Condition dependant effect of spatial structure on cost of male exposure in females

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Abstract:

Spatial structure is a common feature of all naturally occurring populations. Theoretically, spatial structuring of a habitat could modulate the intensity of Interlocus sexual conflict (ISC) in a population, possibly by modulating intersexual encounter rate. We tested this theory using laboratory populations of *Drosophila melanogaster* by measuring male induced fitness decline in females in three-patch habitat systems under two alternative habitat types – structured-interconnected and unstructured. Our results on reproductive and survival costs in females suggested significant costs due to (a) male presence (i.e., ISC) and (b) living on structured habitat. Further, the cost of ISC was found to be higher in structured habitat. Through a follow up experiment we further show that the effect of habitat on ISC was stronger for females raised on poor, nutritionally deprived growth conditions, perhaps due to the compounded adverse effects of cost of dispersal and male interaction.

Introduction

In absence of a true monogamy, males can maximize their fitness by investing in competitive and manipulative traits that increase their mating and/or fertilization success. In contrast, female fitness is usually maximised by achieving longer lifespan, higher fecundity with better-quality eggs, and ensuring higher survival rate of juveniles (Holland and Rice 1999, Arnqvist and Rowe 2013, Adler and Bonduriansky 2014). Such asymmetry in evolutionary interests across the two sexes is commonly referred to as sexual conflict, which is now recognized as a widespread phenomenon affecting many animal and plant taxa (Parker 1979). Theories and empirical evidence suggest that male-benefiting traits that result in better mating and/or fertilisation success can emerge as male reproductive adaptations regardless of their impact on females (Rice 1996, Chapman et al. 2003, Nandy et al. 2013). Often, such male-benefiting traits are detrimental to female fitness as they involve coercing females to mating and/or reproducing at a time and rate which benefits males but is injurious for long-term survival of the females, thus reducing the lifetime progeny output of the females. This sets the stage for the emergence of female counter-adaptations that minimise female susceptibility to male manipulations (Chapman et al. 2003, Wigby and Chapman 2004, Nandy et al. 2014). This form of sexual conflict, commonly referred to as interlocus conflict, can result in intersexual antagonistic coevolutionary arms race (Chapman et al. 2003, Pizzari and Snook 2007).

A crucial aspect of interlocus conflict is the involvement of direct interaction between the sexes such as, persistent and coercive courtship, forced copulation, traumatic insemination, males riding the females or guarding them for long duration, and even modulation of female physiology by the seminal fluid proteins and peptides transferred during mating (Arnqvist 1992, Chapman et al. 1995, Réale et al. 1996, Le Galliard et al. 2005, Adller 2010, Koene 2012). In fruit flies, *Drosophila melanogaster*, females suffer increased mortality and/or reduced life-time progeny production due to coercive male mating behaviour and toxic seminal fluid peptides received during copulation (Chapman et al. 1995, Rice et al.

2006, Wolfner 2009, Nandy et al. 2013). Most theoretical and empirical investigations of interlocus conflict that deal with the intensity of conflict in a population tend to assume, either implicitly or explicitly, an unbridled interaction between the sexes that allow the conflict to operate (Rice et al. 2006). However, most natural populations are spatially structured, and hence, individuals are able to move around across patches seeking or avoiding favourable and unfavourable interactions. The way such population structure affect intensity of conflict in a population is poorly understood.

It is not unreasonable to expect females to avoid patches with a high male congregation to avoid mate harassment. An explicit experimental verification of this theory was first attempted by Rice et al. (2006), wherein *D. melanogaster* females were found to suffer less male harassment (measured as remating rate) when they were allowed to move between two compartments within a culture vial. However, subsequently, more thorough work from the same group did not find the intensity of sexual conflict to be lower when the experimental habitat allowed spatial refuges (Byrne et al. 2008). More recent experiments on *D. melanogaster* showed that the intensity of interlocus conflict is significantly reduced in a large and heterogenous holding environment compared to a small and homogenous one (Yun et al. 2017, Malek and Long 2018). In addition, the scope of hitherto reported adaptive male mating bias, a source of mate harassment, in favour of high fecundity females appeared to have reduced in the large and complex environment (Yun et al. 2018). In water striders of the species *Aquarius remigis*, physical structuring of population modulates males' aggressive behaviour towards females (Eldakar 2009).

Further, individuals in a patch structured population are expected to disperse across population patches, resulting in an added complication due to the ecology of dispersal. Indeed, in *Aquarius remigis*, maintained in a naturalistic laboratory environment that introduces dispersal alters male-female local interactions thereby altering the dynamics of interlocus sexual conflict. For instance, in isolated pools, without dispersal, males tend to gain more mating by being aggressive. Interestingly, in interconnected

pools which allowed individuals to disperse, females were found to disperse away to avoid aggressive male, which in turn affected male dispersal (Eldakar 2009). Besides, if there is inherent difference in dispersal tendency across two sexes, which is prominent in many animals (Trochet 2016, Mishra et al. 2018a), it can heavily influence male-female interactions, thus shaping interlocus conflict and its evolutionary consequences. In addition, it is important to note that dispersal may also impose novel ecological costs. For example, dispersal and other movement related traits such as foraging or exploration, are energetically and/or ecologically costly (Bonte et al. 2012). However, it is not clear how such costs and benefits affect the outcome of interlocus conflict in a population. MacPherson et al. (2018) found low condition females, raised in high larval density, suffered from the cost of increased male exposure, regardless of their holding condition, whereas high condition females were found to be practically immune to mate harm in complex, but not in simple habitat. A patch structured habitat, apart from being relatively complex, may bring in an additional ecological component of dispersal. If both dispersal and resistance to mate harm are energetically expensive, structuring of habitat can impose additional cost on females thereby making them more susceptible to mate harm. However, this expectation will depend on cost of dispersal as well as female condition. Therefore, spatial structuring of a population can clearly impact (a) the level of interlocus conflict in a population, and (b) the evolutionary trajectory of sexually antagonistic traits. However, more investigations are needed to test the theory and assess the general implications. With ever increasing anthropogenic fragmentation of natural populations with varying degrees of connectivity within patches (Rogan and Lacher Jr. 2018), it is becoming increasingly more important to investigate the impact of such changes in the habitat structure on key eco-evolutionary processes, such as, sexual conflict and sexual selection.

Here, we addressed this issue using a *D. melanogaster* laboratory adapted populations. We investigated whether spatial structure could also have a similar complex habitat effect of relaxing intensity of sexual conflict. We mimicked spatial structure by setting up three-patch habitat systems by connecting three standard culture vials with narrow tubes that allow flies to move from one patch (vial) to another. As

controls, three unconnected vials represented similar interacting population units, but not distributed following any spatial structure. The control habitat is similar to the usual laboratory population, without a spatial structure wherein the sexes are free to interact with each other with no scope of escape or refuge. The experimental structured habitat type retained the possibility of identical interaction arena but also allowed scope for individuals, especially females to move around across patches (i.e., vials) and potentially avoid antagonistic male interactions. Individuals within a patch in such a setup, have a higher chance of interacting with each other compared to those from two different patches. We then compared two components of female fitness - fecundity and starvation survival time, following two types of male exposures – limited exposure and continuous exposure, across these two habitat treatment types. We used the results to test the hypotheses on cost of male exposure in females (i.e., the reproductive cost in females arising due to ISC) and cost of dispersal. We found clear evidence of both the costs in females. We also showed that the degree of the cost is affected by the habitat type (i.e., unstructured vs. structured). Further experiments showed that the effect of habitat on such cost was much stronger if the females were nutritionally challenged compared to controls. To the best of our knowledge, this is first explicit experimental test of the emerging theory of habitat type and condition dependence of ISC.

The benign and nutritionally rich laboratory maintenance regime of populations such as, the ones used in our experiments, can often obscure fitness costs, especially those concerning resource allocation trade-offs (Van Noordwijk and De Jong 1986, McCracken et al. 2020, MacTavish and Anderson 2020). Therefore, we further investigated this issue using resource deprived females. To this effect, we first investigated the role of developmental dietary restriction on spontaneous dispersal tendency to determine a level of dietary manipulation that does not significantly alter dispersal but results in significantly resource deprived adults. We then repeated the above-mentioned experiment with an added treatment of the female diet regime wherein standard and nutritionally deprived females were used.

Results

Using laboratory adapted populations of *D. melanogaster*, we conducted two main experiments to investigate the problem at hand. In the first experiment (Experiment 1), we investigated the effect of habitat structure on the extent of interlocus conflict. We measured progeny production and starvation survival time in females under two types of male exposures – limited exposure (LE: females exposed to males for a short duration that ensured only single mating) and continuous exposure (CE: females continuously held with males). The effect of male exposure type treatment in such an assay has been found to be a good measure of interlocus conflict in similar *D. melanogaster* systems (Nandy et al. 2013, 2014). The assay was done under two habitat types – structured and unstructured. The entire experiment was performed in three independent statistical blocks using three randomly chosen replicate base populations (viz., BL_2, Bl_3 and BL_4).

Analysis of the progeny count indicated that effects of both male exposure and habitat type treatment were significant (Table 1). On an average, progeny count from females belonging to the LE treatment was 55% higher compared to that from the CE treatment (Fig. 1a), indicating a significant fitness cost due to continued exposure to the males. Similarly, the progeny output of females from structured setup was on an average 37% lower compared to those from the unstructured setup (Fig. 1a). However, male exposure × habitat type interaction had no significant effect on progeny count (Table 1). Hence, there was no evidence to suggest that the degree of ISC was affected by the habitat type treatment. To further investigate habitat type treatment on fitness reduction due to our male exposure treatment, we computed Relative Reduction (RR) score for each assay population using the block means in the following formula:

$$RR = \frac{mean\ progeny\ count\ in\ LE - mean\ progeny\ count\ in\ CE}{mean\ progeny\ count\ in\ LE}$$

The RR score was then analysed for the effect of habitat type treatment using a GLM. This analysis suggested a significant effect of habitat type treatment, wherein RR scores in structured habitat type was significantly higher compared to the same in the unstructured habitat type (χ^2 = 4.32, df = 1, p = 0.03, Figure 2)

Analysis of the starvation survival time revealed a marginally non-significant effect of male exposure (Table 1), indicating a tentative trend for cost of male exposure for females. Habitat was found to have a significant effect (Table 1), wherein starvation survival time of females from structured treatment was 11% less compared to that of the females from unstructured treatment (Fig. 1b). Male exposure × habitat interaction had no significant effect (Table 1) suggesting that strength of mate harm, measured as post-treatment reduction is starvation resistance in females, was not affected by habitat type also.

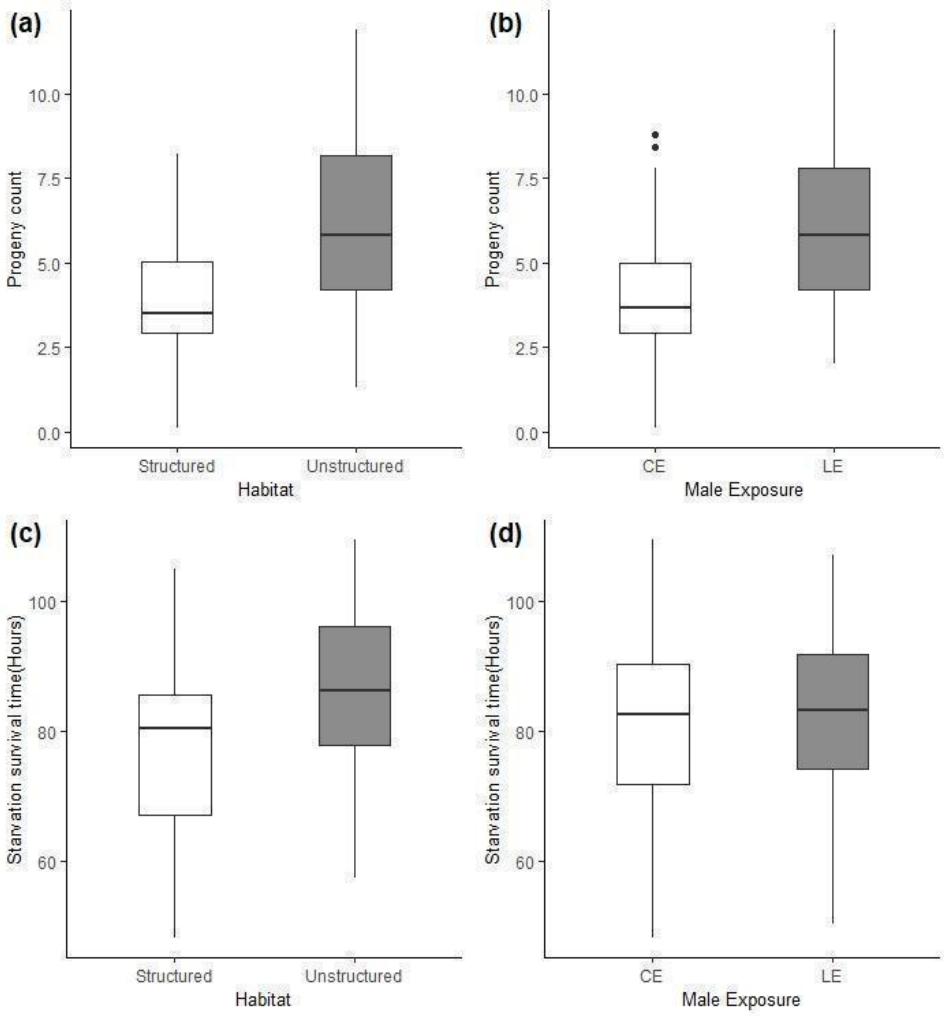

Figure 1: Results from Experiment 1. (a, b) Progeny count, i.e., number of offspring produced by the experimental females during the 18 h window following the experimental treatment. Per capita progeny output was computed as the average progeny output from ten of the fifteen females in a replicate setup. Per capita progeny count was used as the unit of analysis. (c, d) Starvation survival time of the experimental females following the treatment and progeny production. Starvation survival time was computed as the mean of the five randomly selected females within a replicate set. This mean was used as the unit of analysis. The treatment represents a full factorial combination of male exposure (limited exposure, LE and continuously exposed, CE) and habitat (structured and unstructured). The box plots represent data from all three statistical blocks. The figure shows the main effects of the fixed factor habitat (a and c), and male exposure (b and d).

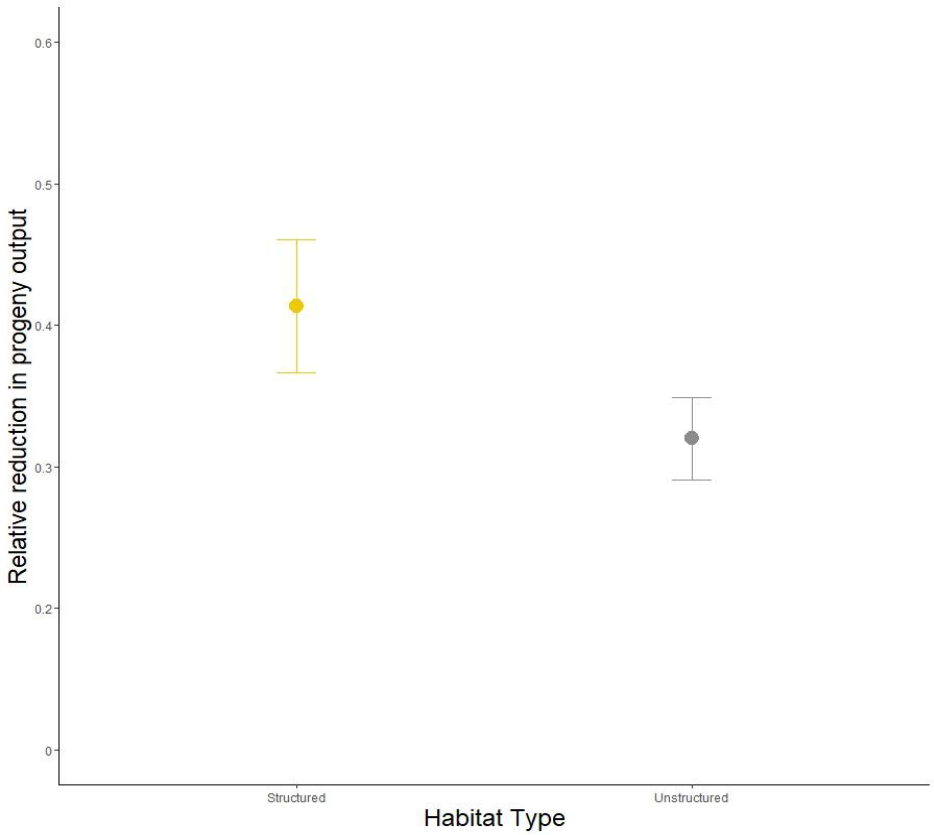

Figure 2: Effect of habitat type treatment on RR scores from the Experiment 1. RR scores were calculated for each block, using the block means of progeny count results (see Results section for the equation). It measures the reduction in progeny production (and hence, fitness) on prolonged exposure to males (limited vs. continued exposure), while considering progeny output under limited male exposure to be an intrinsic progeny production ability. Hence, RR is a measure of cost of male exposure to the females. Error bars represent standard error.

Table 1: Summary of the analyses of results from Experiment 1 and 2. The results were analysed using linear mixed-effect models where male exposure, habitat type, female type (for Experiment 2) were fitted as fixed factors. Block, and all interactions that included it were fitted as random factors. Statistically significant p-values are mentioned in bold font style.

Trait	**Effects**	**SS**	**DF**	**MS**	**F**	**P**

Experiment 1						
Progeny count	Male exposure	119.62	1	119.62	40.68	**0.02**
	Habitat	168.927	1	168.92	57.45	**<0.01**
	Male exposure × Habitat	1.336	1	1.336	0.45	0.50
Starvation survival time	Male exposure	271.49	1	271.49	3.65	0.057
	Habitat	2838.83	1	2838.83	38.26	**<0.01**
	Male exposure × Habitat	71.80	1	71.80	0.96	0.32
Experiment 2						
Progeny count	Female type	211.51	1	211.51	20.34	**0.04**
	Male exposure	219.29	1	219.29	21.09	**0.04**
	Habitat	234.66	1	234.66	22.57	**<0.01**
	Female type × Male exposure	90.74	1	90.74	8.73	**<0.01**
	Female type × Habitat	20.78	1	20.78	1.99	0.15
	Male exposure × Habitat	2.74	1	2.74	0.26	0.60
	Female type × Male exposure× Habitat	9.59	1	9.59	0.92	0.33
Starvation survival time	Female type	543.8	1	543.8	5.04	0.07
	Male exposure	1284.9	1	1284.9	11.91	**0.01**
	Habitat	3493.3	1	3493.3	32.40	**<0.01**
	Female type × Male exposure	51.5	1	51.5	0.47	0.51

(Female)	Female type × Habitat	59.7	1	59.7	0.55	0.47
	Male exposure × Habitat	28.2	1	28.2	0.26	0.60
	Female type × Male exposure× Habitat	110.3	1	110.3	1.02	0.31
Starvation survival time (Male)	Female type	529.12	1	529.12	0.85	0.45
	Habitat	2306.32	1	2306.32	3.719	0.20
	Female type × Habitat	30.99	1	30.99	0.05	0.82

Since dispersal tendency and susceptibility to mate harm in females are known to be condition dependent, and the relatively rich nutritional environment of our standard laboratory regime can obscure the fitness costs that depend on resource allocation trade-offs (Van Noordwijk and De Jong 1986, McCracken et al. 2020, MacTavish and Anderson 2020), we repeated the above experiment with an added treatment - dietary condition. However, *a priori* information was needed on female response to nutritional manipulations. Hence, we first conducted an assay to measure the effect of manipulation of developmental diet on female dispersal tendency (see supplementary material). The results suggested that a nutritionally diluted developmental diet, even up to 40% reduction compared to the standard diet, does not affect female dispersal tendency (Fig. S6). Part of these results however, also suggested that such dietary treatment significantly reduces female quality as measured by lower body mass at eclosion (also see Poças et al. 2022). Such females are considered resource deprived or poor condition females.

We conducted a second experiment (Experiment 2) that involves poor and standard condition females. Additionally, in this experiment, we also assessed the cost of dispersal for males. The design of this experiment was similar to that followed in Experiment 1, except the additional treatment for female condition. We use nutritionally challenged females (poor condition: larval development in 40% diluted

food) and those raised on standard food (control: larval development in standard banana-jaggery-barley-yeast food). The detailed composition of the food types is mentioned in the supplementary information (Table S2).

Results of analysis on progeny count indicated significant effects of all three main effects, viz., female type, male exposure and habitat (Table 1). As expected, poor condition females produced, on an average, 42% less progeny than standard females (Fig. 2b). On an average, progeny count of females from the structured setup was on average 29% less compared to those from the unstructured setup, indicating cost of dispersal that comes from living in a structured habitat (Fig. 2c). Progeny count from females belonging to the LE treatment was on an average 65% higher compared to that from the CE treatment, replicating the finding from Experiment 1, indicating significant reproductive cost of male exposure (Fig. 2d). Interestingly, female type × male exposure interaction was found to have significant effect on progeny count (Fig. 2a). This suggested that the reproductive cost of male exposure, the measure of ISC, is dependent on female type. Pairwise comparisons (Table S4) revealed a significant reduction of progeny count in CE treatment compared to the LE treatment only for standard females, but not for females raised in poor diet (i.e., poor female type). However, the effects of female type × habitat interaction as well as the male exposure × habitat interaction were not significant (Table 1). Therefore, the analysis did not reveal any evidence suggesting that the male induced fitness reduction is affected by habitat type treatment. We computed the RR score just like we did for the Experiment 1. Analysis of the RR score indicated significant effects of habitat type (Figure 2), consistent with the analysis of the results from Experiment 1. Here, we also found the effect of female type × habitat interaction to be significant (Table 2). The main effect of female type was not significant (Table 2). Therefore, the analysis of RR scores suggested that (a) male induced fitness cost in females was significantly higher in structured habitat type treatment, and further, (b) the habitat effect itself is dependent on female type. Fig. 3 shows that the effect of habitat type is much stronger when the females were poor quality. The effect of female type on RR score was not significant.

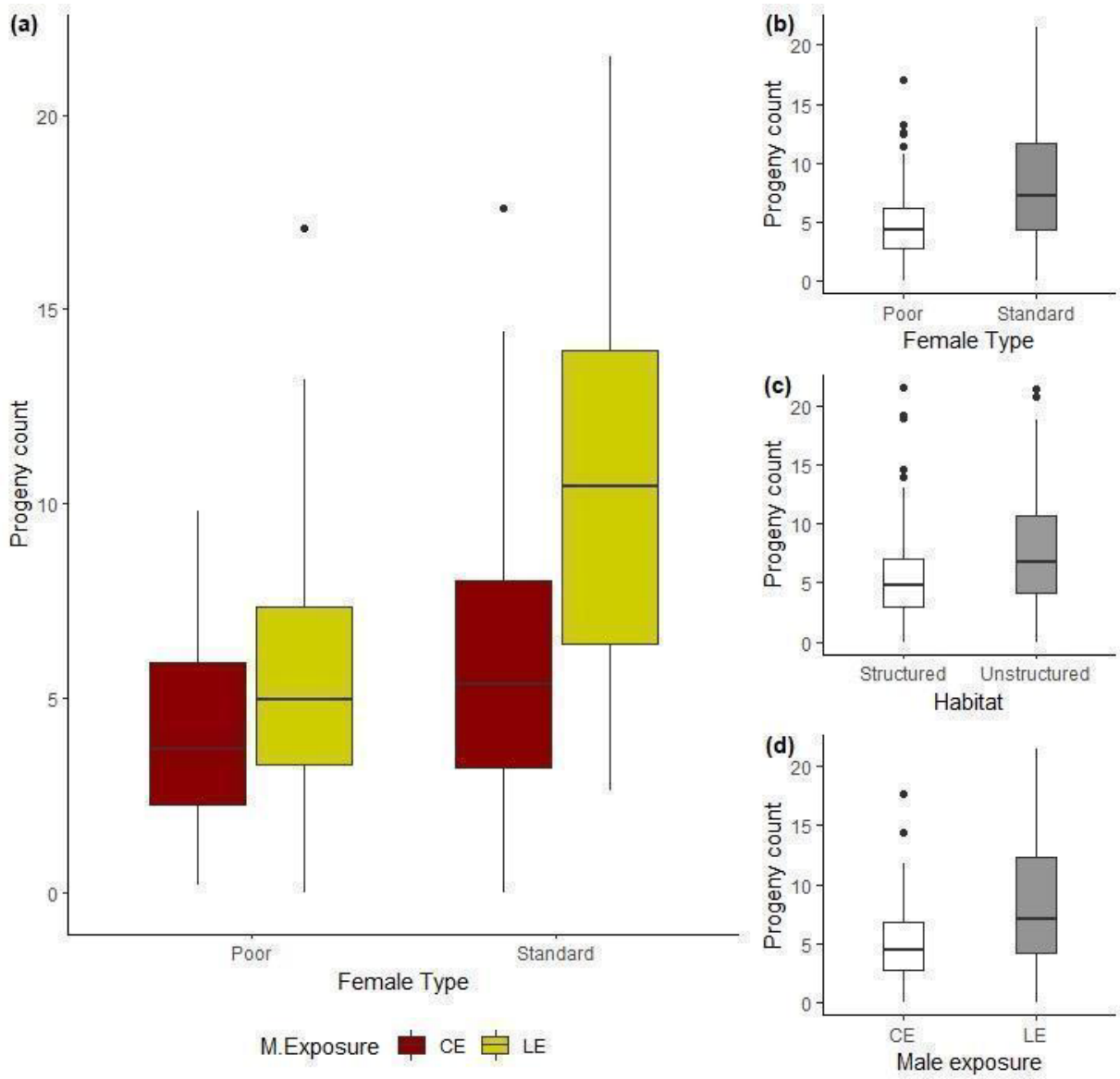

Figure 2: Progeny count results from Experiment 2. Progeny count, i.e., number of offspring produced by the experimental females during the 18 h window following the experimental treatment. Per capita progeny output was computed as the average progeny output from ten of the fifteen females in a replicate setup. Per capita

progeny count was used as the unit of analysis. The treatment represents a full factorial combination of female type (poor and standard), mate exposure (limited exposure, LE and continuously exposed, CE) and habitat t (structured and unstructured). The box plots represent data from all three statistical blocks. The figure shows the main effects of the fixed factors female type, (b), habitat (c), male exposure (d), and the effect of female type × male exposure interaction (a).

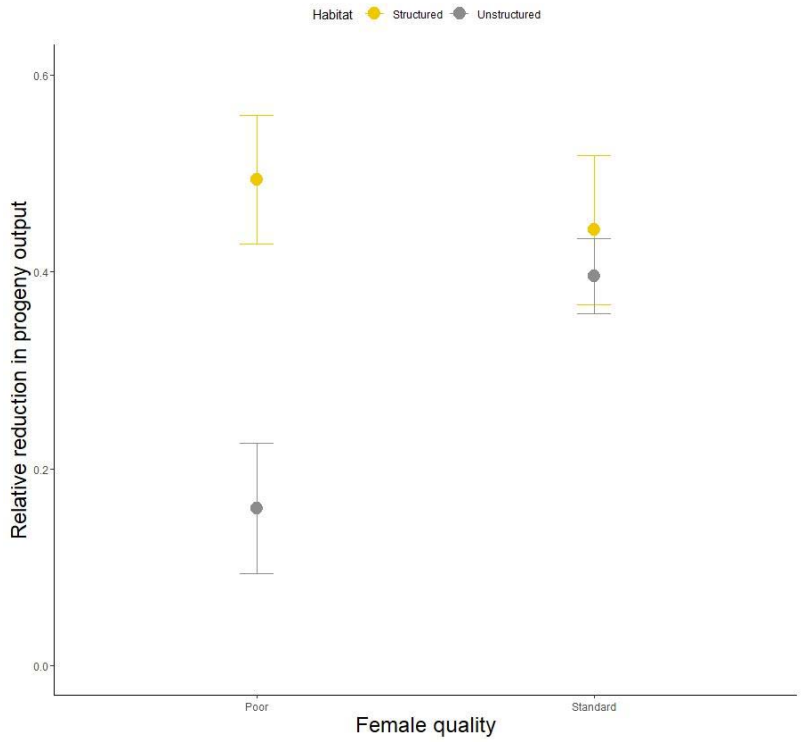

Figure 3: The Relative reduction (RR) scores from Experiment 2. RR scores were calculated for each block, using the block means of progeny count results (see Results section for the equation). It measures the reduction in progeny production (and hence, fitness) on prolonged exposure to males (limited vs. continued exposure), while considering progeny output under limited male exposure to be an intrinsic progeny production ability. Hence, RR is a measure of cost of male exposure to the females. Effect if habitat type treatment for poor and standard females are shown. Error bars represent standard error.

Table 2: Summary of the analysis of results RR score from Experiment 2. The RR score were analysed using a general linear mixed-effect model (GLMM) where Female and Habitat were fitted as fixed factors. Block as random factors. Statistically significant p-values are mentioned in bold font style. RR score indicated significant

effects of habitat type and the female type × habitat interaction, whereas the main effect of female type was not significant.

Contrast	χ^2	DF	p-value
Female	0.08	1	0.76
Habitat	6.16	1	**0.01**
Female × Habitat	4.27	1	**0.03**

Analysis of the starvation survival time of the females revealed significant main effects of male exposure and habitat (Table 1). Effect of female type was marginally non-significant (Table 1, Fig. 3a). Starvation survival time of females from structured treatment was on an average 12% less compared to that of the females from unstructured treatment (Fig. 3b), replicating the results from Experiment 1. Females that received continuous male exposure (i.e., CE) died under starvation on an average 6% faster than females that received limited male exposure (Fig. 3c). None of the two-way and three-way interactions was found to have a significant effect on starvation survival time of the females (Table 1). For males, neither the main effects (viz., female type and habitat) nor the interaction between them had a significant effect on starvation survival time (Table 1), indicating that male starvation resistance depends neither on the type of females they are exposed to nor the presence of habitat structure. Assuming that the males disperse between the three patches in our structured setup, there was no evidence of a survival cost of such spontaneous dispersal in males.

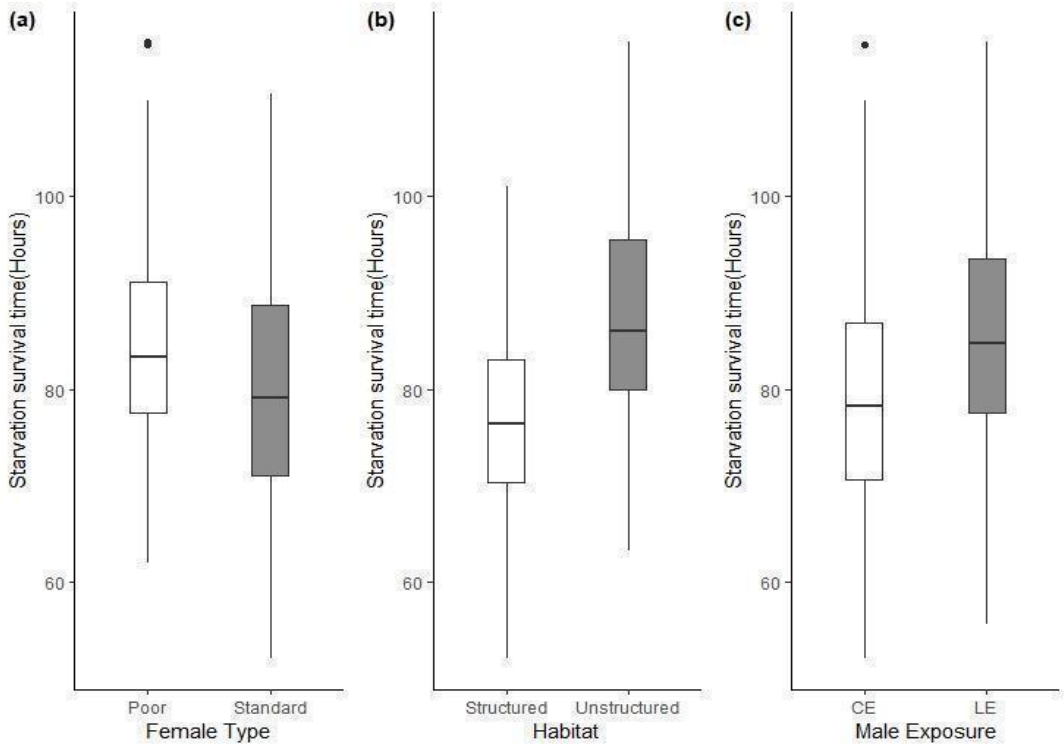

Figure 4: Female starvation survival time results from Experiment 2. Starvation survival time of the experimental females following the treatment and progeny production. Starvation survival time was computed as the mean of the five randomly selected females within a replicate set. This mean was used as the unit of analysis. The treatment represents a full factorial combination of female type (poor and standard), male exposure (limited exposure, LE and continuously exposed, CE) and habitat (structured and unstructured). The box plots represent data from all three statistical blocks. The figure shows the main effects of female type (a), habitat (b), and male exposure (c) on starvation survival time of the experimental females.

Discussion:

Regardless of the experimental habitat type we found females to bear a substantial fitness cost due to continuous exposure to males (i.e., due to ISC). We found such a cost to be expressed as short term reproductive and survival costs. Importantly, given their laboratory ecology, these costs are good measures of the actual fitness cost. However, we found the reproductive cost of male exposure to be absent for poor condition females giving rise to a significant effect of the female type × male exposure

interaction on progeny count data in our Experiment 2. The main aim of the current investigation was to understand how spatial structuring might affect these costs. We found that spatial structuring has a significant negative impact on fitness components in females (but not in males) – possibly indicating the cost of dispersal. Though unsupported by the progeny count analysis, our analyses using the RR scores (a matric designed to capture the fitness cost of male encounter in females) suggested that reproductive cost for females due to ISC was significantly higher in our experimental structured habitat compared to unstructured habitat. This is contrary to the expectations from the hitherto reported complex environment effect on sexual conflict wherein spatial complexity is expected to reduce ISC (Yun et al. 2017, Malek and Long 2019). Further, we also found our observed habitat type effect to be dependent on female condition. The observed habitat effect on female reproductive cost attributable to ISC appeared to be much stronger for poor condition females. We discuss the broader implications of our findings in light of existing understanding of sexual conflict, dispersal and life history theories.

Yun et al. (2017) reported that physical complexity of the habitat can modulate the intensity of ISC - bringing down male-female interaction rate, giving females opportunity to seek refuges, and even changing the outcome of male mate choice for high fecundity females. This and the subsequent follow-up study from the same group was an important step towards bridging the intellectual gap between sexual conflict research and the nuanced ecology of a natural population. We investigated another component of a natural population that is usually simplified in laboratory microcosm setups - fragmented, discontinuous nature of a habitat. In our experimental setups, we found clear evidence of ISC, but unlike Yun et al. (2017), no evidence indicating a decline in ISC in structured habitat. On the contrary females suffered an increased cost of male exposure under such a habitat. Since a substantial amount of movement of individuals was shown to be a common feature in such a habitat (see supplementary information), such observation is unlikely to be due to the lack of movement. Further, we found significant detrimental effects of habitat type on female progeny output as well as starvation survival time. Such negative impacts of alteration in habitat (viz., from unstructured vs. structured) was

most likely due to the cost of dispersal across the three vials during the assay period (Mishra et al. 2022). Though we did not record the degree of dispersal of individuals across the vials during our assay, we have ample data on the movement tendencies of flies under similar setups, and that using a two-patch system (see supplementary information for experimental details of dispersal tendency). Further, the sex specific effect of habitat type (see results of Experiment 2) wherein only females, but not males, were found to show fitness costs of living in the fragmented habitat, is perfectly compatible with our findings from (a) a two-patch setup, and (b) over representation of females in the connecting corridors in three-patch experimental setups - both suggesting a female biased spontaneous dispersal tendency.

Theoretically, in a fragmented habitat, females can escape male congregation by moving to a patch with less male abundance. Interestingly, when Mishra et al. (2018b) found density dependent dispersal in female *D. melanogaster*, such density dependence was present only when the sexes were held together, and not when they were kept apart. These results seem to suggest a role of the presence of males on female dispersal, potentially invoking the mate harm avoidance hypothesis. Thus, in a habitat that allows such movement opportunities, females can be expected to escape mate harm, at least to some extent, by moving to a neighbouring patch potentially resulting in reduction in population level intensity of ISC. However, such male-avoidance related female dispersal can result in temporary shortage of females in a patch or temporary increase in the intensity of male-male competition resulting in increased ‘mate-finding dispersal’ in males (Mishra et al. 2020). This can in turn result in the equalisation of sex ratio across habitat patches, ameliorating the advantage of female biased dispersal, if any. Indeed, we have observed a substantial temporal and spatial variation in sex ratio in our pilot assay, results of which have been included in the supplementary information. It is quite possible that reduction in ISC under a complex environment, as reported by Yun et al. (2017) and Malek and Long (2018), critically depends on the availability of refuge to the females such that unless spatial structuring also allows access to refuge, it would not lead to reduced ISC. However, Byrne and Rice (2008) did not

find any evidence of access to refuge leading to reduction in ISC. Importantly, in natural populations habitat structure is usually accompanied by a number of other ecological variables, such as, heterogeneity in resource availability, abundance of natural enemies. It is possible that the presence of such heterogeneity in cost and reward of different habitat patches have direct or indirect implications in determining the strength of ISC. For example, the detrimental effect of larger males of *Aquarius remigis* seems to be stronger in habitats with high risk of predation (Sih and Krupa 1992). In the Trinidadian guppies, *Poecilia reticulata*, males in habitat patches with high risk of predation tend to be more persuasive towards females, resulting in increased mate harassment (Magurran and Seghers 1994). Hence, in such a species both predation and habitat structure seem to inflate the effect of ISC, which can in turn have important consequences on dispersal across habitat patches (for example, see Rowe et al. 1994).

Though it did not seem to result in an escape from harmful effects of male exposure, females in our experiments possibly showed greater degree of dispersal compared to the males. At least, data from parallel assays that used a two-patch and three-patch setups, seem to suggest such a female-biased dispersal (see supplementary information for details). This is not surprising and has been previously reported in several animals (Iliadi et al. 2002, Mishra et al. 2018a, b). As it turns out, female-biased dispersal is fairly common in birds (Clarke et al. 1997), and also seen in mammals (Favre et al. 1997), fishes (Yue et al. 2012) and invertebrates (Caudill 2003, Sundström et al. 2003, Beirinckx et al. 2006). Female biased dispersal is more commonly associated with competition for resources instead of mates, which is a common driver of male biassed dispersal (Li and Kokko 2019). An interesting exercise is to consider the consequences of female philopatry. There are two main costs of philopatry for *D. melanogaster* females, which live on ephemeral food patches - (a) competition for food (Bath et al. 2018), which is a primary determinant of female reproductive output, and (b) increasingly unfavourable environment for offspring development (Botella et al. 1985). Incidentally, these are the two main components of female fitness. Male fitness, on the other hand, depends primarily on mating success

(Bateman 1948), and all else being equal, it is much harder for males to increase their chances of mating by simply dispersing to a different patch, unless the resident patch turns out to be mate deprived (Joshi et al. 1999). Thus, it is reasonable to suggest that a female biased dispersal rate is perhaps an adaptive demographic outcome given the behaviour and ecology of *D. melanogaster*. However, as our results suggest, females suffer a significant negative impact of living in a fragmented habitat - possibly due to costs of dispersal. In the long run, this should strengthen natural selection acting on females. Though our results rule out condition-dependence of sex-biased dispersal tendency (see supplementary information), it will be interesting to find out if such sex biased dispersal persists under a variety of other conditions. There is already some evidence that population density can break down this pattern (Mishra et al. 2018b).

Counterintuitively, reduction in progeny production on extended male exposure (i.e., LE vs. CE comparison) was found to be nonsignificant for poor quality females (i.e., females raised on a nutritionally impoverished diet), whereas the same was significant for standard females. This could be due to (a) poor condition females being closer to the fitness lower bound (i.e., zero or very low progeny output) – making the detection of fitness reduction upon prolonged male exposure much harder to detect, or (b) poor condition females being actually harassed less by the males. The latter is possible if males had preference against poor condition females. Male mate choice in *D. melanogaster* is well documented, and indicates adaptive choice towards female qualities associated with higher fecundity (Byrne and Rice 2006, Nandy et al. 2012, Edward and Chapman 2013, Arbuthnott et al. 2017). Long et al. (2009) showed non-virgin females of higher quality (viz., larger females) to receive greater male courtship compared to that received by low quality females. Such differential courtship resulted in high quality females suffering higher cost of male exposure (Long et al. 2009). Though our results fit this theory, we currently lack data required for a more direct test. However, analysis of our relative fitness reduction (i.e., RR score) suggested that the extent of fitness reduction due to extended male exposure for poor condition females in unstructured habitat type was equivalent to that observed in standard

condition females. Hence, low RR score was unique for the poor condition females held in structured habitat type. If males harmed poor condition females less, such an observation would be unlikely. It is more likely that poor condition females held in structured habitat produced very few offspring due to nutritional deprivation and the cost of dispersal regardless of their male exposure status. Even if male harm is an additional cost to these females, its impact is likely to be significantly less compared to the other conditions. Importantly, it is not possible to completely rule out the ISC-reducing effect of structured habitat in this case. Therefore, based on our observations, if there is such an effect of structured habitat, it is dependent on female condition.

In conclusion, our results are, to the best of our knowledge, the first clear empirical test of the effect of spatial structure on the population level of interlocus sexual conflict. Our results demonstrate that spatial structuring by itself may not be sufficient to reduce the ISC – such an effect being dependent on female condition. Habitat structure can, however, impose additional fitness costs if it results in inter-patch dispersal, and dispersive behaviour itself is costly. Such a fitness cost can be sex specific, if there is sex difference in dispersal tendency. Future investigations should assess the generality of these findings under variability of different components of environment, including patch quality, predation risk, intensity of competition risk.

Material and methods:

We used flies from a set of large, outbred, wild-type, laboratory adapted populations of *Drosophila melanogaster* (BL_{1-5}), maintained under a 14-day discrete generation cycle, 24 h light, at 25 °C (±1) temperature on standard banana-jaggery-yeast food medium. Flies are grown in culture vials (25 mm diameter × 96 mm height) at a density of ~70 per 6-8 ml food in each vial, forty such vials make up one population. On day 12 post-egg collection, and after all the adults have emerged from pupae, flies are transferred into a population cage and thereafter maintained in the cage as a population of ~2,800 individuals. On day 14, eggs are collected on a fresh food plate within a time window of 18 h. These

eggs are cultured in fresh food vials following the above-mentioned density to start the next generation. The details of the history and maintenance of the populations can be found in Nandy et al. 2016 and Dasgupta et al. 2022. Randomly chosen three out of the five BL populations, viz., BL_2, Bl_3 and BL_4 were used to conduct the experiments.

Structured and unstructured habitats and the experimental setup:

As mentioned above, the base populations are maintained as large unstructured island populations, either as a single large unit with ~2,800 individuals (i.e., the cage phase), or 40 small unconnected units each with ~70 individuals (i.e., the vial phase). For the purpose of the experiments, we used a simple three-patch setup. We created experimental patch-structured habitat systems (hereafter simply referred to as "structured" habitat type), by connecting three culture vials with narrow tubes (length 12.5cm, diameter 0.6 cm, hereafter referred as "corridor") that allowed flies to move from one vial to another in either direction (see Fig. S1). Results from pilot studies conducted prior to the assays suggested that both density of flies (individuals per patch) and sex ratio in each patch show substantial spatial and temporal variation (Figure S2a and S2b) within the framework of the assay conditions mentioned below. Further details regarding the dispersal behaviour of flies held in such an experimental setup can be found in the Supplementary information.

We used three standard culture vials, without connection between them, as controls (Fig. S1). Hereafter, we refer to this habitat type as "unstructured"). We opted for this design, since we aimed to compare structured vs. unstructured habitats controlling for the population size (i.e., large vs. small) effect. In the structured setup, because individuals were free to move around, the density across the three patches are expected to change (also see SI). However, the average density (number of individuals per vial) was expected to remain the same between the two habitat types.

Experiment 1:

To generate experimental flies, eggs were collected from the baseline populations in standard food vials (~8ml food) with a density of ~70 eggs/vial. After egg collection, vials were kept in controlled laboratory condition and monitored carefully for the onset of emergence of adults. Following the onset of eclosion, flies were collected every six hours. Experimental flies were collected as virgins from the peak of eclosion during which flies were separated by sex and housed in same sex vials at a density of 5 females/vial and 10 males/vial. The assays were performed on 2-3 days (post-eclosion) old flies. This adult age was chosen because it is the most relevant to adult fitness in this system (see description of maintenance regime above and Nandy et al. 2016).

On day 12, 10 virgin males and 5 virgin females were introduced in each vial for both S and US setups. An adult sex ratio of 2:1 was taken to ensure higher male inflicted harm on females, thereby helping in the resolution of even relatively smaller differences in female performance across treatments. For the unstructured habitat type, a set of three identical vials with 10 males and 5 females in each vial (i.e., 30 males and 15 females for a set) constituted a replicate set. For the structured habitat type, the same numbers of sexes (i.e., 10 males and 5 females) were introduced in each of the three interconnected vials. Twenty-four replicate sets for each of the unstructured and structured habitat types were established. These twenty-four replicates were further subdivided into limited exposure (LE) and continuous exposure (CE), with twelve replicate sets in each. Thus, our assay design involved a 2×2 fully orthogonal combination of the two treatments (viz., habitat and exposure type). For the LE subset, one hour following the introduction of experimental flies, males were removed (using light CO_2 anaesthesia) whereas females were retained in the same setup. In the CE subset, males and females were allowed to remain in their respective setups for ~48 hours. To equalise the handling, flies in the CE setup were also exposed to mild dosage of CO_2 roughly after 1 hour after the initial introduction of the sexes in the setups. On day14, i.e., following ~48 hours of the initial combination of the sexes, flies were anaesthetized and ten randomly chosen females were transferred to oviposition tubes. For both unstructured and structured setups, females from the three component vials in a replicate setup were

combined and 10 females (out of 15) were randomly chosen for oviposition. Males were discarded at this point. A fixed window of 18 hours was allowed to the females in the oviposition tubes (1 female in each oviposition tube) for laying eggs. Following this, the females were removed from the tubes; the tubes were then incubated for offspring emergence. Per capita progeny output was calculated for a given replicate as the average number of offspring produced by these ten females. Following oviposition in tubes, 5 females were randomly chosen out of 10 and were regrouped and transferred into a non-nutritive agar vial to measure starvation survival time. Female survival in these vials were recorded every six hours. Mean survival time was calculated as the average survival time of five females for a given replicate vial.

Experiment 2:

Flies were generated using a method similar to that followed in Experiment 1. However, as mentioned above, for this experiment, two types of females - poor and standard, were raised and collected as virgins (see Experiment 1). Followed by this, the experimental flies were subjected to the assay setups. However, unlike Experiment 1, this was done on 3-4 days old flies to account for the difference in development time of the poor and control females. We subjected the females to two habitat treatments - unstructured and structured, as mentioned in Experiment 1. These setups were further subdivided into two mate exposure treatments - limited exposure and continuous exposure, i.e., the LE and CE vials as mentioned in experiment 1. All males used in this stage were raised on a standard diet. Subsequently, we measured female progeny output and starvation survival time following the protocol similar to that followed in Experiment 1. We had 8 replicates for each female type × mate exposure × habitat treatment, and number flies in each vial/setup were identical across Experiment 1 and 2, with the latter being also performed in randomised block design with three blocks (three randomly chosen BL populations - BL_3, Bl_4 and BL_5). Per capita progeny output and mean starvation survival time for the experimental females were computed following the same method adopted in Experiment 1.

Statistical analysis:

Progeny count (i.e., per capita progeny output) and mean starvation survival time results were analysed using a linear mixed-effect model using `lmer` function of the `lme4` package in R (R Version 4.2.0). In the models, progeny count or starvation survival time were taken as response variables, habitat, male exposure, and female type (only for Experiment 2) were treated as fixed factors, while block and all interactions concerning block were modelled as random factors. In order to test for the effect of random factors we ran separate models by dropping a random factor term of our interest and compared it with the global model using `Anova` function. We did not find any significant effect of block and all interaction terms concerning block (see Table S6, for details of the results of this analysis). Following the analysis of variance using `Anova` function, pairwise multiple comparisons were done with `pairwise` function in package `emmeans`. In both experiments, the RR score (see Results section for the method of computation) was analysed using Generalized linear mixed effect model using `glmer` function of the `lme4` package. In Experiment 1, female type was modelled as fixed factor and block and the block × female type interaction as random factors. In Experiment 2, female type and habitat type were modelled as fixed factors, and block and all interactions involving block were modelled as random factors.

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References:

Adler, M. (2010). Sexual conflict in waterfowl: why do females resist extrapair copulations? *Behavioral Ecology*, *21*, 182-192.

Adler, M. I., & Bonduriansky, R. (2014). Sexual conflict, life span, and aging. *Cold Spring Harbor perspectives in biology*, *6*, a017566.

Alvarez, B., & Koene, J. M. (2021). Sexual conflict in nonhumans. *Encyclopedia of Evolutionary Psychological Science*, 7333-7351.

Arbuthnott, D., Fedina, T. Y., Pletcher, S. D., & Promislow, D. E. (2017). Mate choice in fruit flies is rational and adaptive. *Nature Communications*, *8*, 1-9.

Arnqvist, G. (1992). Pre-copulatory fighting in a water strider: inter-sexual conflict or mate assessment? *Animal Behaviour*, 43, 559-567.

Arnqvist, G. (2006). Sensory exploitation and sexual conflict. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*, 375-386.

Arnqvist, G., & Rowe, L. (2013). Sexual conflict. In *Sexual Conflict*. Princeton university press.

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity*, *2*, 349-368.

Bath, E., Morimoto, J., & Wigby, S. (2018). The developmental environment modulates mating□induced aggression and fighting success in adult female Drosophila. *Functional Ecology*, *32*, 2542-2552.

Beirinckx, K., Van Gossum, H., J. Lajeunesse, M., & R. Forbes, M. (2006). Sex biases in dispersal and philopatry: Insights from a meta□analysis based on capture–mark–recapture studies of damselflies. *Oikos*, *113*, 539-547.

Bonduriansky, R., Maklakov, A., Zajitschek, F., & Brooks, R. (2008). Sexual selection, sexual conflict and the evolution of ageing and life span. *Functional ecology*, 443-453.

Bonte, D., Van Dyck, H., Bullock, J. M., Coulon, A., Delgado, M., Gibbs, M., ... & Travis, J. M. (2012). Costs of dispersal. *Biological reviews*, *87*, 290-312.

Botella, L. M., Moya, A., Gonzalez, M. C., & Mensua, J. L. (1985). Larval stop, delayed development and survival in overcrowded cultures of *Drosophila melanogaster*: effect of urea and uric acid. *Journal of Insect Physiology*, *31*, 179-185.

Byrne, P. G., & Rice, W. R. (2006). Evidence for adaptive male mate choice in the fruit fly Drosophila melanogaster. *Proceedings of the Royal Society B: Biological Sciences*, *273*, 917-922.

Byrne, P. G., Rice, G. R., & Rice, W. R. (2008). Effect of a refuge from persistent male courtship in the Drosophila laboratory environment. *American Zoologist*, 48, e1-e1.

Caudill, C. C. (2003). Measuring dispersal in a metapopulation using stable isotope enrichment: high rates of sex□biased dispersal between patches in a mayfly metapopulation. *Oikos*, *101*, 624-630.

Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F., & Partridge, L. (1995). Cost of mating in Drosophila melanogaster females is mediated by male accessory gland products. *Nature*, *373*, 241-244.

Chapman, T., Arnqvist, G., Bangham, J., & Rowe, L. (2003). Sexual conflict. *Trends in Ecology & Evolution*, 18, 41-47.

Clarke, A. L., Sæther, B. E., & Røskaft, E. (1997). Sex biases in avian dispersal: a reappraisal. *Oikos*, 429-438.

Edward, D. A., & Chapman, T. (2013). Variation in male mate choice in *Drosophila melanogaster*. *PloS one*, *8*, e56299.

Eldakar, O. T., Dlugos, M. J., Pepper, J. W., & Wilson, D. S. (2009). Population structure mediates sexual conflict in water striders. *Science*, 326, 816-816.

Favre, L., Balloux, F., Goudet, J., & Perrin, N. (1997). Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *264*, 127-132.

Harrison, X. A., Donaldson, L., Correa-Cano, M. E., Evans, J., Fisher, D. N., Goodwin, C. E., ... & Inger, R. (2018). A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ, 6, e4794.

Holland, B., & Rice, W. R. (1999). Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proceedings of the National Academy of Sciences*, *96*, 5083-5088.

Iliadi, K. G., Iliadi, N. N., Rashkovetsky, E. L., Girin, S. V., Nevo, E., & Korol, A. B. (2002). Sexual differences for emigration behavior in natural populations of *Drosophila melanogaster*. *Behavior genetics*, *32*, 173-180.

Jones, T. M., Elgar, M. A., & Arnqvist, G. (2010). Extreme cost of male riding behaviour for juvenile females of the Zeus bug. *Animal Behaviour*, *79*, 11-16.

Joshi, A., Do, M. H., & Mueller, L. D. (1999). Poisson distribution of male mating success in laboratory populations of *Drosophila melanogaster*. *Genetics Research*, *73*, 239-249.

Koene, J. M., & Schulenburg, H. (2005). Shooting darts: co-evolution and counter-adaptation in hermaphroditic snails. *BMC Evolutionary Biology*, *5*, 1-13.

Koene, J. M. (2012). Sexual conflict in nonhuman animals

Krupa, Jakub & Travers, Steven. (1990). An Experimental Study on the Effects of Predation Risk and Feeding Regime on the Mating Behavior of the Water Strider. *The American Naturalist* - Amer Naturalist.

Le Galliard, J. F., Fitze, P. S., Ferriere, R., & Clobert, J. (2005). Sex ratio bias, male aggression, and population collapse in lizards. *Proceedings of the National academy of Sciences*, 102, 18231-18236.

Li, X. Y., & Kokko, H. (2019). Sex□biased dispersal: A review of the theory. *Biological Reviews*, *94*, 721-736.

Long, T. A., Pischedda, A., Stewart, A. D., & Rice, W. R. (2009). A cost of sexual attractiveness to high-fitness females. *PLoS biology*, *7*, e1000254.

MacPherson, A., Yun, L., Barrera, T. S., Agrawal, A. F., & Rundle, H. D. (2018). The effects of male harm vary with female quality and environmental complexity in *Drosophila melanogaster*. *Biology Letters*, 14, 20180443.

MacTavish, R., & Anderson, J. T. (2020). Resource availability alters fitness trade□offs: implications for evolution in stressful environments. *American Journal of Botany*, *107*, 308-318.

Magurran, A. E., & Seghers, B. H. (1994). Sexual conflict as a consequence of ecology: evidence from guppy, Poecilia reticulata, populations in Trinidad. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *255*, 31-36.

Malek, H. L., & Long, T. A. (2019). Spatial environmental complexity mediates sexual conflict and sexual selection in *Drosophila melanogaster*. *Ecology and evolution*, 9, 2651-2663.

McCracken, A. W., Adams, G., Hartshorne, L., Tatar, M., & Simons, M. J. (2020). The hidden costs of dietary restriction: implications for its evolutionary and mechanistic origins. *Science advances*, *6*, eaay3047.

Mishra, A., Tung, S., Shree Sruti, V. R., Srivathsa, S., & Dey, S. (2020). Mate□finding dispersal reduces local mate limitation and sex bias in dispersal. *Journal of Animal Ecology*, 89, 2089-2098.

Mishra, A., Tung, S., Shreenidhi, P. M., Aamir Sadiq, M., Shree Sruti, V. R., Chakraborty, P. P., & Dey, S. (2018). Sex differences in dispersal syndrome are modulated by environment and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*, 20170428.

Mishra, A., Tung, S., Sruti, V. S., Sadiq, M. A., Srivathsa, S., & Dey, S. (2018). Pre□dispersal context and presence of opposite sex modulate density dependence and sex bias of dispersal. *Oikos*, 127, 1596-1604.

Mishra, A., Tung, S., Sruti, V. S., Shreenidhi, P. M., & Dey, S. (2022). Desiccation stress acts as cause as well as cost of dispersal in *Drosophila melanogaster*. *The American Naturalist*, 199, E111-E123.

Nandy, B., Joshi, A., Ali, Z. S., Sen, S., & Prasad, N. G. (2012). Degree of adaptive male mate choice is positively correlated with female quality variance. *Scientific Reports*, *2*, 1-8.

Nandy, B., Gupta, V., Sen, S., Udaykumar, N., Samant, M. A., Ali, S. Z., & Prasad, N. G. (2013). Evolution of mate-harm, longevity and behaviour in male fruit flies subjected to different levels of interlocus conflict. *BMC evolutionary biology*, *13*, 1-16.

Nandy, B., Gupta, V., Udaykumar, N., Samant, M. A., Sen, S., & Prasad, N. G. (2014). Experimental evolution of female traits under different levels of intersexual conflict in *Drosophila melanogaster*. *Evolution*, *68*, 412-425.

Parker, G. A. (1979). Sexual selection and sexual conflict. *Sexual selection and reproductive competition in insects*, *123*, 166.

Pizzari, Tommaso & Snook, Rhonda. (2007). Sexual conflict and sexual selection: Measuring antagonistic coevolution. *Evolution*. 58. 1389 - 1393.

Poças, G. M., Crosbie, A. E., & Mirth, C. K. (2020). When does diet matter? The roles of larval and adult nutrition in regulating adult size traits in Drosophila melanogaster. *Journal of insect physiology*, 104051.

Rankin, D. J., Dieckmann, U., & Kokko, H. (2011). Sexual conflict and the tragedy of the commons. *The American Naturalist*, *177*, 780-791.

Reale, D., Bousses, P., & Chapuis, J. L. (1996). Female-biased mortality induced by male sexual harassment in a feral sheep population. *Canadian Journal of Zoology*, 74, 1812-1818.

Rice, W. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* 381, 232–234 (1996)

Rice, W. R., Stewart, A. D., Morrow, E. H., Linder, J. E., Orteiza, N., & Byrne, P. G. (2006). Assessing sexual conflict in the *Drosophila melanogaster* laboratory model system. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361, 287-299.

Rogan, J. E., & Lacher Jr, T. E. (2018). Impacts of habitat loss and fragmentation on terrestrial biodiversity.

Rossi, B. H., Nonacs, P., & Pitts-Singer, T. L. (2010). Sexual harassment by males reduces female fecundity in the alfalfa leafcutting bee, *Megachile rotundata*. *Animal Behaviour*, 79, 165-171

Rowe, L., Arnqvist, G., Sih, A., & Krupa, J. J. (1994). Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends in ecology & evolution*, *9*, 289-293.

Siva-Jothy, M. T. (2006). Trauma, disease and collateral damage: conflict in cimicids. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*, 269-275.

Sundström, L., Keller, L., & Chapuisat, M. (2003). Inbreeding and sex□biased gene flow in the ant *Formica exsecta*. *Evolution*, *57*, 1552-1561.

Trochet, A., Courtois, E. A., Stevens, V. M., Baguette, M., Chaine, A., Schmeller, D. S., ... & Wiens, J. J. (2016). Evolution of sex-biased dispersal. *The Quarterly Review of Biology*, 91, 297-320

Van Noordwijk, A. J., & de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *The American Naturalist*, 128, 137-142.

Wigby S, Chapman T. Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution*. 2004 May; 58:1028-37**.**

Wolfner, M. F. (2009). Battle and ballet: molecular interactions between the sexes in Drosophila. *Journal of Heredity*, *100*, 399-410.

Yue, G. H., Xia, J. H., Liu, F., & Lin, G. (2012). Evidence for female-biased dispersal in the protandrous hermaphroditic Asian seabass, *Lates calcarifer*. *PloS one*, *7*, e37976.

Yun, L., Chen, P. J., Singh, A., Agrawal, A. F., & Rundle, H. D. (2017). The physical environment mediates male harm and its effect on selection in females. *Proceedings of the Royal Society B: Biological Sciences*, *284*, 20170424.