Sex in Space! Does Sexual Selection Accelerate or Hinder Adaptation?

by

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Abstract

Theory suggests sexual selection should accelerate adaptation in novel environments. A number of past studies used Drosophila species to test this prediction, and yielded inconsistent result. However, these experiments were all conducted in settings where the test populations are being housed in spatially restricted, simple mating environments. The use of high-density, small and physically simple arenas can exacerbate the effect of male harassment, which reduces both selection on high condition females, and selection against low condition males. Adult fly interaction in large and complex mating environment is predicted to alter selection by alleviate the intensity of male harassment, and increase selection on males. As a necessary extension to past studies, we give evidence suggesting that sexually selected populations evolving under complex ecological environment yields fitness measure more consistent with the alignment of natural and sexual selection.
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**Introduction**

Natural selection generally facilitates adaptation to novel environments via the increase in frequency across generations of alleles that increase survival and/or fecundity (i.e. non-sexual fitness). The effect of differential mating and reproductive success (i.e., sexual selection) on non-sexual fitness, and hence on adaptation, however, is unclear. On one hand, if alleles responsible for higher reproductive success also increase nonsexual fitness, sexual selection will accelerate adaptation by favouring the same alleles as natural selection, causing natural and sexual selection to be aligned (Darwin 1871). Studies inquiring about the effect of sexual selection with respect to nonsexual fitness (i.e., opposing or reinforcing) are limited and results are mixed (see below). Understanding the interaction between reproductive success and nonsexual fitness would give the field of evolutionary biology more predictive power on the topic of adaptation, as well as the purging of deleterious alleles and potentially the evolution of sex.

In theory, natural and sexual selection should align if reproductive success and, hence, the sexually selected traits underlying it, are condition dependent. According to theory (Proulx et al. 2002), traits that are the target of persistent directional sexual selection should evolve heightened condition dependence. In turn, traits affecting survival and fecundity should therefore be highly condition dependent. Hence, alleles that decrease condition will decrease not only survival and fecundity, but they will also decrease reproductive success (Rowe & Houle 1996). Therefore, selection should favour high condition individuals, as fitness is an increasing function of condition. This is evident when a population is adapting to an environment where nonsexual fitness starts out away from the optimum (Long et al. 2012), as large portions of the segregating alleles are maladaptive, selection on these alleles are then predicted to be directional,
and the trait expression of these alleles are thus condition dependent. Consequently, individuals of high reproductive success (sexual fitness) also constitute high relative values of other components of fitness (nonsexual fitness) — natural selection and sexual selection are aligned.

Empirical results testing for the effects of sexual selection on adaptation are mixed. Partridge (1980) pioneered laboratory manipulative experimentation using *Drosophila melanogaster* by permitting or preventing sexual selection (i.e. by allowing mate choice or not) in separate laboratory populations. Larval viability in a population that had mate choice was a little more than 1% higher compared to a population in which female choice was prevented, suggesting an indirect benefit of sexual selection to nonsexual fitness. A number of later studies using *Drosophila melanogaster* also support the result by Partridge (1980), demonstrating that sexual selection increases nonsexual fitness or vice versa (Dolgin et al. 2006; Hollis et al. 2009; Hollis & Kawecki 2014; Promislow et al. 1998; Sharp & Agrawal 2008). However, alleles that confer higher reproductive success may act in opposition to natural selection by negatively impacting nonsexual fitness. For example, such alleles may be responsible for the costly expression of elaborate display traits, or bestow deleterious sexually antagonistic effects to individuals of the opposite sex, and studies that suggest sexual selection acts in opposition to natural selection are just as numerous (Arbuthnott & Rundle 2012; Chenoweth et al. 2015; Correia et al. 2010; Holland 2002; Holland & Rice 1999; Rundle et al. 2006). These past experiments employed experimental evolution on treatment of randomly assigned monogamous pairs as a proxy to measure the effect of natural selection (NS), while both the effect of sexual selection combined with natural selection (SS) were exercised by putting males and females in a small, structurally simple container. Some studies made fitness comparisons between the SS and NS treatments and found the SS treatment obtained an equal or lower fitness measure (Holland
2002; Rundle et al. 2006). This suggests a potentially strong role of sexual conflict, where adaptation is hindered by divergent reproductive interests between the sexes.

Sexual conflict can be divided into two genetic forms (Arnqvist & Rowe 2005). Whenever expression of alleles at a given locus cause opposing fitness outcomes depending on the sex of its bearer there is intralocus sexual conflict. Intralocus sexual conflict will almost always give way to sexual and nonsexual selection being in opposite directions, because sexual selection is often sex-specific, such that females that mate with high fitness males will produce daughters of low fitness, and females that mate with a low fitness male will produce daughters of high fitness. If sexual dimorphism does not evolve to resolve the conflict over sex-specific fitness optima, intralocus sexual conflict will impede adaptation via the presence of a gender load—fitness cost associated with sexually antagonistic alleles preventing both sexes from reaching their optimal phenotype (Pischedda & Chippindale 2006). Interlocus sexual conflict on the other hand, can be viewed as conflict over a shared trait (such as mating rate) that is controlled by different loci in males and females. For example, optimal mating rate for males and females often differs due to the nature of anisogamy (Dawkins 1976; Trivers 1972) with selection favoring lower mating rate in females and higher mating rate in males. If mating rate is determined by the interaction between a set of loci (loci A) expressed in males, but a different set of loci (loci B) expressed in females, then any allelic expression of loci A that enhances male fitness by increasing the mating rate will sequentially incur mating cost to females. As a result, any counter adaptation at loci B expressed in females is under positive directional selection to reduce the harm caused by male courtship, while simultaneously generating the opportunity for selection on new alleles at loci A to increase male mating rate. Sexual selection (interlocus sexual conflict) can hinder natural selection because the courtship cost females bear may lower
their fitness below what it would be in the absence of such conflict. For example, interlocus sexual conflict can drag females away from their optimal mating frequency via persistent male courtship, then the realized fitness for these females are always lower than their potential fitness. In addition to directly reducing female fitness via persistent male courtship (Chapman et al. 1993; Fowler & Partridge 1989), interlocus sexual conflict can also lead to the reduction of the variance in realized female fitness (see below).

We propose that interlocus conflict may be playing a particularly large role in many of the previous experiments using Drosophila. Typical fly experiments examine the effect of sexual selection in small and highly simplified environments where females are constantly exposed to male courtship, potentially amplifying the cost of sexual interactions. Long et al. (2009) found a reduction in the realized variance in female fitness as males preferentially harass high condition females in a population of both large and small females (size is a proxy for condition). The benefit of high condition in females is then discounted due to the unequal distribution of cost incurred from males, thereby reducing the fitness differences between high and low condition females (i.e., sexual conflict reduces selection on females). Hence sexual conflict—a consequence of sexual selection—can impede adaptation by reducing (or possibly reversing) the inherent advantage of high condition females compared to low condition females. In nature, however, females may have the leisure of leaving male aggregations and thereby avoid harm caused by persistent male courtship (Byrne et al. 2008). Thus, investigations on the alignment of natural and sexual selection in a typical lab experiment may be studying the problem in a context where the fitness effects caused by sexual conflict are exaggerated.
Another equally feasible explanation supporting the heightened role of sexual conflict in experimental population adapting to novel environment relies on the different strength of selection on male condition between the natural and lab settings. In nature, mating success requires many steps that may not be required, or may be easier, in a simple laboratory environment (i.e. locating and persistently courting a potential mate). High condition males likely perform better at these requirements, so we predict reproductive success should depend strongly on condition. A simple lab environment is comparatively benign for the *Drosophila* males as both food and females are readily available. Consequently, the association between male reproductive success and condition may break down, i.e., weaker selection on condition in males. In turn, the number of loci contributing to overall condition decreases. However, the number of loci under antagonistic selection may remain reasonably constant between vials and nature (perhaps because such loci are involved in the direct interaction between males and females), resulting in a larger portion of the segregating variance being sexually antagonistic (Long et al. 2012). This would make the net effect of sexual selection less likely to be favourable for female adaptation.

We hypothesize that the use of vials (or other high-density, small and physically simple arenas) have underestimated the beneficial effects of sexual selection in *Drosophila* for the two reasons outlined above: (i) excessive male harassment weakens selection on females, and/or (ii) weaker selection on condition in males. To gain insight into this, we employed experimental evolution in *D. melanogaster* to test how the environment in which mating interactions occurs influences the effect of sexual selection on adaptation. Three different sexual selection treatments are utilized to compare the effect of spatial complexity on population fitness: minimal sexual selection (-SS), sexual selection in a simple environment (+SS), and sexual selection in a
complex mating arena (+SSc). The experiment was run for 25 generations. To get a better sense of the effect of sexual selection and mating environment on adaptation, we performed three separate evolution experiments, each involving adaptation to a different abiotic environment: (I) cornstarch food with cold shock; (II) ethanol-enriched food with heat shock; and (III) salt-enriched food at constantly elevated temperature (28 °C). Within each evolution experiment, seven replicate populations were assigned to each of the three sexual selection treatments (-SS, +SS, +SSc).

Using these populations, we performed productivity measurements at different time points to estimate differences in fitness among the sexual selection treatments. In addition, after 14+ generations we conducted assays to measure the three major components of fitness - viability (larval), mating/reproductive success (male), and fecundity (female).
Methods

Derivation and Maintenance of Experimental Populations

The *Drosophila melanogaster* population we used was sampled from Agrawal Lab Similkameen (SIM) stock, which was collected in the Similkameen Valley, British Columbia in 2005. This population had been maintained in a constant environment (cornmeal-yeast media; 25°C; 12L:12D photoperiod; 50% RH) at ~2000-4000 adults per generation. For at least four years prior to the start of this experiment, this stock has been maintained in discrete, non-overlapping 2-week long generations.

In September 2014, separate samples (21 independent populations per week for three weeks; 140 males and 140 females in a given population) of flies from the SIM population were collected to undergo evolution experiment to different environments consisting of various abiotic stresses while being maintained under different mating regimes (sexual selection treatments). Each evolution experiment (novel abiotic environment) consisted of 21 populations, each assigned to one of three different sexual selection treatment (7 per treatment). For the first six generations, our evolution experiments consisted of the following three abiotic environments: (I) standard cornmeal medium supplemented with 5% salt and constant exposure to 28 °C (rather than the standard 25 °C); (II) medium based on cornstarch (rather than cornmeal) and a 2-hour heat shock (37 °C) to 3-day old larva; and (III) standard cornmeal medium supplemented with 10% EtOH and a 2-hour cold shock (4 °C) to 3-day old larva. To ensure continuing directional selection on these populations, we increased the salt concentration to 6% in (I) and the duration of heat and cold shock to 4 hours after the sixth generation in (II) and (III), respectively.
For each evolution experiment, the replicate populations were assigned to one of three different sexual selection treatments: minimal sexual selection (-SS), sexual selection in a simple environment (+SS), and sexual selection in a complex mating arena (+SSc).

The -SS treatment consists of randomly enforced monogamous pairs (140 male/female pairings per population) held in wide straws (radius = 6.35mm; height = 88.9mm) and inserted into a 3 oz dixie cup filled with 25mL of ancestral food (28 straws per cup). The food surface within each straw is supplemented with ~1-2 yeast pellets for the flies to feed on. For the +SS treatment, 35 males and 35 females are placed in a standard fly vial (28.5mm x 95mm Drosophila vial filled with 10 mL of ancestral food media); the food surface is supplemented with ~30-50 dry yeast pellets; 4 such vials are created for each population (totalling 140 adults of each sex). For the +SSc treatment, 35 males and 35 females are placed in a 1.65L cylindrical plastic Ziploc container; 4 such containers are created for each population (totalling 140 adults of each sex). Within each container, there are five separate food sources (three 3oz Dixie cups containing 25mL of ancestral media, each divided into two by a plastic divider inserted into the median of the food; and two smaller 1oz cups containing 7.5mL of ancestral media) and pipe cleaners dangling from the lid. The food surface in each 3oz dixie cup is supplemented with ~10-15 yeast pellets, and ~5-10 yeast pellets is placed onto each 1oz cup food surface. This arena design was chosen to reduce density and increase spatial complexity, allowing flies to be out of sight from one another and providing multiple food sources so that all females are not forced to a single locale to feed and lay eggs.

Adult flies were held in their respective mating arena for a total of 6 days to feed, interact, and mate. Because females lay eggs in the food during this time, to avoid high larval
density that may alter adult use of the food and hence mating interactions, adult flies were
transferred via light anesthesia to fresh mating arenas supplied with new food on the third day.
After 6 days, we anaesthetized flies in the arenas and randomly sampled 105 females from each
population for egg laying. These females were evenly distributed into seven Drosophila vials
with ~3–5 yeast pellets to lay eggs for 24 hours, the vials were filled with the novel abiotic food
media (i.e. I; II; or III) corresponding to the evolution experiment. Eleven days later, we
collected the adult offspring into holding vials separated by sex (35 flies per vial) and were held
for 72 hours before repeating the above mating protocols for the next generation. Each non-
overlapping generation lasted three weeks. Each set of 21 populations for a single evolution
experiment was offset from the other two sets by 1 week in the maintenance cycle.

Throughout the evolution experiment, a separate population of the SIM stock (~2000
flies) was maintained in parallel (we call this population 'Ancestral'). This population was
sampled at the same day as the start of experimental evolution procedure and maintained the
same way as the regular SIM stock.

In March 2015, a sample of the Ancestral population had the DsRed marker introgressed
into their genetic background by mating 100 DsRed males to 100 Ancestral virgin females, and
backcrossing the F1 offspring to DsRed males once more. For four generations after the initial
population backcross, we artificially selected for the DsRed marker until it was fixed in the
population. The genetic marker is a dominant mutation that allows all bearers to emit red light
under a fluorescent microscope. The population was maintained on a two week cycle in
cornmeal-yeast media: 25°C; 12L:12D photoperiod; 50% RH, i.e., as the regular SIM stock.
These marked flies are competitor fly strain for some of the assays listed below.
Productivity Assay

A productivity (fecundity + viability) assay was performed at generations 4, 7, and 10 for each evolution experiment. In a given assay, 50 males and 50 females were sampled from each population and distributed into two standard cornmeal-food vials (25 of each sex per vial) for 24 hours. Females were then separated to create 20 laying vials of 2 females per vial. All the vials contained the appropriate novel abiotic food (i.e. (I), (II), or (III) above) supplemented with ~5-10 pellets of live yeast. Laying females were discarded after 24 hours and vials were treated to the appropriate temperature regime according to the abiotic environment in which they have been evolving. In addition, we set up 50 laying vials for each of the abiotic environments containing 2 ancestral females per vial. The ancestral flies in this assay were exposed to the novel abiotic environment one generation prior to control for maternal effects. Productivity was measured as the total number of flies counted on day 14 after oviposition. Because this assay was used to track changes in fitness in a qualitative manner, formal statistical tests were not performed.

Viability Assay

At generation 14, we measured larval viability relative to the DsRed-marked ancestral SIM population as a competitor (see above in method). We conducted this assay separately for each evolution experiment e.g., (I), (II), (III) above in methods. On day 11 of the maintenance protocol, we first sampled 300 male and 300 female adults from each population, placing 10 adults of each sex into holding vials containing the ancestral media for 24 hours. These flies were then transferred into a new set of vials containing the novel abiotic environment
corresponding to the evolution experiment and ~3-5 pellets of live yeast to oviposit for 24 hours. These vials were held under the appropriate temperature regime. Eleven days later, we released all emerged flies into a cage (one cage per population; ~5000 flies per cage). Within each cage, six petri dishes (60mm x 15mm) filled with the appropriate novel abiotic environment and covered in yeast paste were placed for flies to lay eggs for 15 hours. The plates were then harvested using an egg washing protocol (Wang et al. 2014; Yun & Agrawal 2014). Using a pipetting technique, ~100 focal eggs + ~100 competitor (DsRed homozygotes) eggs were placed into a vial filled with the appropriate novel food. A maximum of 10 replicates per population was set up and the corresponding novel temperature regime was applied to eggs and larva on the appropriate days. We then scored the number of emerging focal (wild-type) and DsRed flies eleven days later. In addition, we performed the same protocol for the Ancestral population (30 replicates for each evolution experiment) to detect signals of larval adaptation. We scored all the emerged wild-type and DsRed adults 11 days later.

**Male Reproductive Success Assay**

At the onset of generation 16, we performed an assay to measure male mating success for each of the three evolution experiments (abiotic environments). For each, we used a 2x2 factorial design for males from each sexual selection treatment (-SS, +SS, and +SSc) under each possible “assay mating environment” (e.g., single pair straws, multi-fly vials, multi-fly containers). On day 11 of the experimental protocol above, we collected focal males from their emergence vials and placed 10 males into holding vials (15 vials per population). DsRed competitor males and ancestral females of the same age were also collected and placed into separate holding vials (25 DsRed males per vial and 35 ancestral females per vial). The number
of DsRed and ancestral holding vials matched the number of focal holding vials and all consisted of the ancestral cornmeal media. Approximately 70 hours later, we assigned the 10 focal males within each holding vial into one of three assay mating environment (5 replicate vials per population), along with 25 DsRed males and 35 ancestral females. For the single pair straws (enforced monogamy), ancestral females were paired with either a focal male from an experimental population or a DsRed male. In the multi-fly vials and containers, groups of males from an experimental population competed with DsRed males for mates. Each replicate was given the same opportunity to mate according to the maintenance protocol. We then took the females out of each replicate and distributed them among three Drosophila vials (28.5mm x 95mm) filled with 10mL ancestral food media to lay eggs. The females were then cleared 4 hours later so that egg density was well below the carrying capacity (to minimize larval selection as much as possible). Scoring of non-DsRed (sired by focal males) and DsRed (sired by competitors) offspring was done on day 11 and day 12, accounting for ~ 95% of the emerged flies. Within each assay, we also set up 30 replicates with ancestral males (10 ancestral male + 25 DsRed male + 35 ancestral female per replicate) for each assay mating environment. All ancestral males used in this assay were raised in the test abiotic environment (e.g., (I), (II), (III)) one generation prior.

Female Fecundity Assay

At the onset of generation 22, we sampled 100 males and 100 females from each experimental population and distributed them evenly among 10 vials with the appropriate novel abiotic environment, the flies were allowed 24 hours to interact and oviposit. Temperature regimes were then applied to these vials matching the novel abiotic environment. We also
sampled the Ancestral population by taking 500 males and 500 females, they were distributed into 50 vials and raised in the same manner as the experimental population. Female offspring collected eleven days later from these vials were utilized for fecundity measures. To do so, we employed a 2x2 factorial design by allocating females sampled from each sexual selection treatment population into one of three assay mating environments (similar to the male assay).

For the monogamous (single pair straw) mating environment, females from each sexual selection treatment (-SS; +SS; +SSc; Ancestral) were sampled and paired individually with DsRed males into straws (n=35 per experimental population; n= 210 for the Ancestral population). They were allowed to go through the same mating interaction as maintenance protocol mentioned above. Females were then collected and put singly into a laying vial containing ancestral food with ~1-3 yeast pellets. For the multi-fly vial and multi-fly container mating environments, we created 10 replicates from each experimental population (n=60 for ancestral) and assigned half to each of the two assay mating environments. Each replicate consisted of 10 focal females; 25 DsRed females; and 35 DsRed Males. Each replicate went through the same mating interaction duration (6-day period) as in the maintenance protocol. A maximum of 7 focal females from each replicate was sampled; females were put singly into laying vials containing ancestral food with ~1-3 yeast pellets. Oviposition for all of the singly held females was timed for 24 hours; we then scored the number of pupa and emerged flies on day 12.

Data Analysis

Each evolution experiment (i.e. novel abiotic environment) was analyzed separately. Larval viability was calculated as the number of wild type flies out of 100 possible emerged by day 11. (Viability of competitor DsRed was low across all replicates (<40%), so we elected to
not measure viability of focal flies relative to the competitor.) Male mating/reproductive success was measured as the percentage of wild type (non-DsRed) out of total fly emergence. Female fecundity was scored as the total number of pupa and emerged flies 12 days after oviposition. For each assay above, we provide a qualitative comparison of the fitness measurements between sexual selection treatments (−SS; +SS and +SSc) to the Ancestral to assess adaptation; with only one ancestral population, we lacked replication for formal tests. We made formal comparisons among sexual selection treatments using population as the unit of replication, and employed one or two-way ANOVA (outlined below) to quantify the effect of sexual selection and spatial difference on fitness.

Viability of the ancestral population in all three evolution experiments was calculated as the mean out of 30 replicates. For the Ancestral population, we calculated 95% confidence interval via bootstrapping (x10000). We also calculated Ancestral male mating/reproductive success and female fecundity with the same method.

For the viability assays, we compare the difference in viability among all sexual selection treatments by fitting one-way ANOVA and Tukey HSD tests within each evolution experiment (abiotic environment) (R version 3.0.3). For the male and female assays, we fitted separate two-way ANOVAs with the factors being sexual selection treatment and assay mating environment. Because of apparent interactions between these factors, one-way ANOVA and Tukey HSD tests were used within each assay mating environment to detect any significant differences among the sexual selection treatments.
Results

Productivity Assay

In the first two abiotic environments ((I) NaCl/28°C and (II) cornstarch/heat shock), flies evolved greater productivity across all three mating environments (Figure 1a and b), suggesting increased adaptation compared to the Ancestral stock. In environment III ((EtOH/cold shock; Figure 1c), only the +SSc flies showed a pattern consistent with adaptation. Populations from the +SSc environment tended to show the highest productivity after 10 generations. The weaker signal of adaptation in the -SS and +SS treatments might be attributed to a plastic response in egg laying rate that was generated by the experimental protocol. More specifically, prior to oviposition, flies in the productivity assay experienced a common garden environment to mate instead of their evolved mating environment, and both fly mating behaviour and egg laying rate can be highly dependent on the social context (Billeter et al. 2012). Particular caution is needed in interpreting the results of the assay using ethanol. Despite the adaptive history of a population, female egg laying preference is also highly skewed towards food media supplemented with EtOH (Zhu & Fry 2015), causing the non-EtOH adapted females to lay a large number of eggs. To get a clearer picture on whether our experimental populations had undergone adaptation, or whether differences in the opportunity for sexual selection influences adaptation, we look to results from later assays.

Viability Assay

All of the evolved populations show a strong signal of adaptation in that average viability is substantially higher than that of the Ancestral population in their novel environment (Figure 2). There is significant variation in viability among mating treatments in the NaCl/28°C and
cornstarch/heat shock environments (F$_{2,18}$ = 3.55; p = 0.02 and F$_{2,18}$ = 3.55; p = 0.03 respectively, Figure 2a, b). While adaptation was considerable in the EtOH environment, differences among sexual selection treatments were not significant. However, the highest estimates of larval viability came from the +SSc treatment in all three adaption experiments (Figure 2a-c), with +SSc being significantly better than +SS in two of the three.

Male Reproductive Success

In all three evolution experiments, both -SS and +SSc males performed better than Ancestral males in their "native" assay mating environments (single pair straws and multi-fly containers, respectively; Fig. 3). In contrast, the Ancestral males performed just as well as the +SS males in the multi-fly vial assay mating environment. Initial two-way ANOVAs for each abiotic environment revealed interactions between sexual selection treatments and the assay mating environment (Table 1), so we examined the data separately for each assay mating environment. Male success differed significantly among sexual selection treatments in all assay mating environments (one-way ANOVA: p<0.05 for each). Among each assay mating environment, average reproductive success among the sexual selection treatments was consistent across all three abiotic environments (Figure 3). In the single pair straw environment, -SS males performed better than +SS males, which was expected as -SS males were tested in the same assay mating environment under which they evolved. However, -SS males were not significantly better than +SSc males in two out of three abiotic environments (Figure 3a, d), and were significantly worse in the EtOH environment (Figure 3g; p<0.05, Tukey HSD). In the multi-fly (vial and container) assay mating environments, +SSc males sired the largest
percentage of offspring, significantly so for most comparisons (Figure 3 b, c, e, f, h, i), while -SS males sired the lowest percent of offspring among all sexual selection treatments.

**Female Fecundity Assay**

There was much less evidence of adaptation in female fecundity (Figure 4). +SSc females produced more offspring in their native mating environment (multip-fly container) than Ancestral females (Figure 4 c, f, i), while -SS and +SS female fecundity did not differ from the Ancestral. With respect to the comparison among evolved populations, the interaction between sexual selection treatment and assay mating environment was significant in one of the abiotic environment (Cornstarch). While no interaction was detected, the main effects of both sexual selection treatment and assay mating environment were significant in the EtOH environment. In the third (NaCl) abiotic environment, only the main effect of assay mating environment was significant although the sexual selection treatment approached significance (Table 2). Given the presence of these results, we again analyze the graphs separately by assay mating environment. There was significant variation among sexual selection treatments in 3 out 9 instances (Figure 4 e, f, i). Variation among sexual selection treatments is significant in the multi-fly container environment for two out of three cases (Figure 4 f, i), with +SSc females producing the greatest number of offspring (p<0.05, Tukey HSD), and this pattern also exists in the NaCl environment although the difference was not significant (Figure 4 c).
Discussion

Theory suggests sexual selection should accelerate adaptation as populations adapt to a novel environment (Lorch et al. 2003; Proulx 2001; Whitlock 2000). However, empirical studies testing for this prediction provide mixed results, as sexual selection occasionally aligns with natural selection to increase mean population fitness, while at other times oppose natural selection and impedes adaptation (see Introduction). The majority of these past studies involving Drosophila species were conducted in a laboratory setting where the test populations were housed in spatially restricted and ecologically simple mating environments (i.e. bottle or vial). We hypothesize the the use of high-density, small and physically simple arenas has underestimated the beneficial effects of sexual selection. Such ecological aspect can exacerbate the effect of excessive male harassment, which weakens selection on high condition females (Long et al. 2009), and weaker selection against low condition males (Whitlock & Agrawal 2009). Adult fly interactions in a large and complex mating environment is predicted to alter selection to alleviate excessive male harassment, allowing females to escape male courtship, at the same time impose strong selection against low quality males via intraspecific competition. As a necessary extension to past studies, we predict sexually selected populations evolving under a large and complex ecological environment should yield results more consistent with the alignment of natural and sexual selection than studies using simple environments.

In our study, we conducted three separate evolution experiments. Each was carried out by rearing D. melanogaster populations in a stressful larval environment, while adults of each evolution experiment went through different sexual selection regimes (minimal sexual selection,
-SS; sexual selection in a small simple environment, +SS; and sexual selection in a large complex environment, +SSc). During the course of experimental evolution, we compared fitness components of the evolved flies to ancestral flies for a signal of adaptation. We also examined the variance among the three sexual selection treatments. These comparisons were made from the following major fitness components: larval viability (generation 12); male reproductive success (generation 15); and female fecundity (generation 22).

There were strong signals of adaptation in larval viability for all three evolution experiments (Figure 2). Though adaptation occurred in all sexual selection treatments, the highest levels of adaptation were observed in the +SSc treatment in all three evolution experiments (and the treatment effect was significant in some contrasts). Female adaptation was evident in only two out of nine assayed cases (Figure 4 f, i), and adaptation was associated with +SSc females tested in their evolved mating environment. Both results is consistent with an important role of sexual selection in accelerating adaptation.

The fact that there was not stronger evidence of adaptation with respect to fecundity may be due to selection acting on different sexes, past studies have demonstrated alleles that are deleterious to both sexes tend to be purged through higher selection on males (Mallet & Chippindale 2011; Sharp & Agrawal 2008; Sharp & Agrawal 2013; Zikovitz & Agrawal 2013). Because of competition for mates, the variance in male reproductive success is expected to be greater than that of females (Bateman 1948). Sexual selection can thus result in a high variance in male reproductive success, and in turn selection on male condition is stronger than that of females.
Plasticity in egg laying behaviour can also explain a lack of variation in female fecundity among the sexual selection treatments. In the fecundity assay, focal females laid eggs in vials containing ancestral food media (to minimize subsequent larval mortality). However, during the evolution experiment, females laid on the novel media. Moreover, during experimental evolution the females were provided ancestral food during the mating period, and eggs laid during that period did not contribute to the next generation. Thus, these females may have been selected to put egg laying on hold when exposed to ancestral food, until the appropriate novel food was encountered (i.e., NaCl, cornstarch, or EtOH). Behavioural plasticity in egg laying may have resulted in a reduction in the performance in the fecundity assay for all sexual selection treatments.

Comparing among the sexual selection treatments, we found a significant benefit of sexual selection for viability (Figure 2 a) and fecundity (Figure 4 f), as these fitness measures tend to be higher in populations with sexual selection. Benefits of sexual selection are primarily associated with the complex mating environment. In populations evolved in the simple environment (+SS) however, the effect of sexual selection on nonsexual fitness was beneficial in only one case (Figure 4 e), but was negligible in almost all other cases. This was consistent with our prediction that sexually selected populations in a large and complex environment would be more likely to yield results reflective of the alignment of natural and sexual selection than populations evolving in a simple environment. The fact that different mating treatments showed different levels of adaptation with respect to larval viability indicates pleiotropic effects between larval and adult fitness components. This is consistent with Rowe & Houle (1996) that traits under constant directional selection (such as fitness components) will evolve to be more
condition dependent, so that loci that are responsible for increasing one component of fitness are also likely to increase other fitness components.

With respect to sexual fitness (male reproductive success), males evolved under the presence of sexual selection (+SS and +SSc) performed significantly better than males evolved under the single pair monogamous regime (-SS) when there was competition for mates, i.e., assays performed in multi-fly vials or containers. Furthermore, evolution with environmental complexity enhanced this effect greatly, as +SSc males outperformed +SS males, even when tested under mating conditions from which +SS males evolved (multi-fly vials). This indicates +SSc males are better at obtaining fertilizations (either via getting more mates or via better performance in sperm competition), and suggest stronger selection on males in the complex mating environment.

Our results suggest sexual selection generally promotes adaptation in the ecologically complex environment, whereas it failed to do so under the "standard" laboratory test conditions. In the Introduction we proposed that mating interactions in a simple lab environment (rather than a complex environment) can lead to weaker selection on male and preferential harassment of high-fitness females, weakening total selection. While our study does not distinguish between these two (non-exclusive) explanations, the results support the prediction that mating in a large complex ecological environment is more likely to generate a net positive effect of sexual selection. However, we note that the single pair straw and multi-fly container mating environments are physically different from the standard lab environment (multi-fly vial), thus selection may differ among these environments for abiotic reasons rather than because of how intersexual interactions occur within these environments (i.e. differences in natural selection).
Hence, differences in evolutionary response among the sexual selection treatments may be partially attributed to this environmental effect.

In the absence of any competition for mates (i.e. assays in single pair straws), variation in reproductive success can be attributed to how a male affects the fecundity of the mate. In all three evolution experiments, -SS males performed better in their "native" environment compared to +SS males (Figure 3 a, d, g). Consistent with past results (Holland & Rice 1999; Holland 2002; Pitnick et al. 2001), these results imply that males evolved under the simple lab environments (multi-fly vial) are quite harmful to females and, in comparison, -SS males evolved to be less harmful to their mates. Surprisingly, +SSc males performed significantly better than -SS males in two out of three cases. This result suggests +SSc males are even less harmful than males evolved under forced monogamy. One possibility is that females can better escape unwanted attention in the larger, more complex environment, thus mating rates are substantially lower such that a partially "monogamous" mating system is achieved. This effect should generate selection in males to be less harmful to females, at least relative to +SS males. Furthermore, +SSc males may be under selection to efficiently identify receptive females and not waste time or energy harassing unreceptive females with mating attempts when more receptive females may be available. If these +SSc males are selected to seek out females that are more receptive to mate (perhaps when the effect of sex peptide from previous mating had worn off), and leave recently mated females alone to alleviate energetic expense of sexual interaction, then this could result in the evolution of lower harassment in the complex environment than under enforced monogamy. Evidence from a recent study suggests males evolved under low density mating environments (with sexual selection) tend to take shorter time to identify and narrow in courtship behaviours upon virgin female under a mixed-female population (Hollis & Kawecki
2014), whereas males evolved from the monogamous mating environment tend to take a significantly longer time to differentiate female mating status. Thus, different learning capabilities between populations evolved from different sexual selection treatments can lead to differential fitness consequences in mating/reproductive success. If, as we suspect, +SSc males evolved to be more benign to females, the cost of female attractiveness due to preferential male harassment is expected to decrease. In turn, the variance in fitness between high and low condition females will not be suppressed by biased male harassment, allowing sexual selection to positively affect nonsexual fitness. In a separate study (Yun et al., in prep), we have found direct evidence that male harassment creates a cost of sexual attractiveness in vials but this cost is absent in the type of complex containers used here.

Our study helps clarify some of the ambiguities surrounding whether sexual selection accelerates or hinders the rate of adaptation to a novel environment. Consistent with our expectations, the spatial complexity of a large mating environment can positively affect the alignment of natural and sexual selection. Future investigations on the alignment of natural and sexual selection in controlled laboratory settings should also consider the ecological factors in which mating interactions take place.
References


Table 1. Two-way ANOVA tests for the male reproductive success assay. The tests were performed separately for each evolution experiment (listed in columns below) across factors of assay mating environment and sexual selection treatment. Values highlighted in bold are significant with p < 0.05

<table>
<thead>
<tr>
<th>Male Mating Success</th>
<th>NaCl/28°C</th>
<th>Cornstarch/Heat Shock</th>
<th>EtOH/Cold Shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Mating Environment</td>
<td>2.49E-11</td>
<td>0.6267</td>
<td>6.59E-13</td>
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<tr>
<td>Sexual Selection Treatment</td>
<td>1.17E-11</td>
<td>2.26E-13</td>
<td>1.07E-12</td>
</tr>
<tr>
<td>Interaction</td>
<td>9.73E-10</td>
<td>2.95E-05</td>
<td>1.35E-05</td>
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</tbody>
</table>
Table 2. Two-way ANOVA tests for the female fecundity assay. The tests were performed separately for each evolution experiment (listed in columns below) across factors of assay mating environment and sexual selection treatment. Values highlighted in bold are significant with p < 0.05.

<table>
<thead>
<tr>
<th>Female Fecundity</th>
<th>NaCl/28ºC</th>
<th>Cornstarch/Heat Shock</th>
<th>EtOH/Cold Shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Mating Environment</td>
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<td><strong>4.42E-10</strong></td>
<td><strong>2.3E-29</strong></td>
</tr>
<tr>
<td>Sexual Selection Treatment</td>
<td>0.088</td>
<td><strong>4.51E-4</strong></td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>Interaction</td>
<td>0.719</td>
<td><strong>0.001</strong></td>
<td>0.208</td>
</tr>
</tbody>
</table>
Figure 1. Productivity measures on generation 4, 7, and 10 in abiotic environments - a) NaCl/28°C; b) cornstarch/heat shock; c) EtOH/cold shock. Error bars represents standard error calculated using the means of population replicates.
**Figure 2. Average larval viability (%) of experimental populations.** Each graph depicts sexual selection treatment (-SS; +SS; +SSc) vs. viability. Each point represents the mean larval viability of a given mating regime, using population as the unit of replication (n = 7). The error bars are SEs. The grey horizontal line represents the mean viability of the Ancestral population; the dashed lines on each graph are 95% confidence interval for the average Ancestral viability (Bootstrap x10000). Different letters indicate significantly different means based on Tukey HSD tests.
Figure 3. Average male reproductive success (% of offspring sired by focal males) of experimental populations. The rows are evolution experiments (abiotic environments (I), (II), and (III) in Methods), columns are assay mating environments. Each point represents mean sire percentage between population replicates (n=7). Error bars are standard errors. Horizontal grey line represents the mean reproductive success of the Ancestral population treating replicates, the dashed lines on each graph are 95% bootstrap confidence interval for the Ancestral. Different letters indicate significant different means based on Tukey HSD tests.
Figure 4. Average female fecundity of experimental populations. The rows are evolution experiments (abiotic environments (I), (II), and (III) in Methods), columns are assay mating environments. Each point represents mean female fecundity between population replicates (n=7). Error bars are standard errors. Horizontal grey line represents the mean mating/reproductive success of the Ancestral population treating replicates, the dashed lines on each graph are 95% bootstrap confidence interval for the Ancestral. Different letters indicate significant differences via Tukey HSD tests.