

Title

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

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Abstract

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

Keywords

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

Introduction

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

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

Investment in mate searching behaviours frequently results in males having larger home ranges than females, or being more mobile than them. For example, male moose and wood mice have much larger home ranges than females as they move more to find females (Attuquayefio et al. 1986; Cederlund and Sand 1994). Furthermore, sex-specific selection for traits associated with mate searching often

results in sexual dimorphism, so that males can have larger eyes to detect females more effectively (e.g., Thornhill and Alcock 1983; Eberhard et al. 1998).

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

(Gage 1995), which mirrors the theoretically predicted trade-off (Parker 1978; Simmons and Parker 1996; Simmons et al. 2017). However, to date there are no studies directly demonstrating that selection on movement reduces investment in sperm competitiveness.

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

competition trade-off, than a trade-off between dispersal and reproduction. However, from hereon we refer to a general movement – sperm competitive trade-off to allow for the possibility that greater walking distance has purposes other than mate searching.

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

Materials and Methods

Insects and culture

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

The *T. castaneum* beetle culture used in this study has been maintained in the laboratory for more than 30 years, reared with a mixture of whole meal (Yoshikura

Shokai, Tokyo) enriched with brewer's yeast (Asahi Beer, Tokyo) and maintained at 25°C with a 16 h photoperiod (lights on at 07:00, lights off at 23:00). Since this species is a stored-grain pest, these lab conditions closely mirror the native environment of these beetles.

Artificial selection for walking distance

To generate each treatment, replicate populations were established. Each beetle was sexed conducted at pupal stage, and males and females were separated to avoid mating until the experiments. Briefly, 75 virgin males and 75 virgin females (21–28 days old) were randomly collected from a stock culture, and the distance each beetle walked in 30 minutes was measured using an image tracker (Digimo, Osaka, Japan). The 10 males and 10 females with the longest walking distance were selected to propagate the longer-distance walking lines (Long searchers: L treatment), and the 10 males and 10 females with the shortest walking distance were selected to propagate the shorter-distance walking line (Short searchers: S treatment). To propagate a Control (C) treatment (i.e. a population without selection), 10 males and 10 females were randomly selected from the stock culture. For this procedure, 10 males and 10 females were housed together to reproduce until the emergence of the next generation of beetles (about 40 days) in a plastic cup (diameter 70mm × height 25mm). This procedure was repeated, so that three replicate populations for each selection regime (i.e. L x 3, S x 3) were generated (hereafter, these replicate populations are referred to as treatment replicates). Selection continued for 22 generations. By generation 15, selection had successfully generated populations that

differed in walking distance (Matsumura and Miyatake 2015), and this remained the case at generation 22 (L treatment males walked further than S treatment males: $X_{1,448} = 254.54$, $P < 0.001$; Fig. 1 and S1). For more information on responses to selection including direct responses (walking distances) and correlated responses (mate searching and predator avoidance), at generation 15, see Matsumura and Miyatake (2015).

Mating behavior

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

Sperm competitiveness

To compare sperm competitiveness among treatments selected for more or less movement (i.e. L vs. S), we measured sperm defense (P1 – fertilization success when the focal males is the first of two males to mate with a female) and sperm offence

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

We then conducted similar experiments but where focal males were paired with both L and S females (as opposed to C-females) to test whether any effects detected in the previous experiment were specific to C-females. Again virgin males aged 14–21 days old, from L or S treatments, were paired with females from each treatment in a fully factorial fashion (i.e., $L\text{♂} \times L\text{♀}$, $L\text{♂} \times S\text{♀}$, $S\text{♂} \times L\text{♀}$, $S\text{♂} \times S\text{♀}$) and allowed to

mate once and copulation duration was measured. Here only P2 was assessed, following the methods described above.

Leg morphology

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

Statistical analysis

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

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

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

Results

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

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

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

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

Discussion

In general, we expect a trade-off between investment in reproductive effort, and investment in movement. Indeed, there is evidence for this trade-off in female *T. castaneum* (Matsumura and Miyatake 2018). Theory also predicts a trade-off between traits associated with mate searching and sperm competitiveness (Parker 1978). Although this trade-off has been found in some species (e.g., Gage 1995; Simmons et al. 2017), there are few direct tests of this theory. Here we tested how selection on walking distance in red flour beetles affected male sperm competitiveness. Walking is the main means of local mate searching in this beetle, and previous work shows that males selected to walk further had significantly higher mating success than males that walk shorter distance (Matsumura and Miyatake 2015). Therefore, artificial selection on walking distance provides one way to test for a trade-off between mate searching (mating success) and sperm

competitiveness (siring success). In keeping with theoretical predictions (Simmons et al. 2017), males selected to be more mobile, mated for less time and invested less in copulatory courtship. We also found evidence that males selected for increased mobility were poorer sperm competitors, as predicted by theory. Additionally, males selected for low mobility had significantly longer legs than controls and males selected for high mobility, illustrating a surprising relationship between mobility, mating and morphology. We discuss these findings in turn.

Consistent with general predictions, males selected for decreased movement outperformed males selected for increased movement in a number of mating assays. They mated for significantly longer and also rubbed females more frequently during mating, thereby performing better in copulatory courtship. Furthermore, when males from decreased movement populations were mated with control females, they also secured greater fertilization success, siring more offspring than competing males. This is consistent with a mobility/sperm competitiveness trade-off and reflects findings in moths and species with alternative mating tactics where there are frequently trade-offs between pre- and post-copulatory traits involved in male-male competition (e.g., Gage 1995; Simmons et al. 1999). While less mobile males also mated for longer with females from selected populations (i.e., females from the short and large movement treatments), this resulted in higher sperm offence, especially in females from the high mobility populations, but this effect was not significant. It is not immediately obvious why there was this difference across experiments (when males were paired with control vs. selection females). However, we note that the largest siring advantage to males selected for limited movement

when mating with the control females was in sperm defense (P1) rather than in sperm offence (P2). Thus the lack of P2 effect with selected population females may simply be a power issue: we needed more populations (the unit of replication) to detect the smaller effect size. Importantly, and as noted, the trend for a difference was in the same direction - males selected to disperse less tended to be better at sperm offence. So while results were not quantitatively identical, the overall direction of effects across experiments is consistent with a trade-off between walking (which is associated with mate searching) and sperm competitiveness.

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

We cannot definitively say why males from shorter-movement populations performed better in sperm competition when paired with control females (and

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

The improved siring success of less mobile males could also result from greater cryptic-female-choice for these males. Female *T. castaneum* exert control over sperm numbers transferred during mating and can use this as a mechanism for biasing the fertilization success in favor of preferred males (Fedina 2007). Accordingly, it is possible that the improved fertility of males from reduced movement populations represents cryptic-female-choice based on their increased copulatory courtship -

they rubbed females more during mating. Copulatory courtship is wide-spread (Eberhard 1994) and thought to be a major female determinant of siring success (Eberhard 1996). For example, male stimulation of females during mating enhances sperm transfer in crickets (Wulff et al. 2017) and siring success in spiders (Peretti and Eberhard 2009). The effect of rubbing behavior on siring success is less clear in *T. castaneum* with positive correlations between rubbing rate and paternity success reported (Edvardsson and Arnqvist 2000) and no association also found (Fedina and Lewis 2006). In any case we generally find males that move less tend to be better sperm competitors and certainly copulate for longer and engage in more copulatory courtship. Irrespective of the mechanism responsible, our results are therefore broadly consistent with a trade-off between post-copulatory sexual selection and mate searching. Although as noted, our results were not entirely consistent across experiments in statistical significance terms but trends were in the same direction.

In addition to differences in movement, mating behaviors and sperm competitiveness, selection also altered leg morphology. Males selected for shorter walking distances evolved the longest legs, but this difference was not seen in females. This result contrasts with previous work in *T. castaneum* showing that individuals with greater walking ability have relatively longer legs (Arnold et al. 2017). Our result is also counterintuitive because longer legs are associated with reduced energy expenditure in men and women walking quickly (Salamuddin et al. 2014) and leg length increases running speed in the fastest terrestrial animal (relative to its body size) (Rubin et al. 2016). All else being equal, long legs should positively correlate with mobility. However, theory predicts that longer legs only

increase mobility if the proportions of each leg segment do not change (i.e. the tarsus, tibia and femur all increase in length to the same degree (Leurs et al. 2011)). Here we see shifts in the relative proportions of male leg segments, suggesting that changes in leg length may be for a purpose other than improved mobility. Legs in the beetles are also associated with copulatory courtship (see above), which could explain why selecting for more and less movement resulted in different leg morphology of males, but not in females. We need additional detailed studies examining the relationship between male's leg length (in particular tarsus) and other reproductive traits in the future.

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

In conclusion, selection for reduced walking distance was effective, but somewhat counter-intuitively, this was associated with the evolution of longer legs and shifts in the relative proportions of leg segments. These morphological changes were correlated with improved male mating ability, increased copulation duration,

greater copulatory courtship and increased sperm competitiveness was evident at least some times. These results are more or less consistent with theoretical predictions of mate-searching/sperm competition trade-offs, but additional research is warranted to test this fundamental prediction.

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figure legends

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

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

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

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

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

Fig. 5. Results of fully-factorial selection matings for beetles from treatments selected for increased movement (L) and those selected for less movement (S). A shows the duration of mating. B shows the proportion of offspring sired (P2). Numbers in parenthesis show sample size. Error bars show standard error.

tables and table legends

Table 1. Results of MANOVA for each morphological trait. Significant values are 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

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

Leg	Factor	<i>d.f.</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		

Table 3. Results of GLMM for paternity success when males competed with 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		

Table 4. Results of GLMM for duration of copulation and P2 of males when 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		

SUPPLEMENTARY INFORMATION

1 **Table S1.** Mean ($\mu\text{m} \pm \text{SE}$) of length of each leg morphology. Letters indicate significant differences at $P < 0.05$ (Tukey's HSD test).
 2 For femur, L and W show length and width, respectively.

Leg	Segment	Male			Female		
		Long	Short	Control	Long	Short	Control
Foreleg	Tarsus	333.00 \pm 2.69 ^b	348.03 \pm 2.63 ^a	330.33 \pm 2.62 ^b	329.68 \pm 2.73 ^b	329.21 \pm 2.70 ^b	321.73 \pm 2.60 ^c
	Tibia	483.12 \pm 2.08 ^c	496.36 \pm 2.03 ^a	484.49 \pm 2.02 ^{bc}	482.90 \pm 2.12 ^c	487.85 \pm 2.09 ^{bc}	489.40 \pm 2.00 ^b
	Femur: L	584.23 \pm 2.37	586.78 \pm 2.32	581.05 \pm 2.31	577.13 \pm 2.40	577.34 \pm 2.38	576.80 \pm 2.29
	Femur: W	216.01 \pm 1.70 ^b	220.52 \pm 1.69 ^a	216.81 \pm 1.68 ^b	212.54 \pm 1.71 ^c	215.28 \pm 1.71 ^{bc}	217.15 \pm 1.68 ^b
Middle leg	Tarsus	397.29 \pm 4.07 ^b	406.29 \pm 4.02 ^a	393.25 \pm 4.01 ^{bc}	387.56 \pm 4.03 ^{cd}	386.06 \pm 4.08 ^{cd}	385.16 \pm 4.00 ^d
	Tibia	547.90 \pm 2.91	552.81 \pm 2.86	545.34 \pm 2.85	544.79 \pm 2.93	548.07 \pm 2.92	546.69 \pm 2.83
	Femur: L	614.67 \pm 2.42	616.52 \pm 2.36	616.67 \pm 2.34	611.23 \pm 2.45	608.75 \pm 2.44	610.71 \pm 2.32
	Femur: W	204.91 \pm 1.01	204.28 \pm 0.98	204.31 \pm 0.97	203.76 \pm 1.02	201.76 \pm 1.02	204.22 \pm 0.96
Hind leg	Tarsus	439.65 \pm 3.00 ^{bcd}	457.67 \pm 2.95 ^a	442.08 \pm 2.93 ^b	433.81 \pm 3.04 ^d	442.48 \pm 3.04 ^{bc}	435.02 \pm 2.91 ^{cd}
	Tibia	653.19 \pm 3.17	664.22 \pm 3.11	653.42 \pm 3.10	651.25 \pm 3.21	656.06 \pm 3.20	653.46 \pm 3.08
	Femur: L	733.15 \pm 2.48 ^{bc}	742.84 \pm 2.43 ^a	736.89 \pm 2.41 ^{ab}	728.35 \pm 2.53 ^c	729.69 \pm 2.52 ^c	729.77 \pm 2.39 ^c
	Femur: W	238.10 \pm 2.19	239.19 \pm 2.18	234.85 \pm 2.18	234.99 \pm 2.20	235.33 \pm 2.20	232.60 \pm 2.17

4 **Table S2.** ANCOVA as post-hoc test for results of MANOVA (L: length, W: width).

Leg	Segment	Factor	<i>df.</i>	<i>F</i>	<i>P</i>	
Foreleg	Tarsus	Treatment	2	6.87	0.0268	
		Sex	1	57.89	< 0.0001	
		Treatment*sex	2	11.82	< 0.0001	
	Tibia	Treatment	2	6.26	0.0306	
		Sex	1	1.27	0.2613	
		Treatment*sex	2	13.77	< 0.0001	
	Femur: L	Treatment	2	0.54	0.6095	
		Sex	1	36.85	< 0.0001	
		Treatment*sex	2	2.03	0.1329	
	Femur: W	Treatment	2	1.34	0.3316	
		Sex	1	24.26	< 0.0001	
		Treatment*sex	2	9.99	< 0.0001	
	Middle leg	Tarsus	Treatment	2	0.82	0.4857
			Sex	1	79.21	< 0.0001
			Treatment*sex	2	7.85	0.0004
Tibia		Treatment	2	0.85	0.4741	
		Sex	1	2.93	0.0875	
		Treatment*sex	2	2.45	0.0872	
Femur: L		Treatment	2	0.06	0.9386	
		Sex	1	19.55	< 0.0001	
		Treatment*sex	2	0.96	0.382	
Femur: W		Treatment	2	0.73	0.5214	

		Sex	1	4.81	0.0286
		Treatment*sex	2	1.75	0.1741
Hind leg	Tarsus	Treatment	2	7.26	0.0252
		Sex	1	37.71	< 0.0001
		Treatment*sex	2	3.92	0.0204
	Tibia	Treatment	2	2.20	0.1936
		Sex	1	4.95	0.0265
		Treatment*sex	2	2.98	0.0514
	Femur: L	Treatment	2	1.62	0.2749
		Sex	1	36.81	< 0.0001
		Treatment*sex	2	3.39	0.0343
	Femur: W	Treatment	2	0.77	0.5023
		Sex	1	23.48	< 0.0001
		Treatment*sex	2	0.62	0.5373

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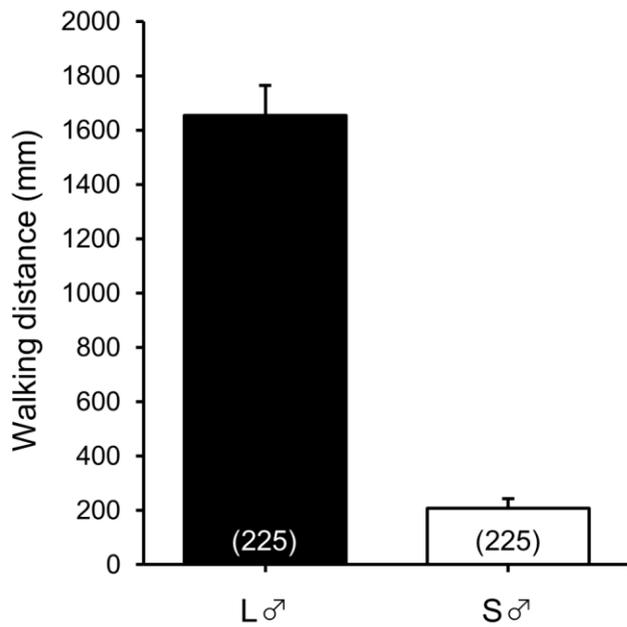
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10 **Fig. S1.** Direct responses to artificial selection for walking distance (30 min) in
11 males of *T. castaneum* during 22 generations. Solid and broken lines show L and S
12 treatments. Error bars show SE.

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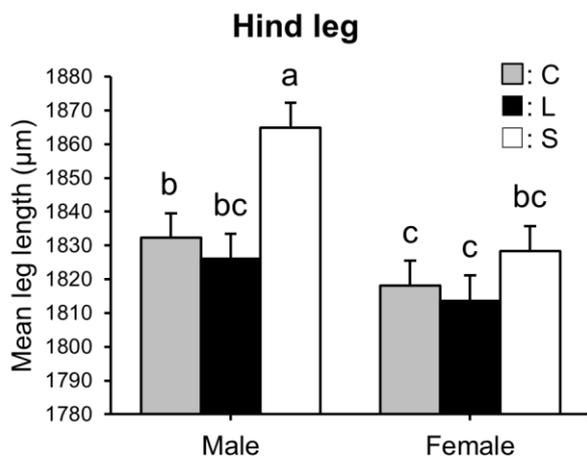
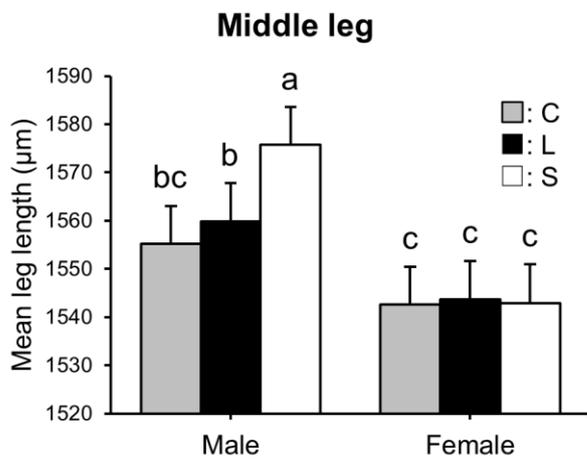
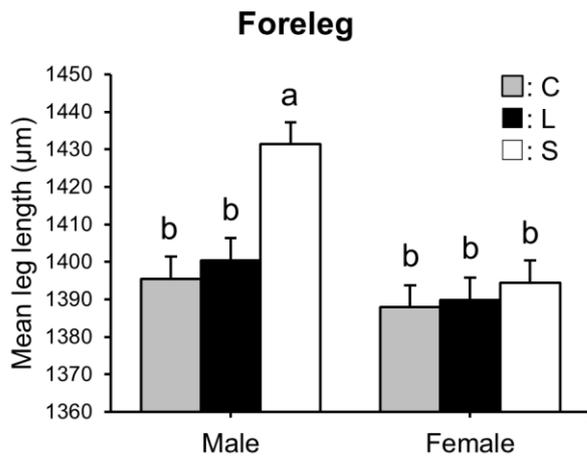
14 **Figure S2.** Average length of the tarsus (triangles), tibia (square) and femur
15 (circle) for males (A) and females (B) from control populations (C) (grey points),
16 and populations selected for increased mobility (L) (black points) and populations
17 selected for reduced mobility (S) (open points). Error bars show standard errors
18 around the mean.

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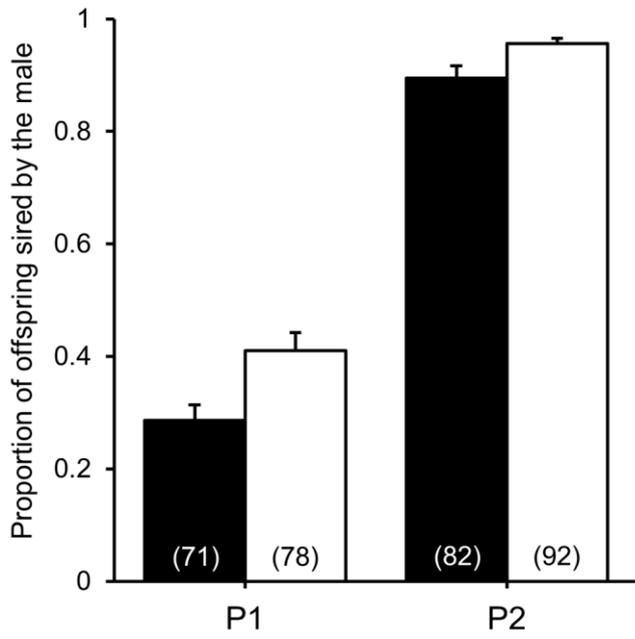
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21 Fig. 1



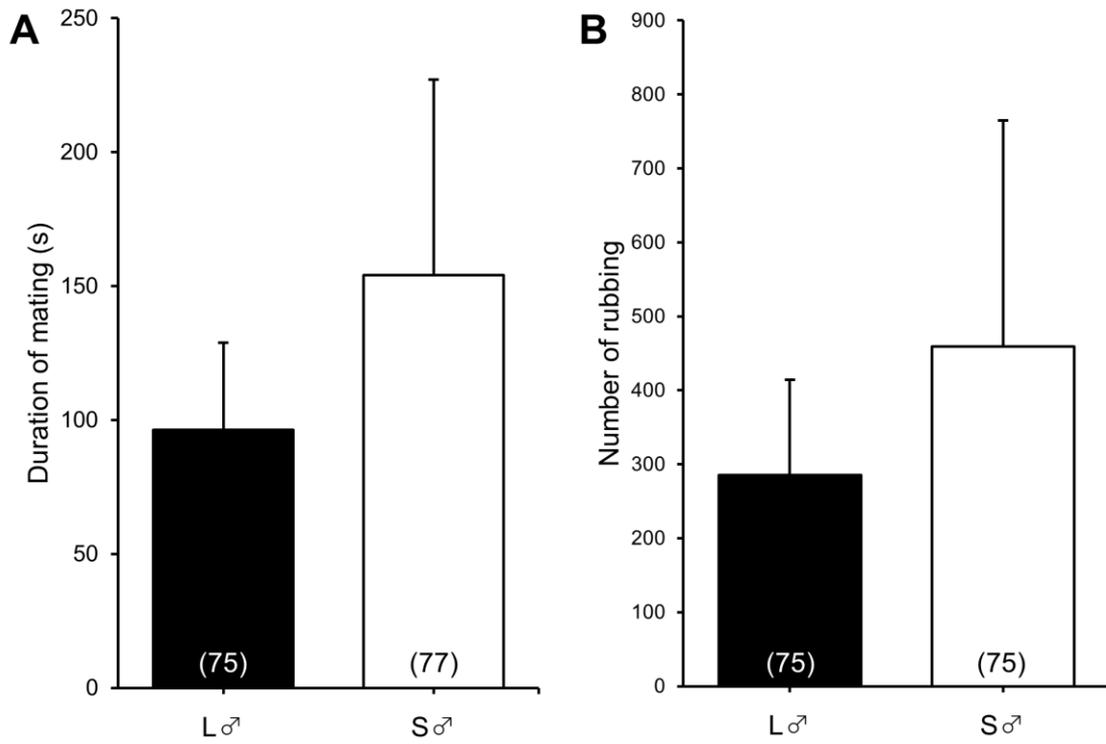
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23 Fig. 2



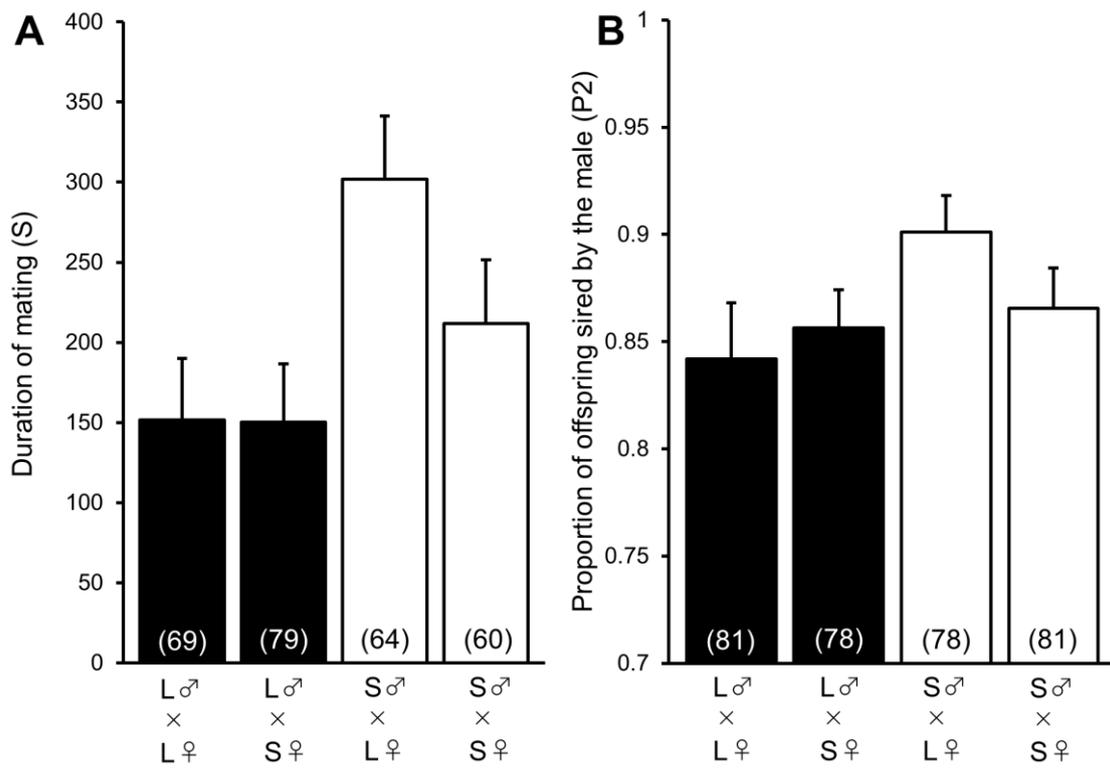
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25 Fig. 3



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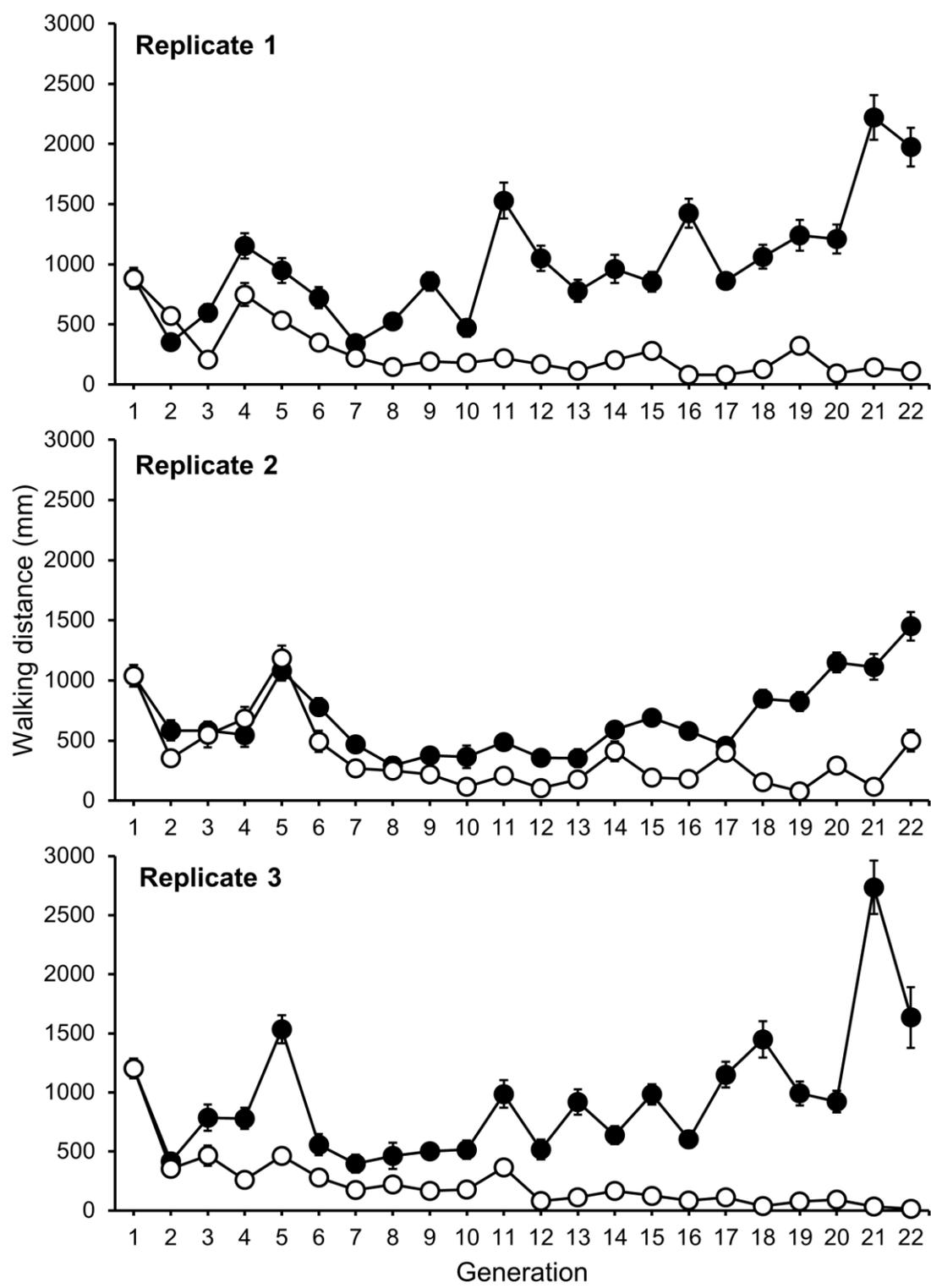
27 Fig. 4



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29 Fig. 5

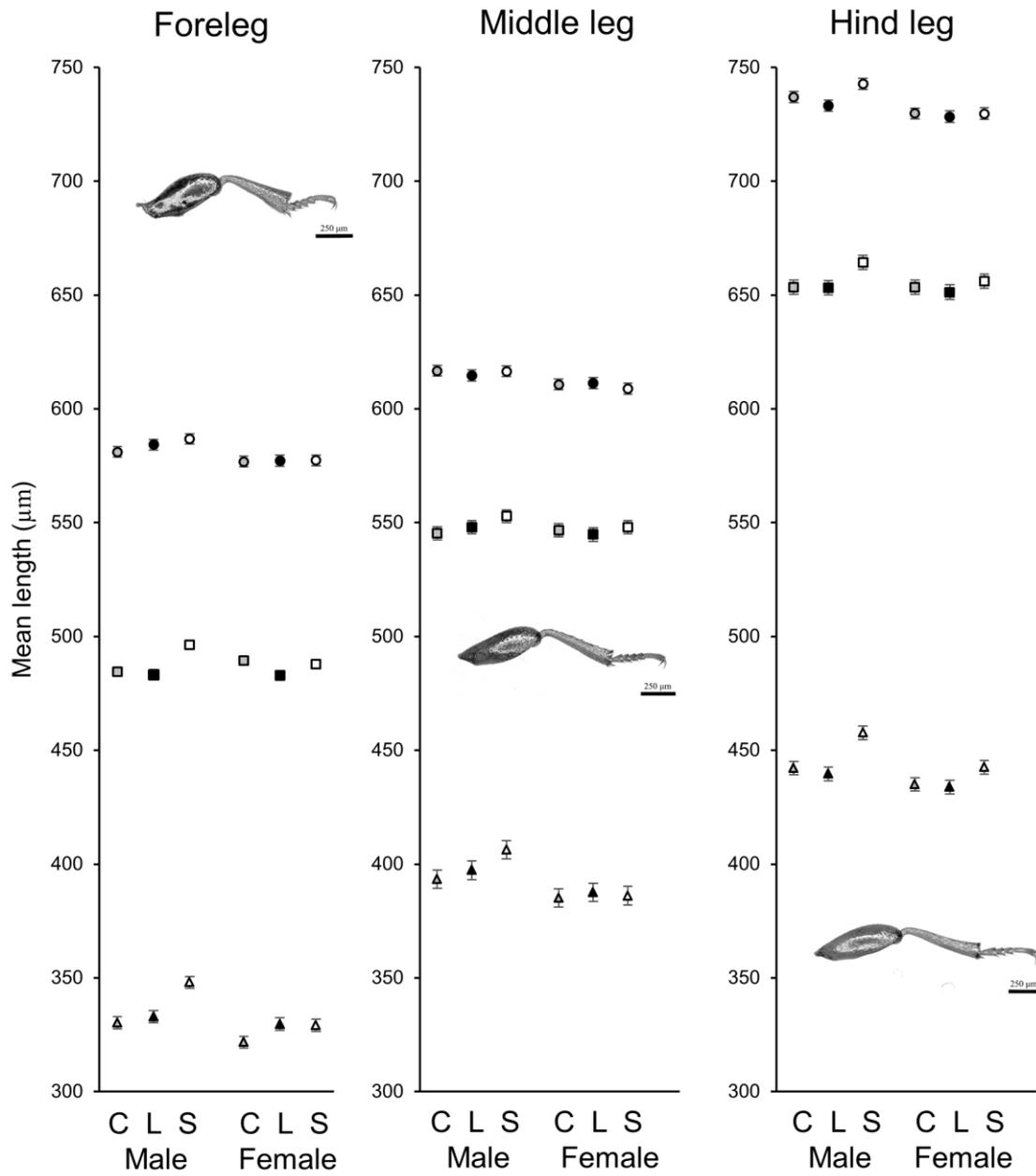
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33 S1 Fig.



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35 S2 Fig.

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