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**Costs and benefits of giant sperm and sperm storage organs in *Drosophila*
*melanogaster***

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1 **ABSTRACT**

2

3 In *Drosophila*, long sperm are favoured in sperm competition based on the length of the
4 female's primary sperm storage organ, the seminal receptacle (SR). This sperm-SR
5 interaction, together with a genetic correlation between the traits, suggests that the
6 coevolution of exaggerated sperm and SR lengths may be driven by Fisherian runaway
7 selection. Here, we explore the costs and benefits of long sperm and SR genotypes, both
8 in the sex that carries them and in the sex that does not. We measured male and female
9 fitness in inbred lines of *D. melanogaster* derived from four populations previously
10 selected for long sperm, short sperm, long SRs, or short SRs. We specifically asked: what
11 are the costs and benefits of long sperm in males and long SRs in females? Furthermore,
12 do genotypes that generate long sperm in males or long SRs in females impose a fitness
13 cost on the opposite sex? Answers to these questions will address whether long sperm
14 are an honest indicator of male fitness, if male post-copulatory success is associated with
15 male pre-copulatory success, if female choice benefits females or is costly, and whether
16 intra-genomic conflict could influence evolution of these traits. We found that both sexes
17 have increased longevity in long sperm and long SR genotypes. Males, but not females,
18 from long SR lines had higher fecundity. Our results suggest that sperm-SR coevolution
19 is facilitated by both increased viability and indirect benefits of long sperm and SRs in
20 both sexes.

21

22 **Keywords:** fecundity; Fisherian runaway selection; good genes; intralocus sexual
23 conflict; longevity; mating success; pre-copulatory sexual selection; post-copulatory
24 sexual selection; sperm; sperm storage organ

25 INTRODUCTION

26 Foundational questions in sexual selection ask how female preferences for elaborate male
27 ornaments can evolve. That is, how do females benefit from these preferences, and what
28 are the associated costs? There is ample evidence that, as predicted by theory (Zahavi,
29 1975), ornaments are costly to produce and thus serve as signals of genetic quality (e.g.,
30 Godin and McDonough, 2003; Kotiaho, 2000; Manica et al., 2016; Mobley et al., 2018; Zuk
31 et al., 1995). Females will gain indirect benefits from mating with high-condition males
32 by having high-condition offspring (good genes; Fisher, 1958; Zahavi, 1977), if condition
33 is heritable. If ornament phenotype is also heritable, females will additionally benefit by
34 producing sexy sons, and if female preference is heritable, a choosy female will have
35 choosy daughters, who will also gain these indirect benefits. Likewise, males would
36 benefit by mating with females exhibiting preference through also having choosy
37 daughters.

38 In addition, intralocus conflict for either the trait that is exaggerated in males or its
39 female preference (Lande, 1980; Rice, 1984) will constrain the evolutionary benefit of
40 advantageous ornament or preference genotypes in males or females, respectively, by
41 incurring fitness costs when those genotypes are expressed in the other sex
42 (Bonduriansky and Chenoweth, 2009; Chippindale et al., 2001; Cox and Calsbeek, 2009;
43 Pischedda and Chippindale, 2006). Thus, the benefit of being a successful male may be
44 limited by any costs of also having unfit daughters (Foerster et al., 2007), and any benefit
45 of choosy daughters may be limited by low fitness of a female preference genotype in
46 males.

47 Principles of female preference and male ornament evolution can apply to traits
48 under post-copulatory sexual selection, which is mediated by sperm competition (Parker,
49 1970) on the one hand and cryptic female choice (Eberhard, 1996; Firman et al., 2017) on
50 the other. These two processes occur after mating in an analogous fashion to male-male
51 competition and female choice, which comprise pre-copulatory selection that acts before
52 mating. Male traits under pre-copulatory sexual selection often take the form of elaborate
53 visual, audible, tactile, and/or chemical displays, and female preferences for them are
54 based on sensory perception that leads to behavioral decisions (Candolin, 2003; Jennions
55 and Petrie, 1997). In contrast, female preference under post-copulatory sexual selection
56 occurs when female-mediated behavioral, morphological, or physiological processes bias
57 paternity in favor of certain males (Pitnick and Brown, 2000), based on pre-copulatory
58 (Pilastro et al., 2004; Sbilordo and Martin, 2014) or post-copulatory male traits
59 (Wojcieszek and Simmons, 2012). Whether acting before or after copulation, female
60 preference evolution follows similar expectations predicted under runaway selection
61 (Fisher, 1958; Kirkpatrick, 1982), good genes (Iwasa and Pomiankowski, 1991; Zahavi,
62 1975), or sexy son (Pomiankowski et al., 1991)/sexy sperm (Keller and Reeve, 1995)
63 hypotheses.

64 In *Drosophila*, the correlated evolution of sperm and sperm storage organs has
65 become a model system in which to study the evolution of traits under post-copulatory
66 sexual selection. In this lineage, sperm reach extraordinary lengths (Pitnick et al., 1995),
67 driven by length of the female's primary sperm storage organ, the seminal receptacle
68 (SR), which can be even longer (Pitnick et al., 1999). Long sperm have a competitive

69 fertilization advantage against shorter sperm (Lüpold et al. 2012; 2016; Miller and Pitnick,
70 2002), but primarily within long SRs (Miller and Pitnick, 2002). This long sperm
71 advantage occurs through as yet undescribed fluid dynamic processes during the
72 displacement stage of sperm competition (Manier et al., 2010; 2013). Thus, variation in SR
73 length is a proxy for the strength of cryptic female choice for sperm length, with longer
74 SRs being more selective, or “choosier”, based on the size of the post-copulatory male
75 ornament, sperm length.

76 Male ornaments are typically considered to evolve under pre-copulatory sexual
77 selection, because females are assumed to be agnostic to their mate’s sperm traits, and it
78 is difficult to imagine a sperm phenotype being “preferred” by that female. If *Drosophila*
79 sperm length can be considered to be a male ornament, a number of patterns would be
80 expected. (1) If this exaggerated trait has evolved under runaway selection, the male
81 ornament and female preference should coevolve and be genetically correlated. (2) If long
82 sperm carry indirect benefits consistent with a good genes model of ornament evolution,
83 they should also be costly and condition-dependent (Zahavi, 1977), and possibly trade
84 off with other male traits (reviewed in Manica et al., 2016). Finally (3), we could expect
85 long sperm to display strong positive allometry (disproportionally longer for a given
86 body size; Bonduriansky, 2007; Kodric-Brown et al., 2006; Voje, 2016), particularly if
87 sperm length could be considered a “weapon” rather than a “display” (Eberhard et al.,
88 2018).

89 In support of these predictions, (1) sperm length and SR length are coevolving both
90 among species (Pitnick et al., 1999) and among populations within *D. mojavensis* (Pitnick

91 et al., 2003), and there is a significant genetic correlation between the two traits (Lüpold
92 et al., 2016). (2) Long sperm are also costly in terms of time required to reach reproductive
93 maturity (Miller and Pitnick, 2002; Pitnick et al., 2003; Pitnick et al., 1995), and sperm
94 length trades off with sperm number across species (Pitnick, 1996). Moreover, condition-
95 dependence of sperm length increases in species with longer sperm (Lüpold et al., 2016),
96 and (3) as expected for certain male ornaments, sperm length has the strongest positive
97 allometry with body size ever measured for a sexually selected trait (Lüpold et al., 2016).

98 In order to better understand how male ornaments and female preferences
99 coevolve, we need to elucidate the fitness consequences of genotypes controlling these
100 traits for both the sex in which they are expressed and the sex in which they are not.
101 Previous research on the fitness consequences of long sperm and long SRs have shown
102 that production of long sperm incurs costs in delayed male reproductive maturity (Miller
103 and Pitnick, 2002; Pitnick et al., 2003; Pitnick et al., 1995) while also conferring a
104 fertilization advantage during sperm competition (Lüpold et al., 2012; 2016; in review;
105 Miller and Pitnick, 2002). Similarly, long SRs are associated with extended development
106 times and decreased longevity but increased fecundity (Miller and Pitnick, 2003, 2002).
107 Moreover, females that experience low-quality environments as larvae have shorter SRs
108 (Amitin & Pitnick, 2007), suggesting that the production of long SRs is metabolically
109 costly and requires adequate resources.

110 Despite these advances, much remains unknown. Do males with long sperm also
111 have higher pre-copulatory success (increased attractiveness, mating success), fecundity,
112 or viability? Sperm size could be correlated with these traits due to genetic linkage with

113 viability alleles (Gilbert and Uetz, 2016; Head et al., 2005; Svobodová et al., 2018), or there
114 may be significant trade-offs (Ball and Parker, 1996; Dines et al., 2015; Foo et al., 2018) or
115 even no relationship (Travers et al., 2016), depending on a range of ecological factors
116 (Evans and Garcia-Gonzalez, 2016; Lüpold et al., 2014; Parker et al., 2013; Simmons et al.,
117 2017). In addition, do females benefit from bearing SRs that select for longer sperm, or
118 does cryptic female choice carry a cost? In order for female preference for a male trait to
119 evolve, it must be strong enough to outweigh any costs associated with that preference,
120 even if the male trait is not at first linked to viability (Chandler et al., 2013; Mead and
121 Arnold, 2004). Therefore, quantifying the costs of female preference is critical for
122 understanding preference-trait coevolution. Finally, is there evidence for intragenomic
123 conflict at loci controlling sperm length and SR length, or do both sexes benefit from
124 exaggerated reproductive traits? The strength and direction of female-male coevolution
125 may be affected by fitness consequences incurred by trait genotypes in the sex not
126 expressing the trait (Chenoweth et al., 2008; Chippindale et al., 2001; Cox and Calsbeek,
127 2009, Pischedda and Chippindale, 2006).

128 Here, we investigate the costs and benefits of long sperm and long SRs that may
129 influence how they coevolve. This system has a unique advantage in that the female
130 “preference” (SR length) is an easily and consistently quantifiable morphological trait,
131 rather than a behavioral or cognitive process that may be more difficult to measure and
132 is potentially affected by social learning (Danchin et al. 2018; Dion et al., 2019; Monier et
133 al., 2019). We measured male mating success, male and female fertility, and male and
134 female longevity in isofemale lines derived from populations that had been

135 experimentally evolved to have long sperm, short sperm, long SRs, or short SRs. This
136 experimental design allowed us to examine costs and benefits of long sperm and long SR
137 genotypes both in the sex expressing the trait and in the opposite sex. Fitness
138 consequences of exaggerated trait genotypes manifested in either sex could influence the
139 dynamic of sperm-SR coevolution, either by reinforcing selection in the same direction
140 on both sexes or imposing an antagonistic relationship between selection on males and
141 females.

142

143

144 **METHODS**

145

146 **Experimental populations**

147 To determine fitness effects of sperm length or SR length, we quantified mating success,
148 fecundity, and longevity in inbred isofemale lines derived from four *D. melanogaster*
149 populations that had been previously selected for long sperm, short sperm, long SRs, or
150 short SRs (initially reported in Miller and Pitnick, 2002; 2003). Briefly, these populations
151 underwent 17 generations of selection for sperm length, and at least 30 generations of
152 selection for SR length. For each generation of sperm length selection, males were
153 dissected, and sperm length was measured after breeding with virgin females. Progeny
154 of sires with the longest or shortest sperm contributed to the next generation. For each
155 generation of SR length selection, females were transferred to individual vials after group
156 mating, where they laid eggs before dissection and SR measurement. Progeny of dams

157 with the longest or shortest SRs contributed to the next generation (for details see Miller
158 and Pitnick, 2002; 2003).

159 Approximately 300 generations after the initial selection experiments described
160 above, the long and short SR lines underwent eight additional generations of selection in
161 order to re-establish highly significant divergence in SR length. For each generation of
162 this second selection regime, 75 virgin pairs were housed in individual food vials
163 (standard cornmeal-yeast-agar-molasses diet). After laying eggs for several days, females
164 were dissected, and SR length measured. We selected the highest 5 or lowest 5 families
165 for the next generation. All 5 families contributed equally (15 males and 15 females), and
166 we avoided pairing siblings by mating Family 1 females with Family 2 males, Family 2
167 females with Family 3 males, etc. Eight generations of selection yielded SR lengths with
168 non-overlapping distributions between the high line (mean = 2946 μm ; min-max = 2552-
169 3574 μm) and low line (mean = 2150 μm ; min-max = 1841-2507 μm).

170 We then proceeded to generate panels of isolines for sperm and SR length selection
171 regimes through 10 generations of full-sibling inbreeding for sperm lines and 15
172 generations for SR lines. Inbreeding of the SR lines began immediately after the second
173 round of selection, and approximately 330 generations following initial sperm length
174 selection. For each panel, the four most extreme isolines were identified and used for this
175 experiment. For each isoline, a minimum of five female SRs and, on average, five sperm
176 cells (range: 2-11 sperm) from each of at least four males (range 4-8, average: 5.56) were
177 measured. To measure sperm, seminal vesicles from mature virgin males (5 days post-
178 eclosion) were dissected into a large droplet of 1X phosphate buffered saline (PBS) on a

179 glass slide, ruptured, and dragged several times to release the live sperm. The droplet
180 was dried down at 55 °C, and sperm were fixed in 3:1 methanol:acetic acid, mounted in
181 glycerol, and the slide sealed with nail polish. Sperm were visualized on a Nikon Ni-U
182 upright light microscope at 200X magnification under darkfield. Images were captured
183 with an Andor Zyla 4.2 camera and measured using the segmented line tool in ImageJ
184 (<https://imagej.nih.gov/ij/>).

185 SRs were measured from mature virgin females (5-7d post-eclosion) that were
186 stored frozen (-20°C) until dissection. Female reproductive tracts were dissected into 1X
187 PBS, the SR gently unraveled with a fine insect pin, and the sample mounted under a
188 coverslip, such that the SR was two-dimensional but not over-compressed. SRs were
189 visualized at 100X magnification under phase contrast, and images were captured and
190 measured as outlined above.

191 From across three experimental blocks conducted at different time points, a total
192 of $N = 1151$ males and $N = 1298$ females were included in the final analyses. All stocks
193 were maintained at ambient room temperature and light regime in polyethylene fly vials
194 with cornmeal agar yeast molasses medium supplemented with live yeast. Experimental
195 flies were reared by pairing 2-3 day old female and male flies from the same isoline for
196 48 hours, and emerging offspring were collected as virgins. All reproductive and
197 behavioral assays were performed at the same time of day to reduce circadian rhythm
198 effects. All individuals were collected as virgins under light CO₂ anesthesia, maintained
199 in same-sex vials with densities of 10 females or 20 males, and were 2-5 days old when
200 first mated.

201

202 **Mating success**

203 We observed male mating behavior to assess attractiveness (latency to mate), copulation
204 duration, and the proportion of successful matings for males from each of the four
205 selection regimes: long sperm, short sperm, long SR, and short SR. A subset of ten
206 randomly selected males from each of three replicate group vials from each of the 16
207 isolines ($N = 460$) were tested for five consecutive hours (or until successful mating) each
208 week over a period of six weeks. Individual males were transferred without anesthesia
209 into a mating arena consisting of a polyethylene vial with a foam plug in the bottom to
210 enhance visibility, containing a single 5 day old wild type (LHm) virgin female. For each
211 mating arena, the cotton plug was pushed halfway down the vial, leaving approximately
212 2.5 cm of vertical space, to stimulate male-female interactions. For each successful mating,
213 latency to mate and mating duration were recorded, after which males were returned to
214 their original group vial. Males were transferred to new food vials three times a week,
215 and dead males were removed without replacement, with date of death recorded for
216 longevity analyses.

217

218 **Fecundity**

219 To measure female fecundity, experimental virgin females 2-3 days post-eclosion were
220 paired individually with a wild type LHm male (5 to 7 days old) for 48 hours, after which
221 the male was removed ($N = 160$). Each week, we subsampled progeny produced within
222 a 24 hr period for each female over the course of her life (see Longevity, below).

223 Specifically, we allowed the eggs that had been laid within the specified weekly 24 hr
224 period to develop, and counted the number of eclosed and unclosed pupae, four days
225 after the flies in a given vial had started hatching. All weekly counts from each female
226 were summed to approximate lifetime reproductive output.

227 For male fecundity, we counted progeny produced by up to two randomly
228 selected successfully mated males from each replicate group vial ($N_{\max}/\text{week} = 96$) for
229 each week of the mating success assays (see above). Specifically, LHm females were
230 separated from the males directly after mating, and transferred to a new individual food
231 vial, where they were allowed to deposit eggs for 48 hours before being discarded. Adult
232 offspring were counted as a proxy for male fecundity. In contrast to females, male
233 offspring data were not measured on the same individuals over time, as the individual
234 identity of males within a given vial was unknown. Any measure of fecundity is subject
235 to both male and female effects, but by using standard wild type females we aim to
236 distribute female effects in an unbiased way across male treatments and factors (selection
237 regime, block, line).

238

239 **Longevity**

240 Males were kept in cohorts of initially 20 same-sex flies per vial (three replicate vials per
241 isoline, populated one day post-eclosion, $N = 48$ vials). We checked for survival every
242 two days, when flies were transferred to a new food vial. We tested how selection regime
243 affected survival using Cox proportional hazard models (function *coxph*; Therneau, 2015),

244 separately for each sex and each selected trait. Females were maintained individually to
245 assess female reproductive success (10 replicates each, conducted in blocks 1 & 2).

246

247 **Statistical Analyses**

248 To analyse male fecundity and mating behavior, we used general linear mixed models
249 (Bates and Maechler, 2009) and *lmer*test (Kuznetsova et al., 2017) to calculate *p*-values,
250 tested with line fitted as a random effect. Degrees of freedom were based on the
251 Satterthwaite approximation. In some cases, the response variable was square root
252 transformed to satisfy model assumptions. Binomial data (mating success) were checked
253 for overdispersion, and *p*-values were calculated in *afex* (Singmann et al., 2016). All
254 analyses were performed using R (v 3.4.0, R Core Team, 2017).

255

256

257 **RESULTS**

258

259 **Sperm and SR length**

260 Long sperm lines had significantly longer sperm than short sperm lines (long mean \pm SE:
261 1934.00 ± 10.78 , $n = 86$ sperm cells; short: 1673.97 ± 10.46 , $n = 115$ sperm cells; $t_{192.94} =$
262 17.313 , $P = 2.2e-16$). Likewise, long SR lines had significantly longer SRs than short SR
263 lines (long: $2504.72 \pm 58.74 \mu\text{m}$, $n = 22$ SRs; short: $1640.56 \pm 29.83 \mu\text{m}$, $n = 31$ SRs; $t_{31.85} =$
264 13.17 , $P = 1.98e-14$). However, sperm lengths were not significantly different between SR
265 selection regimes (long: $1840.02 \pm 6.83 \mu\text{m}$, $n = 119$ sperm measurements; short: $1855.07 \pm$

266 6.76 μm , $n = 117$ sperm cells; $t_{234} = -1.567$, $P = 0.1185$). Similarly, SR length in sperm
267 selection treatments did not differ (long: $2138.50 \pm 94.97\mu\text{m}$, $n = 6$; short: 2237.69 ± 51.78
268 μm , $n = 10$; $t_{8.02} = -0.917$, $P = 0.39$).

269

270 **Fitness**

271 In the sperm selection lines, long sperm males had lower mating success ($\chi^2 = 4.35$, $\text{df} =$
272 1 , $P = 0.037$; Fig 1a), suggesting that there is a pre-copulatory cost to the post-copulatory
273 long sperm advantage found in previous studies (Miller and Pitnick, 2002). However,
274 there were no differences in male attractiveness (mating latency; $F_{1,211} = 2.270$, $P = 0.133$;
275 Fig 1c) or copulation duration ($F_{1,192} = 0.553$, $P = 0.458$; Fig 1e). Both males and females
276 from long sperm lines trended toward higher fecundity, though this pattern was not
277 statistically significant (males: $F_{1,5.8} = 3.997$, $P = 0.094$; Fig 2a; females: $F_{1,6} = 3.560$, $P =$
278 0.108 ; Fig 2c). We standardized fecundity within sex and selected trait (sperm or SR) by
279 subtracting the mean and dividing the difference by the standard deviation, to directly
280 compare fitness for both males and females (see Fig. 3). Standardized fitness did not differ
281 between males and females for short sperm ($F_{1,54.9} = 0.119$, $P = 0.731$) or long sperm
282 lines ($F_{1,53.6} < 0.001$, $P = 0.988$; Fig. 3a). We did find a longevity advantage to long sperm
283 genotypes in both sexes (males: $\chi^2 = 32.50$, $\text{df} = 1$, $p = 0.001$; sperm selected, females: $\chi^2 =$
284 9.13 , $\text{df} = 1$, $P = 0.003$; Fig 4a, c). Higher survival specifically occurred for older females
285 (Fig 4c) and at all ages for males (Fig 4a).

286 In the SR selection lines, short SR males were more attractive (shorter mating
287 latency; $F_{1,569} = 8.727$, $P = 0.003$; Fig 1d) and copulated for longer ($F_{1,536} = 91.261$, $P < 0.0001$;

288 Fig 1f), but long SR males ultimately had higher mating success ($\chi^2 = 5.82$, $df = 1$, $P =$
289 0.0158; Fig 1b) and higher fecundity ($F_{1,5.8} = 6.118$, $P = 0.049$, see Fig 2b). Females had
290 higher relative fitness than males in short SR lines ($F_{1,52.4} = 10.419$, $P = 0.002$; Fig. 3b) and
291 males had higher relative fitness than females in long SR lines ($F_{1,55.2} = 7.485$, $P = 0.008$;
292 Fig. 3b). Interestingly, long SR females did not produce more offspring ($F_{1,6} = 0.413$, $P =$
293 0.544; Fig 2d), but they did live longer ($\chi^2 = 4.64$, $df = 1$, $p = 0.031$; Fig 4d), primarily at
294 intermediate ages (Fig 4d). Male longevity was marginally longer between short and long
295 selection regimes in SR selection lines ($\chi^2 = 2.88$, $df = 1$, $P = 0.090$; Fig 4b).

296

297

298 **DISCUSSION**

299 In our study, genotypes producing long sperm or SRs confer multiple fitness benefits and
300 few costs for both sexes (Table 1), suggesting that higher genetic quality is required to
301 produce these traits. In particular, long selection lines for both sperm and SR phenotypes
302 had increased longevity in males and females. By selecting for longer sperm, long SRs
303 might also select for higher fitness genotypes in sons and daughters. Thus, the evolution
304 of long sperm and long SRs may be driven by both viability selection (e.g., increased
305 longevity) and indirect benefits (long SRs select for longer sperm, which confer fitness
306 benefits to both sons and daughters). Together with a genetic correlation between the
307 traits (Lüpold et al., 2016), these fitness benefits may aid in fueling a Fisherian runaway
308 process. An alternate explanation for our results is that the selection and inbreeding
309 history of the populations used in this experiment has led to the capture of genes

310 conferring increased longevity in long sperm and long SR lines. It is important to note
311 that increased longevity in both males and females is not necessarily indicative of
312 increased lifetime reproductive success, which was not quantified here. Evaluation of
313 fitness in unrelated populations with known sperm and SR phenotypes will be required
314 to determine if sperm length and SR length are actually linked to “good genes”.

315 We unexpectedly found that long SR genotypes in females confer increased
316 longevity with no fecundity benefit, in contrast to previous work that showed that
317 females with long SRs have higher reproductive output but at a cost to survival (Miller
318 and Pitnick, 2003). These previous results may be due to increased storage capacity of
319 both sperm and detrimental male ejaculate proteins (Chapman et al., 1995). In that study,
320 long SRs were 40% longer than those reported here (3.5 mm vs 2.5 mm) and unlikely to
321 occur naturally, perhaps because of these costs. Our more moderate SR lengths are
322 comparable to those found in local wild *D. melanogaster* in the Washington, D.C. area
323 (mean 2.5 mm, unpubl. data), and also on par with SR phenotypes shown to select for
324 longer sperm (Miller and Pitnick, 2002). These moderately long SRs come with a
325 longevity benefit, while also mediating sperm choice for longer sperm, perhaps reaping
326 viability benefits for both sons and daughters. We thus find that both long sperm and
327 long SRs may be honest signals of genetic condition.

328 Our results identified a tradeoff in males between long sperm and mating success,
329 suggesting evolutionary modularity for traits under pre-copulatory versus post-
330 copulatory sexual selection. In other words, long sperm confer only a post-copulatory
331 advantage through sperm competitive outcome, with no premating benefits with regards

332 to mating success. For males with long SR genotypes, reproductive success was mixed,
333 with decreased attractiveness and copulation duration but increased mating success. This
334 outcome may be due to more persistent courtship by long SR males, despite lower
335 attractiveness, though we did not quantify courtship effort. At the same time, females
336 mated to less attractive long SR males produced more progeny, suggesting a disconnect
337 between male attractiveness and male fecundity. Higher fecundity in long SR males also
338 further supports the hypothesis that genotypes associated with long SRs are of higher
339 quality.

340 Most studies that examine the relationship between pre-copulatory and post-
341 copulatory processes ask if mating success and attractiveness (pre-copulatory) is
342 correlated with paternity outcome (post-copulatory). This study flips that question by
343 starting with traits associated with paternity success (sperm and SR length) and looking
344 for an association with premating outcome. We would not necessarily expect to find a
345 difference between comparisons of pre-copulatory success with post-copulatory
346 outcome, as opposed to associating post-copulatory outcome with pre-copulatory
347 success. However, most studies in other species have found that pre-copulatory success
348 is a good predictor of post-copulatory outcome (Evans et al., 2003; Hosken et al., 2008;
349 Lewis and Austad, 1994; Polak and Simmons, 2009; Sbilordo & Martin, 2014; McDonald
350 et al., 2017), though it matters which traits are considered (Ala-Honkola and Manier,
351 2016). Here, however, we did not find an association between sperm length and
352 premating success, in concordance with Droge-Young et al. (2012) and Travers et al.
353 (2016). It is possible that pre-copulatory and post-copulatory effort trade off in *D.*

354 *melanogaster* (Filice and Dukas, 2019), and that both are so costly that males may invest in
355 only one or the other.

356 In conclusion, sperm length and SR length in this system do not appear to have
357 fitness costs in the opposite sex. Rather, both long sperm and long SR phenotypes seem
358 to confer fitness advantages to both males and females (with few costs). Long SR females
359 and long sperm males lived longer (viability benefits), and by selecting for long sperm,
360 long SRs in females may provide indirect benefits through increased longevity in both
361 sons and daughters. These results suggest not only that long sperm are indeed an honest
362 signal of good genes, but that female preference can also be an indicator of female quality.
363 The costs and benefits incurred by female preferences have received less empirical
364 attention than selection on male traits, primarily because female preferences (and
365 concomitant costs and benefits) are more difficult to measure. Our work here suggests
366 that selection driving male-female coevolution is not always antagonistic and can actually
367 align to benefit both sexes.

368

369

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371

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541 **Tables**

542

Table 1. Summary of results showing fitness benefits (+) or costs (-) of long phenotypes. Parentheses indicate a marginally insignificant trend, and NS indicates no significant difference between long and short phenotypes.

| | Sperm | | SR | |
|---------------------|-------|-----|-----|----|
| | ♂ | ♀ | ♂ | ♀ |
| Mating success | - | | + | |
| Latency | NS | | - | |
| Copulation duration | NS | | - | |
| Fecundity | (+) | (+) | + | NS |
| Longevity | + | + | (+) | + |

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547 **Figure captions**

548

549 FIGURE 1. Mating success (A, B), mating latency (C, D), and copulation duration (E, F) in
550 males from the sperm selected (A, C, E) and SR selected (B, D, F) lines.

551

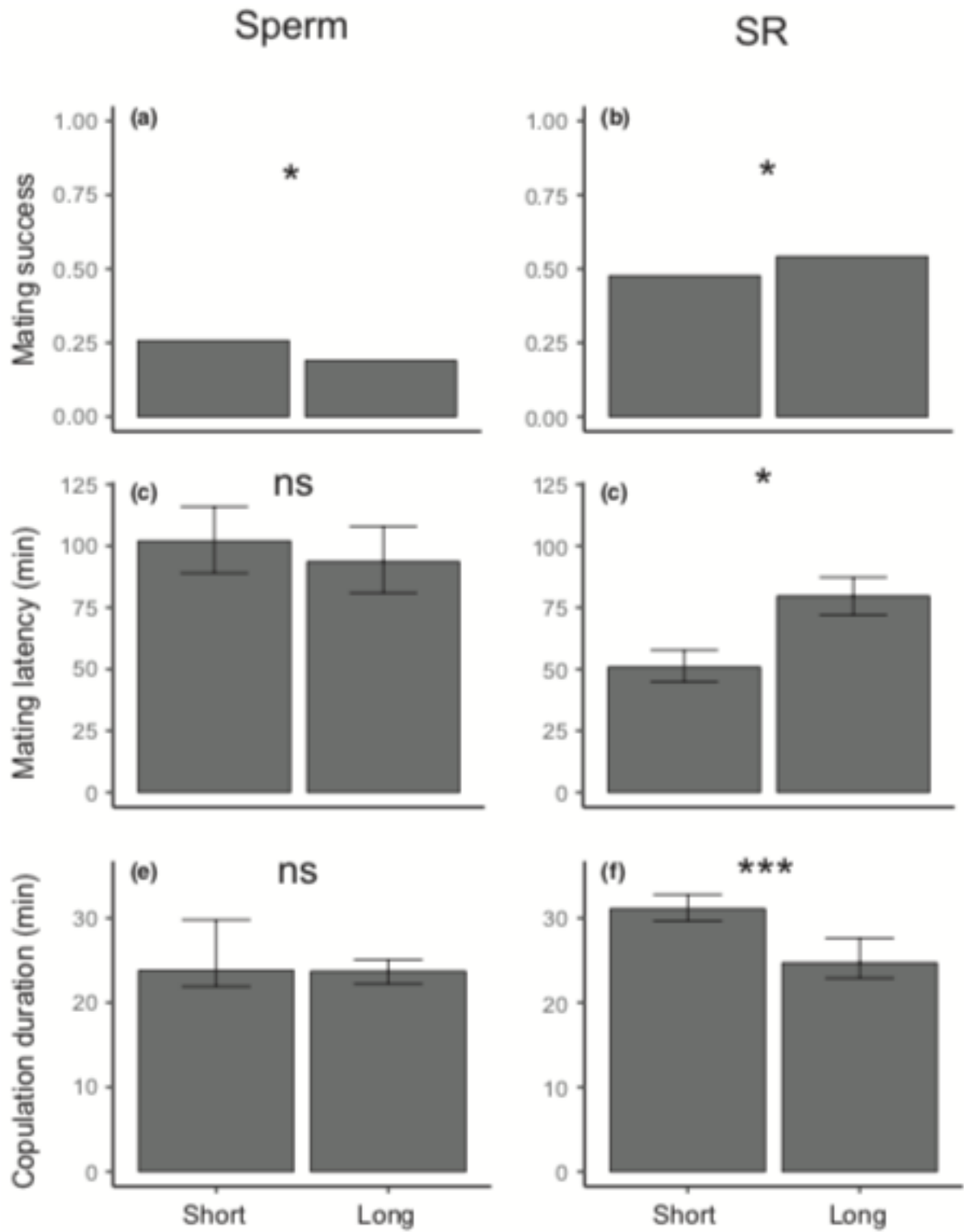
552 FIGURE 2: Number of offspring produced after mating trials by subsamples of males from
553 long sperm (A), long SR (B), short sperm (C), and short SR (D) lines.

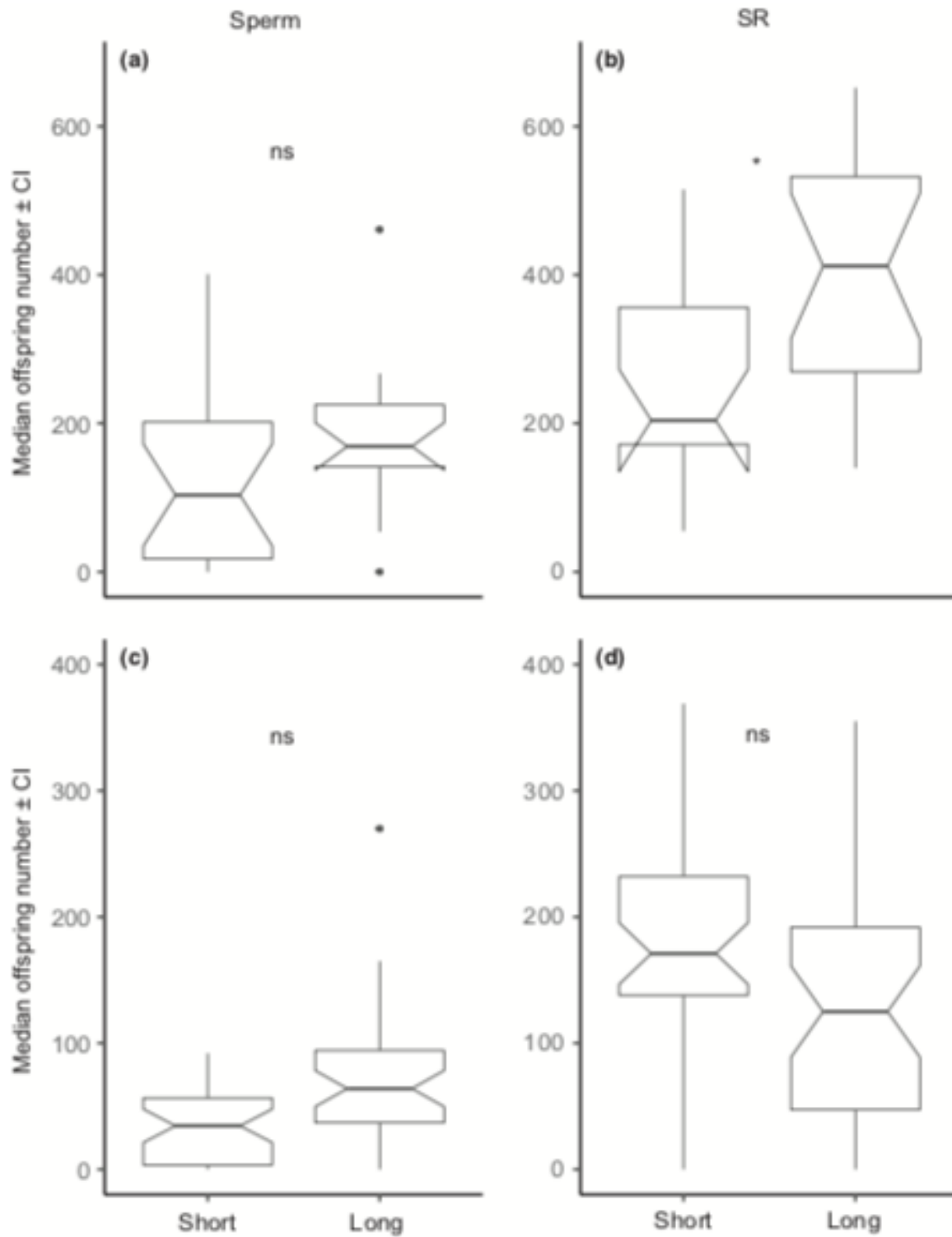
554

555 FIGURE 3: Standardized male and female fitness (mean \pm bootstrapped 95% CI), for A)
556 sperm and B) SR lines. Blue: male; red: female.

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558 FIGURE 4. Survival curves of males from sperm selected lines (A) and of SR selected lines
559 (B) and of females from sperm (C) and SR selected lines (D). Line colours represent
560 selection regime (blue: short trait values; red: long trait values). Shaded areas represent
561 95% confidence intervals. Age refers to adult age





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