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Environmental complexity and the purging of deleterious alleles

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Sexual interactions among adults can generate selection on both males and females with genome-wide consequences. Sexual selection through males is one component of this selection that has been argued to play an important role in purging deleterious alleles. A common technique to assess the influence of sexual selection is by a comparison of experimental evolution under enforced monogamy versus polygamy. Mixed results from past studies may be due to the use of highly simplified laboratory conditions that alter the nature of sexual interactions. Here, we examine the rate of purging of 22 gene disruption mutations in experimental polygamous populations of *Drosophila melanogaster* in each of two mating environments: a simple, high-density environment (i.e., typical fly vials), and a lower density, more spatially complex environment. Based on past work, we expect sexual interactions in the latter environment to result in stronger selection in both sexes. Consistent with this, we find that mutations tend to be purged more quickly in populations evolving in complex environments. We discuss possible mechanisms by which environmental complexity might modulate the rate at which deleterious alleles are purged and putatively ascribe a role for sexual interactions in explaining the treatment differences in our experiment.

KEY WORDS: ***Drosophila melanogaster*, monogamy, mutation, polygamy, sexual selection.**

The mating process generates selection on both sexes that can affect the entire genome. Sexual selection, typically through males, has received the most attention in this respect. Assuming a male's overall health or condition is positively related to his reproductive success, and that the majority of the genome affects health/condition, it follows that most genes will be subject to sexual selection that should aid natural selection in spreading beneficial alleles and in purging deleterious alleles (Houle 1991; Rowe and Houle 1996; Whitlock 2000; Whitlock and Agrawal 2009). (Sexually antagonistic alleles are an obvious and important exception but appear to be rare among new mutations.) Consistent with this line of thinking, laboratory studies in insects (primarily *Drosophila*) demonstrate that mutations are typically selected in the same direction between the sexes but more strongly in males (Sharp and Agrawal 2008; Mallet et al. 2011; MacLellan et al. 2012; Sharp and Agrawal 2013; Grieshop et al. 2016; but see Prokop et al. 2017).

One common design for testing the effects of sexual selection compares the outcome of experimental evolution under monogamy versus polygamy, as the opportunity for sexual selection is absent in the former but present in the latter. While a benefit of sexual selection has been detected in some cases (e.g., Hollis et al. 2009; Jarzebowska and Radwan 2010; Almbro and Simmons 2014), others have found little evidence for this (Rundle et al. 2006; Hollis and Houle 2011; Arbuthnott and Rundle 2012, 2014; Chenoweth et al. 2015). For those addressing adaptation from standing genetic variation, loci with sexually antagonistic effects may disproportionally contribute to the response which might explain the negative results (e.g., Radwan 2004; McGuigan et al. 2011; see Whitlock and Agrawal 2009). However, this is not an issue for studies testing the purging of individual deleterious alleles, yet the largest such study to date found little evidence of a benefit of sexual selection under polygamy when testing six different mutations (Arbuthnott and Rundle 2012).

A possible explanation may be found in the work of Long et al. (2009) who showed that, when male sexual attention is

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harmful (as a result of interlocus sexual conflict) and males preferentially court and mate high quality females, the differential harm this induces can reduce the intrinsic fitness advantage of high over low quality females. They termed this a "cost of attractiveness" to females and showed the effect could be very large. The population-level manifestation of such differential male harm is a reduction in the variance of female nonsexual fitness (e.g., fecundity and/or survivorship) and thus a weakening of natural selection through females. It follows that when the opportunity for male choice exists (as it does under polygamy but not monogamy), male harm arising from intense interlocus sexual conflict can result in a dramatic reduction in natural selection through females that hampers purging and adaptation. Only two of the monogamy–polygamy studies that failed to detect a benefit of polygamy investigated why and both found evidence in support of such a cost of sexual attractiveness (Arbuthnott and Rundle 2012; Chenoweth et al. 2015).

Thus, there may be two important and possibly opposing changes in selection when comparing polygamy to monogamy. Total selection through males should strengthen because of the addition of sexual selection, but natural selection through females may weaken because of differential male harm. The magnitude of each of these effects is likely to depend on the physical environment in which adults interact. Most of the monogamy–polygamy experiments have been conducted in *Drosophila* using typical fly culture conditions, which are high density, spatially simple environments. We suspect that in such environments the benefit of sexual selection through males is relatively small and the negative effect of preferential male harm in weakening natural selection through females is relatively large.

The harm caused to females by males is substantial in the simple environments in which *Drosophila* are typically kept in the laboratory (Fowler and Partridge 1989; Partridge and Fowler 1990; Rice et al. 2006) and the opportunity for male choice may also be exaggerated. Under these conditions, differential harm of high over low-quality females may be large, thereby considerably weakening natural selection on females (Long et al. 2009; Arbuthnott and Rundle 2012; Chenoweth et al. 2015). However, we recently examined male harassment in two different settings (Yun et al. 2017): (i) standard fly vials representing a high density, spatially simple mating environment; and (ii) small population cages designed to include features of most natural environments including lower density, more spatial structure, and multiple food patches. For convenience, we refer to these as "simple" and "complex" environments but note that these mating environments differ in multiple ways (e.g., density/volume, total food abundance, material composition). We have shown that, in this more complex environment, sexual interactions are reduced in frequency and are unbiased with respect to female quality (Yun et al. 2017), presumably because females can more easily evade males and choose among alternative food patches so as to minimize the harassment they experience. The effect of exposure to males on female fitness (i.e., male harm) was lower in the more complex environment relative to the simple one. Moreover, the opportunity for male choice (as occurs under polygamy) weakened viability selection through females in the simple environment (consistent with Long et al. 2009) but had the opposite effect in the complex environment (Yun et al. 2017).

With respect to sexual selection on males, locating and mating females may be relatively easy in a simple environment so there may be comparatively weak selection on the multitude of genes that directly or indirectly affect male reproductive success. Indeed, MacLellan et al. (2009) found that sexual selection against deleterious alleles in *Drosophila* was stronger on average when assays were conducted in large rather than small arenas. (The "large" arenas used by MacLellan et al. were not the same as the complex containers used by Yun et al. (2017) and which we also use here, but share the feature of having more space in which to move than vials.)

As stated earlier, Arbuthnott and Rundle (2012) failed to detect a benefit to purging under polygamy relative to monogamy. However, for the reasons outlined above, we believe the simple environment they used may have both exaggerated the cost of sexual attractiveness and underestimated the benefits of sexual selection compared to what would occur in a more complex environment. The ideal test would be a 2×2 factorial manipulation of mating system (monogamy vs. polygamy) and environment (simple vs. complex). However, it is logistically prohibitive to maintain monogamous populations in replicate complex cages. Instead, we compared rates of purging under polygamy in simple versus complex environments, allowing us to test many more mutations. This design cannot tell us whether polygamy per se accelerates purging relative to monogamy, but rather tests the prediction that the benefits of polygamy should be greater in a complex compared to a simple environment. Specifically, we predict faster purging of deleterious mutations in the complex environment used by Yun et al. (2017) because this environment has been shown to eliminate (indeed reverse) the cost of sexual attractiveness, and it might also strengthen sexual selection through males (MacLellan et al. 2009), the net result of which should be stronger selection against deleterious mutations. We test this prediction for a set of 22 separate gene disruption mutations on the second chromosome of *D. melanogaster*, using experimental evolution to compare the rate of purging under polygamy when adults are kept in either "simple" or "complex" environments.

As predicted, mutations were typically purged faster in the complex compared to the simple mating environment. Previous results strongly imply that this result arose at least in part from a change in the cost of attractiveness (i.e., eliminating the cost of attractiveness; Yun et al. 2017). Additional work

would be needed to determine whether and how the environment altered sexual selection through males, or if other abiotic differences between simple and complex containers also contributed to the result in a manner independent of sexual interactions.

Methods

We tested the effects of sexual selection on purging for 22 separate gene-disruption mutations on the *D. melanogaster* second chromosome (Table S1). These mutations were randomly selected from a larger set originally created by the Exelixis Corporation via transposon insertion mutagenesis (Thibault et al. 2004). We screened the Exelixis collection for unique insertions on chromosome 2, excluding those that were not available at the Bloomington Stock Center or were denoted as homozygous lethal. Insertions were originally performed on an isogenic background carrying the X-linked recessive w^{1118} allele and were balanced over a *curly of Oster* (*CyO*) chromosome. w^{1118} produces a white-eye phenotype but wild-type eye color is partially to fully restored by a *mini-white* cDNA sequence of the wild-type *white* gene (w^{+mC}) that resides within the transposon construct. For each mutation and the isogenic background, a series of crosses with different genotypes was performed to remove the *CyO* balancer and the w^{1118} allele, thereby recovering wild-type eye color (Fig. S1). The resulting populations were isogenic for their respective Exelixis second chromosome but were segregating variation for the rest of the genome.

To create experimental populations, males from each of the 22 mutant populations were separately crossed with virgin females from the isogenic background-derived population to create heterozygotes. At the same time, homozygotes for each mutation were created by crossing mutant males and females separately for each mutation. From these individuals, six experimental populations were founded for each mutation, each consisting of 120 mutant homozygotes and 120 heterozygotes (with equal sex ratios), yielding an initial mutant allele frequency of 0.75 in each. Three of these populations were assigned to each of the two treatments that differed in size and structural complexity of their mating arena following Yun et al. (2017). The "simple arena" was a standard "wide" plastic *Drosophila* culture vial (28.5 × 95 mm; approximately 60 mL) that contained 10 mL of food with live yeast sprinkled on top. The "complex arena," in contrast, was a cage made from a 1650-mL plastic *Ziploc*® food storage container that had two pipe cleaners protruding down from the lid and that contained five cups with yeasted food (Figs. S2 and S3).

Populations were maintained via nonoverlapping generations on a 12L:12D cycle at 25°C and 70% humidity using a standard (2:1 sugar:yeast) media sprinkled with live yeast. For a given population each generation 100 females were allowed to oviposit for 24 h in five vials (20 females/vial), after which they were discarded. Twelve days later the adult offspring were mixed among the vials and then 120 females and 120 males were collected using light CO_2 anesthesia. These individuals were held separately by sex in vials (30 individuals/vial) for two days (to maintain a three-week generation time to facilitate blocking), after which they spent six days in their respective mating arena (i.e., vials or cages), with 30 males and 30 females together in each of four replicate arenas. Fresh media was provided on day four in the mating arenas. After six days in the mating arenas, 100 females were randomly chosen from among the survivors for egg laying. The experiment was conducted in three blocks with all six replicate populations of a given mutation within the same block (Table S1). In the majority of cases, populations were maintained for 10 generations although three mutations (2, 6, and 16) were terminated after eight generations due to the loss of multiple populations for unknown reasons.

During experimental evolution, mutant allele frequency in each population was estimated three to four times (i.e., generations; Table S1) by individually pairing (for four days) each of 75 males (50 males in gen. 4 of Block 1) from a given experimental population with a single virgin female from a separate stock that was homozygous for the X-linked recessive *white* (w^{1118}) allele. Paternal genotype was inferred from the eye color of the male offspring: if the father carried 0/1/2 mutant allele(s), respectively, then none/some/all male offspring would express the partially rescued wild-type eye color due to the *mini-white* allele within the transposon. Vials with fewer than eight male offspring were discarded, as were population estimates for a given generation if based on fewer than 10 male–female pairs. An exact binomial test (two-tailed) was used to determine whether the sign of the difference in final allele frequency between the simple and complex environments deviated from random expectation. A two-tailed nonparametric Wilcoxon signed-rank test was also used to determine whether the difference in average allele frequency (simple−complex, treating populations as replicates) deviated significantly from zero. All tests were performed in *R* version 3.2.2 (R Core Team 2016).

Any variance in fitness due to sources (both genetic and nongenetic) other than the focal mutation will increase the amount of drift experienced by the focal mutation, that is, variance in fitness reduces the effective population size (N_e) experienced by the focal mutation. It is possible that the variance in fitness (from sources other than the focal mutation), and thus drift, differs between environments. Using a standard Wright–Fisher model with diploid selection, we performed simulations that mimicked conditions in our evolution experiment (22 mutations tested in each of two environments with three replicated populations per treatment for 10 generations). Our goal was to evaluate whether differences

in drift alone (rather than differences in selection) could cause differences in purging similar to what we observed so the simulations assumed the two environments had the same strength of selection but differed in N_e (we used N_e values in the set {20, 40, 60, . . . ,240}). Each simulated experiment was replicated 2500 times for each combination of N_e values and for each combination of dominance coefficients ($h = 0.25$ and 0.5) and selection coefficients ($s = 0.0$, 0.01, 0.05, and 0.1). We then calculated the proportion of the 2500 simulated experiments (for each parameter set) that resulted in a deviation from the null expectation for each test (binomial and Wilcoxon) that was as, or more, extreme as that observed in our empirical evolution experiment. All simulations and analyses were performed in *R* 3.2.2 (R Core Team 2016; see Supporting Information "Simulation Methods" for code).

Results

Average mutant frequency was lower in populations evolving in the complex as compared to the simple environment for 18 of the 22 mutations (Fig. 1), representing a significant deviation from expectation under the null hypothesis of an equal probability of either treatment having a lower average (binomial exact test: $P = 0.0043$). Across mutations, the difference in average mutant frequency (simple−complex) was also significantly greater than zero (Wilcoxon signed-rank test, $V = 237$, $P < 0.0001$), indicating more rapid purging of the mutations in the complex than in the simple environment (Fig. 2). We used computer simulations to investigate whether treatment level differences in N_e alone could have generated results similar to these. We found little evidence that differences in N_e alone could have produced our empirical results. For every parameter set examined, the frequency of observing an effect in simulated data as large as or larger than what we observed in the real data was less than 1%.

Discussion

Our study compared the rate of purging under polygamy in two different mating environments in which we have previously studied male harm and its effect on selection through females (Yun et al. 2017). Stronger selection in a complex environment would be expected if biased male harm of females was reduced and/or male mating success was more selective. Consistent with this, final deleterious allele frequency in populations evolving in the spatially complex environment was lower than in spatially simple mating environment for 18 of the 22 mutations assayed after 8–10 generations of experimental evolution (Fig. 1). The difference in the average frequency of the deleterious allele at the end of experiment was also significant in the direction indicative of stronger selection in the complex environment (Fig. 2). These results may be conservative because some of the gene disruptions are likely to be effectively neutral in the laboratory setting (e.g., possibly mutations 18 and 21; Fig. 1) and there is no expectation in terms of directionality of treatment effects for neutral alleles.

Previous studies had shown that when the opportunity for male choice exists (i.e., polygamy), biased male harm substantially weakens selection on females in simple environments (Long et al. 2009) and this reduces the rate of purging (Arbuthnott and Rundle 2012). However, Yun et al. (2017) showed this suppression of selection through females via male harm did not occur, and in fact reversed, when adults interacted in the more complex environment we used here (and which we speculate is more representative of most natural settings). Yun et al.'s (2017) results strongly suggest that the more rapid purging in cages observed in the current study arose at least in part due to a strengthening of selection through females. In addition, we suspect selection through males was stronger in the complex versus simple environment. The complex cages used here were larger than the simple vials and MacLellan et al. (2009) found evidence of stronger selection through males in "large" versus "small" arenas. A proper test of selection through males would require performing a study like that of MacLellan et al. (2009) but using the specific environments studied here.

Our simple versus complex environments differed with respect to multiple abiotic factors (e.g., volume, structural complexity, food distribution, and availability) and we do not know whether any of these also contributed to our results independent of effects mediated by sexual interactions. Environmental changes often alter the strength of selection on individual mutations but these effects vary among genes, that is, selection becomes stronger on some mutations but weaker on others (see reviews by Martin and Lenormand 2006; Agrawal and Whitlock 2010). Because there is a difference in the rate of purging, on average, across the set of mutations studied here, we are interested in factors that could alter the average strength of selection. A commonly held view is that selection becomes stronger under "stress," but there is little support for this. Nonetheless, in some cases selection is stronger, on average, in one environment than another for reasons that remain unclear (Agrawal and Whitlock 2010). We cannot exclude that such an effect contributes to our result. However, we know that our simple and complex environments differ in important ways with respect to sexual interactions in a manner that would produce the observed results. This implies that any direct effects of the abiotic environment on selection would be in addition to, rather than in place of, those mediated by sexual interactions.

Although our study was motivated by an extensive body of *Drosophila* laboratory research, the underlying ideas are likely to be relevant across a variety of taxa in nature. Our intention was to introduce a small degree of ecological realism to the laboratory

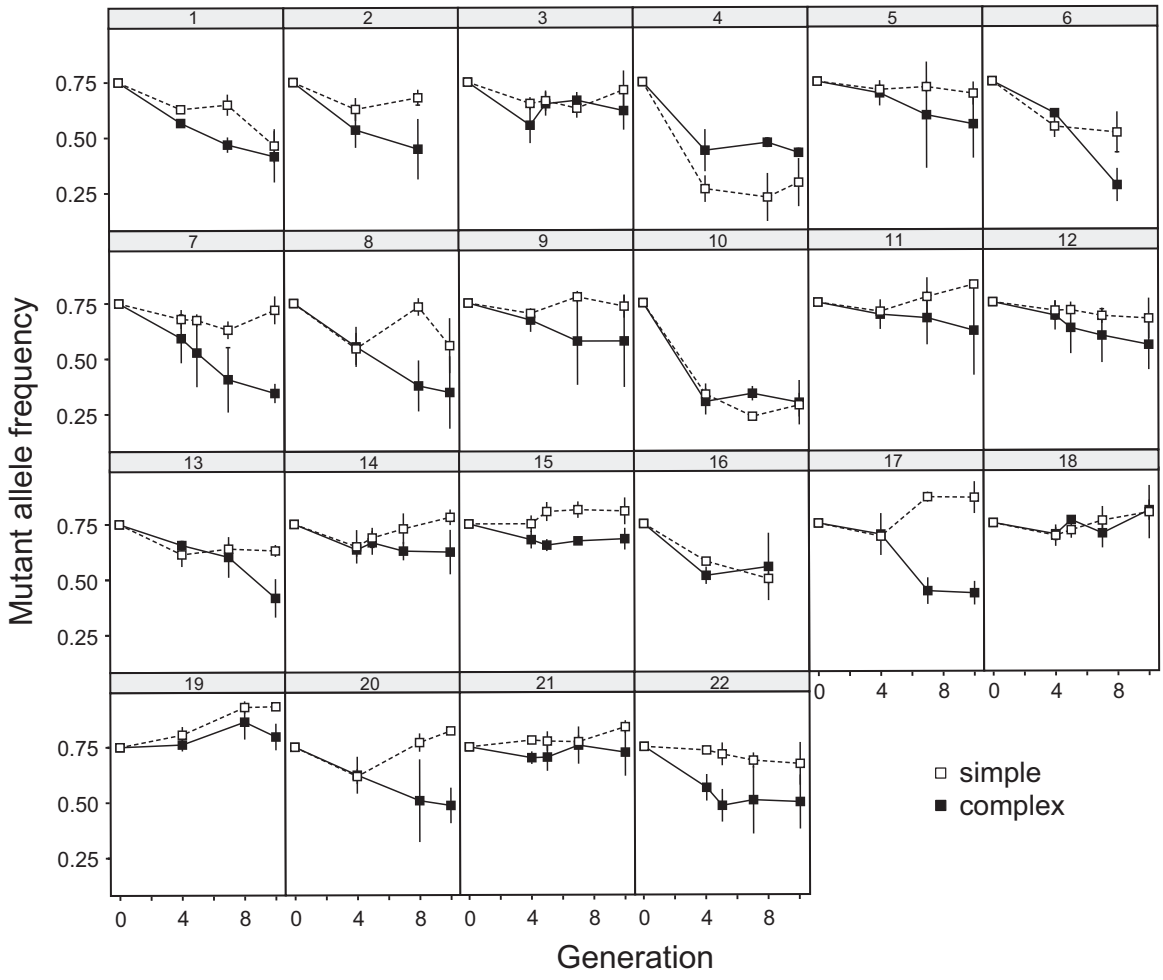

Figure 1. Mean (±SE) mutant frequency of replicate populations evolving in either simple (open squares, dashed lines) or complex (closed squares, solid lines) mating environments for each of 22 *Exelixis* gene disruption lines. Mutations are identified by arbitrary ID numbers (Table S1).

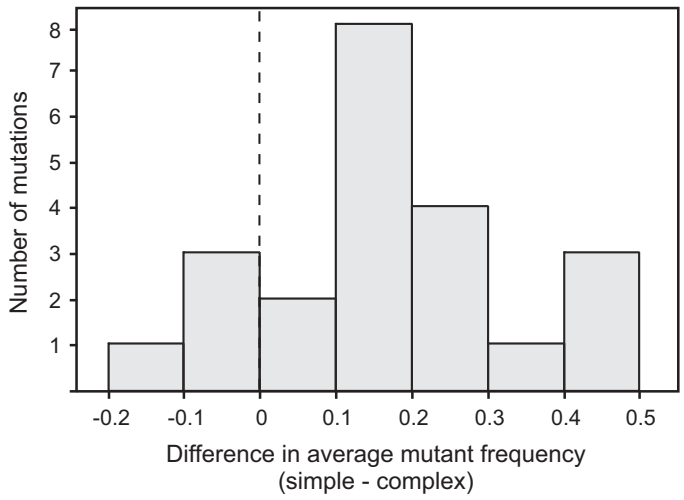

Figure 2. Distribution of the difference in average frequency (simple – complex) of each of 22 mutations in replicate populations that had evolved for 8–10 generations in a simple compared to complex mating environment.

environment: spatial complexity and variation in density are conditions that are likely representative of most, but not necessarily all, natural systems. Populations/species in nature can also experience spatial and temporal differences in the environments in which they breed. Alongside other impacts, such changes in environment (e.g., density, food availability, predation risk, and spatial complexity) may alter selection arising through behaviors associated with sexual conflict. For example, hunger levels affect female resistance to males, exerting stronger selection on males (Rowe 1992; Ortigosa and Rowe 2002). In guppies, female preference for bright males is stronger when predators are absent than when they are present (Godin and Briggs 1996), while in stickleback fish, female preference for red coloration in males is reduced in densely vegetated habitat (Candolin et al. 2007). Such changes may play a key role in mediating the contribution of sexual selection through males to purging deleterious alleles and the extent to which male harassment weakens purifying selection through females.

We were motivated by a body of work that has used monogamy versus polygamy experiments to make inferences about the alignment of sexual and natural selection (e.g., Rundle et al. 2006; Candolin et al. 2007; Hollis et al. 2009; Hollis and Houle 2011; Arbuthnott and Rundle 2012; Almbro and Simmons 2014; Arbuthnott and Rundle 2014; Chenoweth et al. 2015). As discussed, monogamy versus polygamy studies are nonideal for isolating the effects of sexual selection because of the potential for biased male harm to alter selection in females. Many of these studies used simple environments and we speculate their results were strongly affected by treatment differences in selection through females rather than through males, as intended (see Arbuthnott and Rundle 2012; Chenoweth et al. 2015). While monogamy versus polygamy studies have value in assessing the net effect of polygamy, our knowledge will be richer if we understand the underlying components (e.g., selective effects through each sex), the mechanisms (e.g., behavioral interactions) responsible, and how these are mediated by the environment. Changes in selection through females may be less of a problem in systems with little scope for male harm. Zuk et al. (2014) have argued that in insect taxa where females do not mate, feed, and lay in the same location (e.g., many orthopterans and coleopterans), females may have more control over sexual interactions and thus be unlikely to suffer male harm. Moreover, a variety of alternative experimental approaches have been used to test the alignment of sexual with natural selection. For example, some studies have compared monogamy to polyandry (e.g., Holland 2002; Fricke and Arnqvist 2007; Power and Holman 2015), while others have experimentally removed the variance in female reproductive success within a mating system manipulation (e.g., Radwan 2004; Radwan et al. 2004; McGuigan et al. 2011; Pelabon et al. 2014). Such approaches limit the scope for a "cost of attractiveness" but may require unnatural social settings in which sexual selection occurs (e.g., highly male-biased sex ratios) and a "cost of attractiveness" may still arise through differential viability effects of males on females. In general, any manipulation of the mating system should be carefully considered with respect to unintended changes in selection and how this might be mediated by the physical and social environment in which it assessed.

AUTHOR CONTRIBUTIONS

AFA and HDR conceived the project and AS conducted the experiment and did the simulations. All authors contributed to data analysis and writing the paper.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Information about the 22 gene-disruption mutations tested.
Figure S1. Crossing scheme used to remove the *CyO* balancer and the w^{1118} allele from each *Exelixis* line, and to create lines homozygous for the *Exelixis* (*Ex*) gene disruption allele on chromosome 2.
Figure S2. Comparison of the containers used for the simple (left) and complex (right) mating arenas.
Figure S3. Details of the complex mating arena.