ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

Assessing putative interlocus sexual conflict in *Drosophila melanogaster* using experimental evolution

by

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Electronic Appendices

Appendix A: These protocols and data show that live-yeast is a limiting resource to female reproduction and that female density does not significantly affect female fecundity.

Appendix B: These protocols and data show that female mating success in 'initial mating' vials is nearly complete.

Appendix C: These protocols and data show that most females remate during their time in the 'male-female interaction' vials.

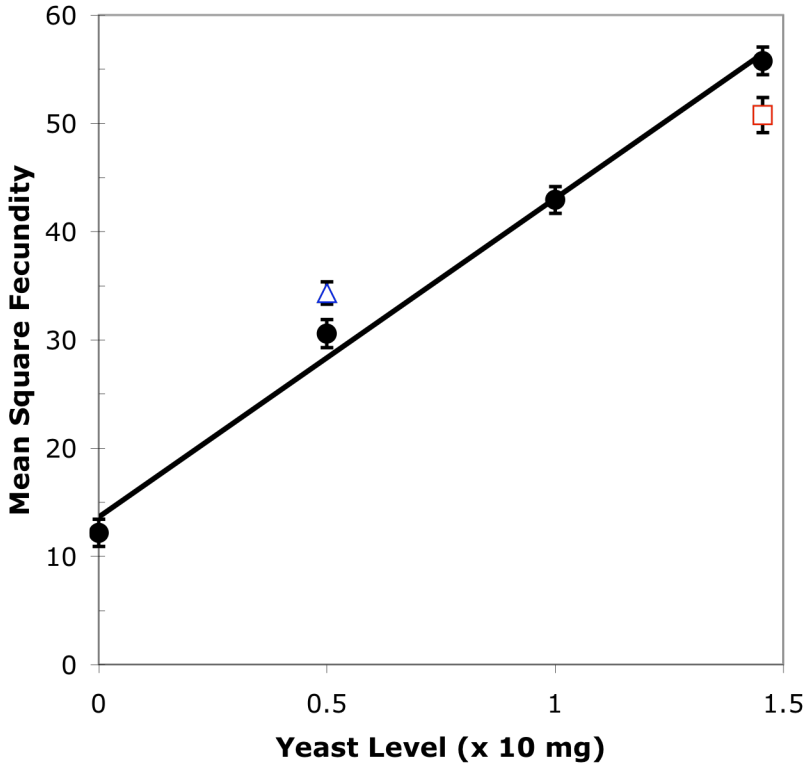
Appendix D: These protocols and data show that the relative fitness of brown-eyed (*bw/bw*) females and red-eyed (bw/bw^{+}) females are approximately equal, while those of brown-eyed and red-eyed males significantly differ.

Appendix E: These protocols and data show that males do not significantly influence female fecundity by either consuming or fouling the limiting resource, live-yeast.

Appendix A

To measure the effect of fly density on female fecundity we first determined whether or not the amount of live-yeast applied to the surface of the killed-yeast medium was a limiting resource to female lifetime reproduction. Five replicate adult competition vials each with 16 mated females (with no males) were constructed with 0, 0.5, 1.0, and 1.45 times the normal level of yeast used to propagate the LH_M laboratory population (10 mg/16 females). A simple linear relationship was found between average female fecundity and the level of live-yeast provided, indicating that live-yeast was the major factor limiting female lifetime fecundity (Appendix A: Figure 1, black circles, $r^2 = 99.3\%$, $P = 0.0037$). To test for an effect of density besides its influence on the amount of live-yeast available per capita, we constructed five additional replicate vials of 11 females per vial (45% more yeast available per capita per vial) and 32 females per vial (50% less yeast available per capita per vial). We found neither a significant difference between the average fecundity of females at double density with normal yeast level (blue triangle in Appendix A: Figure 1) and females at normal density with 50% less yeast, nor between females at normal density with 45% more yeast and females at 31% lower density with normal yeast level, such that per capita yeast availability was increased by 45% (red square in Appendix A: Figure 1; $P > 0.05$, Tukey-Kramer multiple comparisons test). These data indicate that the effect of changing density is the same as a corresponding change in the level of the limiting resource, live-yeast. There was, however, a non-significant trend toward increased density reducing female lifetime fecundity less than would be expected by changes in per capita yeast consumption alone (blue triangle in Appendix A: Figure 1). There was also a non-significant trend where decreased density resulted in lower lifetime female fecundity than would be expected by changes in per capita yeast consumption alone (red square in Appendix A: Figure 1).

We suspect that these patterns, if real, are probably due to flies spreading new yeast colonies on their feet to additional locations on the surface of the killed-yeast medium in the vials. This effect of density, over and beyond that of the consumption of live-yeast, would make our 'unprotected' environment a conservative estimate of male-induced harm (see electronic Appendix E).



Appendix A: Figure 1) Relationship between the quantity of live-yeast provided during culture and average female fecundity in *Drosophila melanogaster* from the base LH_M population (16 females per vial; black circles). Also shown is the average female fecundity under double density (32 females per vial; blue triangle) and under 45% reduced density (11 females per vial; red square). All mean squares are depicted ± standard error.

Appendix B

To determine the proportion of virgin females that mated during the 'initial mating' stage (Figure 1, left) a separate control experiment was conducted. Without anesthesia, 16 virgin females (*bw/bw*) were combined with 24 males (*bw/bw*) and allowed to mate for 2 hours (as in the main experimental protocol). After the initial mating, all males were removed under brief anesthesia and discarded. Females were placed in 'male-female interaction' vials (but without males) with limited yeast for two days. After two days, females were placed into individual 'oviposition' vials for 18 hours, then discarded. After 6 days, all oviposition vials were scored for the presence/absence of larvae. This process was replicated 20 times for a total of 320 females. During the two-hour 'initial mating' period we found that nearly all females were mated (96.8 ± 1.1% [mean ± SE]).

Appendix C

To estimate the level of remating in the male-female interaction vials, flies from the brown-eyed replica of the base population (LH_M-*bw*) were used. On day-12 of its 2-week generation cycle 16 vials each containing 16 females were made.

In 8 of the vials, we measured whether or not females had mated by the time that they were transferred to the male-female interaction vials. For each of these vials, the 16 brown-eyed females (*bw/bw*) were individually placed in oviposition vials for 18 hours then removed. Six days later we scored the vials for the presence/absence of larvae – indicating that the female had mated – and found that 95.3% of females were mated at the time of transfer to the male-female interaction vials.

In the 8 remaining vials we measured the remate rate. In each vial, 16 red-eyed (bw^+/bw^+) males from the LH_M population were added to the 16 brown-eyed females (*bw/bw*). Any remating that occurred in these male-female interaction vials was apparent since any brown-eyed females that remate with these red-eyed males will produce red-eyed offspring. After 2-days, individual females were placed in oviposition vials for 18 hours, then removed. Twelve days later the offspring were screened for red-eyed flies. A total of 94.1% of the females produced at least one red-eyed offspring. After adjusting for the females that had not mated at the time of transfer, we estimate that 89.4 ± 2% (mean ± SE) of females remate in male-female interaction vials. Additionally, because of the high level of sperm displacement in *D. melanogaster* (approximately 85% in the LH_M population) most offspring are sired from matings that occur in the male-female interaction vials.

Appendix D

In order to determine the cause of the rise of the red allele in control populations, two experiments were conducted to determine the relative fitness between the brown eye-color and red eye–color alleles when present in both males and females.

Males:

In the first experiment, we measured the fitness, within the context of sexual selection, of red-eyed males compared to brown-eyed males when they both competed for mating opportunities. A total of 16 replicate vials were assayed. In each replicate, 24 males (12 brown-eyed [*bw/bw*] and 12 red-eyed [bw^{+}/bw]) were placed in an initial mating vial with 16 brown-eyed females (*bw/bw*) for 2 hours. Then, under light anesthesia, the number of males was reduced to 16 (8 each) and transferred with the 16 females to 'male-female interaction' vials, containing a limited amount of live-yeast. After the two days, females were transferred to individual oviposition vials for 18 hours, then removed. After twelve days all progeny were scored for eye color to determine male parentage.

Females:

In the second experiment, we measured the fitness, in the context of natural selection, of red-eyed females compared to brown-eyed females when they both competed for a limiting resource linked to reproductive success (live-yeast; see electronic Appendix A). A total of 20 replicate vials were assayed. In each replicate 16 females (8 red-eyed and 8 brown-eyed) were placed in an initial mating vial with 24 males (12 brown-eyed and 12 red-eyed) for two hours. Then, under light anesthesia, the number of males was reduced to 16 (8 each) and transferred with the 16 females to male-female interaction vials,

containing a limited amount (10 mg) of live-yeast. After the two days, females were transferred to individual oviposition vials for 18 hours, then removed. After twelve days all progeny were counted.

We found that while there was no difference between the lifetime fecundity of brown-eyed females and red-eyed females (mean fecundity brown-eyed ± SE = 26.13 ± 1.21 vs. red-eyed = 26.71 ± 1.20; t_{paired} = 0.493, d.f. 19, P = 0.627), there was a significant difference in mating success between brown-eyed and red-eyed males (mean proportion brown-eyed offspring ± SE = 0.682 ± 0.0151 vs. red-eyed = 0.318 ± 0.0151, where expected proportions are 0.75 vs. 0.25; t = -4.516, d.f. = 15, P = 0.0004). We estimate that red-eyed males have a 27.2% competitive advantage over brown-eyed males. These data indicate the pleiotropic natural/sexual selection that we observed on the bw^+ allele was manifested through males but not through females.

Appendix E

To determine if males can affect female fecundity in a non-direct manner (i.e., by eating or fouling the environment/live-yeast), we pretreated male-female interaction vials for 8 hours with either i) 16 males, ii) 16 females, or iii) no flies, and then removed them before the vials were used to culture 16 singly mated females in a manner otherwise identical to that used during the male-female interaction phase of the experiment. Sample sizes for each pretreatment were 12 vials of 16 females each. Average fecundities of females after feeding within the pretreated vials were (mean ± SE) i] 31.30 ± 0.71, ii] 21.86 ± 0.71, and iii] 31.16 ± 0.71, respectively. Pretreatment of the male-female interaction vials with 16 males had no measurable effect on female fecundity (no flies vs. 16 males, delta = -0.131 ± 1.003, t = -0.131, d.f. = 533, P = 0.896). In sharp contrast, pretreatment with 16 females produced a 30% reduction in the fecundity of females that subsequently fed in these vials (no flies vs. 16 females = 9.301 ± 1.002, t = 9.286, d.f. = 533, $P < 0.000001$). Interestingly, females eat all of the live-yeast within the first 24 hours, so the nearly one-third reduction in fecundity after an 8-hour exposure of live-yeast to competitor females is in accordance with expectations based upon the linear relationship between quantity of live-yeast and fecundity (see electronic Appendix A).