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Competition for mates and the improvement of nonsexual fitness

Li Yun[a,b], Patrick J. Chen [a,b], Kevin E. Kwok[b],

Christopher S. Angell[b], Howard D. Rundle[b,*] & Aneil F. Agrawal[a,*]

[a]Department of Ecology & Evolutionary Biology, University of Toronto, 25 Willcocks St., Toronto, ON, Canada, M5S 3B2

[b]Department of Biology, University of Ottawa, 30 Marie-Curie Priv., Ottawa, ON, Canada, K1N 6N5

[*]These authors contributed equally

Short title: Mate competition, adaptation and purging

Corresponding Author: Aneil Agrawal, Department of Ecology & Evolutionary Biology, University of Toronto, 25 Willcocks St., Toronto, ON, Canada, M5S 3B2; Tel. 416-946-5563; email: a.agrawal@utoronto.ca

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Significance

Competition for mates is often intense. The resulting selection can have pervasive effects across the genome, potentially affecting components of nonsexual fitness. There is controversy over whether these effects on nonsexual fitness occur and, if so, their direction. Past studies have yielded variable results but without providing insight into why outcomes vary. Here we show that when mate competition occurs in an environment in which male harassment is weak, there are substantial benefits in terms of the rate of adaptation to novel larval conditions as well as the purging of inbreeding depression. In contrast, these benefits are absent when mate competition occurs in an environment in which male harassment is strong.

Abstract

Competition for mates can be a major source of selection, not just on secondary sexual traits but across the genome. Mate competition strengthens selection on males via sexual selection, which typically favours healthy, vigorous individuals and, thus, all genetic variants that increase overall quality. However, recent studies suggest another major effect of mate competition that could influence genome-wide selection: sexual harassment by males can drastically weaken selection on quality in females. Because of these conflicting effects, the net effect of mate competition is uncertain, though perhaps not entirely unpredictable. We propose that the environment in which mate competition occurs mediates the importance of sexual selection relative to sexual conflict, and hence the net effect of mate competition on nonsexual fitness. To test this, we performed experimental evolution with 63 fruit fly populations adapting to novel larval conditions where each population was maintained with or without mate competition. In half the populations with mate competition, adults interacted in simple, high-density environments. In the remainder, adults interacted in more spatially complex environments in which male-induced harm is reduced. Populations evolving with mate competition in the complex environment adapted faster to novel larval environments than did populations evolving without mate competition or with mate competition in the simple environment. Moreover, mate competition in the complex environment caused a substantial reduction in inbreeding depression in larval viability relative to the other two mating treatments. These results demonstrate that the mating environment has a substantial and predictable effect on nonsexual fitness through adaptation and purging.

Keywords: adaptation, purging, sexual conflict, sexual selection.

Darwin (1859) noted that mate competition can generate strong selection on males such that "…sexual selection will give its aid to ordinary selection, by assuring to the most vigorous and best adapted males the greatest number of offspring." (1) Most genes are likely to affect male reproductive success because a male must be healthy to succeed in the competition for fertilizations and most genes have some effect on the overall health of an individual (2, 3). This leads to a general expectation that mate competition will be an important source of selection genome-wide, and will typically reinforce natural selection on most alleles. Sexually antagonistic alleles (4) are an important exception but these appear to be rare among new mutations. Experiments show that most new mutations are selected in the same direction in the two sexes and that selection is typically stronger through males, likely because of sexual selection (5-7). However, mate competition can do more than create sexual selection on males; it can also substantially alter natural selection on females.

In the last 25 years, it has become clear that, in many taxa, sexual conflict over mating interactions is at least as important as traditional forms of sexual selection (8). As a by-product of sexual selection to outcompete their rivals, traits can evolve in males that inadvertently harm females, but not all females may be equally affected. A recent study with *Drosophila* showed that males, when given the choice, preferentially direct their sexual attention, and thus harm, towards the best (most fecund) females (9). The intrinsic fitness difference between high- and low-quality females can be dramatically reduced or eliminated because of the attention shifted away from the latter and directed towards former. Thus, biased male harm can weaken natural selection through females, possibly genome-wide. Two recent evolution experiments implicated this as the reason that evolutionary responses were not more adaptive in populations with mate competition than those without (10, 11).

Competition for mates allows for sexual selection, which strengthens selection through males, as well as interlocus sexual conflict, which may weaken selection through females. Each of these effects can vary in strength. Under some environmental conditions females experience substantial harassment and the effects of harm can thus be large, providing

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scope for major changes to selection on females via biased male harm. Similarly, some types of environments may impose a stronger selective sieve on the genetic quality of males that achieve the greatest mating success. The physical environment is therefore likely to play a key role in mediating the relative importance of sexual selection and sexual conflict and thus, whether mate competition helps or hinders the improvement of nonsexual fitness.

In the last few decades, lab experiments with insects have strongly shaped views on the evolutionary consequences of mate competition (12-18); work with *Drosophila* has been particularly influential, highlighting a major role for sexual conflict (19-21). Results from these studies are mixed; in several cases mate competition did not improve nonsexual fitness (10, 22-24). Little attention has been given to understanding this variation in outcomes. The vast majority of this work with *Drosophila* has been performed with mate competition occurring under 'standard lab conditions', which involve small, high-density containers with low spatial complexity. While this may be representative of some natural settings, other environments will have lower density and greater spatial structure. Such environmental differences are important because they can alter both sexual selection and sexual conflict (25). In larger, more spatially complex environments, it is more challenging for males to find mates, so sexual selection operates more strongly on many mutations (26). Further, in larger, more complex environments females can evade males, reducing harm overall as well as the opportunity for males to bias harm (27). For these reasons, we predict that the benefits of mate competition on the genetic improvement of nonsexual fitness are likely to be more evident if mate competition occurs in a larger, more complex environment than in a small simple environment.

To test this, we compare evolution in populations with enforced monogamy ('mate competition absent', MC_{absent}) to two versions of evolution with mate competition occurring in either spatially simple or complex environments ('mate competition simple' and 'complex', MC_{simple} and $MC_{complex}$, respectively). Mate competition occurs in a standard high-density fly vial in the MC_{simple} treatment, but in a larger cage that includes multiple food patches and additional spatial structure in the $MC_{complex}$ treatment (27).

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While there may be small differences in abiotic selection due to the physical differences between the MC_{simple} and MC_{comlex} mating environments, we know from a previous study that male-female interactions differ between these specific environments in a manner that alters biotic selection, generating a strong *a priori* expectation. In particular, in the complex environment harassment rates are lower and less biased, female fitness is less affected by males, and the opportunity for male choice (i.e., biased harm) no longer weakens selection in females (27). In the current study, we have 63 evolving fly populations, divided equally into three "larval adaptation" sets. Each set was maintained in a different but novel larval rearing environment to which it could adapt (Set 1 cornstarch-based larval medium (rather than cornmeal) and a 2-4 h heat shock (37 °C) to 3-day old larvae); Set 2: elevated ethanol in the larval medium with a 2-4 h cold shock (4 °C) to 3-day old larvae; Set 3: elevated salinity of larval medium with constantly elevated rearing temperature (28 °C)). Within each larval adaptation set, the 21 populations were divided evenly among the three mating treatments (MC_{absent}, MC_{simple}, $MC_{complex}$) that differed in the mating environment, while all populations within a set experienced similar larval rearing conditions (see Materials and Methods). Our goal is to test how mate competition, and the environment in which it occurs, affects the genetic improvement of nonsexual fitness. To do so, we assess the effects of mating treatment both with respect to adaptation and purging. By having independent sets of populations adapting to three different novel larval environments, we gain insight as to whether any observed effect of mating treatment is specific to adaptation to one particular environment or represents a more general result.

Results and Discussion

Within each larval adaptation set, we examine adaptation by measuring egg-to-adult survivorship in the appropriate novel larval environment at generations 14 and 50. Egg-to-adult viability is a major component of nonsexual fitness that is both directly affected by the novel larval environment and is fully comparable across mating treatments. In all three sets, there is clear evidence of adaptation: when tested in their respective novel larval environments, populations from all three mating treatments have substantially higher viability than the ancestral population (i.e., average survivorship in each treatment

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is greater than the ancestor by at least 1.40-fold in generation 14 and 1.46-fold in generation 50). However, our key interest concerns variation among the mating treatments in the degree of adaptation. Analyzing across generations and adaptation sets, there is significant variation among mating treatments ($F_{2,108} = 13.69$, $P < 0.0001$; Table S1); adaptation in the treatment where mate competition occurs in the complex environment is greater than the other two treatments, which do not differ significantly from one another ($MC_{complex} > MC_{absent}$, MC_{simple}; Fig. 1A). This pattern is present when examined separately by adaptation set and generation; in all cases, the point estimate of mean viability in $MC_{complex}$ is higher than the other two treatments, although the effect is small in some cases (Fig. 2A). Averaging across adaptation sets, the amount of adaptation at generation 14 (measured as *viability of evolved populations – viability of ancestor*) is 1.40-fold greater in $MC_{complex}$ than MC_{absent} but only 1.07-fold greater in MC_{simple} than MC_{absent}. At generation 50, adaptation is 1.19-fold greater in $MC_{complex}$ than MC_{absent} whereas adaptation in MC_{simple} is 0.89-fold (i.e., less than) that of MC_{absent}.

Though alleles with beneficial effects specific to a given larval rearing environment likely underlie much of the observed adaptation, fitness in all populations will also be affected by the presence unconditionally deleterious alleles segregating at mutation-selection-drift equilibrium. When selection is stronger, these deleterious alleles should be rarer. In an experiment such as ours, it is extremely difficult to identify which genetic variants have detectable deleterious effects. However, useful inferences about the genetic load of deleterious mutations can be made from studying inbreeding depression (28). In comparison to a population that experiences a smaller magnitude of inbreeding depression, a population with greater inbreeding depression harbors a higher frequency of deleterious alleles, presumably due to weaker selection. We measured inbreeding depression for egg-to-adult survivorship in the ancestral larval environment so these measures of inbreeding depression likely reflect the effects of unconditionally deleterious alleles. We find significant variation among the mating treatments in inbreeding depression ($F_{2,54} = 15.64$, $P < 0.0001$; Table S2). In all three larval adaptation sets, inbreeding depression in the MC_{absent} and MC_{simple} treatments are similar, while that in the $MC_{complex}$ treatment is significantly lower (Fig. 1B). This pattern is also present when

examined separately by adaptation set (Fig. 2B). Averaging across larval adaptation sets, inbreeding depression is 36% as large in $MC_{complex}$ as in MC_{absent} whereas it is 90% as large in MC_{simple} as in MC_{absent}. Low inbreeding depression can occur either because both outbred and inbred offspring are reasonably fit or because both offspring types are unfit. In all three larval adaptation sets, the $MC_{complex}$ populations have low inbreeding depression because both outbred and inbred offspring are fit (Fig. S1). This suggests that purging of deleterious alleles has been more efficient in the $MC_{complex}$ treatment than in the other mating treatments.

Several recent studies have reported that mate competition hinders the improvement of nonsexual fitness. We replicate these results with respect to adaptation to three distinct novel larval environments, as well as purging, for populations evolving in simple mating environments. Crucially, we also show that this result changes dramatically and predictably in a slightly more complex—and arguably more realistic—mating environment, with mate competition now promoting more rapid adaptation and more efficient purging. Such environmental effects on the relative importance of sexual conflict and sexual selection are consistent with predictions from mechanistic studies in *D. melanogaster* (26, 27). Our results are supported by experiments with other fly populations examining the purging of large-effect marker mutations (29, 30) in similar mating environments.

The idea that genome-wide consequences of mate competition can vary predictably with environment is appealing and may be general. Sexual conflict, for instance, been shown in a variety of taxa to vary with population density, sex ratio, the availability of refuges, resource levels, predation risk, and other factors(31-34) which, in turn, are likely to vary with the environment. Likewise, the intensity of sexual selection on traits likely reflecting male quality has been shown to vary with the environment (35-37). On the other hand, environment may have little effect in some systems. For example, in some taxa females have more control over sexual interactions and thus are unlikely to suffer male harm(38) regardless of environmental conditions. Nonetheless, our work demonstrates that the environment can play a key, and even predictable, role in determining the effect of mate

competition on nonsexual fitness in some systems, and points to the need for caution in interpreting results from simple lab environments.

Materials and Methods

Creation and Maintenance of Experimental Populations.

The source for our experimental populations were ~2000 *Drosophila melanogaster* collected in the Similkameen Valley, British Columbia in 2005. This population was originally 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, stock maintenance has involved discrete, non-overlapping 2-week long generations. Throughout the evolution experiment described below, a copy of the stock population (~2000 flies) was maintained under its original conditions (i.e., two-week generation time on cornmeal-yeast based larval food, maintained at 25°C in bottles). We refer to this copy as the 'ancestor'.

In September 2014, separate samples of flies from the stock population were collected to form replicate experimental populations. Specifically, 21 independent populations per week over three weeks (63 populations in total) were established; 140 males and 140 females were sampled to found each population. As described below, experimental populations were maintained on a three-week life cycle. All 21 populations from each establishment week were assigned to the same "larval adaptation" set (i.e., novel larval rearing conditions). Corresponding to each of the three weeks of population establishment, there were larval adaptation sets involving: (I) larval medium based on cornstarch (rather than cornmeal) and a 2-hour heat shock (37 °C) to 3-day old larva; (II); standard cornmeal larval medium supplemented with 10% ethanol and a 2-hour cold shock (4 °C) to 3-day old larva; and (III) standard cornmeal larval medium supplemented with 5% salt and constant exposure to 28 °C (rather than the standard 25 °C). To ensure continuing directional selection on these populations, we increased the salt concentration to 6% (set III) and the duration of heat (set I) and cold shock (set II) to 4 hours after the sixth generation.

Within each larval adaptation set, the replicate populations were divided equally amongst three different mating treatments: 'mate competition absent' (MC$_{absent}$), 'mate competition simple' (MC$_{simple}$) and 'mate competition complex' (MC$_{complex}$). The MC$_{absent}$ treatment involved keeping flies each generation with enforced monogamy, achieved by creating randomly chosen pairs (140 male/female pairings per population), each held in wide straws (radius = 6.35 mm; height = 88.9 mm) that had been inserted into a 3 oz Dixie cup filled with 25 mL of ancestral food (28 straws per cup). Straws were used to minimize the space requirements and maintenance costs associated with this treatment given the scale of the experiment. The food surface within each straw was supplemented with 1-2 yeast pellets. For the MC$_{simple}$ treatment, 35 males and 35 females were placed in a standard fly vial (28.5 mm x 95 mm *Drosophila* culture vial filled with 10 mL of ancestral food supplemented with 30-50 dry yeast pellets). Four such vials were created for each population (totaling 140 adults of each sex) each generation. For the MC$_{comlex}$ treatment, 35 males and 35 females were placed in a 1.65L cylindrical plastic *Ziploc®* food storage container. Four such containers, hereafter "cages", were created for each population (totaling 140 adults of each sex) each generation. Within each cage there were five separate food sources (three 3 oz Dixie cups containing 25 mL of ancestral media, each divided into two by a plastic divider inserted into the food, and two smaller 1 oz cups containing 7.5 mL of ancestral media) and pipe cleaners protruding from the lid into the interior. The food surface in each 3 oz Dixie cup was supplemented with 10-15 yeast pellets and 5-10 yeast pellets were placed onto the food surface in each 1 oz cup. This cage design was chosen to reduce density and increase spatial complexity, allowing flies to be out of sight from one another and providing multiple food patches so that all females are not forced to a single locale to feed and lay eggs. The cages are pictured in Figure S1 of Yun et al. (27). We have previously used these types of mating 'arenas' (i.e., cages and vials) in more mechanistic studies of behaviour and selection (27). For simplicity in the main text, we refer to vials and cages as "simple" and "complex" environments, respectively; however, we note that, in addition to spatial complexity, there are additional differences including fly density and the availability of food and laying sites.

In all mating treatments, adult flies were held in their respective mating arena for a total of six days to feed, interact, and mate. Because females lay eggs in the food during this time, developing larvae may alter adult use of the food and mating interactions, and/or simply cause the food to become "soupy" and hazardous for adults. To minimize this issue, adult flies were transferred via light CO_2 anesthesia to fresh mating arenas (i.e., straw, vial, or cage) supplied with new food on the third day. After six days (day 20 of the maintenance protocol), we anaesthetized flies in the arenas and randomly sampled 105 females from each population for egg laying. These females were evenly distributed into seven standard fly vials with 3-5 yeast pellets to lay eggs for 24 ± 3 hours; the vials contained the appropriate novel abiotic larval medium (i.e., from set I, II, or III). After the egg-laying period, adult females were discarded. Vials were visually inspected and egg density was lowered to approximately 200 eggs by scraping to reduce variation in egg densities among vials. Eleven days later, we collected the adult offspring into holding vials separated by sex (35 flies per vial) and stored them for three days (for logistical reasons to give a 21 day generation time) before repeating the above mating protocols for the next generation. Note that populations in the same larval adaptation set but in different mating treatments were maintained in the same manner at all stages except during the "mating phase" (Day 14-19) of the life cycle. Each non-overlapping generation lasted three weeks (Day 0/21: egg-laying mothers removed from larval vials; Day 11: adults collected from larval vials and placed in holding vials; Day 14: adults placed in mating environments; Day 17: adults transferred to mating environments with fresh media; Day 20: females collected from mating environments and placed in egg-laying vials). As noted above, larval adaptation sets I-III (each consisting of 21 populations) were offset from one another by one week each.

In March 2015, an autosomal *DsRed* marker (39) was introgressed into a sample of the ancestral population by mating 100 *DsRed* males to 100 virgin ancestral females, and backcrossing the F1 offspring to *DsRed* males once more. The *DsRed* marker is a dominant mutation that causes all bearers to emit red light under a fluorescent microscope. Following the backcross, 100 families were created, each consisting of a virgin red female and male. These families were inbred for at least four generations and

families that produced any individuals of wild-type (i.e. non-red) phenotype during this time were discarded; those that did not produce any individuals of wild-type phenotype (around 30 families) were pooled to create a population in which the *DsRed* marker was likely fixed. The population was maintained on a two-week cycle in cornmeal-yeast media, matching the ancestral population. These marked flies served as a competitors for the first viability adaptation assay (generation 14) described below.

In November 2016, another competitor population was created by crossing the brown eye recessive (*bw*) marker into the ancestral genetic background. We first sampled 100 *bw/bw* males and 100 ancestral virgin females and mated them in 10 vials containing ancestral food media. The virgin female offspring of these flies were then backcrossed with ancestral males. We allowed the offspring of the resulting backcross to mate with each other for one generation to obtain homozygous recessive *bw* flies. This population was maintained on a two week cycle in cornmeal-yeast media; 25 ℃; 12L:12D photoperiod; 50% RH, matching the ancestral stock. This competitor was used in the second viability adaptation assay (generation 50) and the inbreeding depression assay.

Adaptation Assays.

At generation 14, we measured larval viability of each population in the appropriate novel larval environment and in competition with the *DsRed*-marked ancestral population. We conducted this assay separately in consecutive weeks for each larval adaptation set (I-III above). On day 11 of the maintenance protocol, we sampled 300 male and 300 female adults from each population, placing sets of 10 adults of each sex into holding vials containing the ancestral media for one day. These flies were subsequently transferred into a new set of vials to oviposit for one day. Each vial contained the appropriate novel larval medium corresponding to their adaptation set (with 3-5 pellets of live yeast sprinkled on top) and were held under the appropriate novel temperature regime. Eleven days later, we released all emerged flies into a large ($24 \times 24 \times 35$ cm) Plexiglas® cage (one cage per population; ~5000 flies per cage). Within each cage, six petri dishes (60 mm × 15 mm) filled with the appropriate novel abiotic media and covered in yeast paste were placed for flies to lay eggs for 15 hours. An egg

harvesting and pipetting protocol (40) was then used to collect and allocate ~100 focal eggs and ~100 *DsRed* competitor eggs together into replicate vials filled with the appropriate novel food. An average of 9.97 replicates per experimental population (range 9-10) was set up and the appropriate novel temperature regime was applied to each vial. After 11 days, emerging focal (wild-type) and *DsRed* flies were counted. We included the *DsRed* flies to help homogenize the competitive environment across populations, though our interest is in the survival of the focal flies. In addition, we performed the same protocol for the ancestral population (i.e., unmarked ancestor larvae against *DsRed* competitors); 30 replicate vials were set up at the same time as each of the three separate larval adaptation sets.

The egg-to-adult viability assay was repeated in generation 50. The protocol was the same except that we set up an average of 19.98 (range 19-21) replicates per experimental population, the competitor for this assay was the *bw/bw* population described above, and narrow glass vials were used instead of wide plastic vials. The ancestral population was again tested using the same protocol and an average of 26 (range 20-30) replicates were set up at the same time as each of the three larval adaptation sets. Combining data across the two assays (i.e. generations), variation in egg-to-adult viability was analyzed using a general linear model with the average number of emerging focal (i.e. wild-type) offspring of each experimental population in a given generation as the dependent variable and adaptation set (I, II, III), mating treatment (MC_{absent}, MC_{simple}, $MC_{complex}$), generation, and their interactions (two- and three-way) as independent variables. Results were qualitatively unchanged if significance was determined via a non-parametric permutation procedure.

Inbreeding Depression Assay.
We created replicate inbred and outbred mating pairs from each population and measured the average fitness differences of their offspring with respect to egg-to-adult viability within each population. For each larval adaptation set (I-III), we calculated the average inbreeding depression for each of the three mating treatments as the mean inbreeding depression of the seven replicate populations.

For each population, we set up 30 inbred families by forming 30 male-female virgin pairs from newly emerged adults (10-12 hours post-eclosion) and placed each into a separate vial containing the ancestral (cornmeal-yeast) media with 3-5 yeast pellets. The pairs were allowed to mate and oviposit for 72 hours. After 11 days, we collected single virgin male and female (full-sib) offspring from each family and placed them into a fresh vial to mate and lay eggs for 24 hours. This procedure was repeated for one more generation (for families that had viable progeny from the previous inbreeding generation) to further increase the average inbreeding. The procedure was conducted separately for each larval adaptation set (i.e. 21 populations). The families used for this assay came from the generations 38, 39, and 41 for larval adaptation sets I, II, and III, respectively. For each larval adaptation set, we also attempted to create 40 inbred families from the ancestral population.

After two generations of inbreeding within families, we collected 10 flies of each sex from each family on day 11-12 and placed them in single-sex holding vials. After a three-day holding period, we generated inbred eggs by sampling five males and five females from the same family and put them into a laying vial. Simultaneously, we generated outbred eggs by placing the remaining five females into another laying vial with five males from a separate family in a round-robin design. Each laying vial consisted of the ancestral food media supplemented with blue dye (the dye aids in counting eggs) and 3-5 yeast pellets. Flies were cleared after 8 hours, and immediately after we scraped the food surface to ensure all laying vials contained approximately 50 eggs. To standardize resource competition among all the laying vials, we used the egg harvesting and pipetting technique (40) from the adaptation assay to allocate approximately 100 competitor *bw* eggs to each of the vials.

We counted and phenotyped the number of adults 12 and 15 days after the laying-vial setup; the counts across the two days were summed. Both inbred and outbred eggs produce adults with wild-type eye colour, whereas the competitors have brown eyes. An average (range) of 23.5 (16 – 29) inbred replicates (i.e. vials) were obtained for each

experimental population, and 23.3 (15 – 29) outbred replicates. For the ancestor, we obtained 30 replicate inbred and 30 replicate outbred vials for all three larval adaptation sets with the single exception of 28 replicate inbred vials in set I (cornstarch/heat shock).

We calculated inbreeding depression for each population as $1 - (W_{inbred}/W_{outbred})$, where W (absolute fitness) is the average number of emerged focal flies (inbred or outbred) across the replicate measures for a single population. Standard errors for the inbreeding depression of experimental populations (i.e., Figs. 1 and 2) are parametric based on among-population variation. The ancestral population is unreplicated at the population level, however, so the standard error of its inbreeding depression (Fig. 2) was estimated via a bootstrap procedure with 10,000 bootstrap samples of the vial replicates. In each bootstrap sample, replicate inbred and outbred vials were separately sampled with replacement and then W_{inbred}, $W_{outbred}$, and inbreeding depression, were then calculated. Variation in inbreeding depression was analyzed using a general linear model with the inbreeding depression of each experimental population as the dependent variable and adaptation set (I, II, III), mating treatment (MC_{absent}, MC_{simple}, $MC_{complex}$) and their interaction as independent variables. Results were qualitatively unchanged if significance was determined via a non-parametric permutation procedure.

Data availability.
Data have been deposited in the Dryad Repository (doi:10.5061/dryad.837d96j)

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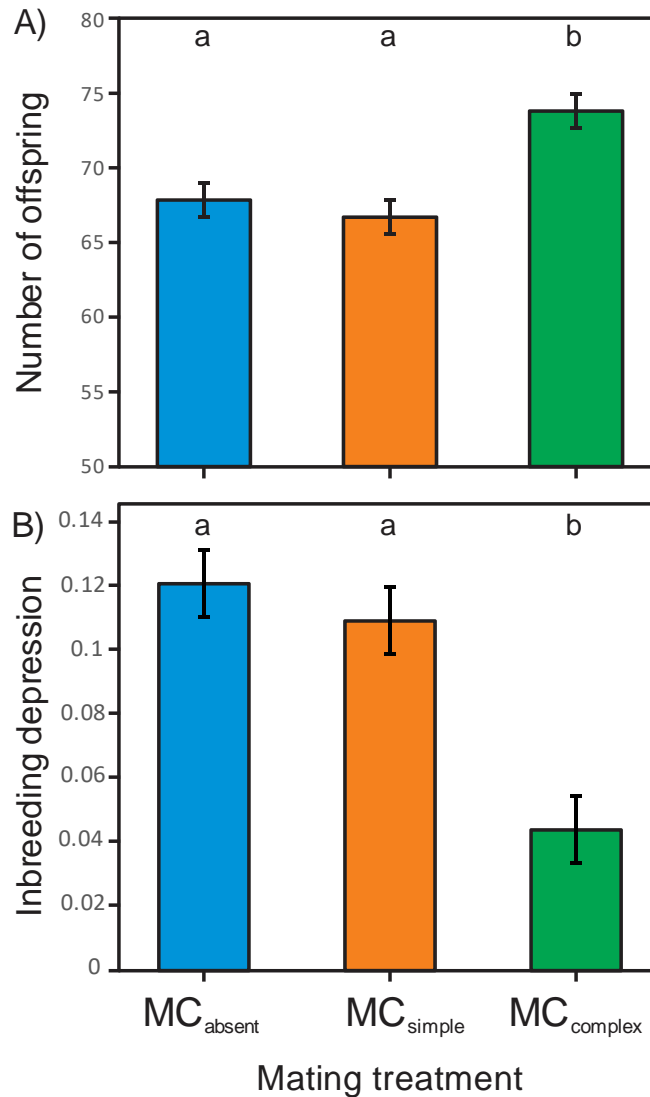
Fig. 1. Quantifying A) adaptation and B) inbreeding depression in the 63 replicate experimental populations from all three adaptation sets evolving under the three different mating treatments. Values are least square means (± 1 SE) for the different mating treatments, treating populations as replicates. Adaptation was measured as egg-to-adult survivorship (quantified as the number of focal adult offspring emerging from a vial) in two competitive assays in the appropriate novel larval environment during generations 14 and 50. Inbreeding depression for egg-to-adult survivorship in the ancestral larval environment was calculated as $1 - (W_{inbred}/W_{outbred})$, where W is the average number of emerged focal flies (inbred or outbred) across the replicate measures for a single population). Treatments with different letters indicate values that are significantly different at $\alpha < 0.05$ using Tukey post hoc test.
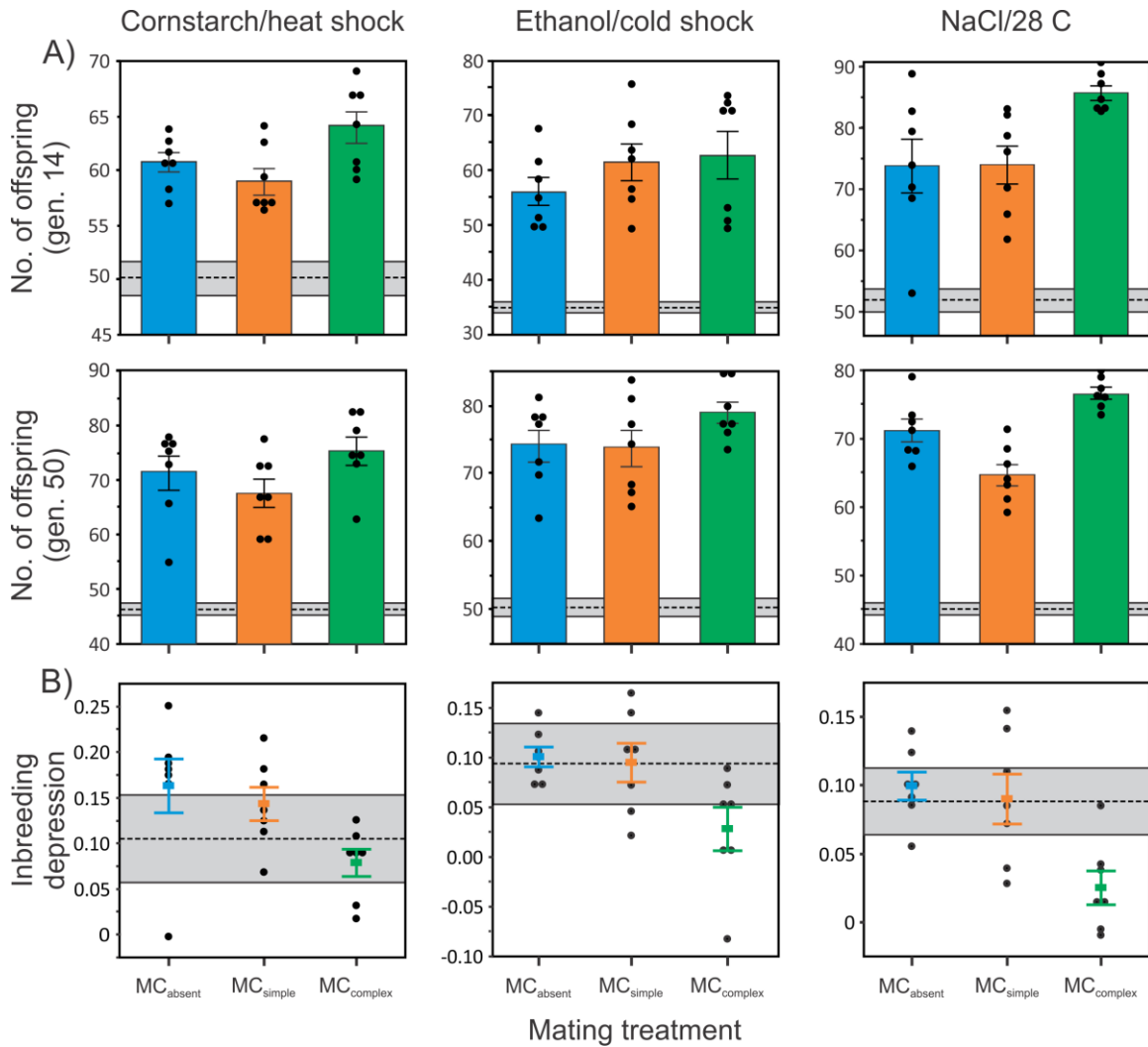
Fig. 2. Quantifying A) adaptation and B) inbreeding depression in the 63 replicate experimental populations evolving under the three different mating treatments separately by adaptation set (columns) and generation (rows; adaptation only). Adaptation and inbreeding depression were quantified as in Fig. 1. Filled circles indicate values for individual populations; means (± 1 SE) are also shown for the different mating treatments. Horizontal dashed lines indicate the value for the ancestral population in each assay, with the grey shading indicating ± 1 SE.

Supplemental Information

Table S1. Results from the statistical analysis of the adaptation assay treating populations
as replicates (average viability ~ adaptation set*generation*mating treatment). For each
population we calculated the average number of emerged focal (i.e. wild-type) flies
across all replicates.

Source	DF	Sum of Squares	F	P
adaptation set	2,108	1549.96	17.30	<0.0001
generation	1,108	1224.15	27.33	<0.0001
mating treatment	2,108	1225.99	13.68	<0.0001
adaptation set × generation	2,108	2893.61	32.30	<0.0001
adaptation set × mating treatment	4,108	326.65	1.82	0.1296
generation × mating treatment	2,108	115.70	1.29	0.2791
adaptation set × generation × mating treatment	4,108	55.76	0.31	0.8700

Table S2. Results from the statistical analysis of inbreeding depression assay treating populations as replicates (inbreeding depression~adaptation set*mating treatment). Inbreeding depression was calculated as $1 - W_{inbred}/W_{outbred}$, where W is the average number of emerged focal flies (inbred or outbred) across all replicates for a single population.

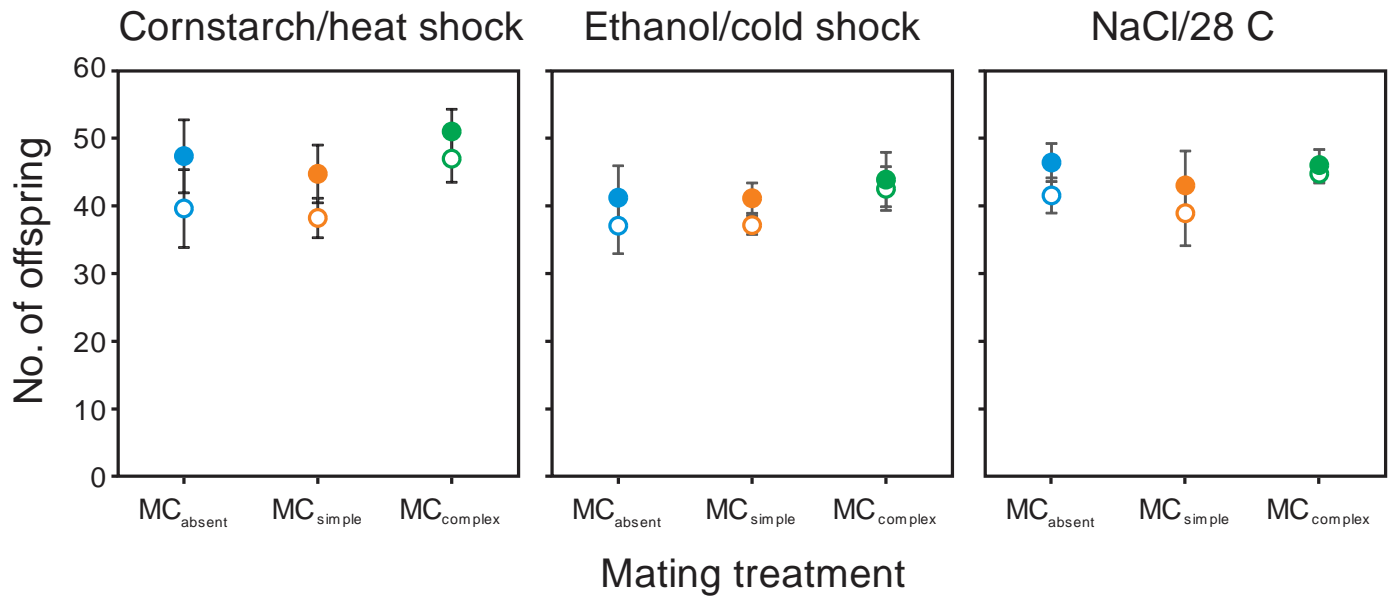| Source	DF	Sum of Squares	F	P
adaptation set	2,54	0.042978	9.29	0.0003
mating treatment	2,54	0.072326	15.64	<0.0001
adaptation set × mating treatment	4,54	0.000443	0.048	0.9955

Fig. S1. Variation in the viability of inbred (open symbols) and outbred (filled symbols) offspring among the 63 replicate experimental populations evolving under the three different mating treatments separately by larval adaptation set (columns). Values are mean number of offspring (± 1 SE), treating populations as replicates.