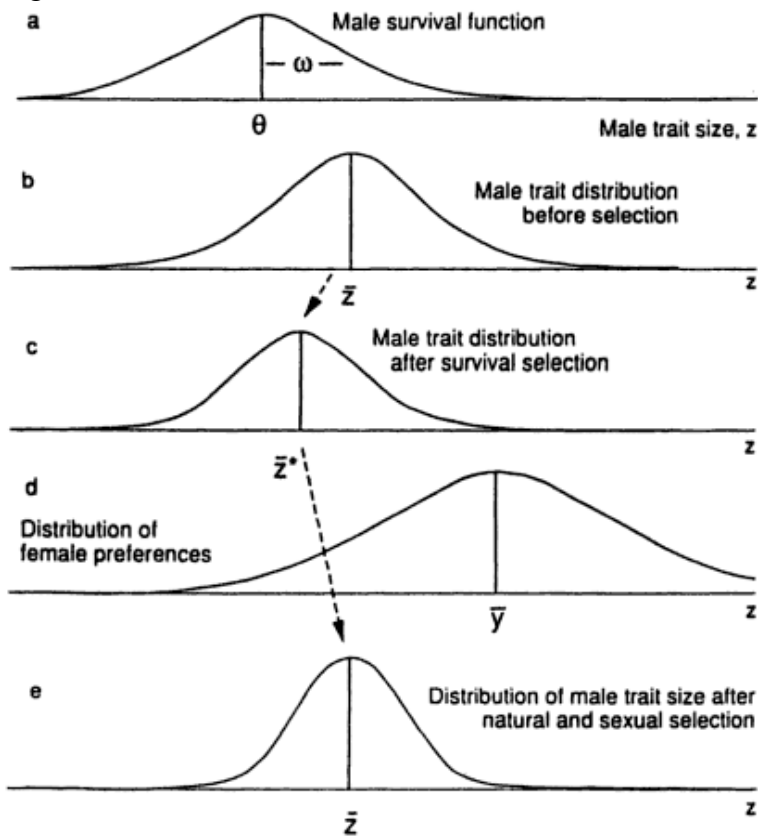


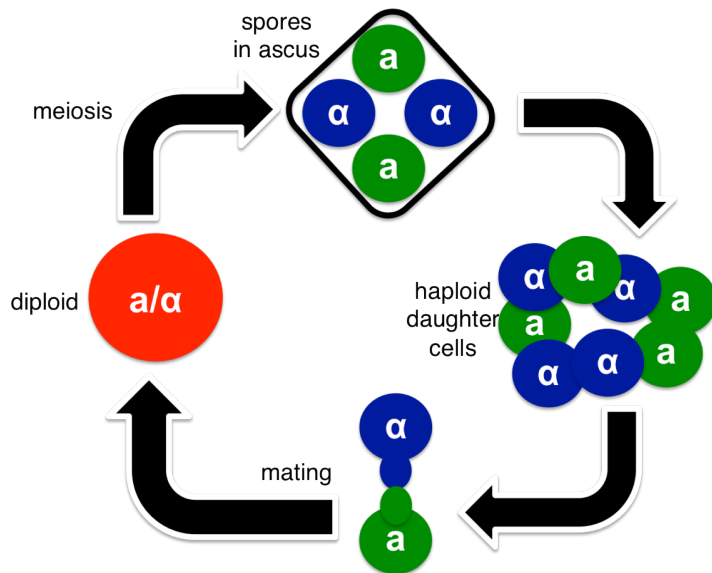
Figure 3 from Ryan 1983. This histogram of number of matings obtained for 617 frogs shows strong reproductive skew: many frogs obtain no mates, while few frogs obtain many mates.

Figure 2



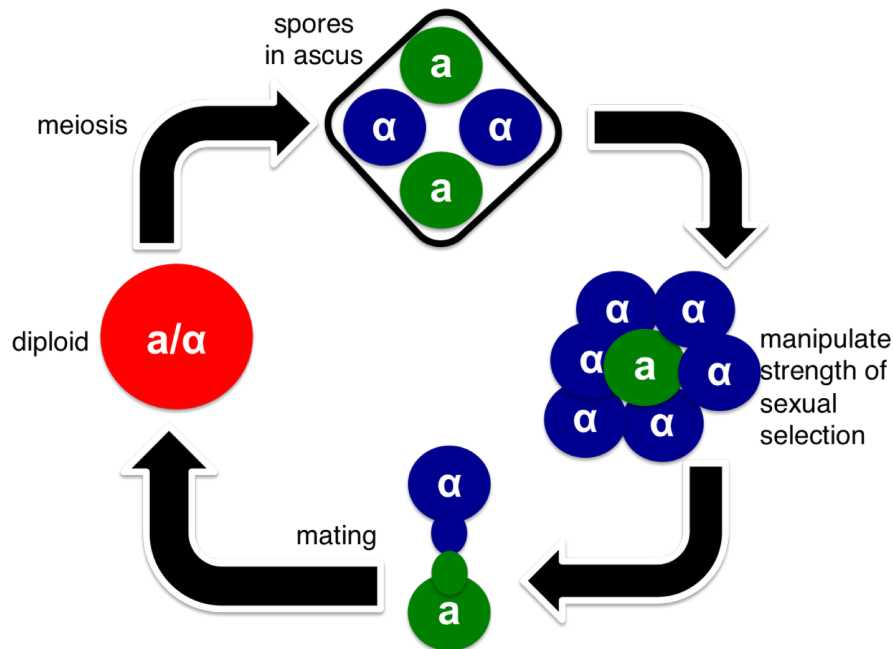
Graphic demonstrating the difference in selective pressures between natural and sexual selection. Note the difference between ω (survival optimum) and \bar{y} (sexually selected optimum). This difference gives rise to the compromise between these two values (\bar{z}) depicted in the final phenotypic distribution of the population in panel e. Taken from Andersson 1994, p.38.

Figure 3



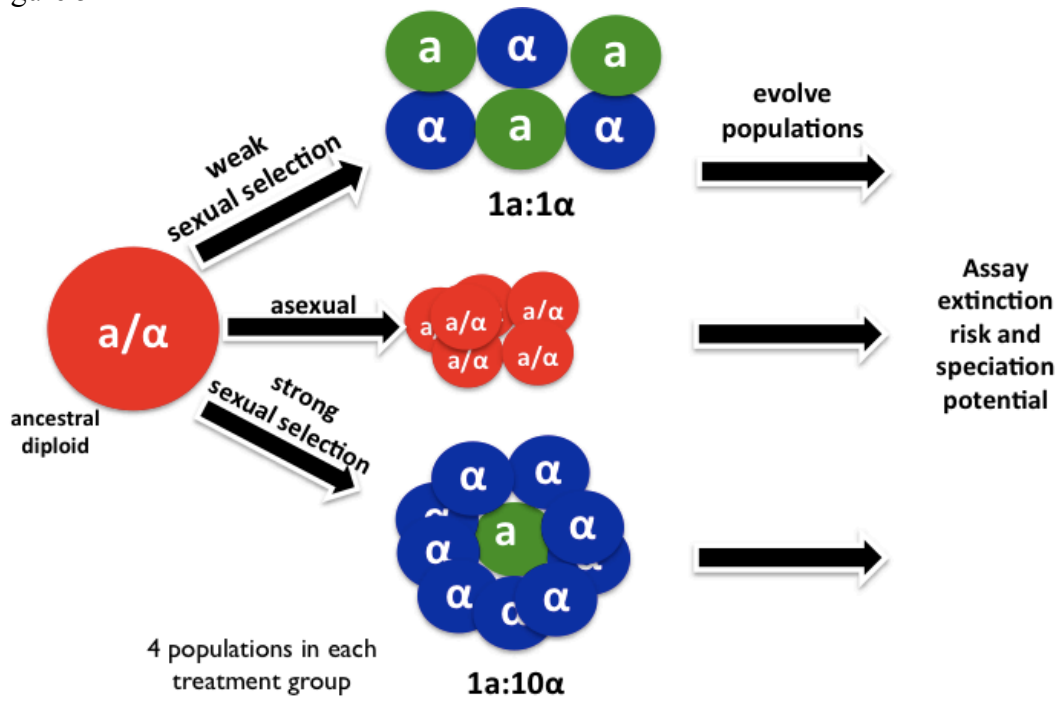
The yeast lifecycle. Yeast in this experiment were kept primarily in the diploid stage. Meiosis and sporulation were induced by placing diploid cultures onto sporulation plates. Blue and green circle represent $MAT\alpha$ and $MATa$ cell types, respectively. Haploids of opposite mating types then mate and fuse to form a diploid ($MATa/MAT\alpha$).

Figure 4



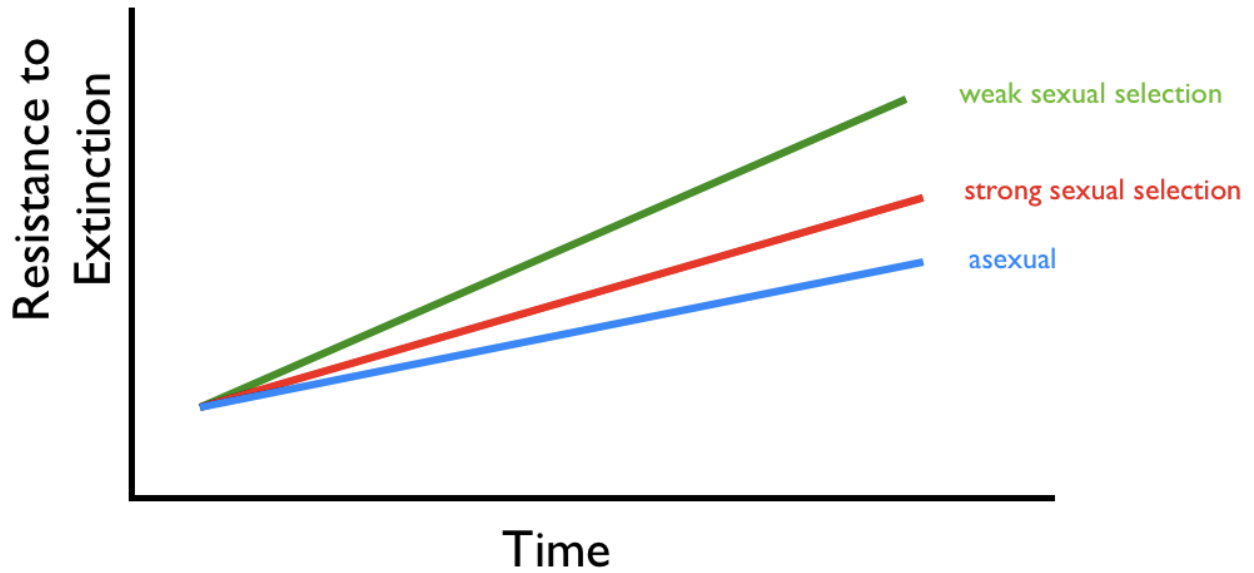
The experimental cycle involves manipulating the strength of sexual selection by recombining $MATa$ and $MAT\alpha$ cells in various ratios.

Figure 5



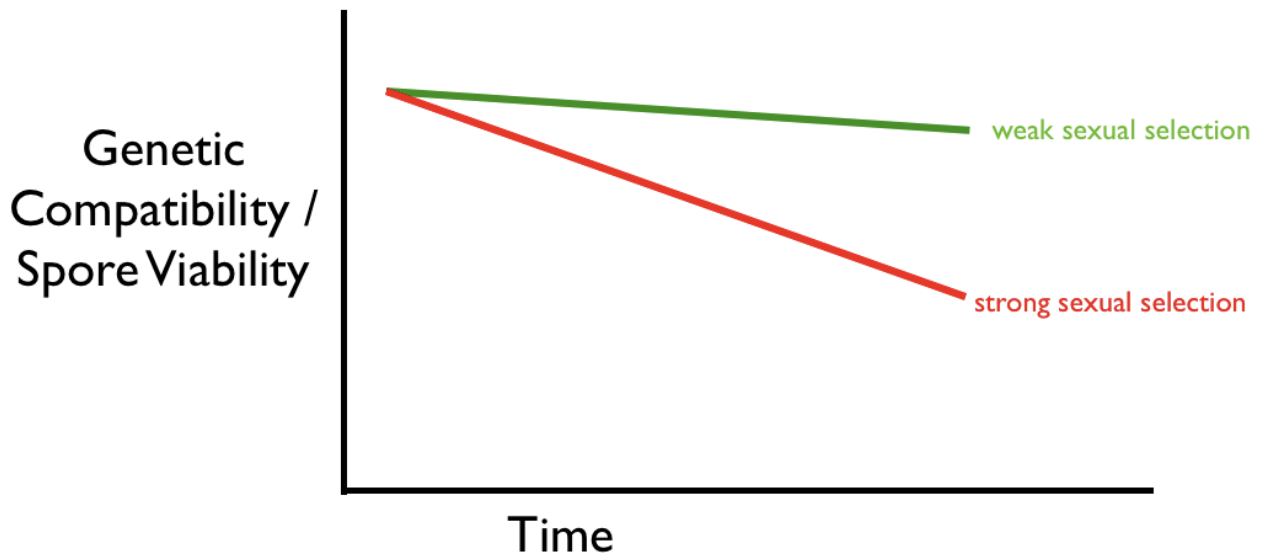
Schematic outlining the experiment. Populations were founded from a single ancestral diploid and evolved experiencing different intensities of sexual selection. Two intermediate treatment groups were also founded, but are not reported here due to time limitations.

Figure 6



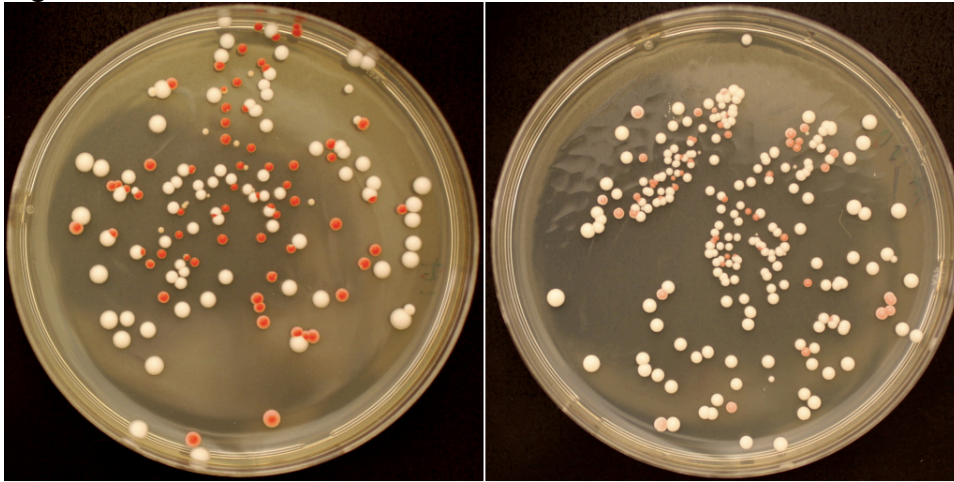
Expected increase in resistance to extinction (relative fitness) between the weak and strong sexual selection group. Both groups should adapt to the EM, but adaptation is predicted to proceed faster in the weak sexual selection group due to the costs of sexual selection. Our goal is to measure that difference if it exists (blue arrow).

Figure 7



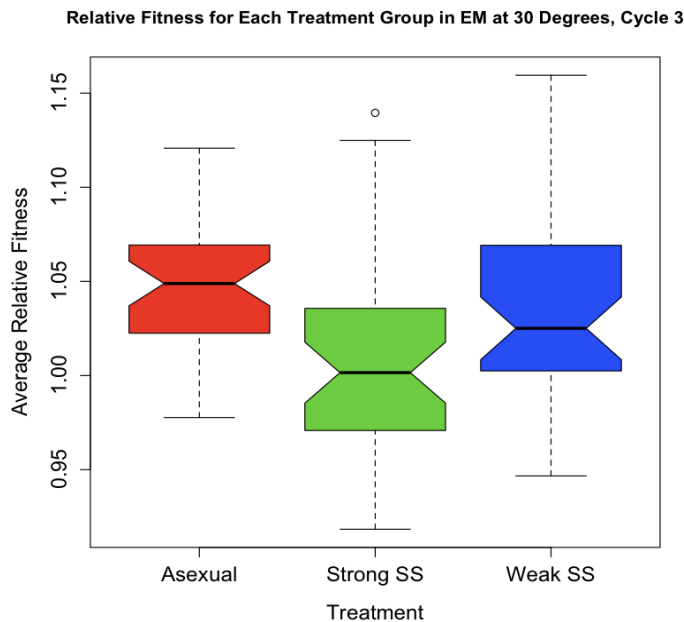
We expect that genetic divergence within the populations experiencing strong sexual selection will be stronger than within those populations experiencing weak sexual selection.

Figure 8



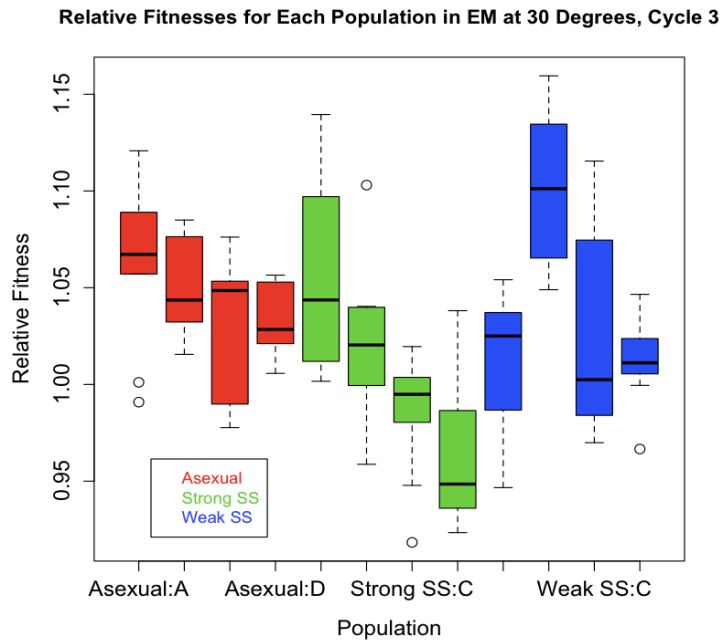
Representative plates from a relative fitness assay. White (evolved) and pink (marked ancestral) colonies were counted using different colored markers and a hand counter. Left: initial number of evolved and ancestral colonies before completion. Right: Colony composition after 48 hours of competition. Samples were diluted 1:5000 with water prior to plating.

Figure 9



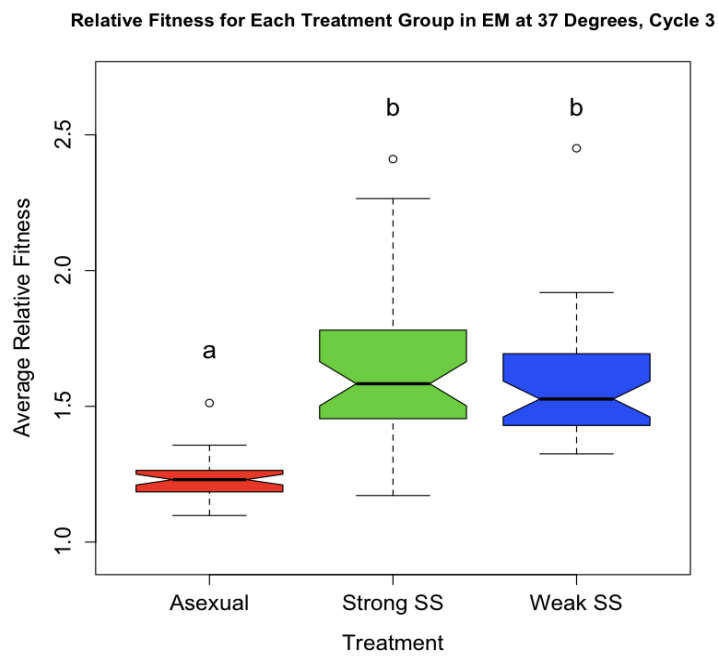
Relative fitness for each treatment group in EM at 30°C at cycle 3. All 40 fitness values (ten per population, four populations per treatment) are pooled for each treatment plot. The ancestral relative fitness is 1.000. Non-overlapping notches between two boxplots is strong evidence that the medians of the two distributions differ.

Figure 10



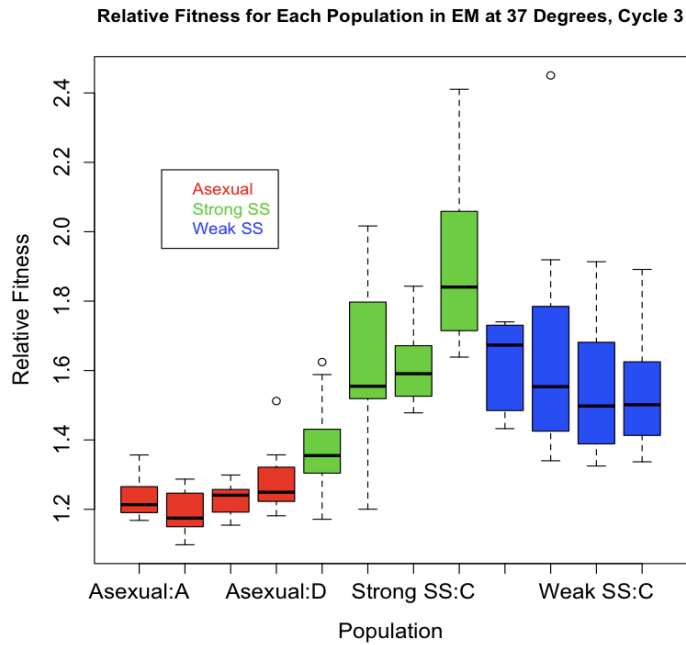
Relative fitness broken down by population in each treatment group in EM at 30°C at cycle 3.

Figure 11



Relative fitness by treatment group in each treatment group in EM at 37°C at cycle 3. The distribution of fitness values for the asexual treatment is different from both the strong and weak sexual selection groups.

Figure 12



Relative fitness broken down by population in each treatment group in EM at 37°C at cycle 3.

TABLES

Table 1

Strain Name	Genotype
YPS3060	MATa, Δho::kan
YPS3340	MATα, YPS 670 hoΔ::nat
YJN20	MATa, Ste3p-hyg, Δhis3::kan, Δho::kan
YJN27	MATa, MFA1p-his3, Ste3p-hyg, Δhis3::kan, Δho::kan
Y8205	MATα, lyp1Δ::Ste3p-leu2, Δleu2, Δura3, Δhis3
YLR11	MATα/MATa, MFA1p-his3, Ste2p-hyg, Δhis3, Δleu2, lyp1Δ::Ste3p-leu2, Δho::nat/Δho::kan
YLR12	MATa, MFA1p-his3, Ste2p-hyg, Δhis3, Δleu2, lyp1Δ::Ste3p-leu2, Δho::nat
YLR13	MATα, Δhis3, Δleu2, lyp1Δ::Ste3p-leu2, Δho::kan
YLR14	MATα/MATa, MFA1p-his3, Ste2p-hyg, Δhis3, Δleu2, ade ⁻ 101 ^{ochre} , lyp1Δ::Ste3p-leu2, Δho::nat/Δho::kan
YLR15	MATa, MFA1p-his3, Ste2p-hyg, Δhis3, Δleu2, ade ⁻ 101 ^{ochre} , yp1Δ::Ste3p-leu2, Δho::nat
YLR16	MATα, Δhis3, Δleu2, ade ⁻ 101 ^{ochre} , lyp1Δ::Ste3p-leu2, Δho::kan
YMZ336	MATα/MATa, ade ⁻ 101 ^{ochre} , ho::nat
Intermediates in producing YLR11, YLR12, YLR13:	
Isolate C	lyp1Δ::Ste2p-leu2, Δhis3, Δleu2
Isolate H	MATa, MFA1-his3, Ste3p-hyg, hoΔ::nat
Isolate J	MATα, lyp1Δ::Ste3p-leu2, Δhis3, Δleu2, Δho::nat
Isolate K	MATa, MFA1p-his3, Ste2p-hyg, Δleu2, hoΔ::nat

Genotypes were confirmed by plating to appropriate media types and patterns of Medelian inheritance where necessary to distinguish between MATa and MATα.

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