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Experimental evolution reveals differences between phenotypic and evolutionary responses to population density

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Abstract

Group living can select for increased immunity, given the heightened risk of parasite transmission. Yet, it also may select for increased male reproductive investment, given the elevated risk of female multiple mating. Trade-offs between immunity and reproduction are well documented. Phenotypically, population density mediates both reproductive investment and immune function in the Indian meal moth, *Plodia interpunctella*. However, the evolutionary response of populations to these traits is unknown. We created two replicated populations of *P. interpunctella*, reared and mated for 14 generations under high or low population densities. These population densities cause plastic responses in immunity and reproduction: at higher numbers, both sexes invest more in one index of immunity (phenoloxidase (PO) activity) and males invest more in sperm. Interestingly, our data revealed divergence in PO and reproduction in a different direction to previously reported phenotypic responses. Males evolving at low population densities transferred more sperm and both males and females displayed higher PO than individuals at high population densities. These positively correlated responses to selection suggest no apparent evolutionary trade-off between immunity and reproduction. We speculate that the reduced PO activity and sperm investment when evolving under high population density may be due to the reduced population fitness predicted under increased sexual conflict and/or to trade-offs between pre- and post-copulatory traits.

Introduction

Population density can have profound effects on multiple life-history traits, potentially altering both individual and population fitness. Two fundamental fitness traits, reproduction

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and immunity, can both show density dependence (Kokko & Rankin, 2006, Wilson & Reeson, 1998). Increases in population size (Bagge et al., 2004) and density may elevate the rate of disease transmission and hence require increased investment in immune activity (Wilson et al., 2003, Wilson & Reeson, 1998, Wilson et al., 2002). Specifically, the density-dependent prophylaxis hypothesis (Wilson & Reeson, 1998) proposes that the risk of parasite transmission increases with population density, or conspecific encounter rates. And, as immune systems are costly to maintain, individuals should strategically tailor their investment into immunity based on predictable cues of parasite and pathogen transmission risk (Wilson et al., 2003, Wilson & Reeson, 1998, Wilson et al., 2002). Yet, population density and increased encounter rates between conspecifics may also increase the possibility of sexual competition between males, requiring increased investment in reproductive function (for a review, see Simmons & Fitzpatrick, 2012).

In general, theory predicts trade-offs between immune investment and reproduction (Lochmiller & Deerenberg, 2000, Sheldon & Verhulst, 1996, Zuk & Stoehr, 2002, Schwenke et al., 2016) given the inherent costs of maintaining and up-regulating immune system function (Kraaijeveld & Godfray, 1997). Indeed, there is now a wealth of empirical research revealing phenotypic trade-offs between immune investment and a suite of pre-copulatory and post-copulatory sexual traits (for reviews, see Lawniczak et al., 2007, Schwenke et al., 2016), although trade-offs are not ubiquitous (Gupta et al., 2013). Furthermore, while there is clear phenotypic evidence that reproductive investment and immune activity independently can display density-dependence, how group living or population density mediates evolutionary trade-offs between immunity and reproduction is unclear. Trade-offs can occur on both phenotypic and evolutionary scales. Within-generational, physiological trade-offs are typically plastic responses to changing socio-sexual and/or environmental conditions. For example, male and female reproductive investment decreases in response to immune activation (Ahmed et al., 2002, McNamara et al., 2012, Simmons, 2012), or immune activity is lower following increased reproductive effort (Fedorka et al., 2004, McKean & Nunney, 2001). Evolutionary trade-offs among populations can occur if there is genetic variation among individuals for allocation between traits. Experimental evolution studies exploring trade-offs between immunity and reproduction have also found that increased allocation to immune activity is negatively genetically correlated with investment in other fitness traits such as larval competitive ability (Kraaijeveld & Godfray, 1997) and fertility (Boots & Begon,

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1993). And conversely, populations with increased reproductive investment demonstrate a reduction in constitutive immunity (Hosken, 2001, McNamara et al., 2013). Yet, evolutionary trade-offs do not necessarily reflect phenotypic plasticity. For example, among primate species those with greater female mating rates (and therefore greater investment in male mating structures) have increased white blood cell counts (an index of immunity). The absence of a reproduction-immunity trade-off here instead suggests that sexually transmitted diseases have been a potent selective force on primate immunology (Nunn et al., 2000). Thus, plastic, phenotypic trade-offs may occur at an individual level, but may not necessarily directly reflect evolutionary trade-offs (Flatt & Kawecki, 2007, Chippindale et al., 1997). Indeed, whether these phenotypic and evolutionary trade-offs share a mechanistic basis remains unclear (Schwenke et al., 2016).

There are also several potential reasons to expect that key fitness traits may not necessarily trade-off against each other, and instead show correlated evolutionary responses. First, there is mounting theoretical and empirical evidence for the ability of sexual selection to reduce population fitness (Holland & Rice, 1999), and even influence extinction rate (Kokko & Brooks, 2003, Holman & Kokko, 2013). In a number of taxa, studies have shown that populations experiencing increased reproductive competition exhibit reduced reproductive output and longevity (Martin & Hosken, 2004) due to increased sexual conflict, and this may result in individuals with reductions in multiple fitness measures (Arnqvist & Rowe, 2005). Second, so-called 'big house, big car' theories of life-history trade-offs have been proposed to explain positive correlations between life-history traits (Reznick et al., 2000, van Noordwijk & de Jong, 1986). Here, it is argued that positive genetic correlations can occur between fitness traits if individuals vary in their ability to acquire resources, and if there is genetic variation in the ability of these individuals to allocate across all fitness components. Thus, evidence as to whether fitness traits evolving under sexual selection should trade-off against each other, or whether they should mirror phenotypic responses remains equivocal.

The Indian meal moth, *Plodia interpunctella,* is an ideal model species to explore the evolutionary relationships between population density, reproduction and immunity. *P. interpunctella*, is mildly polyandrous (mean lifetime female mating frequency = 1.48 (Ingleby et al., 2010)). There is evidence of phenotypic density dependence in both reproductive (Gage, 1995) and immune (Triggs & Knell, 2011) activity. Individuals use population density

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as a proximate cue of sperm competition risk: when larvae are reared at higher population density, males transfer more eupyrene sperm (involved in fertilisation) and apyrene sperm (anucleate sperm thought to function as a physical inhibitor of female post-mating receptivity) (Cook & Wedell, 1999) in their ejaculates (Gage, 1995). Larval rearing environment also affects immune response, but in a complex synergistic fashion with other factors: at high population density and when food restricted, larvae increased their investment into cellular immunity (consistent with the density-dependent prophylaxis hypothesis). However, when provided with low-quality food and when reared at lower temperatures, individuals invested less in immunity (Triggs & Knell, 2011). There is also evidence in this species of evolutionary trade-offs between immunity and reproduction. Populations evolving in the presence of a granulosis virus demonstrated increased resistance to the virus compared to populations evolving in the absence of the virus. This increased resistance, however, came at a cost as these populations also exhibited increased larval developmental durations and reduced fertility as adults (Boots & Begon, 1993). Conversely, evolutionary increases in male mating frequency and thus reproductive investment were traded-off with a reduced constitutive immunity (McNamara et al., 2013). With these strong phenotypic responses to population density, and evidence of evolutionary trade-offs between immunity and reproduction, the Indian meal moth is an excellent model system to explore both the potential evolutionary trade-offs between investment into reproduction and immunity, and also for the potential for genetic and phenotypic responses to differ in intensity and direction.

Experimental evolution is a powerful tool to investigate potential genetic correlations between immune parameters and other life-history traits. We created six populations of *P. interpunctella,* that were reared at two different population densities for 14 generations. After a further two generations in a common garden environment, we examined the longevity, constitutive immune function and male sperm investment of individuals from high- and low-population density evolutionary backgrounds. We measured haemolymph concentrations of the enzyme phenoloxidase (PO), which underlies the cascade that results in encapsulation of foreign bodies (Sugumaran, 2002), and correlates with an organism's ability to resist pathogens and parasites (Rantala & Roff, 2007, Wilson et al., 2001). We also measured male investment in sperm, and male and female longevity, development and somatic investment.

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We predicted that concordant with plastic, within-generational responses in this species that males and females reared in large populations should have a greater investment into immunity and that males should have an increased investment into sperm number. We also predicted that this increased male investment would be costly, manifesting in slower development, reduced body weight, or a reduced longevity.

Methods

P. interpunctella were obtained from several wild-caught populations in Perth, Australia in 2011. Our laboratory population was reared for two generations before commencing the experimental evolution treatments. All individuals were reared on a diet of bran, yeast and glycerol (10:1:1), and maintained at 25°C on a 12:12h light:dark cycle. Wing length was used as a measure of adult body size (see McNamara et al., 2008).

Experimental evolution populations

We established six experimental evolution populations. Here, first-instar larvae were raised in low population density (5 individuals/container) or high population density (20 individuals/container). All containers were 10cm:10cm:5cm (l:w:h), and each larva received 350mg of food. Each treatment had three replicates. Low population density and high population density replicates consisted of 80 and 20 containers, respectively. Adults were removed daily and isolated in 5ml vials until 40 males and 40 females had been collected for each replicate. Then, we placed 40 male and 40 female high-population density individuals in one 2L container, and 20 male and 20 female low population density individuals in each of two 2L containers. Thus, differences in the effective population size between the treatments were minimized, while allowing the adult mating population to approximate the larval rearing population density. First-instar larvae were collected from these mating containers 5 days later to establish the next generation.

After 14 generations of experimental evolution, common garden rearing was used to isolate evolutionary from phenotypic trait responses. Here, 12 first-instar larvae from each replicate were added to 30 containers with 350 mg food/larvae. At adult emergence, 30 males and 30 females were collected (as for experimental lines) and placed in a 2L container

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to mate. Two generations of common garden rearing were conducted to produce all experimental individuals.

Mating frequency

We assessed female mating frequency in our experimental evolution lines at the generation immediately preceding the generation used for this experiment, by dissecting and counting the number of spermatophores in the female's tract (Ingleby et al., 2010). As females were collected once all individuals in the mating container had died, some females had already partially decomposed and the number of spermatophores present in the reproductive tract could not be reliably counted. Thus, we only counted spermatophores for fully intact females, and used the mean number of spermatophores in these females as our measure of female mating frequency for each replicate.

Immune assays

Fifth-instar larvae were removed haphazardly from each of the six replicates and weighed. The time from generation establishment to sampling of the first wandering larvae was used as an estimate of development time (age). 2µl of haemolymph were drawn from decapitated larvae and added to 20µl ice-cold phosphate buffered saline, vortexed and then frozen at -80°C. Phenoloxidase activity was measured using established protocols (Triggs & Knell, 2011). 10µl of haemolymph solution was added to a 96-well plate with 100µl of 5mM dopamine hydrochloride (Sigma-Aldrich H8502). The plate was incubated for 5mins and the absorbance measured at 492nm every minute for 20mins (at 28°C) using a M5 *Spectramax* microplate reader (Molecular Devices, Sunnyvale, CA).

Sperm counting

We assessed sperm investment from each population density treatment. Males from each replicate were mated to females from their own replicate, or from the opposing populations density treatment. Newly-emerged males and females were isolated and randomly paired. Pairs had 5 hours to initiate mating, and were monitored periodically until copulation cessation. After copulation, females were frozen.

Sperm numbers were estimated by releasing the contents of the spermatophore into a well slide containing phosphate buffered saline. The number of eupyrene cysts was

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192 counted at 40× magnification. For apyrene sperm, the sperm solution was diluted with a
193 weighed volume of distilled water and homogenised. Six 10 µl samples were pipetted onto
194 glass slides and air-dried. We counted sperm using dark-field microscopy (400×
195 magnification). Apyrene sperm numbers were calculated by multiplying mean sample values
196 by the dilution factor (McNamara et al., 2010).
197
198 *Longevity*
199 To measure the longevity of males and females, newly-emerged adults were removed from
200 each replicate. Individuals were isolated in 30ml vials (without access to food and water, as
201 they do not feed as adults) until they died.
202
203 *Juvenile development and condition*
204 To examine effects of population density on development and condition, two male and
205 female larvae from each container were removed on the 18th day after the common garden
206 replicates were initiated. The larvae were removed and placed in a 60ml vial with ad libitum
207 food. Latency to adult emergence and adult weight were recorded.
208
209 *Statistics*
210 All analyses were conducted in R 3.2.2 (R Development Core Team, 2015). Mixed-effects
211 models (package "lme4" version_0.999999911–5) were used to account for the random
212 effect of evolution line replicates for all models. All dependent variables were optimally
213 power transformed to maximize normality of residuals, and the exponents used were noted
214 with every analysis. The likelihood of female remating was analysed using a nominal logistic
215 regression. Female mating frequency data were analysed with a mixed model using a
216 negative binomial distribution (package "glmmADMB" version 0.8.0). Non-significant
217 interactions were removed from final models (Engqvist, 2005). Individuals for immune,
218 sperm and longevity analysis were collected from one common-garden generation, and the
219 juvenile development and condition individuals were removed from a separate round of
220 common-garden generations. For each experiment, individuals were not sourced from the
221 same common-garden container, to avoid pseudo-replication.
222
223 Results

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Mating frequency

The likelihood of remating (sperm competition risk) was greater for females from the high population density treatment (proportion remating: high population density = 0.25; low population density = 0.10; χ^2_1 = 8.07, P = 0.004). However, this was not reflected in a difference in mean mating frequencies (sperm competition intensity) between the treatments (mean mating frequency ± standard error: high population density = 1.10 ± 0.05, low population density = 0.95 ± 0.05, z = -1.13, P = 0.26).

Sperm investment

The ejaculates of 45 high population density treatment and 52 low population density males were analysed. The number of eupyrene sperm transferred was higher for low population density males, but was not affected by the population density treatment of the female he was mated with, or the size of the male (Table 1).

The number of apyrene sperm transferred was higher for low population density males, and for larger males. Apyrene sperm number was, however, not affected by the population density treatment of the female with who he was mated (Table 1).

A non-significant male population density treatment by female population density treatment interaction was dropped from both sperm type models.

Juvenile development and condition

Larval development and larval and adult body weight were assessed for 157 high population density and 157 low population density individuals. The time taken to develop from a first instar larva to an adult was not affected by the population density treatment, but was longer for males (Table 1). A non-significant interaction between population density treatment and sex was removed from the final model.

Similarly, an individual's weight at 18 days of development was not affected by population density treatment, but was greater for females (Table 1). A non-significant interaction between population density treatment and sex was removed from the final model.

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Finally, adult weight was not affected by population density, but was greater for females (Table 1). A non-significant interaction between population density treatment and sex was removed from the final model.

Immune activity

Immune activity was assessed for 84 high population density and 85 low population density individuals. PO activity (raised by exponent 0.005) was higher in individuals evolving under low population density and in individuals that were relatively younger. PO activity was affected by an interaction between the individual's sex and weight (Fig. 1). A non-significant population density treatment by sex interaction was dropped from the final model.

Longevity

Adult longevity was assessed for 60 high population density and 81 low population density individuals. Longevity was affected by an interaction between sex and population density treatment (χ^2_1 = 9.11, P = 0.002; Fig. 2), with males evolving under low population density living longer than those evolving under high population density. Longevity was also affected by an interaction between sex and body size (χ^2_1 = 4.83, P = 0.03; Fig. 3).

Discussion

Previous studies have found that *P. interpunctella* reared for a single generation under different larval population densities display clear plastic reproductive and immunological responses to predictable environmental cues of future population demography: males reared in high-density treatments have increased expenditure on reproduction and in one index of immunity (Gage, 1995, Triggs & Knell, 2011). However, our current study has demonstrated that evolutionary responses to these same conditions occur in the opposite direction to within-generation plastic responses.

Females that were reared and mated at a high population density were more likely to remate than females reared and mated at low population densities, but did not have a greater mean mating frequency. Thus, the treatments imposed differences in sperm competition risk, but not intensity. The greater risk of sperm competition in our high population density treatments is consistent with previous experiments examining density-

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dependence of female mating frequency. Gage (1995) reported differences in female mating frequency across three population densities; females reared at 20 individuals/container (sex ratio was not controlled) had a greater mating frequency than those reared at 10 and 5. Similarly, *P. interpunctella* populations that had been evolving under different adult sex-ratio biases for over 80 generations showed divergence in mating frequency; male-biased populations had higher mating frequencies than equal- or female-biased sex ratios (Ingleby et al., 2010). Thus, in this species, female mating rate increases with available mating opportunities. Sperm competition models predict that males should increase their expenditure on the ejaculate under increased risk of sperm competition (Parker et al., 2013).

Despite differences in selection due to differences in female remating probability between our treatments, and thus the greater opportunity for post-copulatory sexual selection in high-population density treatments, males evolving under high population densities transferred fewer sperm than those evolving under low population density. These differences were apparent after two generations of common-garden rearing so that the divergence between populations can be ascribed to genetic divergence between our treatments. Males of many species can respond to both immediate risk (Oppliger et al., 1998, DelBarco-Trillo, 2011, Simmons et al., 2007) and proximate cues of future sperm competition risk to optimise the composition of their ejaculate (Gage, 1995, He & Miyata, 1997, McNamara et al., 2010). Indeed a study of *P. interpunctella* in which moths were reared for a single generation from egg to adult, at the same two larval population densities as used in our study, found that high population density males responded to future sperm competition risk by increasing both apyrene and eupyrene sperm numbers (Gage, 1995). However, it is important to note that without reference to the ancestral population, or the inclusion of an intermediate population density population, it is not possible to confirm whether the divergence we observed among our experimentally evolving populations was due to changes in the low population density, high population density, or both treatments. Thus, any suggestions for causes for the population divergence we observed must remain speculative. Regardless, male evolutionary responses in reproductive expenditure to population density differed in direction to that expected from theory, and from previously reported phenotypically plastic responses. The same was true for expenditure on immunity. Contrary to the density-dependent prophylaxis hypothesis (which predicts increased immunity with group living, or increased encounter rates) both males and females evolved

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reduced, rather than increased PO activity under high population density. We note however, that we only assayed one aspect of immunity, PO. Multiple indices of immunity can trade-off with each other (Cotter et al., 2004a, Cotter et al., 2004b, Cotter et al., 2008), and thus caution must be used when interpreting 'immunity' from only one index. Previous work in this species, using experimental evolution populations to manipulate sexual selection intensity (as in this current study) demonstrated no negative relationship between PO activity and another frequently assayed measure of immunity, lytic activity (McNamara et al., 2013). Whether phenotypic and evolutionary responses in these traits should necessarily track each other is unclear. Indeed, there are relatively few studies, to our knowledge, that have compared trait responses over these two scales. For example, in *Drosophila melanogaster*, exposure to exogenous juvenile hormone increased fecundity at the expense of longevity. However, in populations of flies that were reared over multiple generations in the presence of juvenile hormone, these trade-offs between reproduction and lifespan broke down (Flatt & Kawecki, 2007).

Interestingly, there was no apparent evolutionary trade-off between immunity and reproduction. Males from both treatments showed a consistent relationship between investment into reproduction and constitutive immunity; low population density males invested more in both PO activity and sperm, while high population density males invested less in both traits. Phenotypically, immune challenges at the pre-adult stage reduce male sperm quantity in moths (McNamara et al., 2012) and other insects (Simmons, 2012). Yet, we found for *P. interpunctella* that changes in at least one index of male constitutive immunity did not trade off with sperm quantity. Several researchers have proposed that positive correlations, rather than the negative correlations indicative of trade-offs, can occur between fitness traits if individuals vary in their ability to acquire resources, and if there is genetic variation in the ability of these individuals to allocate across all fitness components (van Noordwijk & de Jong, 1986, Schwarzenbach & Ward, 2006, Reznick et al., 2000). There is support for this theory in a study on the yellow dung fly, *Scathophaga stercoraria*, in which divergence in PO levels was directly selected. The study found no negative genetic correlation between immunity and female reproductive output. Rather, females from lines selected for increased PO also had larger first clutches. However, there appeared to be a longevity cost to higher constitutive immunity: when food restricted, adults from high-PO lines died sooner (Schwarzenbach & Ward, 2006). Interestingly, however, they reported no

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significant effect of selection on adult body size. Thus, it is possible to speculate that the positively correlated responses in PO and reproduction we observed in our study may be indicative of changes in the capacity for resource acquisition between our experimental treatments.

But why should males evolving at low population density invest more, or males evolving under high population density invest less, than their counterparts in multiple fitness traits? One potential explanation for our findings is that increased female remating in our high population density treatment generated an increase in sexual conflict that was responsible for the evolutionary reduction in fitness traits (Holland & Rice, 1999, Martin & Hosken, 2004), although this is not possible to test in the absence of a control, intermediate population. In a number of taxa, studies have shown that populations experiencing increased sexual conflict exhibit reduced fitness, such as female reproductive output and longevity (Martin & Hosken, 2004). Interestingly, there is evidence of strong intra-sexual conflict in this species, demonstrating divergent male and female optima for multiple life-history traits (Lewis et al., 2011). Thus, the increased post-copulatory selection present in the high population density treatments could theoretically explain the reduced longevity, and reproductive and immunological fitness traits in these populations if male adaptation to post-copulatory selection imposes life-history costs. Typically, most studies examining population fitness under sexual conflict have examined female reproductive traits, which were not measured in this study. However, co-evolutionary increases in sexually antagonistic traits can also be costly for males. For example, in cowpea seed beetles, *Callosobruchus maculatus*, males successful in post-copulatory sexual selection were found to sire offspring of reduced fitness (Bilde et al., 2009). High population density may also place increasing selection pressure on the evolution of pre- rather than post-copulatory traits, which we did not assess in this study. Using these same populations, males from our high population density treatments were found to be 14% more likely to locate a female pheromone lure than males from our low population density treatments (Ashman et al., 2016). Although this result fell short of statistical significance it does suggest that the lines may be diverging for pre-copulatory mating effort. Generally, sperm competition models predict negative correlations between male expenditure on pre- and post-copulatory traits (Parker et al., 2013), a prediction for which there is growing support (Dines et al., 2015, Dunn et al., 2015, Fitzpatrick et al., 2012, Lüpold et al., 2014). Future studies should explore how selection has

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affected male pre-copulatory traits used to coerce females into mating, such as male pheromone titre and genital clasper morphology, and how this variation relates to post-copulatory male expenditure.

Interestingly, we did not find any developmental differences between the two treatment populations. Indeed, the absence of divergence in developmental durations between the treatments was not expected, as juvenile rearing environment is predicted to have strong phenotypic effects on larval development and longevity. Typical responses to increased larval population density are prolonged developmental duration and smaller adult body weights (Shenoi et al., 2016, McNamara et al., 2010). Previous studies on *P. interpunctella* and the closely related almond moth, *Cadra cautella*, have shown that juvenile males reared at low population density had a longer juvenile developmental duration and subsequently had a longer adult lifespan (Gage, 1995, McNamara et al., 2010). In contrast, we found no evolutionary divergence in developmental duration or juvenile or adult body weight due to differences in population density. We used two generations of common-garden rearing prior to our experiment, suggesting that, in this species, developmental duration is a plastic trait, which has not undergone evolutionary divergence. Whether divergence in body condition is a requirement for theories of resource acquisition is not apparent, especially as it has not been found in other studies exploring positive genetic correlations among fitness traits (Schwarzenbach & Ward, 2006).

In conclusion, we found that plastic and evolutionary responses to population density differ in their effects on investment in immunity and reproduction. We found no sign of a negative correlated response to selection between two important fitness traits that would suggest a trade-off. Male lifespan, and male expenditure on the ejaculate and PO were lower in populations evolving under high population densities. We suggest that reduced fitness in high population density treatments may have been due to increased sexual conflict within these populations and/or to increased male expenditure on mate acquisition, however, in the absence of a control, intermediate population, this remains speculative.

Data Accessibility

Data to be archived in Dryad.

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412 Table 1. Model summary for analyses of sperm investment, PO activity and developmental
413 duration and condition. Means ± standard errors are provided for significant categorical
414 variables. Significant variables are highlighted in bold.
415

	χ^2	P	Mean trait ± standard error	
			High population density	Low population density
Apyrene sperm				
Male treatment	11.78	**0.0001**	48.68 ± 3.03 (x10^3)	73.04 ± 3.92 (x10^3)
Female treatment	0.14	0.71		
Male density	0.59	0.44		
Eupyrene sperm bundles				
Male treatment	10.16	**0.001**	12.28 ± 0.77	16.52 ± 0.96
Female treatment	0.49	0.48		
Male size	6.38	**0.01**		
PO activity (Vmax)				
Treatment	6.25	**0.01**	4.16 ± 0.80	7.60 ± 1.01
Sex * weight	4.33	**0.04**		
Age	4.29	**0.04**		
			Male	Female
Developmental duration				
Treatment	0.27	0.63		
Sex	7.94	**0.005**	31.42 ± 0.13 days	30.91 ± 0.13 days
Larval weight				
Treatment	0.07	0.80		
Sex	23.68	**<0.001**	11.46 ± 0.40 mg	14.30 ± 0.47 mg

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Adult weight				
Treatment	0.37	0.57		
Sex	988.78	**<0.001**	8.82 ± 0.09 mg	14.17 ± 0.13 mg

Figure legends

Figure 1. The relationship between phenoloxidase activity (Vmax) and body weight for males and females.

Figure 2. Mean ± standard error longevity for males and females from high and low population density treatments.

Figure 3. The relationship between longevity and body size for males and females.

Figure 1.

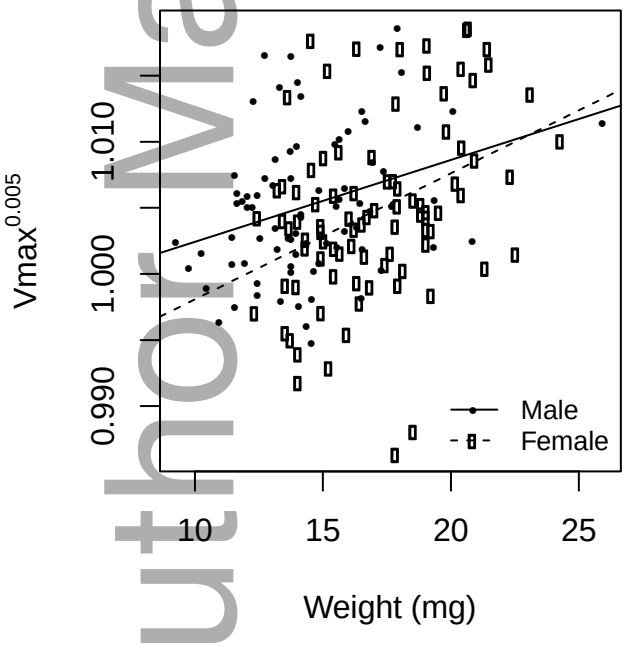


Figure 2.

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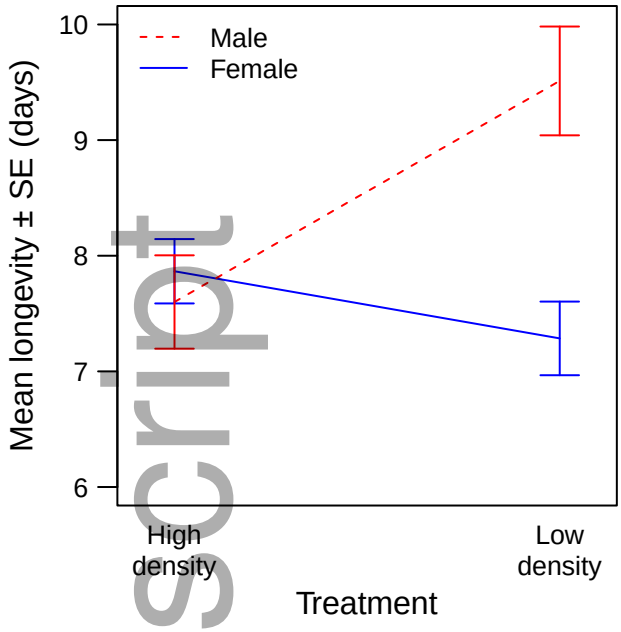
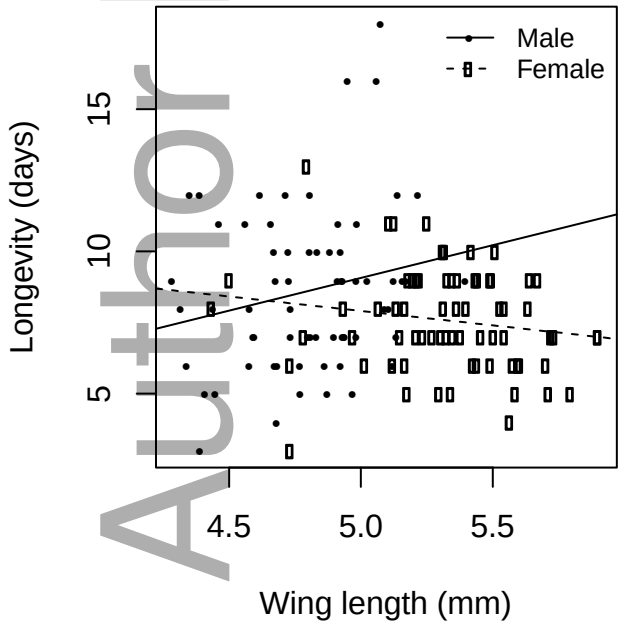


Figure 3.



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