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# Intersexual correlation for same-sex sexual behaviour in an insect

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Same-sex sexual behaviour is widespread across taxa and is particularly common in insects, in which up to 50% of copulation attempts by males are directed towards other males in some species. Research effort has focused on male–male same-sex behaviour and the prevailing theory is that benefits of high mating rate combined with poor sex discrimination explain the high incidence of male–male mounting. However, the evolution of female–female mounting is more enigmatic, since females typically do not mount males in order to mate. Using a full-sib design, we found an intersexual correlation for same-sex mounting in the beetle *Callosobruchus maculatus*. Variation in male–male mounting across families explained over 20% of variation in female–female mounting. Moreover, we found no evidence that same-sex behaviour was related to general activity level in either sex or carried a fitness cost to females. Taken together, our results suggest that female–female mounting is a relatively low-cost behaviour that may be maintained in the population via selection on males.

Same-sex sexual behaviour (SSB) is common in nature and taxonomically widespread (Bagemihl 1999; Sommer & Vasey 2006; Bailey & Zuk 2009; Poiani 2010). A recent realization of this fact has led to a current surge of interest in the evolution and maintenance of this phenomenon as the adaptive value of SSB is not immediately apparent (Gavrilets & Rice 2006; Bailey & Zuk 2009; Poiani 2010). Because different researchers treat SSB differently in a variety of contexts, it is important to define it clearly to avoid confusion. Here we adopt a broad definition of SSB as actions between members of the same sex that are commonly attributed to opposite-sex courtship and mating (Bailey & Zuk 2009).

There is a staggering number of hypotheses proposed to account for the evolution and maintenance of SSB and many of them are not mutually exclusive (Sommer & Vasey 2006; Bailey & Zuk 2009; Dukas 2010; Poiani 2010). While the causes and consequences of same-sex interactions are likely to differ considerably across taxa, genetic studies in model organisms can help identify common patterns in the evolution of these behaviours (Bailey & Zuk 2009). Same-sex mounting is common in insects (Thornhill & Alcock 1983; Maklakov & Bonduriansky 2009), and sometimes up to 50% of all male mating attempts are directed towards other males (Aiken 1981; Serrano et al. 1991, 2000). Such high frequency of male–male mounting is often attributed to poor sex recognition (Parker 1968; Serrano et al. 1991, 2000; Harari et al. 2000; Switzer et al. 2004) combined with the benefits of increased overall mating frequency to males (Thornhill & Alcock 1983). Given high variance in male mating success and a strong relationship between male mating success and male fitness (Bateman 1948; Trivers 1972; Scharer et al. 2012), it is likely that strong selection on mounting behaviour in males will result in perception errors (Thornhill & Alcock 1983; Bailey & French 2012). The notion that SSB in males may commonly be the result of perception errors, rather than a preference for other males, is indirectly supported by the finding that fruit flies, *Drosophila melanogaster*, expressing mutations that reduce their ability to perceive their surroundings increase the instances of male–male behaviour (Zhang & Odenwald 1995; Ryner et al. 1996; Kurtovic et al. 2007). However, while much of the research effort has focused on male–male sexual interactions, females also show SSB in many species (Srivastava et al. 1991; Bagemihl 1999; Sommer & Vasey 2006; Gastal et al. 2007; Poiani 2010), including female–female mounting in insects (Harari & Brockmann 1999; Harari et al. 2000; Stojkovic et al. 2010). The occurrence of female–female mounting is enigmatic since this behaviour is not a part of the typical courtship repertoire during mating (Harari et al. 2000; Stojkovic et al. 2010) and, therefore, cannot be explained as perception errors.

We studied SSB in a widely used model organism for behavioural and life history research, the seed beetle, *Callosobruchus maculatus*. There are several advantages of using *C. maculatus*,

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among them the possibility of conducting experimental laboratory assays under the conditions reflecting the recent evolutionary history of the population (see Methods), as well as the fact that SSB has been observed in different species of seed beetles (Maklakov & Bonduriansky 2009; Stojkovic et al. 2010). There is a close resemblance between female–female mounting and intersexual copulation and even experienced workers can be misled by female behaviour while sexing the animals for experimental work. Despite this, female seed beetles do not mount males in order to mate nor do they actively search for mates in this system. *Callosobruchus maculatus* beetles are protandrous (Fox et al. 2003) such that males emerge earlier than females and patrol the host seeds for newly hatched virgins and mate with all of them (Arnqvist & Tuda 2010). Therefore, virgin females do not search for mates but instead they are surrounded by many males attempting copulations immediately after emergence. It has been shown in a related species of seed beetle that such early mating is in fact disadvantageous for females, which are better off if they start to reproduce a little bit later (Maklakov et al. 2007).

One possibility is that female–female mounting is the consequence of strong selection for mating rate in males and an intersexual correlation for mounting behaviour, combined with a relatively low cost of same-sex interactions for females. This hypothesis is indirectly supported by data from three different species of insects showing that the costs of same-sex interactions are indeed much lower for females than for males (Maklakov & Bonduriansky 2009; Stojkovic et al. 2010). In this study we used a full-sib breeding design to test this hypothesis.

## METHODS

### Study System

*Callosobruchus maculatus* is a world-wide pest of human grain stores. This species is therefore well adapted to environmental conditions that are easy to recreate in the laboratory (Fox et al. 2003; Messina & Karren 2003), which makes it a very popular model organism in evolutionary ecology research, including studies of opposite-sex (Edvardsson & Arnqvist 2005) and same-sex (Maklakov & Bonduriansky 2009) sexual behaviour. This species is easily maintained in the laboratory in part because it is a capital breeder that acquires all of the resources necessary for adult life during the larval stage in the host seed.

We used the well-studied laboratory population SI (South India) of *C. maculatus*, which originated from infested mung beans, *Vigna radiata*, from Tirunelveli, India in 1979 (Messina & Slade 1997) and was obtained from C.W. Fox, University of Kentucky, Lexington, KY, U.S.A. This is a large outbred population (>1500 individuals per generation) that has been maintained in the laboratory for hundreds of generations. The beetles were kept at 30 °C and 50% relative humidity. Using long-term laboratory-adapted outbred populations is advantageous because it allows researchers to measure traits in the environment to which the population is adapted. This approach has been called 'island laboratory analysis' (Rice et al. 2005, 2006) and we have previously described the rationale for measuring focal traits in seed beetles in this context (Maklakov et al. 2007).

### Experimental Procedures

Virgin male and female beetles from the SI population were used to create 80 breeding pairs in two equally sized blocks, from which 77 pairs produced fertile eggs (block 1: $N = 40$; block 2: $N = 37$). Virgin beetles were housed in individual plates before the experiment to eliminate potential effects of early social interactions on the behaviour during the experimental trials. Each pair was allowed to mate and reproduce in individual petri dishes. Larval competition was eliminated by providing beetles with sufficient host beans, since females generally lay only one egg per bean. Upon hatching, the virgin offspring of these pairs were used to estimate the rate of same-sex behaviour in males and females in each family by scoring the total number of mounts in same-sex groups of five beetles placed into a 60 mm petri dish for 10 min. Briefly, same-sex mounting was scored when one beetle had positioned itself behind another beetle, standing on its hindlegs and placing its front legs on the rear abdomen or elytra of the mounted beetle, thus supporting itself in an upright position. The beetle then remained still apart from occasionally striking the back of the mounted beetle with the front legs and antennae. Thus, the mounting beetle stood on its hindlegs next to the genital opening of the mounted beetle, rather than being positioned on its back. This behaviour is indistinguishable from heterosexual mountings and very different from beetles simply crawling over each other or the beans. Genitalia are occasionally protruded but this behaviour was not scored because it was difficult to see without disturbing the beetles. We scored two petri dishes per sex and family and used the mean values of these replicates in the analyses. The experiments were conducted in a climate room at 30 °C and 50% relative humidity and the density in the petri dishes reflected the density in the population cages in which these beetles evolved for hundreds of generations. We used families from the second block only ($N = 35$, two families did not produce enough offspring for both assays) to estimate the general activity rates in each sex by placing five siblings of beetles used in same-sex assays into 60 mm petri dishes (two dishes per sex per family) and scoring the number of moving beetles every 10 min for 1 h. Finally, we ranked the families based on their same-sex performance and used the female siblings from the top eight and bottom eight families from the first block ($N_{\mathrm{total}} = 16$ families) to estimate the relationship between the level of same-sex behaviour and female lifetime realized fecundity. Fecundity was assayed in two replicates per family. In each replicate three individual focal females were kept with three standardized 'tester' males randomly sampled from the source population and all eggs produced by these females until death were scored. Individual beetles were never used more than once in these experiments.

### Statistical Analyses

We used a general linear mixed model (GLMM) with log female–female mounting rate (mean mounting per 10 min per replicate, averaged across two replicates per family) as the dependent variable, log male–male mounting rate as an independent variable and block as a random effect to test for the effect of male same-sex behaviour on female same-sex behaviour across 77 families. We used a paired *t* test to compare the mean rate of same-sex behaviour between the sexes and least-squares regressions to estimate the effect of general activity rate on the same-sex behaviour rate in both sexes, as well as to estimate the effect of female–female mounting on female lifetime fecundity in 16 families.

## RESULTS

Male–male mounting rate was higher than female–female mounting rate (mean ± SE; males: $15.66 \pm 1.09$; females: $5.33 \pm 0.38$; paired *t* test: $t_{77} = 10.76$, $P < 0.0001$). There was a strong effect of male–male mounting rate on female–female mounting rate (Fig. 1; main effect: $F_{1,72.32} = 9.03$, $P = 0.004$). Random block and the random block*male–male mounting interaction were both removed from the final model because neither of these

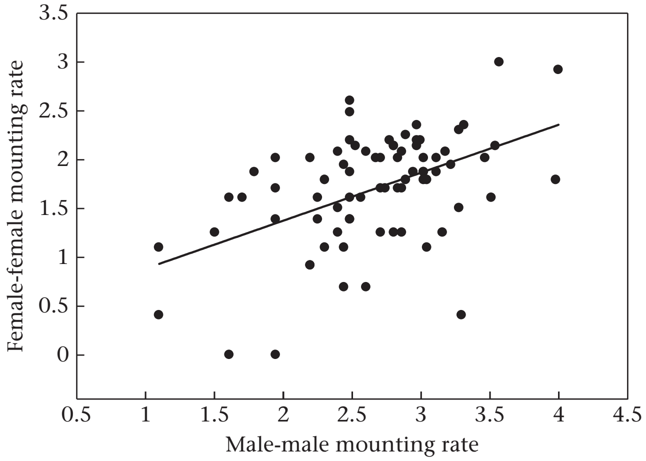

**Figure 1.** The correlation between log-transformed same-sex mounting rate (see Methods) in males and females as measured across 77 families of *C. maculatus* ($r = 0.43$, $P < 0.0001$). The line represents the least-squares regression of female–female mounting on male–male mounting (see Results for statistics).

effects explained any variance in female–female mounting rate (variance component ± 95% confidence interval, CI, for block: 0.061 [−0.131–0.255]; for block*male–male mounting: 0.081 [−0.214–0.377]). We therefore used least-squares regression to estimate that male–male mounting rate explained as much as 22.7% of variation in female–female mounting rate ($F_{1,75} = 23.41$, $P < 0.0001$). We found no effect of general activity rate on same-sex behaviour rate, either in males ($F_{1,33} = 0.003$, $P = 0.956$) or in females ($F_{1,33} = 0.352$, $P = 0.557$). Moreover, there was no relationship between the activity rates of the two sexes ($F_{1,33} = 0.014$, $P = 0.907$; Fig. 2). We also found no effect of same-sex behaviour rate in females on lifetime fecundity ($F_{1,15} = 0.001$, $P = 0.971$).

## DISCUSSION

Female–female same-sex behaviour is a widespread but largely unexplained phenomenon. In this study we found an intersexual correlation for same-sex behaviour across a large number of families in an outbred population of *C. maculatus* seed beetles. We further found that this pattern cannot simply be explained by general activity.

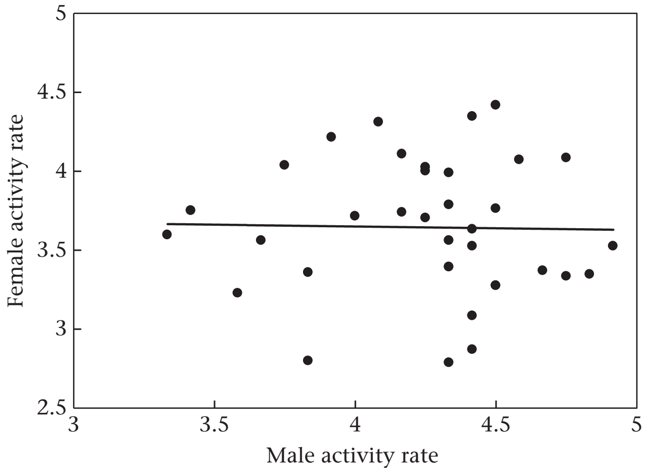

**Figure 2.** The correlation between general activity rate (see Methods) in males and females across 35 families ($r = 0.02$, $P = 0.907$). The line represents the least-squares regression of female activity rate on male activity rate (see Results for statistics).

While female–female mounting is puzzling, earlier studies suggested that females may mount each other in order to attract males (Thornhill & Alcock 1983; Harari & Brockmann 1999). Different versions of this hypothesis suggest that dominant males can be attracted to copulating pairs to drive away subordinate males (Thornhill & Alcock 1983) or because males are attracted to bigger and more fecund females, while copulating pairs appear to look like a very large female (Harari & Brockmann 1999). We do not discuss the relative merits of these hypotheses here but we note that any behaviour aimed at attracting males would be highly detrimental to female fitness in this system (Maklakov & Arnqvist 2009). The costs of mating in seed beetles are extremely high and females spend most of their life physically resisting innumerable mating attempts by persistent males (Maklakov & Arnqvist 2009). It has been shown previously that when female resistance is experimentally increased, resulting in fewer successful matings, it leads to increased net fitness of these females compared to their counterparts with reduced resistance (Maklakov & Arnqvist 2009). While the aforementioned work focused on lifelong effects of male harassment on female fitness in seed beetles, the same logic applies to virgin females. As discussed in the Introduction, female *C. maculatus* in this population do not search for mates because they are approached by multiple males seeking to mate with virgin females directly after emergence (Arnqvist & Tuda 2010). Thus, both virgin and mated females are unlikely to benefit by attracting males in this population.

Earlier studies of male–male mounting in animals suggest that this behaviour has a strong genetic component (Poiani 2010). Artificial divergent selection for male–male mounting in flour beetles, *Tribolium castaneum,* produced a significant response in just two or three generations (Castro et al. 1994). Because males and females share most of their genes, a behaviour selected for in one sex is likely to be present in the other sex unless it is selected against because of the costs associated with bearing the trait. However, the resolution of such intralocus sexual conflict and evolution of either complete or partial sexual dimorphism for a trait is expected to be inherently slow and to depend on the sex-specific genetic architecture and the strength of selection (Lande 1980).

We conducted this study on full-sib families and we therefore cannot irrefutably conclude that the intersexual correlation we found is genetic. A potential alternative hypothesis is that the intersexual correlation is caused by either a maternal or a paternal effect. While both maternal and paternal effects have been widely documented in this species (Fox 1993, 1994; Fox et al. 2003; Bilde et al. 2008), we find it highly unlikely that these effects would change the direction of the underlying genetic correlation. Phenotypic correlations between different traits are, in the vast majority of cases, good estimates of genotypic correlations (Roff 1992, 1995), and a recent study specifically indicates that phenotypic correlations can be used to estimate the direction and the magnitude of genetic correlations for behavioural traits (Dochtermann 2011). Finally, this is, to the best of our knowledge, the first demonstration of intersexual correlation for SSB on the level of a population.

Our results are consistent in several ways with the hypothesis that female–female mounting is maintained in the population because of the selection on males. First, we have shown that male–male mounting rate is a strong predictor of female–female mounting rate across families. Second, we found that female–female mounting is less common than male–male mounting. Third, same-sex behaviour is not associated with a cost in terms of female lifetime reproductive performance. This finding is further supported by the previous studies showing lower longevity costs of SSB in females compared to males (Maklakov & Bonduriansky 2009; Stojkovic et al. 2010). Our study suggests that these longevity costs do not necessarily translate into reproductive costs. Finally, the lack of intersexual correlation for activity levels suggests that our result for same-sex behaviour is directly pertinent to this trait and not a reflection of general locomotory behaviour. This study demonstrates that the rate of same-sex behaviour in one sex can be used to predict the rate of same-sex behaviour in the other sex and we hope it will stimulate further research into the origin and maintenance of female–female sexual behaviour.

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