

1 **Artificial selection on walking distance suggests a**
2 **mobility-sperm competitiveness trade-off**

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4 Behavioral Ecology

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10 Lay summary

11 Animals have a limited resource budget and resources allocated to one trait,
12 cannot be invested in another. This drives trade-offs between traits. One predicted
13 trade-off is between male mobility (for mate searching) and sperm
14 competitiveness. We tested this idea by selecting on walking distance in red flour
15 beetles. We found that less mobile males mated longer, performed better
16 copulatory-courtship and outperformed mobile males in sperm competition. This
17 broadly supports a mate searching, sperm competitiveness trade-off.

18

19 **Artificial selection on walking distance suggests a mobility-sperm**
20 **competitiveness trade-off.**

21

22 **Abstract**

23 Securing matings is a key determinant of fitness and in many species males are the
24 sex that engages in mate searching. Searching for mates is often associated with
25 increased mobility. This elevated investment in movement is predicted to trade-off
26 with sperm competitiveness, but few studies have directly tested whether this
27 trade-off occurs. Here, we assessed whether artificial selection on mobility affected
28 sperm competitiveness and mating behavior, and if increased mobility was due to
29 increased leg length in red flour beetles (*Tribolium castaneum*). We found that in
30 general, males selected for decreased mobility copulated for longer, stimulated
31 females more during mating and tended to be better sperm competitors.
32 Surprisingly, they also had longer legs. However, how well males performed in
33 sperm competition depended on females. Males with reduced mobility always
34 copulated for longer than males with high mobility, but this only translated into
35 greater fertilization success in females from control populations and not the
36 selection populations (treatment females). These results are consistent with a
37 mate-searching/mating-duration trade-off and broadly support a trade-off
38 between mobility and sperm competitiveness.

39

40 **Keywords**

41 leg length, mate searching, morphology, sperm competition, trade-off, *Tribolium*
42 *castaneum*, walking

43 **Introduction**

44 Movement is energetically costly. Thrushes spend 222 calories to travel each of the
45 4800 kilometres on their migration route (Wikelski et al. 2003). Metabolic rate
46 increases by almost four fold when pumas stop talking prey and begin chasing
47 them (Williams et al. 2014). These high costs mean that resources invested in
48 moving rapidly, or over long distances, cannot be allocated to other traits. This
49 drives resource based trade-offs between movement and other costly traits,
50 including reproduction. Movement-reproduction trade-offs are often characterised
51 in terms of dispersal, but mate-searching can also trade-offs with reproductive
52 traits.

53

54 Male fitness is typically limited by access to females (Trivers 1972; Thornhill and
55 Alcock 1983; Powell 1997), or more strictly by access to their eggs, while female
56 fitness is usually limited by resource acquisition rather than sperm limitation
57 (Trivers 1972; Thornhill and Alcock 1983). This is one reason why it is frequently
58 males rather than females that engage in costly activities like mate-searching that
59 increase the likelihood of encountering mates (Parker 1978; Thornhill and Alcock
60 1983; Clutton-Brock and Parker 1992; Andersson 1994; also see Fromhage et al.
61 2016).

62

63 Investment in mate searching behaviours frequently results in males having larger
64 home ranges than females, or being more mobile than them. For example, male
65 moose and wood mice have much larger home ranges than females as they move
66 more to find females (Attuquayefio et al. 1986; Cederlund and Sand 1994).

67 Furthermore, sex-specific selection for traits associated with mate searching often
68 results in sexual dimorphism, so that males can have larger eyes to detect females
69 more effectively (e.g., Thornhill and Alcock 1983; Eberhard et al. 1998).

70

71 While mate searching is important, male fitness does not just depend on finding
72 females and securing matings, it also depends on successfully competing for
73 paternity via sperm competition (Parker 1970). Selection for enhanced mate
74 searching is predicted to come at a cost to sperm production and hence sperm
75 competitiveness (Parker 1978). This is because resources that could otherwise be
76 allocated towards sperm production are instead invested in mate searching
77 (Parker 1978). Simmons and Parker (1996) theoretically explored this trade-off
78 and their analysis indicated that when the fitness returns of mate searching are
79 greater, males should invest less in sperm competitiveness, and conversely, when
80 sperm competition risk is high, males should invest less in mate searching. This
81 logic underlies many models of male reproductive investment that use trade-offs
82 between expenditure on gaining matings and ejaculates to reveal optimal male
83 investment strategies (e.g., Parker 1978; 1998; Alonzo and Warner 1999; Simmons
84 et al. 2017). Thus, theory predicts that there should be trade-offs between
85 ejaculate expenditure and sperm competitiveness, and behaviours associated with
86 mate searching, like male mobility. This trade-off could potentially extend to
87 morphological characters that facilitate mobility such as leg length (e.g., Eberhard
88 et al. 1998). This is essentially what is found in species with alternative male
89 reproductive tactics, where some male morphs invest more in gaining matings and
90 others more in sperm competitiveness (Gage et al. 1995; Simmons et al. 1999;

91 Simmons et al. 2017). Similarly in moths, phenotypes that disperse to find females
92 have larger flight muscles but smaller testis (Gage 1995), which mirrors the
93 theoretically predicted trade-off (Parker 1978; Simmons and Parker 1996;
94 Simmons et al. 2017). However, to date there are no studies directly
95 demonstrating that selection on movement reduces investment in sperm
96 competitiveness.

97

98 Here, we assess the predicted trade-off between male movement and sperm
99 competitiveness in red flour beetles (*Tribolium castaneum*). *T. castaneum* is a
100 model for sexual selection (e.g., Fedina and Lewis 2006; Michalczyk et al. 2010;
101 Demont et al. 2014; Sbilordo et al. 2014; Sbilordo and Martin 2014; Godwin et al.
102 2018). Several studies have demonstrated that *T. castaneum* females are
103 polyandrous, although offspring are largely sired by a female's most recent mate
104 (Schlager 1960; Lewis and Austad 1990; Fedina and Lewis 2004; Sbilordo and
105 Martin 2014) and there is considerable between-male variation in siring success
106 (Arnaud et al. 2001; reviewed in Simmons & Siva-Jothy 1999). While *T. castaneum*
107 do disperse, flying tend to be used in long-distance dispersal (Ridley et al. 2011).
108 Walking is the mechanism by which males locally search for females (Matsumura
109 and Miyatake 2015). A previous study subjected beetles to bi-directional
110 artificial-selection on walking distance, establishing populations that walked
111 longer or shorter distances alongside control populations (Matsumura and
112 Miyatake 2015). Males from population selected for greater walking had
113 significantly increased mating success (measured as the number of females who
114 mated with the male) (Matsumura & Miyatake 2015). Collectively, this suggests

115 that male walking is a key component of mate searching, and accordingly, if there
116 is a trade-off between male movement via walking and sperm competitiveness, it is
117 more likely to reflect a mate-searching, sperm competition trade-off, than a
118 trade-off between dispersal and reproduction. However, from hereon we refer to a
119 general movement – sperm competitive trade-off to allow for the possibility that
120 greater walking distance has purposes other than mate searching.

121

122 If, as predicted, sperm competitiveness trades-off with the ability to search for
123 mates (Parker 1978) and mate searching effort is related to distances males walk
124 (as it should be: more walking requires more energy expenditure), then we should
125 detect differences in sperm competitiveness in populations selected for more or
126 less movement. Furthermore, because sperm competitiveness can be influenced by
127 mating behavior (Eberhard 1996; Pitnick and Hosken 2010), copulatory behaviors
128 may also vary across artificial selection regimes. Additionally, leg length may differ
129 in dispersing versus non-dispersing treatments because longer legs may be
130 correlated with walking ability in this beetle (Arnold et al. 2017). To test these
131 ideas we compared the sperm competitiveness, mating behavior and leg lengths of
132 beetles from populations artificially selected for high or low male mobility.

133

134 **Materials and Methods**

135 *Insects and culture*

136 *T. castaneum* is an insect pest of stored grain. They are highly polygamous, with
137 both sexes mating frequently throughout their adult lives.

138

139 The *T. castaneum* beetle culture used in this study has been maintained in the
140 laboratory for more than 30 years, reared with a mixture of whole meal (Yoshikura
141 Shokai, Tokyo) enriched with brewer's yeast (Asahi Beer, Tokyo) and maintained
142 at 25°C with a 16 h photoperiod (lights on at 07:00, lights off at 23:00). Since this
143 species is a stored-grain pest, these lab conditions closely mirror the native
144 environment of these beetles.

145

146 *Artificial selection for walking distance*

147 To generate each treatment, replicate populations were established. Each beetle
148 was sexed at pupal stage, and males and females were separated to avoid mating
149 until the experiments. Briefly, 75 virgin males and 75 virgin females (21–28 days
150 old) were randomly collected from a stock culture, and the distance each beetle
151 walked in 30 minutes was measured using an image tracker (Digimo, Osaka, Japan).
152 The 10 males and 10 females with the longest walking distance were selected to
153 propagate the longer-distance walking lines (Long searchers: L treatment), and the
154 10 males and 10 females with the shortest walking distance were selected to
155 propagate the shorter-distance walking line (Short searchers: S treatment). To
156 propagate a Control (C) treatment (i.e. a population without selection), 10 males
157 and 10 females were randomly selected from the stock culture. For this procedure,
158 10 males and 10 females were housed together to reproduce until the emergence
159 of the next generation of beetles (about 40 days) in a plastic cup (diameter 70mm
160 × height 25mm). This procedure was repeated, so that three replicate
161 populations for each selection regime (i.e. L x 3, S x 3) were generated (hereafter,
162 these replicate populations are referred to as treatment replicates). Selection

163 continued for 22 generations. By generation 15, selection had successfully
164 generated populations that differed in walking distance (Matsumura and Miyatake
165 2015), and this remained the case at generation 22 (L treatment males walked
166 further than S treatment males: $X_{1,448} = 254.54$, $P < 0.001$; Fig. 1 and S1). For more
167 information on responses to selection including direct responses (walking
168 distances) and correlated responses (mate searching and predator avoidance), at
169 generation 15, see Matsumura and Miyatake (2015).

170

171 *Mating behavior*

172 Copulation duration and male behavior during copulation are major determinants
173 of siring success in many animals (Parker 1970; Eberhard 1996; Singh and Singh
174 2014). In *T. castaneum*, males rub females with their legs during copulation as part
175 of their copulatory courtship (Eberhard 1994; Wojcik 1969; Bloch Qazi 2003).
176 Accordingly, we recorded both rubbing behavior (the number of times males
177 rubbed females on their right side per copulation - we assumed no handedness
178 difference across treatments) and copulation duration for males from L and S
179 treatments when mating to C-females. Each male (14–21 day old virgin) was
180 placed into a petri dish (35×10 mm) and habituated for 5 min, then a female (14–
181 21 day old virgin) was added and the pair was allowed to mate. All observations
182 were carried out at 25°C between 12:00 to 18:00.

183

184 *Sperm competitiveness*

185 To compare sperm competitiveness among treatments selected for more or less
186 movement (i.e. L vs. S), we measured sperm defense (P1 – fertilization success

187 when the focal male is the first of two males to mate with a female) and sperm
188 offence (P2 – fertilization success when the focal male is the second of two males
189 to mate with a female). Competitor (non-focal) males were mutants homozygous
190 for an autosomal, semi-dominant black body color allele – this phenotype is
191 frequently used as a marker in sperm competition studies in *T. castaneum* (Fedina
192 and Lewis 2008). Virgin focal males (14–21 days old) from each treatment were
193 allowed to mate once with a C-treatment female (14–21 days old) either as that
194 female's first or second mate (with a randomly chosen virgin black mutant being
195 the second or first mate respectively). After mating was completed, females were
196 isolated in a plastic container (50×50 mm with enough food) and allowed to lay
197 eggs for 7 days. Progeny were kept at 25°C for 50 days to develop into adults, and
198 adult body color was scored to assign paternity and generate P1 and P2 scores. We
199 used 149 males (L treatment: $N = 71$, S treatment: $N = 78$) to assay P1 and 174
200 males (L treatment: $N = 82$, S treatment: $N = 92$) to test P2, respectively. When
201 either competing male did not sire any offspring (i.e. female fertility was zero)
202 triads were removed from the analysis as we could not be certain copulations were
203 successful and resulted in sperm transfer or storage (i.e. females may also
204 influence sperm retention (Lewis & Austad 1994)). All experiments were carried
205 out in a room maintained at 25°C between 12:00 and 18:00.

206

207 We then conducted similar experiments but where focal males were paired with
208 both L and S females (as opposed to C-females) to test whether any effects
209 detected in the previous experiment were specific to C-females. Again virgin males
210 aged 14–21 days old, from L or S treatments, were paired with females from each

211 treatment in a fully factorial fashion (i.e., $L\sigma^{\text{♂}} \times L\text{♀}$, $L\sigma^{\text{♂}} \times S\text{♀}$, $S\sigma^{\text{♂}} \times L\text{♀}$, $S\sigma^{\text{♂}} \times S\text{♀}$) and
212 allowed to mate once and copulation duration was measured. Here only P2 was
213 assessed, following the methods described above.

214

215 *Leg morphology*

216 At generation 22, adults (L treatment: $N = 90$, S treatment: $N = 100$) were frozen at
217 -20°C for leg measurement. We measured the length of all legs (foreleg, middle leg,
218 and hind leg) of right side, and we also measured the length and width of body of
219 each adult (± 0.01 mm) using a dissecting microscope monitoring system (VM-60,
220 Olympus, Japan). Because three legs were broken from beetles from the L
221 treatment and two legs in S treatment beetles were broken, we removed these data
222 (whole individuals) prior to analyses. The lengths of each of the tarsus, tibia, and
223 femur, and width of the femur were recorded for each leg. Each specimen was
224 carefully positioned so its longitudinal and dorsoventral axes were perpendicular
225 to the visual axes of the microscope eyepiece. Each length was measured as a
226 straight-line distance. We measured each leg segment separately.

227

228 *Statistical analysis*

229 To compare leg morphology among treatments (Treat: selection for long (L) or
230 short (S) movement), we used multivariable analysis of variance (MANOVA) with
231 treatment (i.e., L / S), sex (i.e., M / F), and the interaction between treatment and
232 sex as fixed effects, with replicate populations included as a random effect (and leg
233 segment as the dependent variable). Moreover, we used analysis of covariance
234 (ANCOVA) for each segment (tarsus, tibia, and femur for each leg) with body size

235 as a covariate as a *post-hoc* test for the MANOVA. In each ANCOVA, treatment, sex,
236 and the interaction between treatment and sex was include as fixed effects, and
237 replicate population was included as random effect. If each ANCOVA showed
238 significant effects, we conducted Tukey's HSD test as *post-hoc* test. We also
239 compared leg length (i.e. total of tarsus, tibia, and femur length) in each leg
240 between treatments by ANCOVA.

241

242 To compare walking distance of males among treatments at the 22nd generation,
243 we used a GLMM with treatment (i.e., L / S) as a fixed effect, and replicate
244 population as a random effect. To assess potential differences in mating behavior
245 when males mated with C-females, copulation duration and rubbing behavior were
246 compared among treatments using a GLMM with treatment (L / S) as a fixed effect,
247 and replicate as a random effect. To compare sperm competitiveness, fertilization
248 success was compared among treatments using a GLMM with treatment and
249 mating order (i.e., P1 and P2) as fixed effects, and replicate as a random effect.
250 These models had a binomial error structure. To compare the duration of
251 copulation and paternity success (P2) when focal males were mated to females
252 from the treatment groups (i.e. L/S males mated to L/S females), we used a GLMM
253 with male and female treatment as fixed effects (e.g., L / S), with replicate of assay
254 male and the tester female included as random effects. All of these analyses were
255 carried out using JMP (Ver. 12.2.0, SAS 2015).

256

257 **Results**

258 The responses to selection for walking distance were clear: in each replicate line,
259 after a few generations, walking distances were significantly longer in L than S
260 strains (Fig. 1 and S1).

261

262 In terms of how this affected leg morphology, there were significant effects of
263 treatment, sex, and the interaction between treatment and sex on legs (Table 1).
264 *Post hoc* testing showed significant differences among treatments in lengths of the
265 tarsus and tibia of forelegs, and the tarsus of hind leg (Table S1, S2, and Fig. S2).
266 Moreover, there was a significant interaction between treatment and sex affecting
267 the lengths of various leg segments (Table S2). Effects were primarily driven by
268 males from S populations whose legs (and the segments that contribute to them:
269 Fig. S2) tended to be significantly longer than beetles from all other population
270 (Fig. 2).

271

272 When males were mated with C-females, mean sperm defense (P1: the proportion
273 of offspring sired by the first of two males to mate with females) was significantly
274 lower than sperm offence (P2: the proportion of offspring sired by the second of
275 two males to mate with females). For both measures of sperm competitiveness
276 males from populations selected for reduced movement (S-males) were
277 significantly better sperm competitors than males selected for greater movement
278 (L-males) (Fig. 3, Table 3). Males from short movement populations also copulated
279 for longer ($X^2_{1,150} = 10.68, P = 0.001$) and engaged in more copulatory courtship
280 (rubbed females more during copulation) than males from long movement
281 populations ($X^2_{1,148} = 5.74, P = 0.017$) (Fig. 4).

282

283 When individuals from populations subjected to selection on movement were
284 mated in a fully-factorial design, males from shorter movement populations again
285 copulated for longer than males from greater movement populations, irrespective
286 of which females they were paired with (Fig. 5A, Table 4). There were no
287 significant effects of female treatment on mating duration, and there was no
288 interaction between male and female treatment (Table 4). Qualitatively, P2 was
289 marginally greater again for S-males (short movement populations), but this
290 difference was not significant (Table 4). There were no significant effects of female
291 treatment or an interaction between male and female treatment on P2 (Fig. 5B,
292 Table 4).

293

294

295 **Discussion**

296 In general, we expect a trade-off between investment in reproductive effort, and
297 investment in movement. Indeed, there is evidence for this trade-off in female *T.*
298 *castaneum* (Matsumura and Miyatake 2018). Theory also predicts a trade-off
299 between traits associated with mate searching and sperm competitiveness (Parker
300 1978). Although this trade-off has been found in some species (e.g., Gage 1995;
301 Simmons et al. 2017), there are few direct tests of this theory. Here we tested how
302 selection on walking distance in red flour beetles affected male sperm
303 competitiveness. Walking is the main means of local mate searching in this beetle,
304 and previous work shows that males selected to walk further had significantly
305 higher mating success than males that walk shorter distance (Matsumura and

306 Miyatake 2015). Therefore, artificial selection on walking distance provides one
307 way to test for a trade-off between mate searching (mating success) and sperm
308 competitiveness (siring success). In keeping with theoretical predictions (Simmons
309 et al. 2017), males selected to be more mobile, mated for less time and invested
310 less in copulatory courtship. We also found evidence that males selected for
311 increased mobility were poorer sperm competitors, as predicted by theory.
312 Additionally, males selected for low mobility had significantly longer legs than
313 controls and males selected for high mobility, illustrating a surprising relationship
314 between mobility, mating and morphology. We discuss these findings in turn.

315

316 Consistent with general predictions, males selected for decreased movement
317 outperformed males selected for increased movement in a number of mating
318 assays. They mated for significantly longer and also rubbed females more
319 frequently during mating, thereby performing better in copulatory courtship.
320 Furthermore, when males from decreased movement populations were mated
321 with control females, they also secured greater fertilization success, siring more
322 offspring than competing males. This is consistent with a mobility/sperm
323 competitiveness trade-off and reflects findings in moths and species with
324 alternative mating tactics where there are frequently trade-offs between pre- and
325 post-copulatory traits involved in male-male competition (e.g., Gage 1995;
326 Simmons et al. 1999). While less mobile males also mated for longer with females
327 from selected populations (i.e., females from the short and large movement
328 treatments), this resulted in higher sperm offence, especially in females from the
329 high mobility populations, but this effect was not significant. It is not immediately

330 obvious why there was this difference across experiments (when males were
331 paired with control vs. selection females). However, we note that the largest siring
332 advantage to males selected for limited movement when mating with the control
333 females was in sperm defense (P1) rather than in sperm offence (P2). Thus the
334 lack of P2 effect with selected population females may simply be a power issue: we
335 needed more populations (the unit of replication) to detect the smaller effect size.
336 Importantly, and as noted, the trend for a difference was in the same direction -
337 males selected to disperse less tended to be better at sperm offence. So while
338 results were not quantitatively identical, the overall direction of effects across
339 experiments is consistent with a trade-off between walking (which is associated
340 with mate searching) and sperm competitiveness.

341

342 It is important to note that it is not easy to empirically discriminate between
343 dispersal-reproduction trade-offs and the more specific mate-searching – sperm
344 competitiveness trade-off. However, on balance we believe that our experiments
345 relate to the latter. First, in males selected for longer walking distances we see
346 greater overall mating success – males that walk further win more mates
347 (Matsumura and Miyatake 2015). This is not what we would expect given a general
348 reproduction-dispersal trade-off and shows that greater mobility improves
349 mate-searching and mating success. The reduced sperm competitiveness we
350 observe here, in combination with this improved mate-searching behavior in
351 mobile males, suggests a mate-searching – sperm competitiveness trade-off.
352 Second, dispersal in these beetles tends to be via flight (Ridley et al. 2011) while
353 mate searching tends to occur via walking (Matsumura and Miyatake 2015).

354

355 We cannot definitively say why males from shorter-movement populations
356 performed better in sperm competition when paired with control females (and
357 tended to generally be better mates than longer-movement population males
358 overall). One possibility is the longer legs (and in particular, longer tarsuses) of
359 these males (see below) can better hold onto females, increasing copulation
360 duration and potentially increasing how much sperm was transferred during
361 mating. Sperm numbers are a key determinant of success in sperm competition,
362 particularly when sperm competition occurs via a raffle mechanism (Parker 1998),
363 and this is frequently the mechanism in insects (e.g., Simmons et al. 1996;
364 Simmons and Achmann 2000; also see Birkhead and Moller 1998). In support of
365 this conjecture, longer legs improve how well males secure females in many
366 species (e.g., Zeh et al. 1992; Emlen 2008; Setoguchi et al. 2014). However, in *T.*
367 *castaneum* copulation duration may not be associated with sperm transfer and can
368 be negatively related to paternity success (Bloch Qazi et al. 1996), suggesting that
369 longer copulations may only reflect female resistance to sperm transfer (Lewis and
370 Austad 1990; Bloch Qazi et al. 1996; Fedina and Lewis 2006). Our data indicated
371 that longer copulation duration tends to be associated with greater siring success.
372 The difference across studies may relate to the greater copulatory courtship
373 associated with longer copulations in our study (see below).

374

375 The improved siring success of less mobile males could also result from greater
376 cryptic-female-choice for these males. Female *T. castaneum* exert control over
377 sperm numbers transferred during mating and can use this as a mechanism for

378 biasing the fertilization success in favor of preferred males (Fedina 2007).
379 Accordingly, it is possible that the improved fertility of males from reduced
380 movement populations represents cryptic-female-choice based on their increased
381 copulatory courtship - they rubbed females more during mating. Copulatory
382 courtship is wide-spread (Eberhard 1994) and thought to be a major female
383 determinant of siring success (Eberhard 1996). For example, male stimulation of
384 females during mating enhances sperm transfer in crickets (Wulff et al. 2017) and
385 siring success in spiders (Peretti and Eberhard 2009). The effect of rubbing
386 behavior on siring success is less clear in *T. castaneum* with positive correlations
387 between rubbing rate and paternity success reported (Edvardsson and Arnqvist
388 2000) and no association also found (Fedina and Lewis 2006). In any case we
389 generally find males that move less tend to be better sperm competitors and
390 certainly copulate for longer and engage in more copulatory courtship.
391 Irrespective of the mechanism responsible, our results are therefore broadly
392 consistent with a trade-off between post-copulatory sexual selection and mate
393 searching. Although as noted, our results were not entirely consistent across
394 experiments in statistical significance terms but trends were in the same direction.

395

396 In addition to differences in movement, mating behaviors and sperm
397 competitiveness, selection also altered leg morphology. Males selected for shorter
398 walking distances evolved the longest legs, but this differences was not seen in
399 females. This result contrasts with previous work in *T. castaneum* showing that
400 individuals with greater walking ability have relatively longer legs (Arnold et al.
401 2017). Our result is also counterintuitive because longer legs are associated with

402 reduced energy expenditure in men and women walking quickly (Salamuddin et al.
403 2014) and leg length increases running speed in the fastest terrestrial animal
404 (relative to its body size) (Rubin et al. 2016). All else being equal, long legs should
405 positively correlate with mobility. However, theory predicts that longer legs only
406 increase mobility if the proportions of each leg segment do not change (i.e. the
407 tarsus, tibia and femur all increase in length to the same degree (Leurs et al. 2011)).
408 Here we see shifts in the relative proportions of male leg segments, suggesting that
409 changes in leg length may be for a purpose other than improved mobility. Legs in
410 the beetles are also associated with copulatory courtship (see above), which could
411 explain why selecting for more and less movement resulted in different leg
412 morphology of males, but not in females. We need additional detailed studies
413 examining the relationship between male's leg length and other reproductive traits
414 in the future.

415

416 We should also note concerns were raised that the effective population sizes of
417 populations were small, and therefore drift may have played a role in the observed
418 responses. However, we subjected lines to direct selection and generated
419 treatment-consistent responses to selection, and correlated responses to selection
420 were also largely consistent (i.e. we recorded treatment-specific effects). That is,
421 we selected and generated consistent micro-evolution in the direction of selection.
422 This is not consistent with drift. Employing experimental evolution with these
423 population sizes could cause drift issues, but the consistent responses in the
424 direction of selection in the current study suggest drift was not a major concern.

425

426 In conclusion, selection for reduced walking distance was effective, but somewhat
427 counter-intuitively, this was associated with the evolution of longer legs and shifts
428 in the relative proportions of leg segments. These morphological changes were
429 correlated with improved male mating ability, increased copulation duration,
430 greater copulatory courtship and increased sperm competitiveness was evident at
431 least some times. These results are more or less consistent with theoretical
432 predictions of mate-searching/sperm competition trade-offs, but additional
433 research is warranted to test this fundamental prediction.

434

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436

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- 589

590 figure legends

591

592 **Fig. 1.** Outcome of artificial selection for walking distance (during 30 min: our
593 measure of movement distance) in males of *T. castaneum* after 22 generations. L =
594 males from populations selected for longer movement distances, S = males from
595 populations selected for shorter distances. Numbers in parenthesis show sample
596 sizes. Error bars show SE. Individual population responses are show in
597 supplementary figures.

598

599 **Fig. 2.** Average lengths for front, middle, and hind legs. Grey, black, and open bars
600 show C, L, and S treatments, respectively. Characters on the bar show significantly
601 differences (Tukey's HSD test: $P < 0.05$). Error bars show standard errors.

602

603 **Fig. 3.** Sperm defense (P1) and offence (P2) of focal males in competition against
604 standard black-bodied males in control treatment (C) females, shown as the
605 proportion of offspring sired (i.e. paternity success). Males from treatments
606 selected for increased movement (L males) = black bars and those selected for less
607 movement (S males) = white bars. Note P1 is less than P2, but that males selected
608 to move less (S males) are better sperm competitors. Numbers in parenthesis
609 show sample size. Error bars show standard errors.

610

611 **Fig. 4.** Duration of mating and degree of copulatory courtship (number of rubs) for
612 males from treatments selected for increased movement (L males) and those
613 selected for less movement (S males) when mating with females from control

614 populations (C-females). Numbers in parenthesis show sample size. Error bars

615 show standard errors.

616

617 **Fig. 5.** Results of fully-factorial selection matings for beetles from treatments

618 selected for increased movement (L) and those selected for less movement (S). A

619 shows the duration of mating. B shows the proportion of offspring sired (P2).

620 Numbers in parenthesis show sample size. Error bars show standard error.

621

622 tables and table legends

623

624 **Table 1.** Results of MANOVA for each morphological trait. Significant values are
625 highlighted in bold.

Factor	Statistic	Value	Num DF	<i>P</i>
Treatment	Wilks' Lambda	0.594	28	< 0.0001
Sex	F	0.436	14	< 0.0001
Treatment*sex	Wilks' Lambda	0.890	28	< 0.0001

626

627

628 Table 2. Results of ANCOVA for total length in each leg (front, middle, and hind).

Leg	Factor	<i>df.</i>	<i>F</i>	<i>P</i>
Front	Treatment	2	4.2097	0.0714
	Sex	1	56.4948	< 0.0001
	Treatment*sex	2	15.9292	< 0.0001
	Body length	1	100.0629	< 0.0001
	Body width	1	85.4901	< 0.0001
	Error	581		
Middle	Treatment	2	0.5072	0.6256
	Sex	1	58.3787	< 0.0001
	Treatment*sex	2	5.9092	0.0029
	Body length	1	96.0455	< 0.0001
	Body width	1	97.8003	< 0.0001
	Error	590		
Hind	Treatment	2	4.255	0.0717
	Sex	1	45.5432	< 0.0001
	Treatment*sex	2	6.6547	0.0014
	Body length	1	99.4253	< 0.0001
	Body width	1	105.0414	< 0.0001
	Error	593		

629

630

631 **Table 3.** Results of GLMM for paternity success when males competed with
632 females from control populations.

Factor	<i>d.f.</i>	χ^2	<i>P</i>
Treatment	1	4.72	0.030
Mating order	1	131.77	< 0.001
Treatment × mating order	1	0.34	0.562
Error	319		

633

634

635

636 **Table 4.** Results of GLMM for duration of copulation and P2 of males when
 637 copulated with both female's treatment groups.

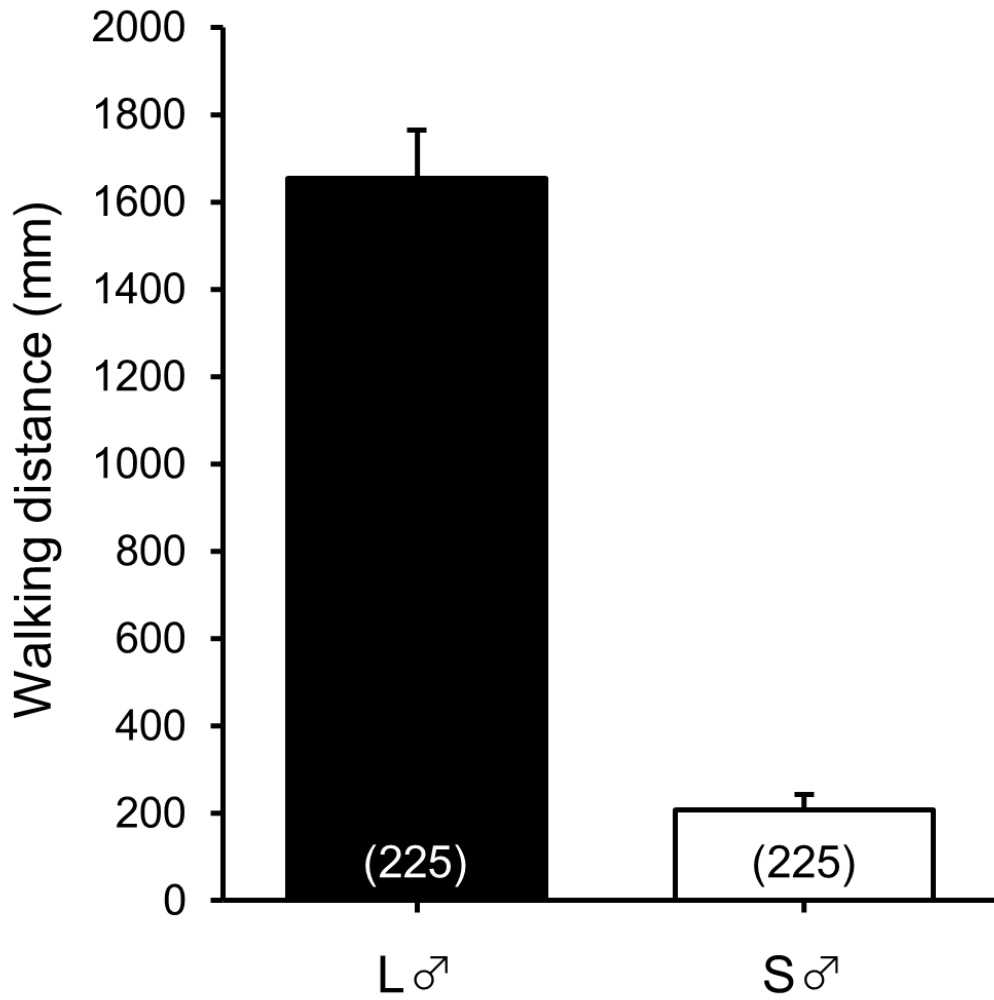
Trait	Factor	<i>d.f.</i>	χ^2	<i>P</i>
Duration	Male treatment	1	7.44	0.006
	Female treatment	1	3.07	0.080
	Male treatment × Female treatment	1	0.47	0.491
	Error	269		
P2	Male treatment	1	1.57	0.210
	Female treatment	1	0.45	0.501
	Male treatment × Female treatment	1	0.56	0.453
	Error	321		

638

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640 figures

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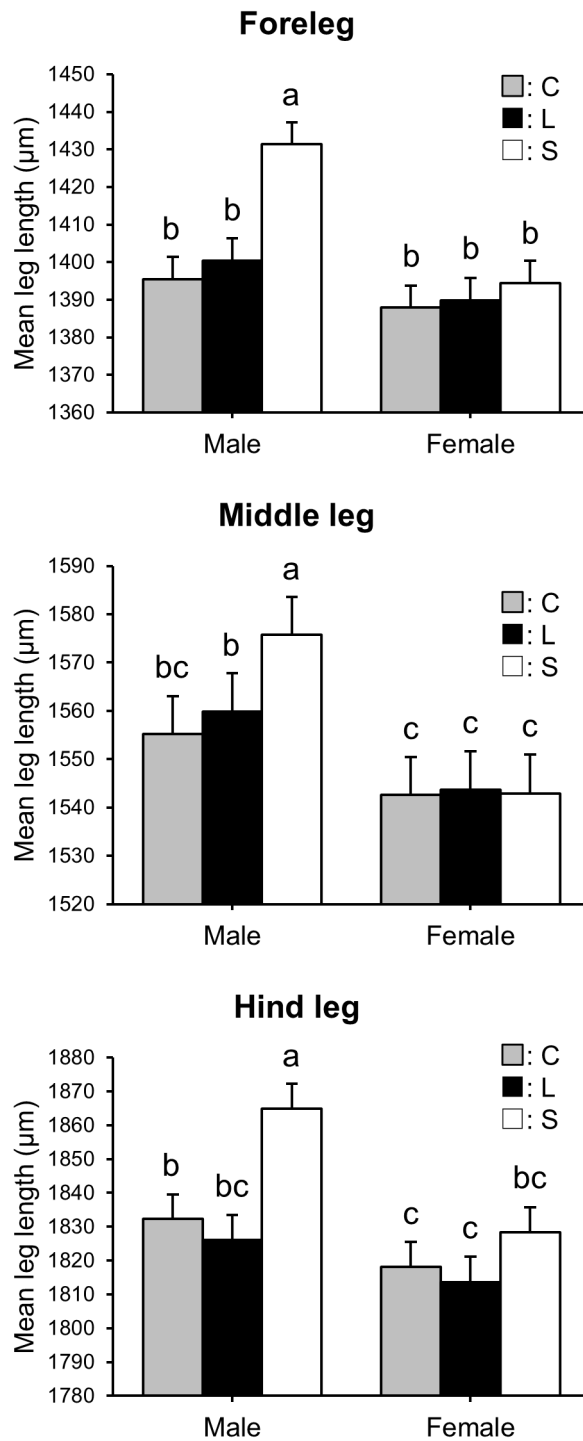
642

643 **Figure 1**

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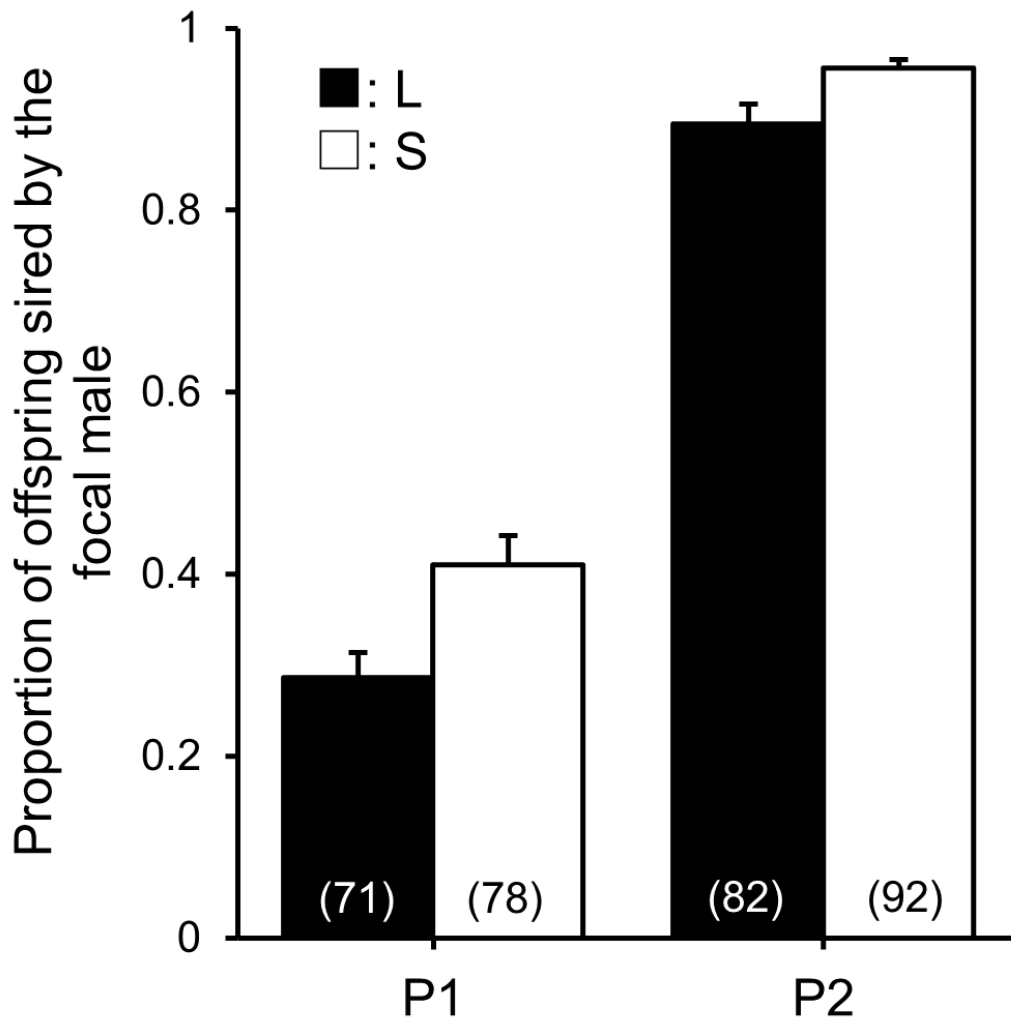
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648 **Figure 2**

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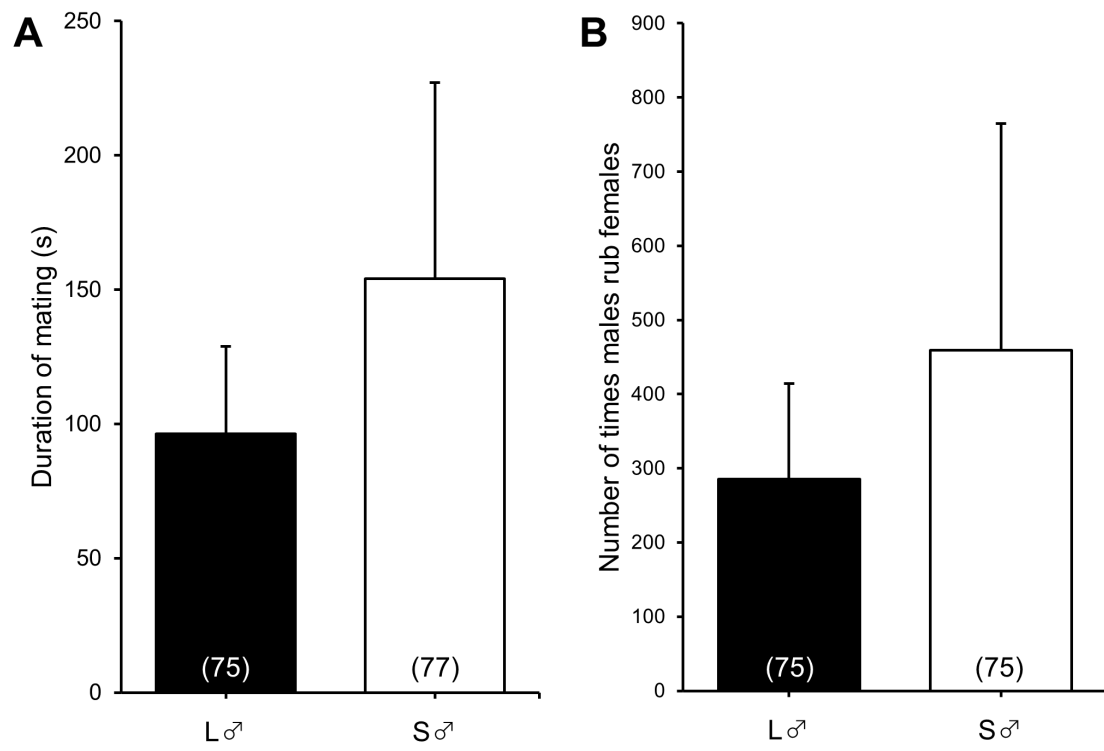


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651 **Figure 3**

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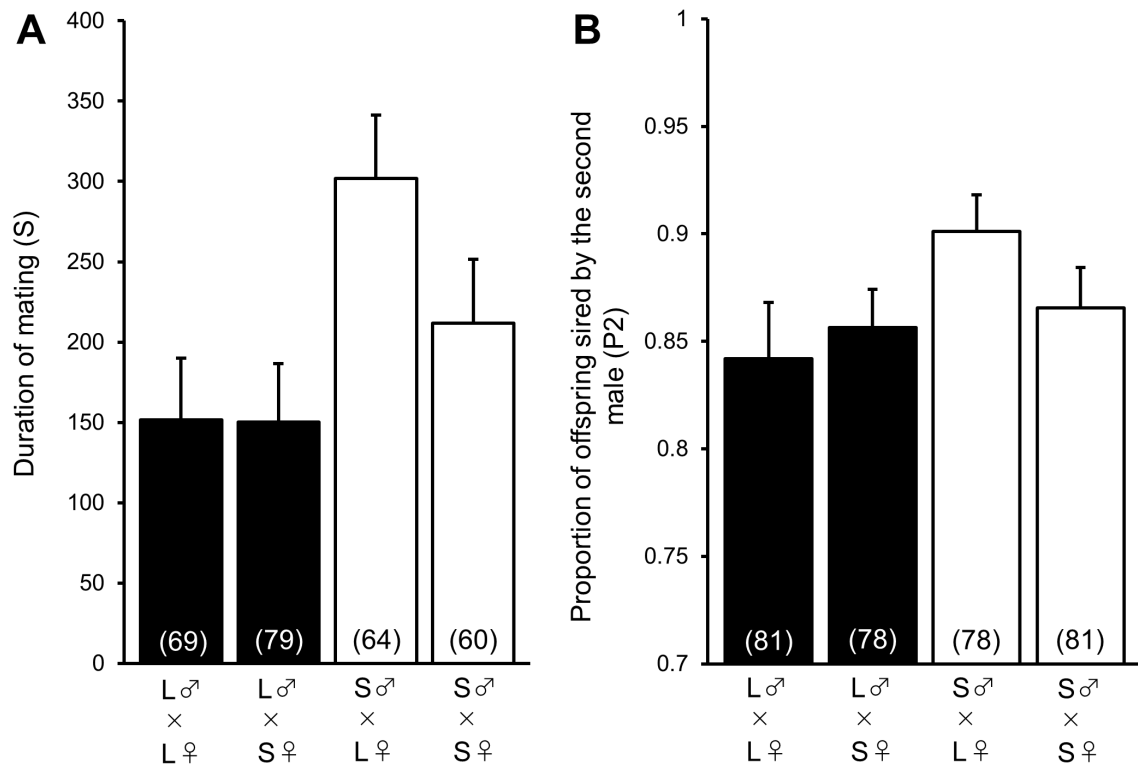
655 **Figure 4**

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661 **Figure 5**

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664 **SUPPLEMENTARY INFORMATION**

665