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Cite this article: Yun L, Chen PJ, Singh A, Agrawal AF, Rundle HD. 2017 The physical environment mediates male harm and its effect on selection in females. *Proc. R. Soc. B* **284**: 20170424.
http://dx.doi.org/10.1098/rspb.2017.0424

Received: 27 February 2017
Accepted: 30 May 2017

Subject Category:
Evolution

Subject Areas:
evolution

Keywords:
cost of attractiveness, environmental complexity, male choice, male harassment, sexual conflict, sexual selection

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Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3805825.

THE ROYAL SOCIETY PUBLISHING

The physical environment mediates male harm and its effect on selection in females

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Recent experiments indicate that male preferential harassment of high-quality females reduces the variance in female fitness, thereby weakening natural selection through females and hampering adaptation and purging. We propose that this phenomenon, which results from a combination of male choice and male-induced harm, should be mediated by the physical environment in which intersexual interactions occur. Using *Drosophila melanogaster*, we examined intersexual interactions in small and simple (standard fly vials) versus slightly more realistic (small cages with spatial structure) environments. We show that in these more realistic environments, sexual interactions are less frequent, are no longer biased towards high-quality females, and that overall male harm is reduced. Next, we examine the selective advantage of high- over low-quality females while manipulating the opportunity for male choice. Male choice weakens the viability advantage of high-quality females in the simple environment, consistent with previous work, but strengthens selection on females in the more realistic environment. Laboratory studies in simple environments have strongly shaped our understanding of sexual conflict but may provide biased insight. Our results suggest that the physical environment plays a key role in the evolutionary consequences of sexual interactions and ultimately the alignment of natural and sexual selection.

1. Introduction

For most genes in the genome, a new mutation that lowers male fitness likely also lowers female fitness, generating a general expectation for the alignment of sexual selection through males with natural selection through both sexes [1–3]. Empirical tests of the benefit of sexual selection usually involve comparisons between monogamous and polygamous mating treatments because sexual selection only occurs in the latter. Results of these studies are mixed [4–6], with several finding no evidence of the benefit of polygamy expected from the addition of sexual selection [7–10]. However, the contrast between polygamy and monogamy involves more than the opportunity for sexual selection. Long *et al*. [11] pointed out that if intersexual conflict leads to the evolution of male traits that are harmful to females, then a novel 'cost of attractiveness' can occur for females under polygamy. The cost arises if males prefer, and thus disproportionally harm, the intrinsically best females. This may reduce the fitness advantage of these females, decreasing the variance in female fitness and thereby weakening natural selection through females. Of those studies that failed to find a benefit of polygamy over monogamy, only two investigated the underlying reason; both found evidence for a weakening of selection through females via such a cost of sexual attractiveness under polygamy [7,12]. These results suggest that there can be a large cost of sexual attractiveness that can overwhelm the benefit of strengthening selection through males that arises

from the addition of sexual selection under polygamy. Given its potential to impede adaptation and purging of deleterious mutations, it is necessary to better understand male harm and how it affects selection on females.

Intersexual conflict is pervasive across the animal kingdom [13] and a weakening of selection through a cost of attractiveness to females could occur in any system involving male harm. However, this cost is unlikely to be large in all cases. First, female counter-adaptations can evolve to reduce the magnitude of harm. Second, this cost arises when male harm is disproportionately allocated to high- over low-quality females, but the extent to which males express such preferences is likely to vary. Finally, Long *et al*. [11] made the sensible argument that males will preferentially interact with, and thus disproportionately harm, the intrinsically best females. However, as noted by Long *et al*. [11], if low-quality females are more sensitive to harm than high-quality females, this could strengthen selection through females by increasing the variance in their fitness. In the end, the net effect of harm on the strength of selection will depend on the extent of harm, the strength of male preference for high- over low-quality females, and the differential sensitivity of females to such harm. These effects likely vary not only among taxa but also within species. In particular, the physical environment in which males and females interact may play a critical (and sometimes predictable) role in mediating both the extent of harm [14–16] and the opportunity for males to express a preference.

Flies of the genus *Drosophila* have been used more extensively than any other taxa in experimental evolution studies of sexual selection and sexual conflict [5,7,12,17–19], and thus have heavily influenced these fields. The majority of 'monogamy versus polygamy' studies use *Drosophila*, including those where a cost of attractiveness has been demonstrated [7,12]. However, standard *Drosophila* laboratory conditions deviate from nature in systematic and potentially important ways [14] and this may influence the outcome of such studies and possibly bias the perspective of the field in general. For example, flies are typically kept at high density in spatially simple environments. Under such conditions, there may be little a female can do to avoid male attention and hence harm, and the opportunity for males to express a sexual preference may be greatly enhanced. Such conditions are likely unrepresentative of many natural systems, but may be representative of others.

Here, we use *Drosophila melanogaster* to assess the frequency and nature of sexual interactions, the extent of male harm, and how male choice changes selection on females, when mating occurs in one of two arena types representing either a smaller and physically simple environment or a larger and physically more complex environment. The small and simple environment is a standard fly vial containing only food, while the larger and more complex environment is a small cage which includes multiple food patches and additional physical structure. (For convenience, we refer to these hereafter as 'simple' versus 'complex' environments, respectively, recognizing that these environments differ by multiple features including size and material composition, as well as complexity.) The natural social and physical environment of *D. melanogaster* is not well known, but we speculate that the cages represent a minor step towards reality as increased space and structural complexity are features common in natural habitats and which may alter the frequency, duration, and fitness consequences of male–female interactions. We find that the frequency of sexual interactions is lower in complex when compared with simple arenas, that females in complex arenas experience less male harm overall and that when males have a choice they disproportionately allocate their sexual attention to high- over low-quality females in the simple but not in the complex environment. Most importantly, we find that male choice weakens viability selection on females in the simple arenas (consistent with past studies), but this effect disappears, and in fact reverses, in the complex arena. These results demonstrate that the physical environment mediates intersexual interactions in ways likely to have fundamental consequences for rates of adaptation and the purging of deleterious mutations.

2. Material and methods

We conducted three separate assays in which we manipulated the physical structure of the mating arena to test the effects of a simple versus complex environment on sexual interactions and their consequences in laboratory-adapted populations of *D. melanogaster*. In each case, the 'simple' arena was a standard wide plastic *Drosophila* culture vial (28.5 × 95 mm; approx. 60 ml) that contained 10 ml of food with abundant live yeast sprinkled on top (live yeast was visible at all times throughout experiments). The 'complex' arena, by contrast, was a cage made from a 1 650 ml plastic *Ziploc*® food storage container that had two pipe cleaners protruding down from the lid and that contained five cups with heavily yeasted food (electronic supplementary material, figure S1). Because a preliminary assay showed reduced sexual interactions in cages compared with vials (electronic supplementary material, 'Preliminary assay' and figure S2), we proceeded to test the effects of these environments on male choice, male-induced harm, and selection through females.

The assays used a population from an ongoing evolution experiment investigating adaptation to a novel environment (28°C and food with 5–6% NaCl added; electronic supplementary material, 'Experimental population'). This population was used because its life cycle includes a 6-day mating phase (which we could manipulate to take place in vials or cages) followed by a 24 h window in which females lay eggs for the next generation (facilitating fecundity measurements). The assays reported here used flies from the population following 28–38 generations of evolution in the novel environment. The assays were performed using the same conditions as the evolution experiment.

(a) Assay 1: effect of the physical environment on male sexual behaviour

We tested the effect of the physical environment on the frequency of male sexual behaviours directed towards large and small females by monitoring replicate mixed-sexed groups of flies (34 males and 34 females) when held in vials versus cages. Ten replicates were set up of each arena type using non-virgin adults that were 3 days post-emergence. Within each replicate, 17 of the females were large and 17 were small. Large and small females were obtained via a density manipulation by adding approximately 100 or 400 eggs, respectively, to a vial using the pipetting technique described in Yun & Agrawal [20]. When created in this way, adults emerging from high-density vials weigh about 25% less on average compared with those from low-density vials (electronic supplementary material, figure S3). Within each replicate, two females of each size had

been marked 10 h earlier by dusting with different fluorescent powders ('Red BNF-RD5813' or 'Green BNF-GR5818', Brilliant Group INC, San Francisco, CA, USA). Colour was balanced such that half the replicates consisted of large red/small green females and the other half consisted of large green/small red females. To facilitate observations during this experiment, the semi-transparent lids of the cages were replaced with plastic wrap and the pipe cleaners were removed.

Individuals were allowed to acclimate to their treatment environment for 1 h prior to commencing behavioural observations. Three observations were made on each replicate per day (commencing at 07.00, 12.00, and 17.00 h; lights turned off at 18.00) for three consecutive days. During each observation, the four focal females (two large and two small) were watched for 1 min each and we scored the total number of sexual activities directed towards her by males, where sexual activity included courtship (i.e. singing, chasing) and copulation (attempted and actual) following Hall [21] and Sokolowski [22]. We also recorded the total duration of all sexual encounters during the 1 min observation, and whether or not the female fed on live yeast at any time during the observation. Because observations within a particular vial or cage are not independent, for large and for small females, we calculated a single sexual activity score for a given replicate that averaged across all observation periods and both females of that size. The difference in the sexual activity score of the two types of females from a single replicate vial or cage (i.e. large–small) was compared between the simple and complex mating arenas using a two-sample *t*-test treating separate vials/cages as replicates. Differences in the total number of times females were observed feeding across all trials were tested using a general linear model with mating arena, female size, and their interaction as fixed effects, fitted via least squares.

(b) Assay 2: effect of the physical environment on male-induced harm

We tested the effect of the environment on male-induced harm to females by comparing the survival and subsequent fecundity of females in a two-way factorial experiment that manipulated their exposure to males (low versus high) and the arena in which this occurred (vial versus cage). Thirty-five replicates consisting of 35 females each were created for each of the four treatment combinations. In the low-exposure replicates, the 35 females were held with 35 males for 3 h in a vial on days 1, 4, and 6 of the mating phase. Outside of these exposure periods, males were removed and the 35 females were held together in their appropriate mating arena (i.e. in a vial or a cage). (Males were stored on fresh food outside of the exposure periods and were used with the same replicate each time.) In the high-exposure replicates, the 35 females experienced the same 3 h exposure to 35 males in a vial on days 1, 4, and 6 of the mating phase, but the males remained present outside of these periods when the females were held in their respective mating arenas, meaning females in these replicates were continually exposed to males. In both exposure treatments, females were handled similarly such that they all received similar exposure to CO_2 anaesthesia throughout the experiment. Immediately after the third male exposure period, females were scored for survivorship and then 10 of the surviving females per replicate were randomly chosen and placed singly into vials to lay eggs for 24 h, matching the timing of the normal maintenance routine for this population. The number of emerged adults and developing pupae produced by each female was scored 11 days later. Throughout, we refer to this as 'fecundity' though this measure will be influenced by larval mortality; however, larval mortality was likely low as larvae were being reared at low density, so most of the variance in this measure is likely from variation in fecundity. As a proxy for 'total' adult fitness, we use the product of viability and fecundity estimates for each replicate. Variation in 'total' adult fitness was analysed using a general linear model with mating arena, male exposure, and their interaction as fixed effects, fitted using least squares.

(c) Assay 3: effect of the physical environment on how male choice alters selection on females

We tested the effect of environment on how male choice alters selection on females in an experiment that compared the survival and subsequent fecundity of large versus small females while manipulating the opportunity for males to choose between them ('no-choice' versus 'choice') and the mating arena in which this occurred. The opportunity for choice was manipulated by holding males with either females of all one size (i.e. all small or all large) or with a mix of half small/half large females (i.e. 'mixed' arenas). In the mixed arenas, males have the opportunity to direct their sexual attention to large over small females, while in the arenas with all small or all large females, the opportunity to do this is greatly reduced. The large and small females were created via a density manipulation as described in Assay 1.

Flies from the density treatments were stored separately by sex for 2 days in holding vials, after which half of the large females and half of the small females were dusted with the pink fluorescent powder (see above) and the other half were dusted with a blue fluorescent powder ('A-19 Horizon Blue', DayGlo, Cleveland, OH, USA). Twenty-four hours later, replicate groups of 34 females (17 blue and 17 pink) and 34 males were set up for the 6-day mating phase in either a vial or a cage. All the males in all arenas were large (i.e. from the low-density rearing vials), whereas the 34 females in a given arena were either all large, all small, or a mixture of 17 large and 17 small. There were 30 replicates for each of the six treatment combinations (two arena types × three female treatments). Half the replicates in the mixed arenas had small pink/large blue females and half had small blue/large pink females. No effect of marking colour was observed on female survival (likelihood ratio test of fixed effect of colour specifying binomial distribution and logistic link function: $\chi^2_1 = 1.44$, $p = 0.230$) or fecundity (two-sample *t*-test, $t_{358} = 0.707$, $p = 0.480$).

The flies interacted for 6 days in their arenas with the food being replaced on day 3 (matching the normal maintenance routine for the population). At the end of the mating phase, females were scored for survivorship and then seven of the surviving females of each colour were randomly chosen from each arena and were put singly into vials to lay eggs for 24 h. The number of emerged adults and developing pupae produced by each female was scored 11 days later.

Our goal was to ask how the opportunity for male choice affects the selective disadvantage of small versus large females. To quantify changes in selection, we calculated selection coefficients (s) quantifying the cost of being small in terms of survival, fecundity, or their product (i.e. 'total' adult fitness). The fitness of small females was defined as $(1 - s)$ times the fitness of large females:

$$s = 1 - \frac{\bar{W}_S}{\bar{W}_L},$$

where $\bar{W}_S$ is the mean survival (or fecundity or their product) of small females from a given treatment combination (i.e. choice or no-choice in vials or cages) and $\bar{W}_L$ is the mean survival (or fecundity or their product) of large females from the same treatment combination. This yielded four selection coefficients: s_{vials/no_choice}, $s_{vials/choice}$, s_{cages/no_choice} and $s_{cages/choice}$. Changes in selection caused by the opportunity for male choice were then calculated separately for vials and cages as $\Delta s = s_{no\_choice} - s_{choice}$,

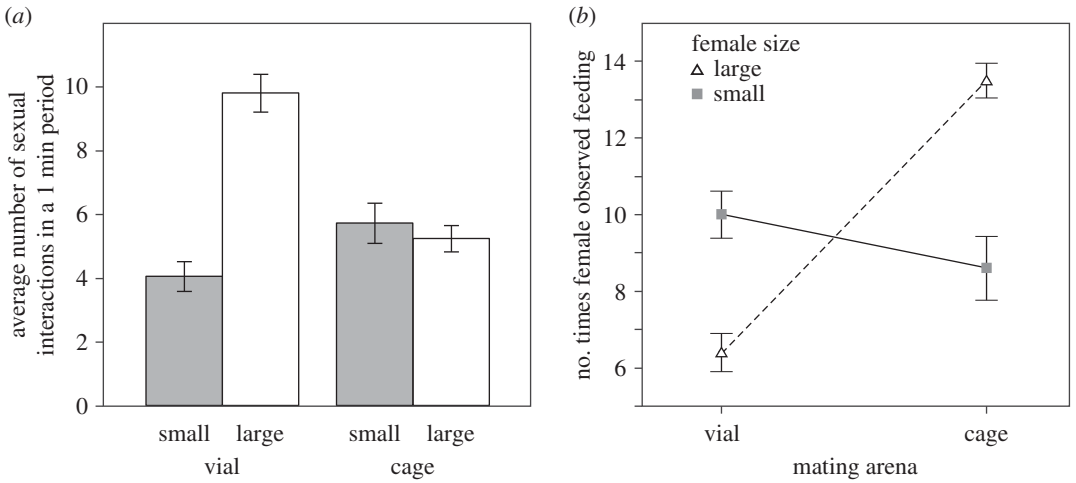

Figure 1. (*a*) Average number of sexual interactions directed towards individual small or large focal females during a 1 min observation period, and (*b*) total number of times the female was observed feeding on live yeast when individuals were held in a mating arena consisting of either a standard *Drosophila* vial ('simple' environment) or a cage ('complex' environment). Individual replicates consisted of 34 males together with 17 small and 17 large females. Error bars are $\pm$ 1 s.e. treating arenas as replicates. Sexual interactions include singing to the female, chasing her, as well as attempted and actual copulations.

such that a positive (negative) value indicates a weakening (strengthening) of selection in the presence of male choice. We used a bootstrap procedure to evaluate the significance of our estimates of s, Δs, and the difference in Δs between mating arenas. Data were sampled 10 000 times with replacement while preserving the structure of the dataset including the treatment combinations (i.e. vial/cages, small/large, mixed/not mixed) and colour markings (i.e. blue/pink). From each resampled dataset, we calculated the mean fitness of the various types, the selection coefficients, Δs values, and the difference in Δs between vials and cages, yielding distributions for each. A value was considered significant if its 95% CI did not overlap zero.

3. Results

(a) Assay 1: effect of the physical environment on male sexual behaviour

Females in the simple arenas (i.e. vials) received more sexual attention than those in the complex arena, consistent with the result of our preliminary assay (electronic supplementary material, figure S2). However, this increase in sexual attention in vials was restricted entirely to large females (figure 1*a*), generating a substantial difference in the average amount of sexual attention experienced by large compared with a small female in vials that was absent in cages. This difference in the amount of sexual attention directed towards large versus small females differed significantly by mating arena ($t_{18} = 5.89$, $p < 0.001$). Results were the same when analysing the total duration of sexual encounters instead of the number (electronic supplementary material, figure S4).

Large females were observed feeding on live yeast more often when they were in cages than when they were in vials (figure 1*b*). Small females, however, showed no such difference, generating a significant mating arena $\times$ female size interaction ($F_{1,36} = 47.36$, $p < 0.001$). Pooling across females, feeding rates were also significantly higher in cages compared with vials (main effect of mating arena: $F_{1,36} = 21.30$, $p < 0.001$; figure 1*b*), but did not differ significantly between large and small females when pooling across arenas (main effect of female size: $F_{1,36} = 1.11$, $p = 0.30$).

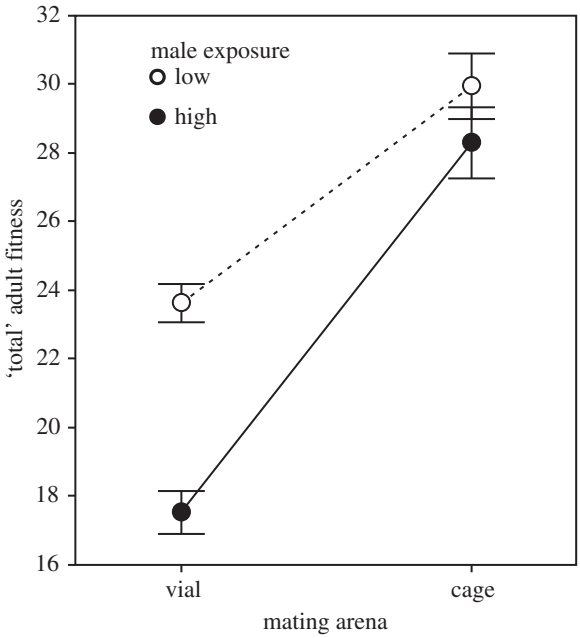

Figure 2. 'Total' female adult fitness (the product of viability and fecundity for each replicate arena) under low versus high exposure to males when held in alternative mating arenas consisting of either a standard *Drosophila* vial (simple environment) or a cage (complex environment; see Material and methods). Points are means $\pm$ 1 s.e. treating separate arenas as replicates.

(b) Assay 2: effect of the physical environment on male-induced harm

'Total' adult fitness (the product of viability and fecundity for each replicate) was greater under low compared with high male exposure, indicating a cost of increased exposure to males (figure 2; exposure effect: $F_{1,134} = 22.70$, $p < 0.0001$). Fitness was also reduced in vials compared with cages (arena effect: $F_{1,134} = 110.43$, $p < 0.0001$). The exposure $\times$ arena interaction was also significant ($F_{1,134} = 7.49$, $p = 0.007$), indicating that the fitness cost of increased male exposure was significantly greater in vials than cages. See electronic supplementary material, figure S5 for the separate effects of these treatments on viability and fecundity.

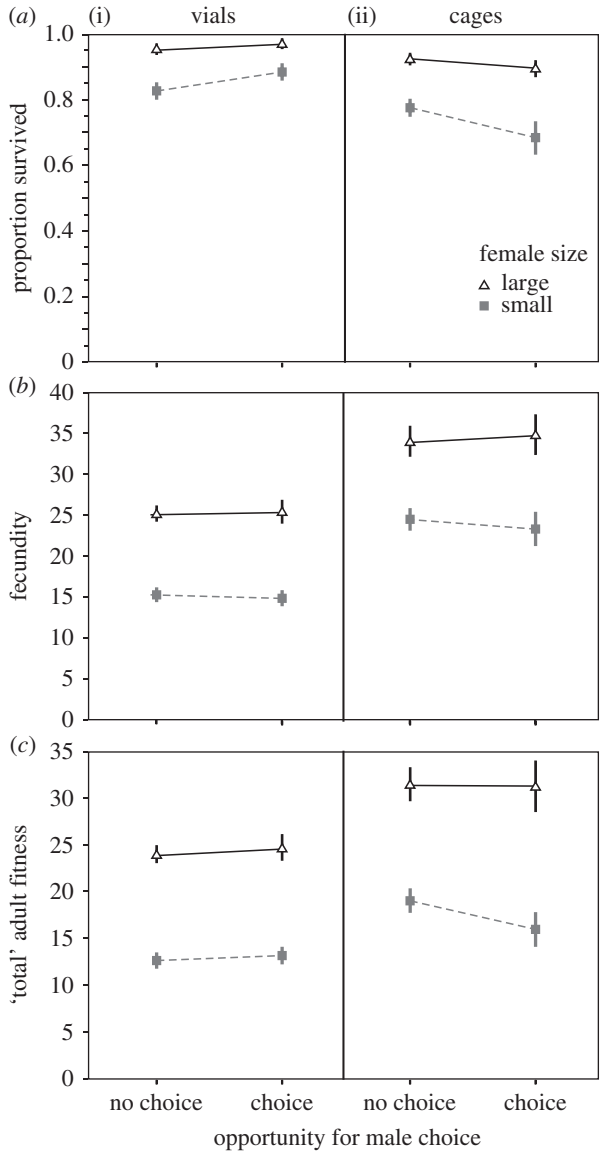

Figure 3. (*a*) Survival, (*b*) fecundity, and (*c*) 'total' adult fitness (the product of viability and fecundity) of large versus small females when manipulating the opportunity for males to choose between them (no-choice versus choice) and the mating arena in which this occurred ((i) simple vials; (ii) complex cages). Points are means ± 95% bootstrap CIs treating separate arenas as replicates.

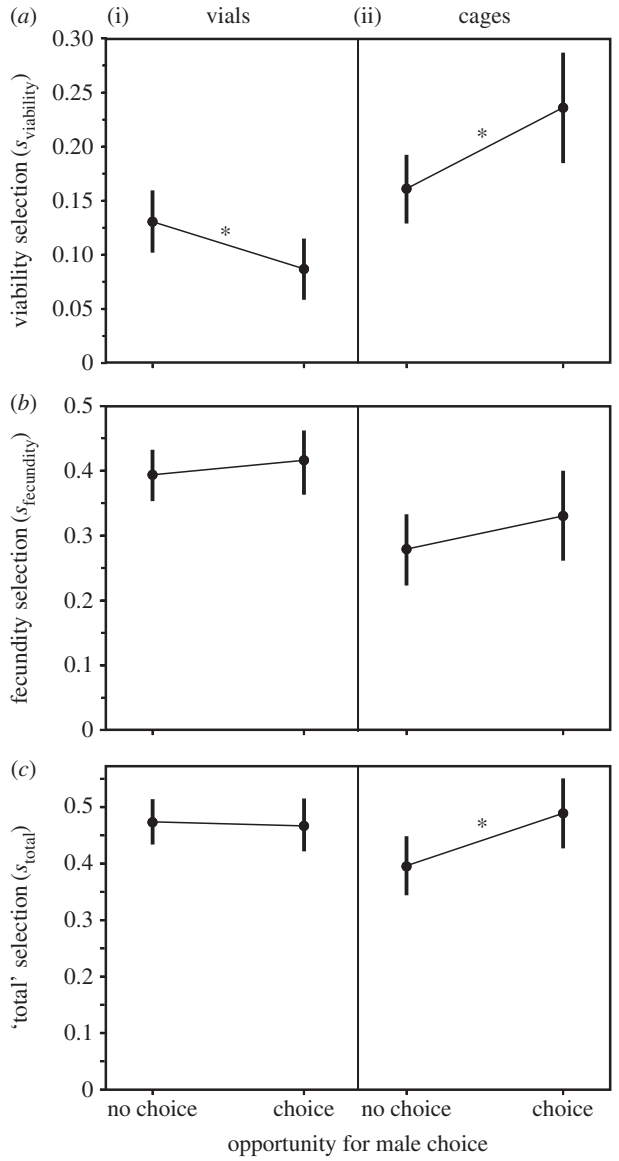

Figure 4. Selection against small females arising from variation in (*a*) viability, (*b*) fecundity, and (*c*) 'total' adult fitness (the product of viability and fecundity) when manipulating the opportunity for males to choose (no-choice versus choice) and the mating arena in which trials occurred ((i) simple vials; (ii) complex cages). Points are means ± 95% bootstrap CIs treating separate arenas as replicates. Asterisks denote instances in which the 95% CI for the mean difference in selection (Δs) does not overlap zero.

(c) Assay 3: effect of the physical environment on how male choice alters selection on females

When males have access to multiple types of females, they can preferentially direct their sexual attention to particular ones, causing disproportionate harm to certain females over others and thus altering selection on females. We measured the fitness of large and small females in the presence versus absence of male choice in both arena types. We quantified how the opportunity for male choice changed selection as $\Delta s_a = s_{a,\text{no\_choice}} - s_{a,\text{choice}}$, where $s_{a,t}$ is the reduction in viability or fecundity of small females relative to large ones as assayed in arena type a (vial or cage) for treatment t ('choice' or 'no-choice').

Large females consistently survived better than small females (figure 3*a*), generating significant viability selection against small females in all treatment combinations (figure 4*a*). The magnitude of this survival difference, however, depended on the opportunity for male choice and the mating arena in which this occurred. In vials, the survival difference between large and small females was reduced when males had an opportunity to choose between them, resulting in a significant weakening of selection in the presence of male choice (mean Δs_{vials} (95% bootstrap CI): 0.044 (0.005, 0.083)); this is the population-level manifestation of the 'cost of attractiveness' described by Long *et al*. [11]. The opposite was true in cages where the survival difference between large and small females was greater when males had an opportunity to choose, causing a significant strengthening of selection against small females in the presence compared with the absence of male choice (mean Δs_{cages} (95% bootstrap CI): −0.075 (−0.134, −0.017)). This difference between arena types in the effect of male choice on selection was itself significant ($\Delta s_{\text{vials}} - \Delta s_{\text{cages}}$, mean (95% bootstrap CI): 0.119 (0.049, 0.189)).

Large females had greater fecundity than small females (figure 3*b*), generating significant fecundity selection against small females in all treatments (figure 4*b*). However, the

magnitude of this fecundity difference did not vary significantly with the opportunity for male choice in vials (mean Δs_{vials} (95% bootstrap CI): −0.029 (−0.092, 0.036)) or in cages (mean Δs_{cages} (95% bootstrap CI): −0.052 (−0.141, 0.034)). The difference in the effect of male choice between arena types was also non-significant ($\Delta s_{vials} - \Delta s_{cages}$, mean (95% bootstrap CI): 0.023 (−0.084, 0.134)).

Estimating 'total' adult fitness as the product of viability and fecundity for each replicate, large females are more fit than small females (figure 3*c*). The formal analysis of selection on 'total' adult fitness has less power because some replicates lack fecundity data (so those replicates are excluded) and there is a compounding of measurement error across fitness components. While we detected significant selection against small females in all treatments (figure 4*c*), we did not observe a significant weakening of selection under male choice (mean Δs_{vials} (95% bootstrap CI): 0.000 (−0.062, 0.064)), perhaps due to reduced power. In cages, the opportunity for male choice significantly strengthened selection (mean Δs_{cages} (95% bootstrap CI): −0.100 (−0.183, −0.020)). The difference between arena types in the effect of male choice on selection was itself marginally non-significant ($\Delta s_{vials} - \Delta s_{cages}$, mean (95% bootstrap CI): 0.100 (−0.001, 0.206)).

4. Discussion

In their efforts to gain fertilizations, males can harm females and thus act as powerful agents of selection [11,13]. We reasoned that the physical environment would mediate the extent of male harm and thereby the selection males impose on females. Using arenas that we postulate to be slightly more representative of natural conditions than typical fly vials, we found significant changes in the frequency and fitness consequences of male–female interactions. In particular, in vials, large females received substantially more sexual attention than did small females, whereas in cages, sexual interactions were less common (electronic supplementary material, figure S2) and involved large versus small females with a similar frequency (figure 1*a*) and duration (electronic supplementary material, figure S4). High exposure to males was also bad for females in both arenas but was worse in vials than in cages, driven by a large effect on fecundity (electronic supplementary material, figure S5). We observed large females feeding less often than small females in vials but more often than small females in cages (figure 1*b*). An interpretation of this result is that the high level of sexual attention directed towards large females in vials (figure 1*a*) reduced their feeding opportunities in that environment. Consistent with this, large females showed a fecundity cost of increased male exposure in vials but not cages (electronic supplementary material, figure S5). Finally, similar to previous work [7,11,12], in vials, we found evidence for a cost of attractiveness (i.e. the survival difference between large and small females was reduced when males had an opportunity to choose; figure 3*a*) and an associated weakening of selection (i.e. the selective advantage of large females is diminished when males can exhibit choice; figure 4*a*). However, this was not the case in cages; rather the difference in survival between large and small females increased in the presence of male choice (figure 3*a*), causing selection against small females to strengthen significantly when males could choose (figure 4*a*). For 'total' adult fitness, we also found that male choice significantly strengthened selection in cages (figure 4*c*). The effect of male choice in vials was non-significant (possibly due to a lack of power from compounding measurement error across fitness components as the effect on viability was quite strong).

Though numerous studies have documented male harm, recently, it was proposed that males may preferentially direct their harassment towards the best females, reducing the intrinsic advantage of these females and thereby weakening selection [11]. This effect was demonstrated in a laboratory population of *D. melanogaster* [11] and has been implicated as a major impediment to selection under polygamy [7,12]. Our results replicate this original finding in a simple environment (at least for viability) but find the opposite in a complex environment, demonstrating that the effect of male mate choice on selection varies considerably across environments. Therefore, with respect to *D. melanogaster*, our results suggest that mate choice is less likely to impede selection when the environment is even slightly more natural than a typical laboratory setting. Below we discuss reasons why the physical environment might alter how male–female interactions influence the strength of selection. For simplicity, we frame our discussion in terms of pre-copulatory forms of male harm. However, seminal fluid proteins affect female behaviour and physiology in a variety of ways, some of which are harmful [23–25]. The consequences of such post-copulatory male harm are also likely to depend on the environment.

The observed difference in the selective consequences of male choice between vials and cages may be due to a 'direct' influence of the physical environment on male–female interactions. The 'complex' arena used here was designed to reduce fly density and increase spatial complexity, which we reasoned would allow females to reduce male harassment by providing both places to hide and choice among multiple food sources to feed and lay eggs. Indeed, fewer male–female interactions were observed in the complex when compared with the simple arenas (electronic supplementary material, figure S2) and most importantly, while intersexual interactions disproportionately involved large over small females in vials, this difference was entirely absent in cages (figure 1*a*). Previous studies [11,26–29] and our Assay 2 (figure 2) have shown such male sexual attention to be harmful. This 'direct' effect of the physical environment on the extent of male harm experienced by large versus small females helps to explain why the cost of attractiveness and the resulting reduction in selection with male choice was greater in vials than cages (i.e. $\Delta s_{vials} > \Delta s_{cages}$). This pattern of biased sexual attention reversed in cages, with small females now experiencing more male courtship and mating attempts than large females (figure 1*a*; electronic supplementary material, figure S4). However, this difference between small and large females in cages was small and non-significant, and it is thus less clear why male choice in cages had the opposite effect of that in vials, strengthening instead of weakening selection (i.e. $\Delta s_{vials} > 0$, $\Delta s_{cages} < 0$).

As initially proposed [11], a cost of attractiveness is likely if high-quality females receive disproportionate sexual attention; males might typically prefer high-quality females because they have greater reproductive output [30]. In their study, Long *et al*. [11] showed that, in vials, males preferentially court/harass larger over smaller females and our

results corroborate this. However, in some taxa or in some environments, males may preferentially harass low-quality females because there is greater opportunity to do so or because the probability of successfully mating with them might be higher than with high-quality females. In our cages, the number and duration of sexual interactions with small females was slightly, though not significantly, greater than that with large females. However, if low-quality females are more sensitive to the effects male harm, they may experience a greater fitness cost even if intersexual interactions are not biased towards them. As hypothesized by Long *et al*. [11], this could serve to strengthen selection through females by increasing the variance in their fitness and this could explain why male choice strengthened selection in cages ($\Delta s_{\text{cages}} < 0$) rather than weakened it as in vials ($\Delta s_{\text{vials}} > 0$).

Differences in the physical environment may have had other unintended 'indirect' effects by affecting fly physiology. When male–female interactions were minimal (i.e. low-exposure trials of Assay 2), vials and cages differed with respect to viability (vials > cages; electronic supplementary material, figure S5) and fecundity (cages > vials; electronic supplementary material, figure S5). Such effects suggest the physical environment alters various aspects of fly physiology (e.g. possibly due to differences in humidity, abundance of food, or other aspects of the environment that are hard to control at the small scale of such containers). These changes may influence the persistence and choosiness of males as well as the resistance and tolerance of females to male harassment, causing an indirect effect of the physical environment on how male choice affects selection in females. Though our observations of flies in cages and vials (including the data presented in figure 1; electronic supplementary material, figures S2 and S4) imply an important role of 'direct' effects of the physical environment on male–female interactions, this also does not preclude 'indirect' effects.

Male harm has been studied intensively in *D. melanogaster* [18,25,26] and we are not the first to investigate potential effects of the physical environment on this. Using a laboratory population, Byrne *et al*. [31] found that the addition of a spatial refuge that was accessible only to females decreased remating rates by approximately 25%, but had no detectable effect on lifetime female fecundity. The refuge, however, lacked live yeast (a key food source). Thus, their arena featured a strong trade-off between avoiding males and accessing live yeast. By contrast, our cages provided females with the opportunity to choose among multiple food patches, potentially allowing them to avoid those where male aggression was most intense and thereby reduce the harm they experienced without forgoing feeding. In nature, spatial variation in local male density (or sex ratio) is not uncommon [32–36] and in some cases has been shown to alter harassment rates [33]. This suggests that females may often have opportunities to reduce male harassment without losing the opportunity to find good feeding and laying sites.

An important consideration in any experiment is the relationship between the assay conditions and the evolutionary history of the population. Results from a population tested in a novel environment may differ from those that would be obtained following adaptation. In our study, vials mirrored the very recent evolutionary history of the population; the cages were novel but were intended to incorporate an aspect of the natural environment in which flies have evolved for millions of generations prior to being brought to the laboratory. One could view our cage results as simply showing an unpredictable response to flies being assayed in a novel environment. Though we cannot definitively rule this out, the motivating ideas behind this experiment generate a series of predictions regarding the frequency of sexual interactions, the extent of male harm, and resulting selection on females, and the observed responses largely correspond with these predictions.

Experimental studies of *Drosophila* have heavily influenced views on how sexual interactions affect adaptation and purging [3]. Some evolution studies have demonstrated that adaptation (or purging) is slower under polygamy than monogamy [7–10]. Rather than the typical interpretation that sexual selection opposes natural selection, evidence from recent studies support an alternative: polygamy provides the opportunity for male choice and the resulting targeted male harm creates a substantial cost of attractiveness that drastically weakens natural selection occurring through females [7,11,12]. Such work highlights the potential for male–female interactions to profoundly influence selection across the genome via harm-mediated selection in females, as well as by sexual selection in males. Both types of selection are likely affected by the environment, but perhaps in very different ways. Sexual selection in males against deleterious alleles tended to be stronger in a large arena compared with a simple vial [37]. In this study, we demonstrated that, relative to vials, complex arenas reduced the frequency of male–female interactions (figure 1*a*; electronic supplementary material, figure S2), eliminated the overrepresentation of high- over low-quality females involved in these interactions (figure 1*a*; electronic supplementary material, figure S4), lessened the harm caused by male exposure (figure 2), and reversed the effects of male choice on selection through females (figure 4).

The generality of our results is not yet known, although we suspect they are broadly applicable because the physical environments appear to play a central role in mediating the frequency and fitness consequences of sexual interactions in many species. For example, aspects of sexual conflict have been shown to vary with population density, sex ratio, the availability of refuges, resource levels, predation risk, and other factors in a range of taxa [33,34,38–42], and these in turn are likely to vary with the physical environment. Whether and how such environmental-dependence alters selection through females in other species is an open question. However, the substantial impact of a small change in arena design in our study suggests that the physical environment may play a key role in determining the effects of male choice and ultimately the alignment (or not) of sexual selection with natural selection.

Data accessibility. Data are available on Dryad (http://dx.doi.org/10.5061/dryad.179h5) [43].

Authors' contributions. A.F.A. and H.D.R. conceived the experiments, analysed the data, and wrote the paper. L.Y. helped design Assays 1–3. L.Y. conducted Assay 1 and with the help of P.J.C. conducted Assays 2 and 3. A.S. conducted the preliminary assay, helped in its design and analysis, and did background research.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada to A.F.A. and H.D.R.

Acknowledgements. We thank Malak Bayoumi, Mackenzie DiGasparro, Laura Weller, and Michelle Yang for help in the laboratory.

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Supplementary Material

Preliminary assay – Effect of the physical environment on sexual activity

We tested the effect of the physical environment on the frequency of male sexual behaviors directed towards females by monitoring replicate mixed-sexed groups of flies (30 males and 30 females) when held in the vials vs. cages. This assay used a lab population originally collected in 1970 from Dahomey, Africa and that has been maintained for 11 years in the Agrawal laboratory under constant conditions (25 °C; 70% relative humidity; 12L:12D photocycle) on a sugar-yeast medium at a moderate population size (1000–3000 adults with a 2-week non-overlapping generation in 177 mL bottles). Ten replicates were set up of each arena type (six in one block, four in a second block six months later) using non-virgin adults that were 4-5 days post-emergence. To facilitate observations, the lids of the cages were replaced with transparent plastic wrap and the pipe cleaners were removed. Within each replicate, three of the females had been marked 4 h earlier by dusting with pink fluorescent powder ('AX-11 Aurora Pink' from DayGlo, Cleveland, OH). (After 4 h females were no longer grooming to remove powder and had returned to normal pre-dusting behaviour.) Individuals were allowed to acclimate to their treatment environment for 2 h prior to commencing observations.

In block 1 (2), five (four) observations were made on each replicate arena approximately hourly starting at 18:45 (19:00). After the lights switched off at 21:00, an ultraviolet A handheld flashlight (405nm λ) was used to locate the marked females. During each observation, the three focal females were watched for 1 min each and we scored the total number of sexual activities directed toward her by males, where sexual activity included courtship (i.e. singing, chasing) and copulation (attempted and actual) following refs. [18] and [19]. During the 1 min

observation, repeated instances of the same activity by a particular male were only counted once. Because observations within a particular vial or cage are not independent, we calculated a single sexual activity score for a given replicate that averaged across all females and observation periods for that replicate. Differences in this score were tested using a general linear model that included the fixed effects of arena (vial vs. cage) and block, fit using least squares.

In both blocks, females in the simple arenas (i.e. vials) received approximately three times the sexual attention from males than did females in the complex arenas (i.e. cages; Figure S2), generating a significant effect of environment ($F_{1,17}$ = 12.51, P = 0.0025).

Experimental population

The population used in our three main assays was founded from a laboratory stock population (SIM) originally collected in 2005 from the Similkameen Valley, British Columbia by S. Yeaman and maintained on standard cornmeal medium at 25 °C at a large population size (N = ca. 1000-3000) with a two week cycle with non-overlapping generations. In September 2014, a sample of 140 males and 140 females from the SIM population were collected and from their offspring we established a new population (as part of a larger, on-going, evolution experiment). Each generation, larvae for this population were reared in standard cornmeal medium supplemented with 5-6% NaCl and constant exposure to 28 °C. Eleven days after eggs were laid, we collected the adult offspring (pooling across laying vials) and stored them in holding vials separately by sex (35 flies per vial). After three days, adults were placed in mating vials, creating four vials each containing 35 males and 35 females. The mating period lasted for 6 days, with

flies transferred to a new vial on the third day. A random sample of 105 females were taken from a pooled collection of the survivors in the four mating vials. They were divided into seven laying vials (15 females/vial) in which they were allowed to lay eggs for 24 hours to establish the next generation. Each non-overlapping generation lasted three weeks. The flies used in assay 2 and 3 of the current study came from generation 28 of this evolution experiment.

A)

B)

Figure S1. Overhead with lid off (A), and side (B) views of the complex environment which consisted of a 1.65 L *Ziploc*® container (S.C. Johnson & Son, Inc., Racine, WI) that held five cups each containing food. These included two 1 oz cups containing 7.5 mL of food each and three larger 3 oz cups containing 25 mL of food each, with the surface of the larger cups being partitioned roughly in half by a plastic divider inserted into the food. Dry yeast pellets were sprinkled on the surface of the food. Two pipe cleaners protruded from the lid of the container into the interior space. The lid itself had two larger holes plugged by foam stoppers (to allow

CO_2 nozzle to be inserted for anaesthetizing flies) and many small holes across its surface to facilitate air circulation (necessary to reduce humidity and prevent condensation on the interior walls).

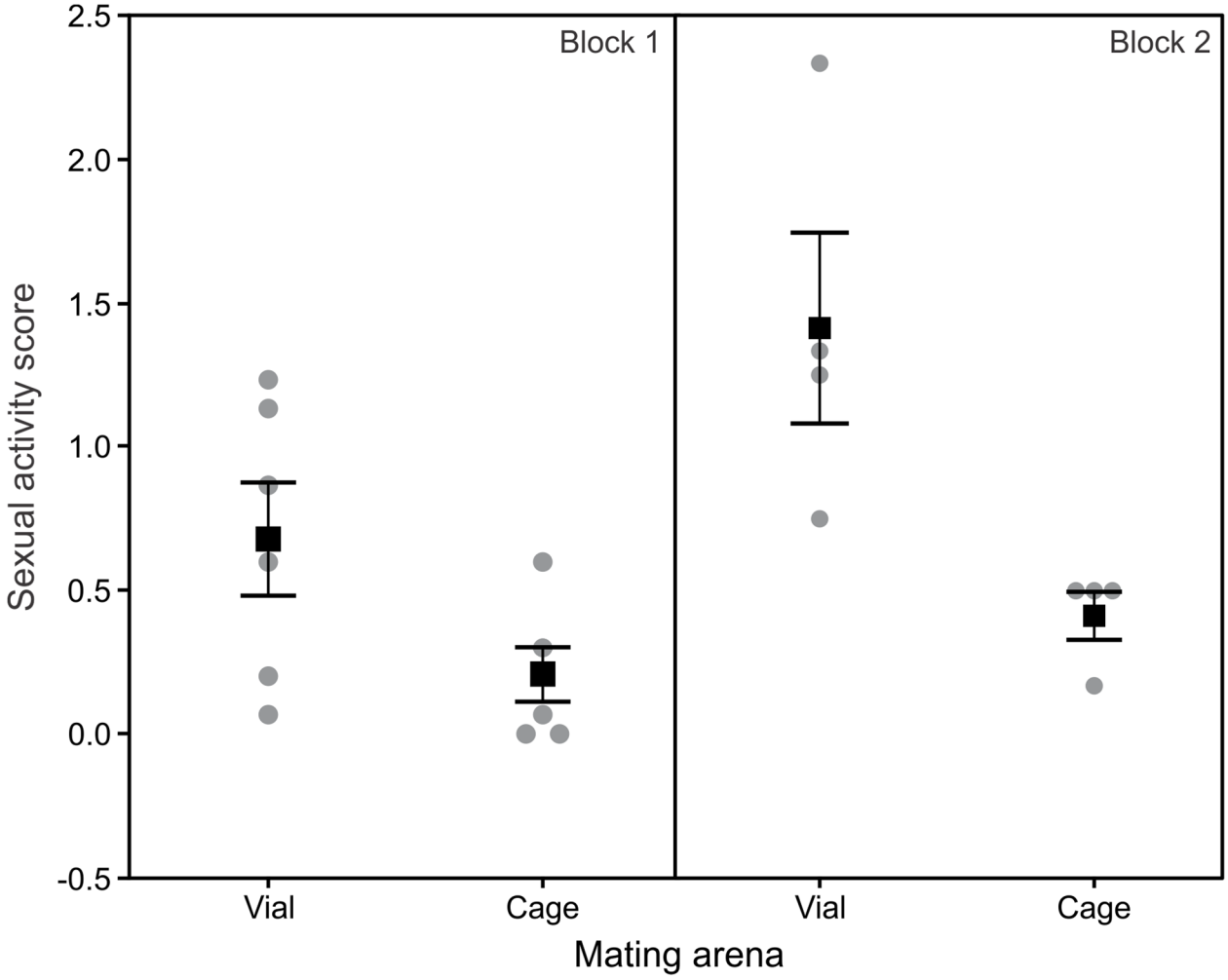

Figure S2. Average number of sexual activities directed toward an individual female during a 1 min observation period for groups of 30 males and 30 females held in alternative mating arenas consisting of either a standard *Drosophila* vial (simple environment) or a cage (complex environment); data from the preliminary experiment. Grey circles depict individual replicates; black squares are means ± 1 SE. Sexual activity includes singing to the female, chasing her, as well as attempted and actual copulations. The experiment was performed in two blocks and sexual activity differed between blocks ($F_{1,17}$ = 5.67, P = 0.0293).

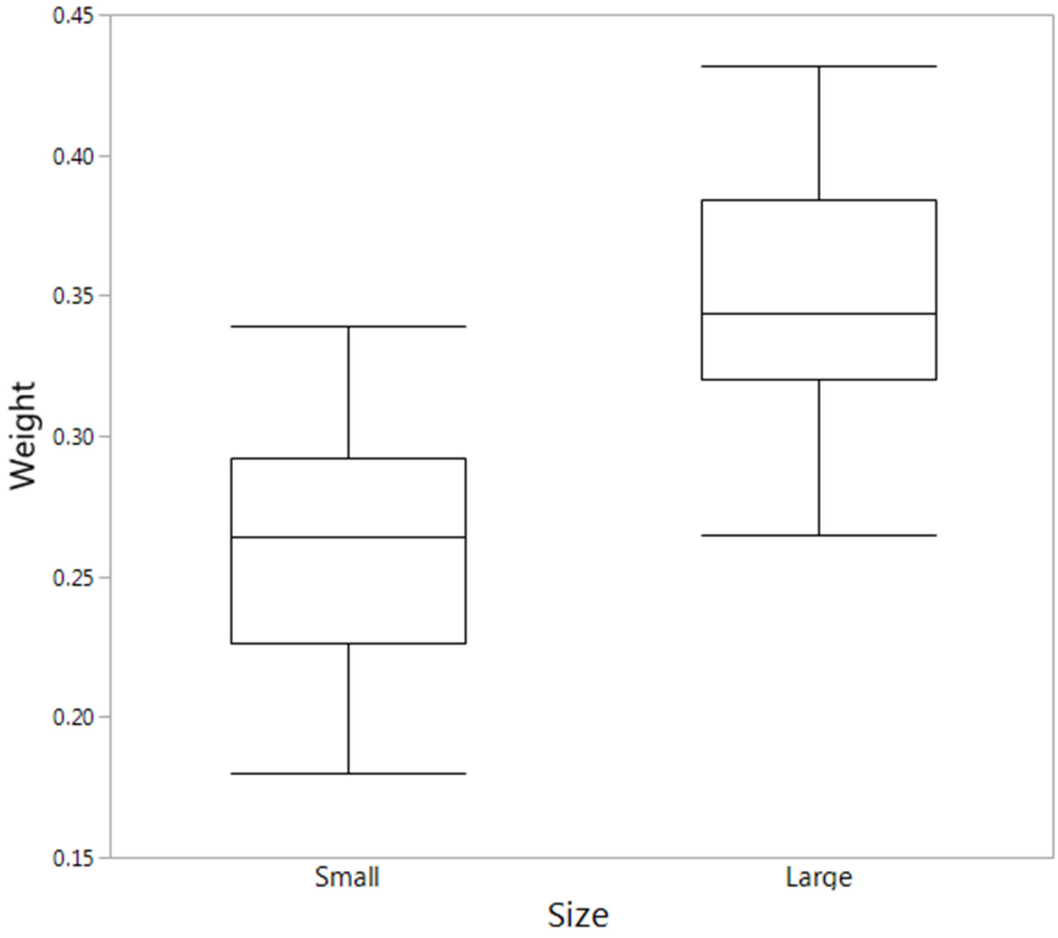

Figure S3. Boxplot of dry weight (mg) of adult females from the high and low density vials in Assay #2. Flies were collected on day 11, dried at 70 °C for 12 h, and then weighted to the nearest 10^{-6} g on a Sartorius microbalance. Flies raised at high density flies were significantly smaller than those raised at low density (two-sample t-test: $t_{74} = 9.46$, $P < 0.0001$).

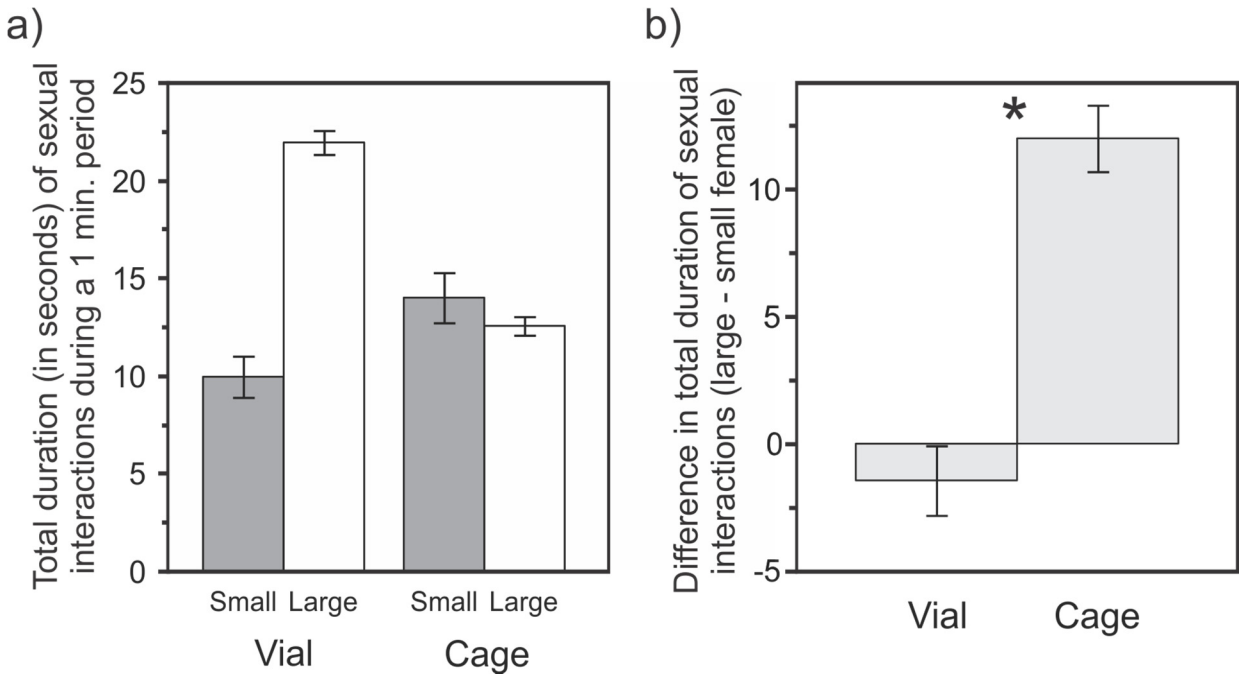

Figure S4. a) Total duration of sexual interactions in Assay #1 directed toward individual small or large focal females during a 1 min observation period, and b) differences in this (large - small) when individuals were held in a mating arena consisting of either a standard *Drosophila* vial ("simple" environment) or a cage ("complex" environment). Individual replicates consisted of 34 males together with 17 small and 17 large females. Error bars are ± 1 SE treating arenas as replicates. Sexual activity includes singing to the female, chasing her, as well as attempted and actual copulations. The asterisks signifies a significant effect of mating arena on the difference (two-sample *t*-test: $t_{18} = 7.11$, $P < 0.0001$).

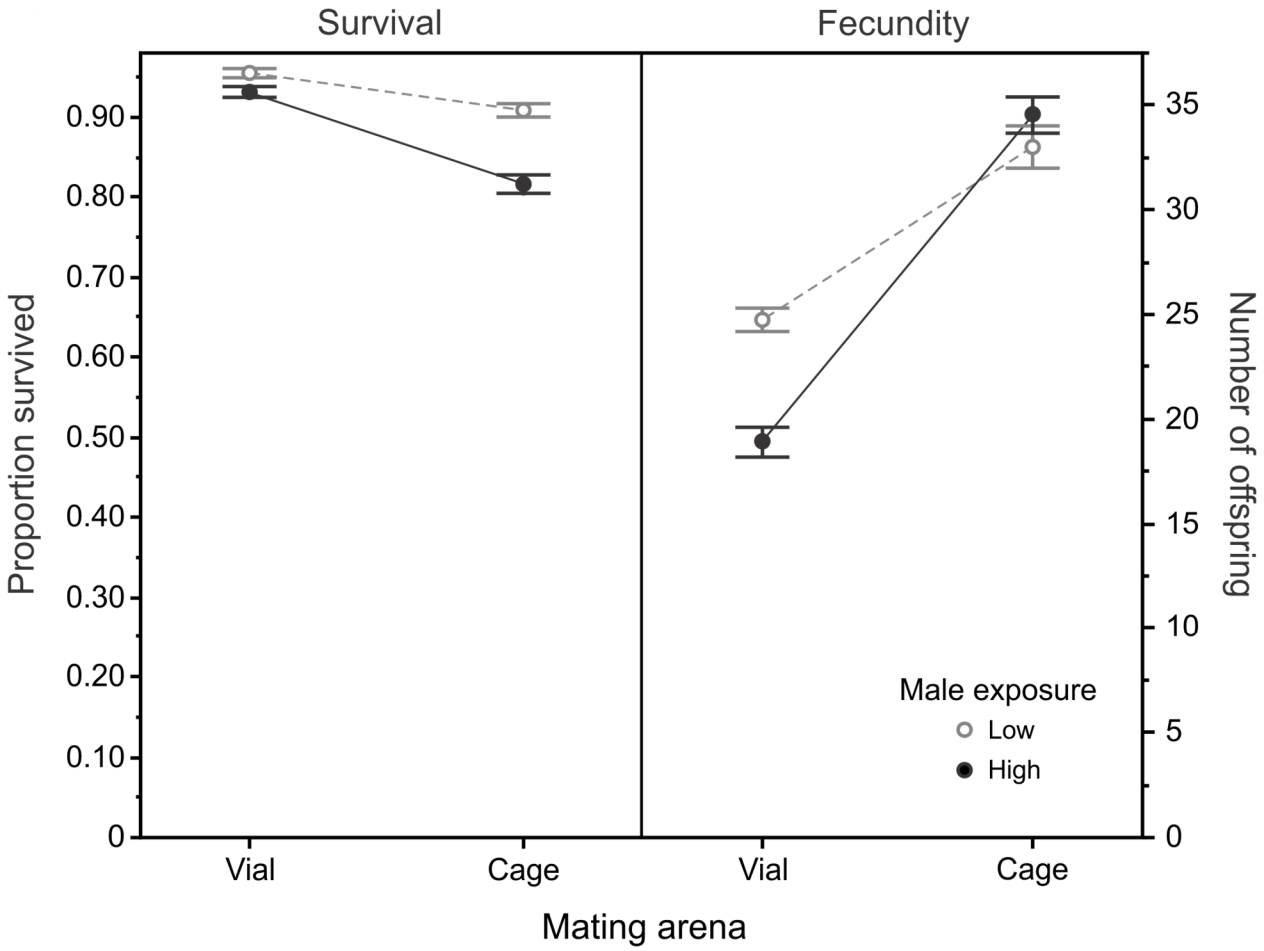

Figure S5. Survival (left panel) and fecundity (right panel) of females under low vs. high exposure to males when held in alternative mating arenas consisting of either a standard *Drosophila* vial (simple environment) or a cage (complex environment; see Methods). Points are means ± 1 SE treating separate arenas as replicates. Differences in viability (i.e. proportion surviving) were tested using a generalized linear model, fit via maximum likelihood, with arena (vial vs. cage), male exposure (low vs. high), and their interaction as fixed effects and employing a binomial error distribution with logistic link function. Survival was greater under low compared to high male exposure (exposure effect: $\chi^2_1 = 35.19$, $P < 0.0001$) and was reduced in

cages compared to vials (arena effect: $\chi^2_1 = 79.55$, $P < 0.0001$). The exposure × arena interaction was non-significant ($\chi^2_1 = 2.55$, $P = 0.111$), providing no evidence that the viability consequences of male-induced harm differed between arena types. Differences in fecundity of surviving females were tested using a general linear model on the average fecundity of the ten females from a given replicate with mating arena, male exposure, and their interaction as fixed effects, fit using least squares. The fecundity of surviving females was significantly higher in cages as compared to vials (arena effect: $F_{1,134} = 220.77$, $P < 0.0001$) and was higher under low as compared to high male exposure (exposure effect: $F_{1,134} = 7.22$, $P = 0.0081$). However, there was a significant exposure × arena interaction ($F_{1,134} = 21.00$, $P < 0.0001$) such that the fecundity cost of increased male exposure was substantial in vials but this cost was entirely absent in cages.