Socio-ecological context modulates significance of territorial contest competition in *Drosophila prolongata*

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Abstract

The intensity and direction of sexual selection is intricately linked to the social and ecological context. Theory predicts that male-biased operational sex ratios (OSR) and high population density should skew resource and mate monopolization toward few successful males, thereby intensifying sexual selection on males. Here, we studied how the mating system of the promiscuous and strongly sexually dimorphic fruit fly *Drosophila prolongata* responds to changes in the OSR and population density. We recorded groups of flies over five days and quantified territory occupancy, mating success, and competitive fertilization success. Although general indices of the intensity of sexual selection supported theoretical predictions, realized selection on morphological traits did not. Rather, we found selection on these traits to intensify under high density, but also under even as opposed to male-biased OSRs. Larger and more territorial males achieved higher mating success, but only under even OSRs. Similarly, competitive fertilization success, mainly determined by sperm precedence, was biased toward large males only under even OSRs. Finally, our combined results support a shift in the mating system from territorial contest competition to scramble competition under male-biased OSRs and potentially at low density, where there was no clear contribution of the measured traits to reproductive success. Our study emphasizes the limitations of traditional selection metrics and the role of the socio-ecological context in predicting adaptation to a changing environment.

Significance

Mating systems are complex and dynamic, adapting to ongoing ecological change. Studies often assume that changes in the socio-ecological context alter the intensity of sexual selection on traits indicating individual fitness, but our work on *Drosophila prolongata* challenges this view. By manipulating operational sex ratio and population density, and jointly investigating territorial behavior and both pre- and post-mating reproductive success over several days, we reveal the plastic mating strategies in this fly. This dynamism underscores the limitations of static classifications and the importance of studying selection across diverse socio-ecological contexts. This broader perspective advances our understanding of the tight connections between environmental change, population demographics, and the evolutionary process.

Introduction

Sexual selection, driven by competition for mates and/or fertilization (1), shapes heritable traits and behaviors that are directly related to reproductive success (2). While often stronger on males (2, 3), sexual selection can also significantly impact females (4–6), with its extent and nature on either sex being intricately linked to the ecological context (7).

One important ecological parameter affecting the intensity of sexual selection and mating system dynamics is the distribution of resources, which shapes the local population composition and so the availability of potential mates [i.e., operational sex ratio, OSR (7)]. Skewed OSRs can lead to monopolization of mating opportunities by a few successful individuals of the competing sex (7). Male-biased OSRs in particular can thus drive selection on traits that are advantageous in competition, including larger body size, the growth of weaponry, or elaborate ornamentation favored by females (7–9).

A meta-analysis across a diversity of animal taxa supports theoretical predictions that OSRs can drive the general intensity of sexual selection (10). However, studies exploring selection on specific phenotypic traits often find no differences between OSRs (11–13), or their results contrast general theory (14–17). For example, in the common lizard (*Lacerta vivipara*), positive selection on male body size is stronger in female-biased than in male-biased OSRs, possibly resulting from higher female costs

under elevated male aggression during mating (14). Overall, the effect of the OSR can vary considerably between species and mating systems (18, 19).

OSRs can also influence post-mating processes, such as competitive fertilization (e.g., refs. 20–22; but see ref. 23), ultimately impacting aspects like ejaculate transfer or mating duration (24). Males tend to invest more in ejaculate production when exposed to a higher risk or intensity of sperm competition (20), often induced by changes in the perceived OSR (25–27). Across generations, this is expected to lead to stronger selection on traits associated with competitive fertilization, such as ejaculate allocation (28) or testes size (29), yet studies on the latter are inconclusive (30–36). In a literature review (30), only one of four studies found an effect of OSR on testes size. This one study showed an increase in testes size for male *Drosophila melanogaster* evolving under an extreme female bias [10:1], suggesting selection against sperm limitation rather than competition (31).

In addition to the OSR, competition for mates can also intensify as populations become denser due to growing numbers of competitors (37). However, studies on the impact of density on metrics of sexual or trait-specific selection have yielded mixed results (supporting: refs. 38–41, opposing: refs. 9, 42–46) or suggest a trade-off between pre- and post-mating sexual selection (36, 47). This implies that the exclusive focus on pre-mating selection in many studies misses this shift toward post-mating competition at higher densities where aggressive strategies might become less effective (36, 37, 48). For example, across anuran species, the trade-off between forelimb muscles and testes size is biased more strongly toward the latter when population density is high and fertilizations are more difficult to monopolize (36).

Beyond the population structure, male reproductive tactics may also be influenced by the distribution of resources (7). While the opportunity for monopolization is minimal when resources are evenly distributed, highly competitive males may gain control over spatially concentrated resources (7). When resource monopolization strongly correlates with mating success (49–51), a skew toward few successful males can be predicted (7). These males may become more attractive to potential mates for their ability to defend a resource or for the benefit the resource provides. For example, in antlered flies, *Phytalmia spp.,* males fight over oviposition sites to which females then gain access through copulation (52). Therefore, females indirectly choose mates with large antlers, which in turn benefit males in these contests (53). However, within a species, diverse reproductive tactics may coexist, depending on demographic,

environmental or individual conditions (37, 43, 49, 54–60). For example, whether a resource is defendable can hinge on the number of individuals competing for it, and switching to an alternative strategy can be advantageous (61–63). One such case is the European bitterling, *Rhodeus sericeus*, in which males abandon their territories as these become indefensible, and join spawning groups instead (63).

Although OSRs and population densities have both been proposed to shape sexual selection and mating systems (7, 37), likely mediated by resource distribution (7), to our knowledge these factors have not been studied jointly, let alone in the context of both pre- and post-mating sexual selection. Here, we investigated these factors in a comprehensive laboratory experiment using the fruit fly *Drosophila prolongata.* This South-Asian species exhibits conspicuous male-biased sexual dimorphism both in body size and in foreleg size and coloration (64), while also showing signals of resource defense when high-quality resources are concentrated (65). Males use their forelegs in both fights against competitors (66, 67) and elaborate courtship behaviors (64). These behaviors also include “leg vibration”, a species-specific behavior that involves a male stimulating the female abdomen and is often displayed immediately before copulation (67), likely to overcome remating suppression by mated females (68, 69). Leg vibration, however, can also trigger an alternative reproductive tactic by a nearby male, namely interception of the female (70). With highly polygamous females (68), this species is ideal for studies of context-dependent pre- and post-mating sexual selection.

Here, we monitored over a five-day interaction period each of 30 genetically diverse groups of *D. prolongata* that varied in their sex ratio (1:1 or 3:1 males:females) and density (12, 16, or 20 flies). Quantifying the distribution of flies around a centrally located resource, pre-mating behaviors, individual copulations, and molecular parentage provided a uniquely comprehensive understanding of reproductive dynamics in this species, highlighting the links between the evolution of sexually selected traits, mating systems, population ecology, and behavioral plasticity.

Results and Discussion

Potential and realized sexual selection. To quantify the selection dynamics in *D. prolongata* under different population compositions, we measured Bateman

136 gradients and the opportunity for sexual selection (I_s) as two commonly used estimates
137 for the intensity of sexual selection (e.g., 10). Whereas the former represents the slope
138 between individuals' number of mates and resulting offspring (ref. 71; i.e., the fitness
139 advantage gained by additional matings), the latter is based on the sex-specific
140 variances in reproductive success (ref. 72; i.e., the opportunity for selection to act on
141 variation). The Bateman gradients for both sexes were positive (Fig. 1; males: X^2_1 =
142 63.65, n = 193, P < 0.001, Table S1; females: X^2_1 = 24.1, n = 175, P < 0.001, Table
143 S2), but more pronounced in males (X^2_1 = 7.42, P = 0.006, Table S3a). The disparity
144 in Bateman gradients between the sexes was greater in the male-biased populations
145 (X^2_1 = 5.6, n = 368, P = 0.018) but unaffected by group density (X^2_2 = 0.23, P = 0.89,
146 Table S3b). Similarly, I_s was higher for males compared to females in all treatments,
147 but particularly pronounced in the male-biased populations (6.2× higher for males)
148 compared to the even OSR populations (2.7× higher for males). Population density
149 again had no significant effect (Table S4, Fig. S1). These findings combined suggest
150 that sexual selection is more pronounced on males than females, and its intensity for
151 males is amplified under a male-biased OSR, as previously shown for a diversity of
152 taxa (10).

153 When comparing selection on specific male traits, results were more complex. For
154 example, we found positive selection gradients on thorax, foreleg and wing length, and
155 composite body size (principal component 1, PC1; see *Methods*) under even OSR,
156 but only on wing length in male-biased populations (Fig. 2, Table S5). Also, contrary
157 to expectations based on I_s, positive selection on all measured traits appeared to
158 intensify with density (Fig. 2, Table S6). This increasing selection on secondary sexual
159 traits with density aligns with theory (37), yet has been reported for only few species
160 (e.g., ref. 40; but see refs. 43, 73). The changing pattern of selection on phenotypic
161 traits across OSRs, however, contradicts both theoretical predictions (7) and insights
162 gained from Bateman gradients and I_s in this study.

163 The lack of positive selection on putative sexually selected traits in male-biased
164 populations might simply indicate insufficient statistical power or that other,
165 unmeasured behavioral or morphological traits would be favored under conditions of
166 mate monopolization (2, 74, 75). However, our findings might also reveal the
167 limitations of using metrics like Bateman gradients, I_s, or the OSR to predict realized
168 selection on specific sexually selected traits, as previously emphasized (18). As our
169 results confirm, these common measures of the intensity of sexual selection do not

170 necessarily correlate with the observed selection on traits and merely set an upper
171 limit to the potential of sexual selection (18). These results underscore the need to
172 integrate different measures to gain a more complete understanding of the dynamics
173 of a mating system. Despite their limitations, Bateman gradients or I_s remain valuable
174 tools for numerically characterizing mating systems. They consider all types of sexual
175 selection (e.g. pre- and post-mating, intra- and intersexual), acting on any relevant
176 morphological or behavioral traits (76). Thus, they accurately capture variation in
177 fitness and allow for comparisons between sexes and populations (76). For example,
178 we showed that reproductive success is more skewed toward a few successful males
179 in male-biased populations than under even OSR. The trait-specific selection
180 gradients indicate, however, that traits selected in male-biased populations are not the
181 morphological traits expected to be involved in physical contest such as resource
182 defense (e.g., forelegs). These insights emphasize the intricate interplay between
183 OSR, density, and sexual selection in shaping mating system dynamics and trait
184 evolution in this species.
185
186 **Territorial behavior & mating success.** To investigate how the territorial behavior
187 previously reported in this species (65) manifests in different group compositions, a
188 resource-dense area (RDA) was established in the center of each experimental unit.
189 This RDA, consisting of highly nutritious yeast paste, covered approximately 16% of
190 the otherwise nutrient-poor arena. Regardless of population composition, males spent
191 more time in the RDA than females (35.6% vs. 12.3%; $X^2_1 = 671.6$, $P < 0.001$, $n = 493$
192 [303 males, 190 females], Table S7), with greater variance in males ($\sigma^2 = 0.016$) than
193 in females ($\sigma^2 = 0.005$). Hence, the RDA appeared to be highly attractive to males, but
194 access was unequal among males as reflected by their varying time spent there
195 (indicating "territorial success"). In addition, individuals of both sexes spent less time
196 (on average) in the RDA at higher densities ($X^2_1 = 22.0$, $P < 0.001$) and under male-
197 biased OSR ($X^2_1 = 21.0$, $P < 0.001$), suggesting intensifying competition for this
198 resource. Although females spent considerably less time in the RDA overall, 69.4% of
199 all matings started there. These matings were also more likely to involve aggressive
200 male-male behavior than matings outside this area ($z = 2.54$, $P = 0.011$, $n = 716$
201 matings across 212 males and 187 females). Both sexes spent more time in the RDA
202 on the first day of recording, when most matings occurred, compared to the
203 subsequent days ($X^2_4 = 313.724$, $P < 0.001$ $n = 2379$ across 5 days and 492

204 individuals; Fig. S2, Table S8). Interestingly, whilst body size (PC1) had no effect on
205 territorial occupancy on the first day, it did on all following days (interaction: $X^2_1 = 5.05$,
206 $n = 1445$, $P = 0.02$, Table S9).

207 Amino & Matsuo (65) report a hierarchy in pairs of males, with one male clearly
208 dominating territory occupancy, particularly when the area is small and easily
209 defendable. Although their findings do not directly demonstrate mating success by
210 territory occupancy, a strong correlation between territory occupancy and courtship
211 rate (65), as well as a mating advantage for winners of contests over territories (77),
212 have been reported for *D. prolongata*. In our study, the RDA was comparable to the
213 larger of the two areas used by Amino & Matsuo (65), but with more males and so
214 reduced ability to monopolize the contested area. Nevertheless, territorial control
215 influenced mating success, albeit with greater complexity at higher numbers of
216 competitors: Compared to 72.5% at an even OSR, only 64.6% of matings were
217 initiated in the RDA at a male-biased OSR. Interestingly, although most matings were
218 initiated in the RDA at both OSRs, overall territory occupancy determined mating
219 success only in even ($t = 2.4$, $P = 0.019$) but not male-biased populations ($t = 0.24$, P
220 $= 0.8$; OSR×territoriality: $t = 1.7$, $P = 0.09$, $n = 290$, controlling for PC1; Fig. 3, Table
221 S10a-b). Similarly, PC1 was the main contributor to mating success under even ($t =$
222 3.7, $P < 0.001$) but not male-biased OSRs ($t = 1.1$, $P = 0.26$, OSR×PC1: $t = 2.15$, $P =$
223 0.03; $n = 290$, Fig. 3, Table S10a-b). In addition to these results, the occurrence of
224 courtship behavior before mating depended on body size and the OSR (PC1×OSR: t
225 $= 2.93$, $P = 0.003$, $n = 760$, Fig. 3, Table S14a), in that large males showed more
226 courtship behavior in the even OSR ($z = 3.20$, $P = 0.001$) but not in the male-biased
227 OSR ($z = -1.27$, $P = 0.20$). These results align with the selection gradients above, but
228 additionally illustrate the changing dynamics of sexual selection in response to varying
229 levels of competition.

230 Our findings support the notion that when competition intensifies (e.g., strongly
231 male-biased OSR), monopolization of resources becomes increasingly difficult,
232 potentially shifting mating strategies (36, 37, 47, 48, 57). However, we neither
233 observed an increase in sneak matings at higher densities nor under male-biased
234 OSRs (both $|z| < 1.2$, $P > 0.26$, $n = 716$, Table S12). In fact, leg vibration, an auditory
235 cue used by sneaky males to intercept a mating attempt, even decreased at high
236 densities ($z = -1.7$, $P = 0.09$) and in male-biased OSR ($z = 1.89$, $P = 0.059$, $n = 716$,
237 Table S11). Courtship was observed less frequently when matings occurred in the

238 RDA (z = -3.3, P < 0.001, Table S14), possibly due to a high local density of
239 competitors and so higher risk of becoming the target of aggression (z = 2.54, P =
240 0.011, n = 716, Table S13) or interception (z = 2.31, P = 0.021, n = 716, Table S13).
241 Similarly, leg vibration was observed less often before matings in the RDA (z = -1.81,
242 P = 0.07, n = 716, Table S11). Large males were more likely to gain matings following
243 leg vibration (z = 2.89, P = 0.004, n = 716), whereas those acquiring matings by
244 interception tended to be smaller males (t = -2.03, P = 0.042, n = 716, Table S12). As
245 shown previously (78), males avoid leg vibration when the risk of interception is high
246 (e.g., at high density). Hence, notwithstanding the lesser importance of resource-
247 defense and body size for mating success in male-biased populations, males did not
248 adopt alternative mating tactics. This raises the question of whether selection might
249 instead favor increased investment in post-mating processes.
250
251 **Fertilization success.** Across all treatments, mating success (MS; 48.9%) explained
252 most of the variation in reproductive success, followed by fertilization success (FS;
253 14.3%) and female fecundity (Fec; 6.4%; Fig. S3, Table S16). This pattern was
254 comparable across social contexts in general, but while mating success explained
255 three times the variation of fertilization success under an even OSR (MS: 42.5%, FS:
256 14.4%, Fec: 9.2%), these variance components were more similar under a male-
257 biased OSR (MS: 37.3%, FS: 25.4%, Fec: 7.2%). Fertilization success may thus gain
258 in relative importance when female monopolization is hampered.
259 Males that were a female's last mate until the end of the experiment (t = 4.21, P <
260 0.001, n = 190, Table S16) and males that were able to delay remating for longer
261 ("remating interval", see *SI Methods;* t = 4.79, P < 0.001) had higher fertilization
262 success (adjusted PCS, see *SI Methods*). Analogous to our findings for mating
263 success, we found that larger males only had higher fertilization success under an
264 even OSR (t = 2.63, P = 0.009), but not under a male bias (t = 0.14, P = 0.89;
265 OSR×PC1: t = 1.79, P = 0.075, n = 190, Table S16). This indicates that rather than
266 counteracting the lack of pre-mating selection on body size pre-mating under male-
267 biased OSRs, the pattern seems to be reinforced in the post-mating phase. Longer
268 mating durations did not significantly contribute to fertilization success (t = 1.62, P =
269 0.11), but males mating more often had decreased fertilization success per mating (t
270 = -2.29, P = 0.02), indicating strategic allocation (79) or sperm depletion (80).

271 In close relatives of *D. prolongata*, the allocation of male seminal fluid proteins
272 (SFPs), such as sex-peptide, can suppress female remating (81, 82). This allocation
273 can vary with a male's social context (82, 83) or his own condition and genotype (84).
274 Although the role of sex peptide in remating suppression is less clear in *D. prolongata*
275 (68), for any SFP delaying remating we would expect enhanced allocation when the
276 perceived sperm competition risk is higher [i.e., higher density or male-biased OSR;
277 (83, 85, 86)]. Yet, while female remating intervals were not affected by density (t =
278 0.69, P = 0.49), they were prolonged under even rather than male-biased OSRs (t =
279 2.14, P = 0.033, n = 190, Table S17). Matings were shorter for large males (t = -3.32,
280 P = 0.001, n = 710, Table S19) and tended to extend remating intervals (t = 1.91, P =
281 0.058), making an overall last-male position more likely (t = 1.97, P = 0.049, n = 211).
282 Male size did not affect remating intervals (t = 0.15, P = 0.88), yet large males were
283 more likely to be a female's last mate overall (t = 2.27, P = 0.024). To the extent that
284 mating duration predicts ejaculate transfer, shorter copulations by larger males can be
285 interpreted as strategic ejaculate allocation in anticipation of more future matings, as
286 shown previously in *D. melanogaster* (79) and supported here by shorter copulations
287 for males with more total matings on that day (t = -2.8, P = 0.005, n = 710).
288 The remating interval only reflects the average delay between two matings and here
289 was not affected by body size. However, the ability to delay remating is captured to a
290 larger extent in the proportion of overall last-male positions held until the end of the
291 experiment. Despite their short mating durations, large males ended up in the last-
292 male position to a higher proportion of their encounters, and remating intervals were
293 extended under even OSR where pre-mating selection is stronger. It thus seems as
294 though females might cryptically bias paternity toward preferred males by delaying
295 remating (87, 88). This scenario is further supported by three arguments: First, with
296 SFPs such as sex-peptide playing a major role in controlling the mating rate
297 *D. melanogaster* (89), a similar function could be predicted for *D. prolongata*.
298 However, considering a much higher mating rate than close relatives [i.e., 2.9±1.4
299 matings on the first day compared to a typical remating interval of 3-5 days in
300 *D. melanogaster* (90)], remating suppression through SFPs may be less effective in
301 *D. prolongata* (68), similar to other species with high mating rates (91). Alternatively,
302 even if sex-peptide does play a limited role, female *D. prolongata* might be able to
303 control the scope of its activity through strategic timing of ejaculate ejection (87).
304 Second, no effect of the OSR or density on mating duration were found (both $|t| < 1.41$,

305 $P > 0.17$, $n = 710$), which could indicate no strategic SFP allocation in response to the
306 level of sperm competition. Third, the remating interval was longer under even OSRs
307 although mating durations remained unaffected. Given the positive pre- and post-
308 mating selection on body size and territoriality under an even OSR, the observed
309 patterns are consistent with females potentially influencing post-mating paternity
310 biases to reinforce pre-mating choices.

311 In sum, our results indicate that intensifying competition does not augment post-
312 mating investment by males. Additionally, post-mating selection does not seem to
313 counteract the lack of pre-mating selection on ostensibly beneficial male traits.
314 Instead, post-mating processes, partially under female control, seem to reinforce the
315 patterns of pre-mating selection.

316

317 **Mating system dynamics.** To synthesize the differences in the mating-system
318 dynamics of *D. prolongata* across different contexts, we built structural equation
319 models. We provide separate visualizations for each density level in Fig. S4 (Tables
320 S22-S24), but Fig. 4 presents a simplified overview of the dynamics under an even
321 and male-biased OSR, respectively. One of the most striking observations was the
322 absence of many positive (green) or negative (red) effects in the male-biased scenario
323 (Fig. 4b, Table S21) compared to that in even populations (Fig. 4a, Table S20). This
324 underlines how both pre- and post-mating sexual selection vary across social contexts.
325 For example, body size, a primary determinant of both pre- and post-mating success
326 in the even population, seemed irrelevant under male-biased OSRs.

327 Even though we documented a larger skew in reproductive success and a higher
328 potential for sexual selection in male-biased populations, we were unable to pinpoint
329 the specific traits under selection. Altered ecological or demographic factors can result
330 in plastic changes in mating systems and ultimately individual strategies (7, 37, 43,
331 57). When the number of rivals increases (e.g., male-biased OSR) or the encounter
332 rate with mates declines (e.g., low density), males might transition from territorial
333 contests to scramble competition (47, 48, 74). In such scenarios, traits like speed,
334 agility or the ability to locate receptive females could be under selection (74).

335 While not systematically quantifying them in this study, we did frequently observe
336 multi-male scrambles [‘love knots’; (75)] when a receptive female had been located,
337 often followed by mating. Consequently, morphological traits linked to speed may also
338 be under selection like their behavioral counterparts (74). Incidentally, of all

339 morphological traits assessed here, the only one exhibiting significant positive
340 selection under male-biased OSR was wing length, a trait often thought to be selected
341 under scramble competition due to its link to speed (74). Similarly, small body size has
342 been proposed to be advantageous in scramble competition (2, 74, 92), with selection
343 on it documented in several arthropods (e.g., refs. 93, 94), including multi-male
344 scramblers (75). Here, we did not measure the behavioral traits potentially under
345 selection and found no significant negative selection on body size under male-biased
346 OSR or low density. It is thus possible that reproductive success became more random
347 in those populations due to a more limited number of selection events (95).
348 Considering the pronounced male-biased sexual size dimorphism in *D. prolongata,*
349 which is unique among Drosophilids (96) and uncommon for species reliant on
350 scramble competition (92, 97), scramble competition might play a minor role in its
351 mating system, triggered under relatively extreme (and potentially unnatural)
352 circumstances. Thus, it is possible that a shift toward scramble competition at low
353 densities or under a male-biased OSR does occur, but our treatments were not
354 extreme enough or the sample size too low to conclusively detect it.
355
356 **Conclusion.** Our findings broaden our current understanding of how mating systems
357 respond to complex socio-ecological pressures. The plastic mating strategies
358 observed in *D. prolongata* challenge the notion of static classifications in favor of a
359 dynamic framework, likely beyond this species. This dynamism emphasizes the
360 importance of studying selection across ecological contexts, such as varying OSRs,
361 population densities or resource distributions to understand the intricate interplay
362 between environmental change, population demographics, and the evolutionary
363 process. Incorporating these factors expands our perspective beyond single-
364 environment selection metrics, with important implications. For example, traits like
365 body size may be associated with dominance in contest competition and so assumed
366 to be under directional selection, but this does not mean that the same traits are also
367 advantageous in other contexts [e.g., dynamic scramble competition (74)]. Our study
368 also highlights that integrating multiple indices of sexual selection intensity and
369 complementing these with measures of selection on phenotypic traits offers a far more
370 comprehensive picture of how sexual selection shapes phenotypes. Finally, the
371 observed link between remating intervals and mating success underscores the need
372 to evaluate selection pressures across episodes of selection. Our integrated approach

373 holds the potential to advance our understanding of evolutionary adaptation and pave
374 the way for the development of more robust predictions about how populations will
375 respond to future environmental challenges.
376
377 **Materials and Methods**
378
379 **Study organism.** The *D. prolongata* flies used in this study were descendants from
380 45 isofemale lines, originally collected in the Sa Pa region in Vietnam (70) maintained
381 in the lab for approx. 30 generations at 20±1°C and 60% relative humidity, on a 14:10
382 light:dark cycle. To avoid potential inbreeding effects and maximize genetic diversity
383 within each experimental unit, virgin males and females were crossed between
384 independent lines, with varying heterozygous genotypes between units. To ensure
385 virginity of experimental individuals, flies were collected by aspiration on the day of
386 eclosion before being kept in sex-specific vials (groups of 5 males or 10 females of the
387 same parental genotypes) on standard fly food (see *SI Methods*) until sexually mature
388 at 5-7 days post-eclosion. For identification, the thorax of each experimental fly was
389 marked with a dot of acrylic paint (Kreul El Greco Acrylic colors) in a unique color
390 within sex and experimental unit, three days before the start of the experiment.
391 Thereafter, flies were housed individually in 20mL vials with approx. 2mL of fly food.
392
393 **Experimental units and procedure.** Each experimental arena (see *SI Methods*)
394 consisted of a Petri dish (ø 9cm) with small ventilation holes in the lid. Petri dishes
395 were filled with orange juice-agar medium (see *SI Methods*) around a central Petri dish
396 (ø 3.5cm) that was filled with standard fly food and covered with yeast paste. This
397 central yeast patch served a resource-dense area (RDA), whilst the peripheral agar
398 medium was considered resource-poor, with small grains of dry yeast sprinkled at a
399 low density (Fig. S1). Each experimental unit (group of flies) was housed in one of
400 these Petri dishes, varying in their operational sex ratio (OSR; 1:1 or 3:1 M:F) and
401 population density (low [12 flies], medium [16], high [20]). Each group composition was
402 replicated five times.
403 Flies were introduced into the plates by aspiration according to the density and sex
404 ratio of their treatment. There was neither an overlap of isoline crosses within a plate
405 nor repeated color within either sex of a plate. Each plate was placed under a
406 Raspberry Pi 3 model B attached to a camera module (V2) in a climate chamber at

407 20°C and 60% humidity with a 14:10 light:dark cycle, and fans near the plates
408 prevented condensation of the lids. Units were photographed from above every two
409 seconds during the light period across five days. On days two to five, groups of flies
410 were moved to fresh plates within the first hour of light. All eggs and larvae of the
411 previous plate were transferred to a fresh plate containing standard fly food and left to
412 develop for 3-4 days before being collected in individual Eppendorf tubes and stored
413 at -20°C for later molecular parentage analysis. On the morning after the fifth night, all
414 experimental flies were removed and anesthetized on ice before separately storing
415 their heads and bodies in Eppendorf tubes at -20°C for later genotyping and
416 morphometry, respectively.
417
418 **Morphological measurements.** For each male and female, thorax length was
419 measured, using the reticular eyepiece of a Leica M60 stereoscope. In males, the left
420 foreleg and wing were removed using forceps and mounted on a microscope slide in
421 a drop of Euparal underneath a coverslip. These body parts were then photographed
422 at 25x magnification using a sCMOS Microscope camera K5 on a Leica M165 FC
423 stereoscope to measure wing length and total foreleg length using Fiji (98) (details in
424 *SI Methods*). Male thorax, wing and foreleg length were further combined in a principal
425 component analysis, with the first principal component (PC1) serving as a proxy of
426 overall body size (see *SI Methods*).
427
428 **Collection of behavioral data.** The images were visually screened for matings. For
429 each mating, we recorded the male and female ID's, along with the start and end times
430 to calculate mating durations and remating intervals. We further noted whether
431 copulations started in the RDA, whether they were attained by an alternative
432 reproductive tactic ["interception", (78)], aggression or courtship, and whether they
433 were preceded by leg vibration (see *SI Methods* for details). In addition to quantifying
434 matings and accompanying behaviors, we recorded every 10 minutes which
435 individuals were present in the RDA (i.e. at least their head or the thorax and abdomen
436 were in the RDA).
437
438 **Parentage analysis.** The parentage analysis was based on six microsatellite loci that
439 we previously determined to be highly variable among isolines (details in *SI Methods*).
440 The larvae or heads of parents were suspended in KAWA buffer (10 mM Tris HCl, 1

441 mM EDTA, 0.5% Tween 20, 50 µg/ml Proteinase K, pH 8.0) and sent to Ecogenics
442 (Microsynth) for DNA extraction, PCR amplification and sequencing. Approximately
443 50% of all larvae from each plate and day were analyzed, selected at random.
444 Parentage assignment was done using CERVUS 3.0.7 (99), which determines the
445 most likely parent-offspring trio by maximum likelihood exclusion based on LOD
446 (logarithm of the odds) scores and their probabilities by simulations on the allele
447 frequencies in the population. Parent-offspring trios were accepted as valid when at
448 least three loci were compared (excluded when more than three were missing), and
449 when these were assigned with a likelihood of 95% ("strict") or 80% ("relaxed"). As all
450 potential parents were known and combinations could be cross-checked with
451 observed matings, trios with 80% likelihood were included.
452
453 **Statistical analyses.** All statistical analyses were conducted in R version 4.3. (100).
454 Linear and generalized linear mixed models were conducted using the package *lme4*
455 (101) with experimental units as a random effect. Where the analysis was done on the
456 level of matings instead of individuals, the male and female ID were added as random
457 effects. Negative binomial model [*glmmTMB* (102)] or observation level random effects
458 (103) were used to control for overdispersion in Poisson or binomial models,
459 respectively. We bootstrapped confidence intervals (n = 1000) for the opportunity for
460 sexual selection and for the variance partitioning using the package *sampling* (104).
461 We used the package *piecewiseSEM* (105) for piecewise structural equation modeling
462 and *diagrammeR* (106) to visualize the structural equation models. A comprehensive
463 description of all statistical methods can be found in the *SI Methods.*
464
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466
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Figures

Figure 1: Bateman gradients for males (blue) and females (red) for each combination of density (low, medium, high) and OSR (even, male-biased) and density.

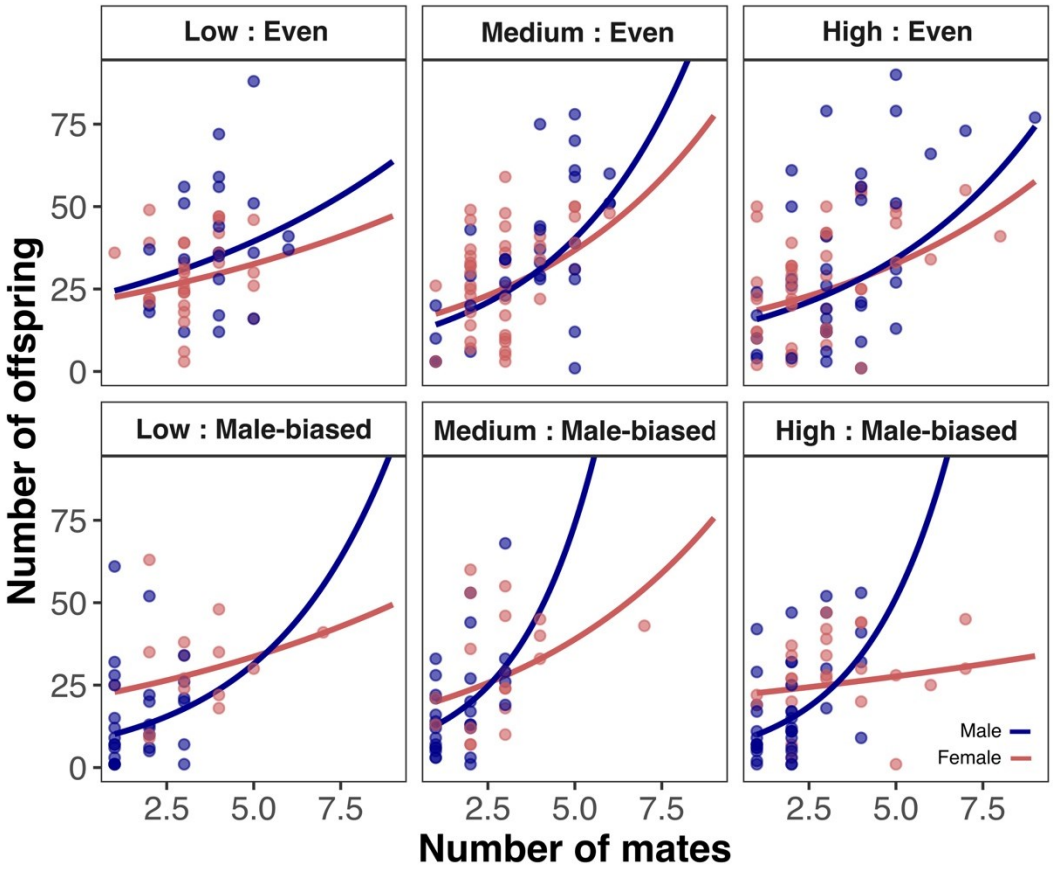
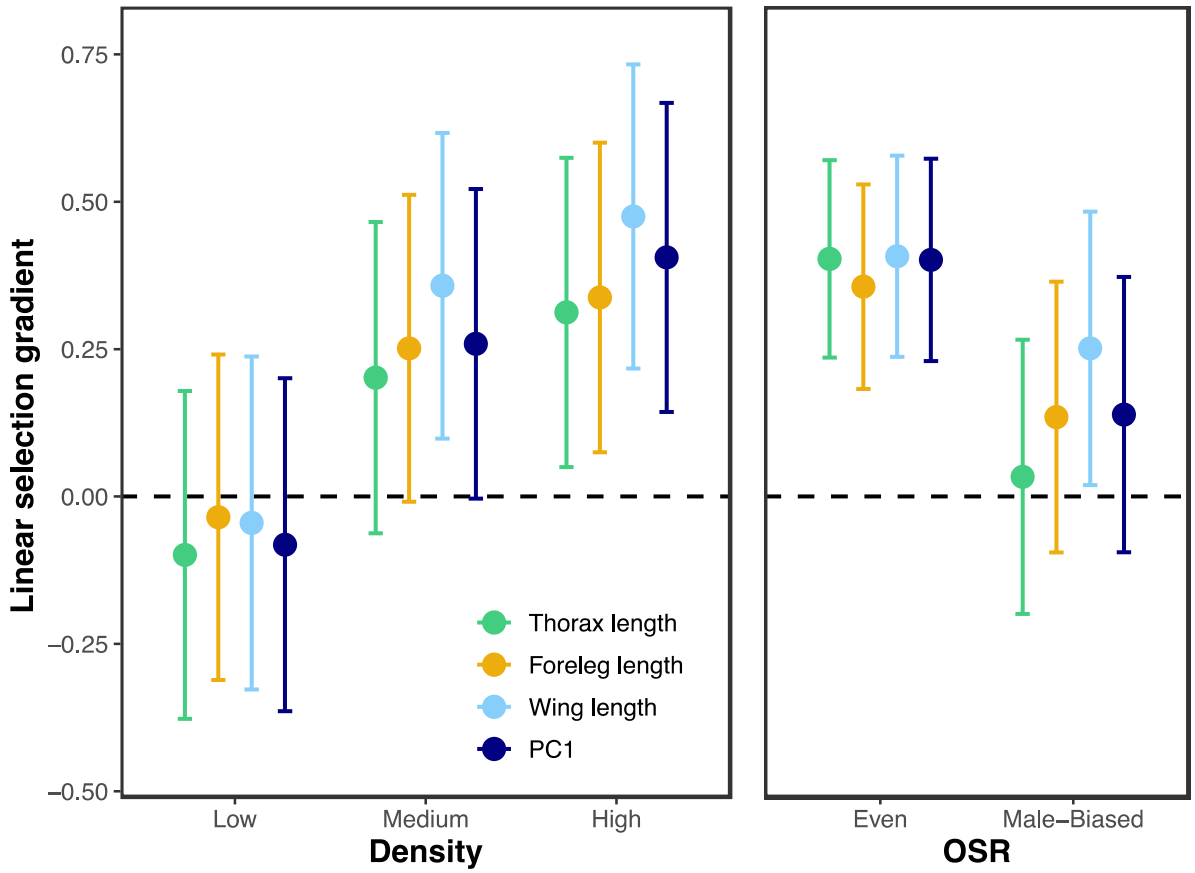
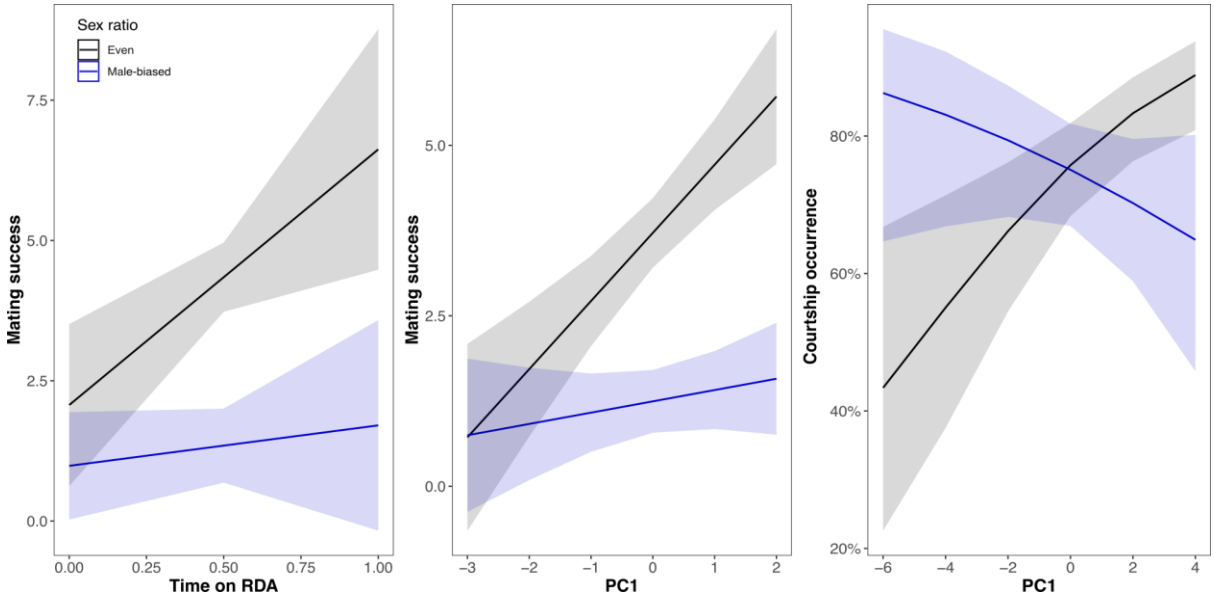


Figure 2: Linear selection gradients with 95% confidence intervals on male morphological traits under different levels of population density and OSRs.



752 Figure 3: The effect of territoriality (time on the resource-dense area; left), and PC1
753 (middle) on mating success depends on the OSR. The effect of PC1 on courtship
754 occurrence also depends on the OSR (right).
755



756

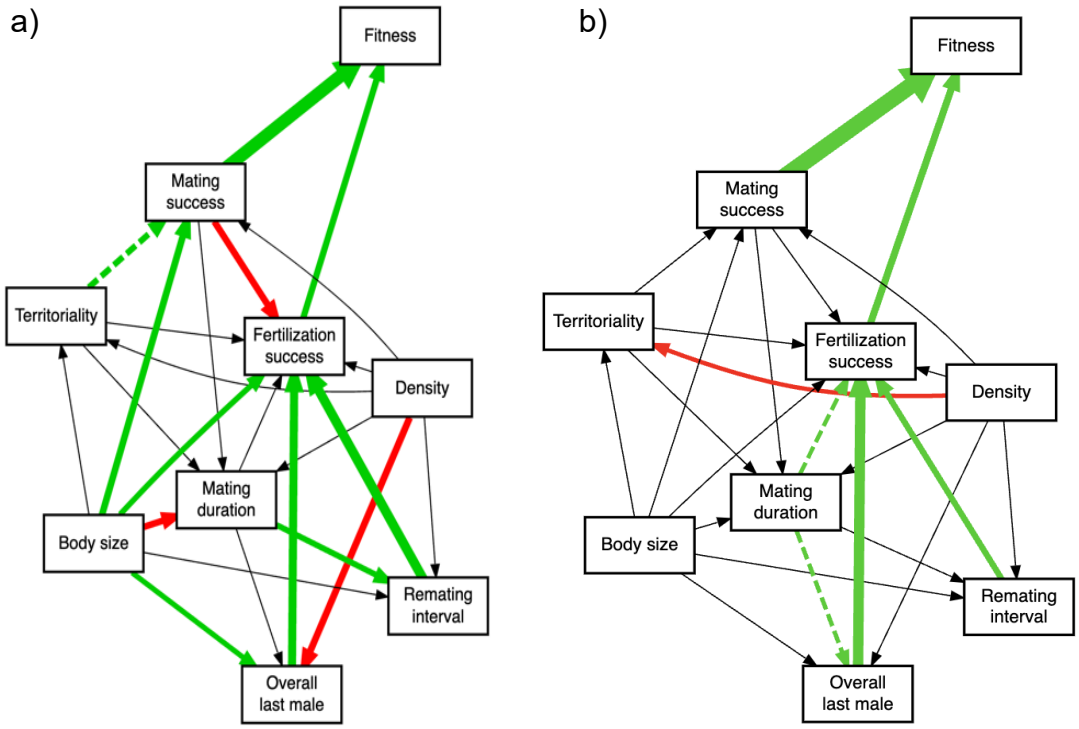



Figure 4: Visualized structural equation model of male reproductive dynamics under (a) an even OSR. Green and red indicate positive and negative relationships, respectively. Black lines indicate neutral relationships. Dashed lines (red or green) indicate trends ($P < 0.1$).