

1 Experimental evolution reveals differences between phenotypic and evolutionary responses
2 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

33 and immunity, can both show density dependence (Kokko & Rankin, 2006, Wilson & Reeson,
34 1998). Increases in population size (Bagge et al., 2004) and density may elevate the rate of
35 disease transmission and hence require increased investment in immune activity (Wilson et
36 al., 2003, Wilson & Reeson, 1998, Wilson et al., 2002). Specifically, the density-dependent
37 prophylaxis hypothesis (Wilson & Reeson, 1998) proposes that the risk of parasite
38 transmission increases with population density, or conspecific encounter rates. And, as
39 immune systems are costly to maintain, individuals should strategically tailor their
40 investment into immunity based on predictable cues of parasite and pathogen transmission
41 risk (Wilson et al., 2003, Wilson & Reeson, 1998, Wilson et al., 2002). Yet, population density
42 and increased encounter rates between conspecifics may also increase the possibility of
43 sexual competition between males, requiring increased investment in reproductive function
44 (for a review, see Simmons & Fitzpatrick, 2012).

45 In general, theory predicts trade-offs between immune investment and reproduction
46 (Lochmiller & Deerenberg, 2000, Sheldon & Verhulst, 1996, Zuk & Stoehr, 2002, Schwenke et
47 al., 2016) given the inherent costs of maintaining and up-regulating immune system function
48 (Kraaijeveld & Godfray, 1997). Indeed, there is now a wealth of empirical research revealing
49 phenotypic trade-offs between immune investment and a suite of pre-copulatory and post-
50 copulatory sexual traits (for reviews, see Lawniczak et al., 2007, Schwenke et al., 2016),
51 although trade-offs are not ubiquitous (Gupta et al., 2013). Furthermore, while there is clear
52 phenotypic evidence that reproductive investment and immune activity independently can
53 display density-dependence, how group living or population density mediates evolutionary
54 trade-offs between immunity and reproduction is unclear. Trade-offs can occur on both
55 phenotypic and evolutionary scales. Within-generational, physiological trade-offs are
56 typically plastic responses to changing socio-sexual and/or environmental conditions. For
57 example, male and female reproductive investment decreases in response to immune
58 activation (Ahmed et al., 2002, McNamara et al., 2012, Simmons, 2012), or immune activity
59 is lower following increased reproductive effort (Fedorka et al., 2004, McKean & Nunney,
60 2001). Evolutionary trade-offs among populations can occur if there is genetic variation
61 among individuals for allocation between traits. Experimental evolution studies exploring
62 trade-offs between immunity and reproduction have also found that increased allocation to
63 immune activity is negatively genetically correlated with investment in other fitness traits
64 such as larval competitive ability (Kraaijeveld & Godfray, 1997) and fertility (Boots & Begon,

65 1993). And conversely, populations with increased reproductive investment demonstrate a
66 reduction in constitutive immunity (Hosken, 2001, McNamara et al., 2013). Yet, evolutionary
67 trade-offs do not necessarily reflect phenotypic plasticity. For example, among primate
68 species those with greater female mating rates (and therefore greater investment in male
69 mating structures) have increased white blood cell counts (an index of immunity). The
70 absence of a reproduction-immunity trade-off here instead suggests that sexually
71 transmitted diseases have been a potent selective force on primate immunology (Nunn et
72 al., 2000). Thus, plastic, phenotypic trade-offs may occur at an individual level, but may not
73 necessarily directly reflect evolutionary trade-offs (Flatt & Kawecki, 2007, Chippindale et al.,
74 1997). Indeed, whether these phenotypic and evolutionary trade-offs share a mechanistic
75 basis remains unclear (Schwenke et al., 2016).

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

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

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

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

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

134

135 Methods

136

137 *P. interpunctella* were obtained from several wild-caught populations in Perth, Australia in
138 2011. Our laboratory population was reared for two generations before commencing the
139 experimental evolution treatments. All individuals were reared on a diet of bran, yeast and
140 glycerol (10:1:1), and maintained at 25°C on a 12:12h light:dark cycle. Wing length was used
141 as a measure of adult body size (see McNamara et al., 2008).

142

143 *Experimental evolution populations*

144 We established six experimental evolution populations. Here, first-instar larvae were raised
145 in low population density (5 individuals/container) or high population density (20
146 individuals/container). All containers were 10cm:10cm:5cm (l:w:h), and each larva received
147 350mg of food. Each treatment had three replicates. Low population density and high
148 population density replicates consisted of 80 and 20 containers, respectively. Adults were
149 removed daily and isolated in 5ml vials until 40 males and 40 females had been collected for
150 each replicate. Then, we placed 40 male and 40 female high-population density individuals
151 in one 2L container, and 20 male and 20 female low population density individuals in each of
152 two 2L containers. Thus, differences in the effective population size between the treatments
153 were minimized, while allowing the adult mating population to approximate the
154 larval rearing population density. First-instar larvae were collected from these mating
155 containers 5 days later to establish the next generation.

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

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

162

163 *Mating frequency*

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

172

173 *Immune assays*

174 Fifth-instar larvae were removed haphazardly from each of the six replicates and weighed.
175 The time from generation establishment to sampling of the first wandering larvae was used
176 as an estimate of development time (age). 2µl of haemolymph were drawn from decapitated
177 larvae and added to 20µl ice-cold phosphate buffered saline, vortexed and then frozen at -
178 80°C. Phenoloxidase activity was measured using established protocols (Triggs & Knell,
179 2011). 10µl of haemolymph solution was added to a 96-well plate with 100µl of 5mM
180 dopamine hydrochloride (Sigma-Aldrich H8502). The plate was incubated for 5mins and the
181 absorbance measured at 492nm every minute for 20mins (at 28°C) using a M5 *Spectramax*
182 microplate reader (Molecular Devices, Sunnyvale, CA).

183

184 *Sperm counting*

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

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

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

224

225 *Mating frequency*

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

232

233 *Sperm investment*

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

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

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

243

244 *Juvenile development and condition*

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

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

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

257

258 *Immune activity*

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

264

265 *Longevity*

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

271

272 Discussion

273

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

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

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

295 Despite differences in selection due to differences in female remating probability
296 between our treatments, and thus the greater opportunity for post-copulatory sexual
297 selection in high-population density treatments, males evolving under high population
298 densities transferred fewer sperm than those evolving under low population density. These
299 differences were apparent after two generations of common-garden rearing so that the
300 divergence between populations can be ascribed to genetic divergence between our
301 treatments. Males of many species can respond to both immediate risk (Oppliger et al.,
302 1998, DelBarco-Trillo, 2011, Simmons et al., 2007) and proximate cues of future sperm
303 competition risk to optimise the composition of their ejaculate (Gage, 1995, He & Miyata,
304 1997, McNamara et al., 2010). Indeed a study of *P. interpunctella* in which moths were
305 reared for a single generation from egg to adult, at the same two larval population densities
306 as used in our study, found that high population density males responded to future sperm
307 competition risk by increasing both apyrene and eupyrene sperm numbers (Gage, 1995).
308 However, it is important to note that without reference to the ancestral population, or the
309 inclusion of an intermediate population density population, it is not possible to confirm
310 whether the divergence we observed among our experimentally evolving populations was
311 due to changes in the low population density, high population density, or both treatments.
312 Thus, any suggestions for causes for the population divergence we observed must remain
313 speculative. Regardless, male evolutionary responses in reproductive expenditure to
314 population density differed in direction to that expected from theory, and from previously
315 reported phenotypically plastic responses. The same was true for expenditure on immunity.
316 Contrary to the density-dependent prophylaxis hypothesis (which predicts increased
317 immunity with group living, or increased encounter rates) both males and females evolved

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

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

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

354 But why should males evolving at low population density invest more, or males
355 evolving under high population density invest less, than their counterparts in multiple fitness
356 traits? One potential explanation for our findings is that increased female remating in our
357 high population density treatment generated an increase in sexual conflict that was
358 responsible for the evolutionary reduction in fitness traits (Holland & Rice, 1999, Martin &
359 Hosken, 2004), although this is not possible to test in the absence of a control, intermediate
360 population. In a number of taxa, studies have shown that populations experiencing
361 increased sexual conflict exhibit reduced fitness, such as female reproductive output and
362 longevity (Martin & Hosken, 2004). Interestingly, there is evidence of strong intra-sexual
363 conflict in this species, demonstrating divergent male and female optima for multiple life-
364 history traits (Lewis et al., 2011). Thus, the increased post-copulatory selection present in
365 the high population density treatments could theoretically explain the reduced longevity,
366 and reproductive and immunological fitness traits in these populations if male adaptation to
367 post-copulatory selection imposes life-history costs. Typically, most studies examining
368 population fitness under sexual conflict have examined female reproductive traits, which
369 were not measured in this study. However, co-evolutionary increases in sexually antagonistic
370 traits can also be costly for males. For example, in cowpea seed beetles, *Callosobruchus*
371 *maculatus*, males successful in post-copulatory sexual selection were found to sire offspring
372 of reduced fitness (Bilde et al., 2009). High population density may also place increasing
373 selection pressure on the evolution of pre- rather than post-copulatory traits, which we did
374 not assess in this study. Using these same populations, males from our high population
375 density treatments were found to be 14% more likely to locate a female pheromone lure
376 than males from our low population density treatments (Ashman et al., 2016). Although this
377 result fell short of statistical significance it does suggest that the lines may be diverging for
378 pre-copulatory mating effort. Generally, sperm competition models predict negative
379 correlations between male expenditure on pre- and post-copulatory traits (Parker et al.,
380 2013), a prediction for which there is growing support (Dines et al., 2015, Dunn et al., 2015,
381 Fitzpatrick et al., 2012, Lüpold et al., 2014). Future studies should explore how selection has

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

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

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

409 Data Accessibility

410 Data to be archived in Dryad.

411

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

415

	χ^2	P	Mean trait \pm standard error	
			High population density	Low population density
<i>Apyrene sperm</i>				
Male treatment	11.78	0.0001	48.68 \pm 3.03 ($\times 10^3$)	73.04 \pm 3.92 ($\times 10^3$)
Female treatment	0.14	0.71		
Male density	0.59	0.44		
<i>Eupyrene sperm bundles</i>				
Male treatment	10.16	0.001	12.28 \pm 0.77	16.52 \pm 0.96
Female treatment	0.49	0.48		
Male size	6.38	0.01		
<i>PO activity (Vmax)</i>				
Treatment	6.25	0.01	4.16 \pm 0.80	7.60 \pm 1.01
Sex * weight	4.33	0.04		
Age	4.29	0.04		
<i>Developmental duration</i>				
Treatment	0.27	0.63		
Sex	7.94	0.005	31.42 \pm 0.13 days	30.91 \pm 0.13 days
<i>Larval weight</i>				
Treatment	0.07	0.80		
Sex	23.68	<0.001	11.46 \pm 0.40 mg	14.30 \pm 0.47 mg

Adult weight

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

416

417

418

Figure legends

419

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

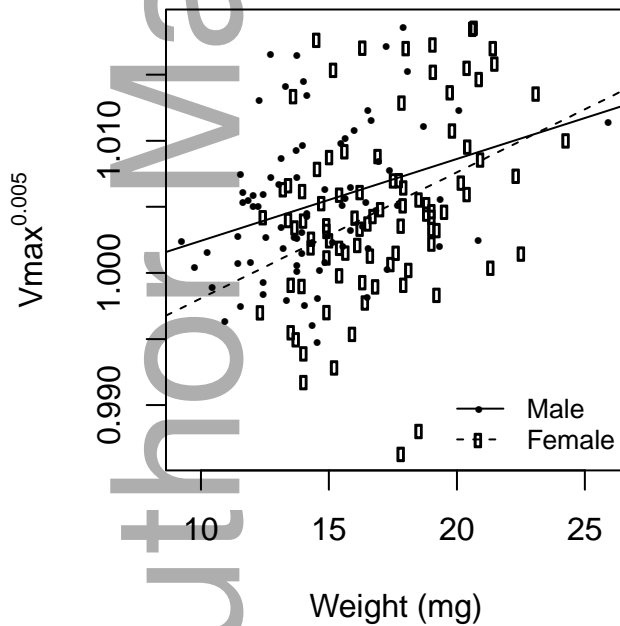
422

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

425

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

427 Figure 1.



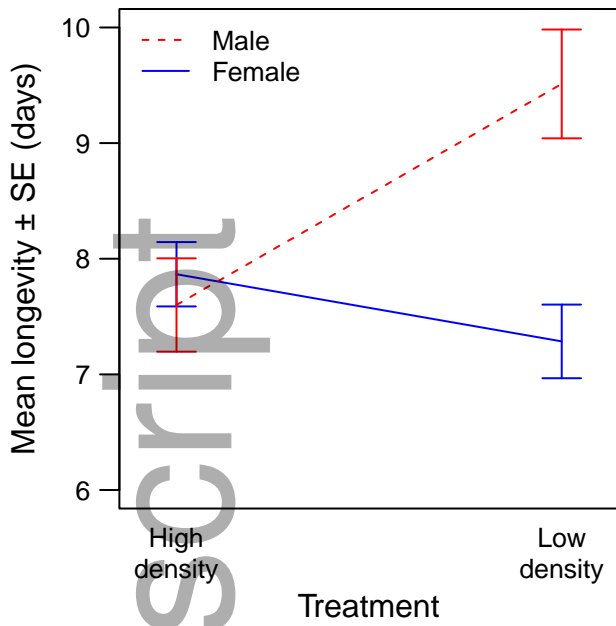
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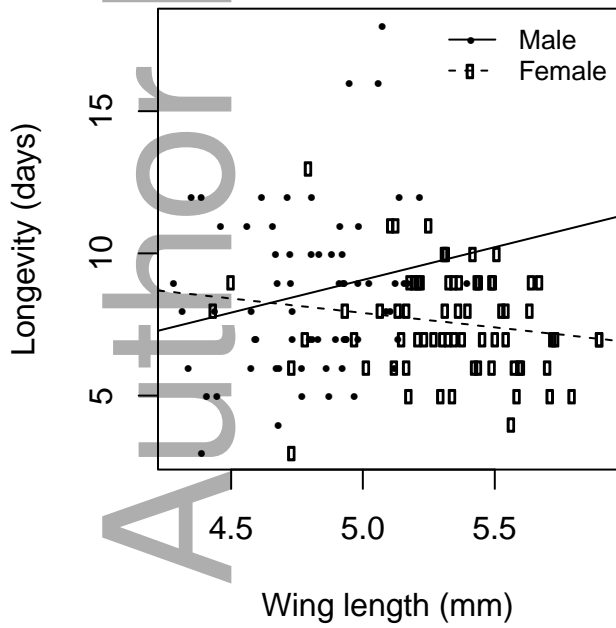
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432 Figure 2.



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Figure 3.



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