Behav Ecol Sociobiol (2009) 63:817–823
DOI 10.1007/s00265-009-0716-7

ORIGINAL PAPER

Repeatability and heritability of sperm competition outcomes in males and females of *Tribolium castaneum*

Tom Tregenza · Fathi Attia · Saleh Salem Bushaiba

Received: 14 June 2008 / Revised: 12 January 2009 / Accepted: 15 January 2009 / Published online: 12 February 2009

Abstract Differences among males in their success in achieving fertilisations when females mate with more than one partner are now recognised as an important target of sexual selection. However, very few studies have attempted to determine whether particular males are consistently successful in sperm competition and whether success in sperm competition is a heritable trait. Additionally, the potential heritability of female traits that influence the outcome of sperm competition has received only limited attention. Using the polyandrous beetle *Tribolium castaneum*, we examined repeatability of male success in sperm competition by mating pairs of males carrying different visible genetic markers to a string of different females. Males showed consistency in their ability to successfully transfer sperm to females, but not in their success in sperm competition. Furthermore, when we independently compared success in sperm competition of fathers with their sons, we found no evidence for heritability of this trait. Similarly, females that exhibited high or low first male sperm precedence did not tend to have daughters that showed the same pattern. Our results suggest that we should be wary of assuming that success in sperm competition is heritable through either sex.

Keywords Polyandry · Mate choice · Post-copulatory · Sexy sperm · P2 · Flour beetle

Communicated by M.-Siva-Jothy

T. Tregenza (✉)
Centre for Ecology and Conservation, School of Biosciences,
University of Exeter,
Cornwall Campus, Tremough,
Penryn TR10 9EZ, UK
e-mail: T.Tregenza@exeter.ac.uk

F. Attia · S. S. Bushaiba
Department of Zoology, Faculty of Science,
University of Garyounis,
P.O. Box 17074, Benghazi, Libya

Introduction

The importance of post-copulatory processes in the reproductive success of males is now well established. The fundamental role of sperm competition in sexual selection is supported by numerous studies revealing variation among males in their adaptations to sperm competition among species (Birkhead and Møller 1998; Simmons 2001). However, there have been few studies examining how consistent individual males are in their success in sperm competition or measuring heritability of sperm competitive ability (see 'Discussion' section). Even less evidence exists in relation to the potential existence of heritable traits expressed in females that affect the outcome of sperm competition between their mates. This is an important question because there are potential costs and benefits to females associated with variation in fertilisation success of males according to the order in which they mate with a female, but we do not know if females can actually respond to selection pressures on these characteristics, as they may be strictly male traits.

If matings have a net cost for females (females that mate fewer times have higher fitness), then females would benefit from having a very high P1 (the proportion of offspring sired by the first male to mate). A very high P1 would mean that males would not gain paternity from mating with non-virgin females and hence could not increase their reproductive success by harassing females, and sexual conflict over mating rate would cease to be expressed. Therefore, it is puzzling that high P2 is much more common than high P1 in the insects (Simmons 2001). There are a number of possible explanations for this. One is that, even if high P1 is advantageous for females, selective constraints prevent them from achieving it. This might be the case because even if a population is characterised by sperm mixing (arguably the simplest scenario), a mutant female with a P1 of 100% would not avoid harassment, because males would not have evolved any mechanism for recognising this trait in females. Additionally, the mutant female would incur the costs of having male offspring who would tend to be less effective at sperm competition because their father would not have been under selection for being good at sperm competition. Hence, the lack of immediate benefits to females of high P1 may prevent high P1 from evolving even if this is possible through changes in sperm storage organ morphology and even if it would be beneficial for females (and would increase the potential fitness of the population relative to populations with more sexual conflict). Another possibility is that there are other benefits of high P2 such as the possibility that it allows females to avoid using older sperm (Siva-Jothy 2000). Alternatively, it is possible that females have no control over the level of sperm precedence and that it is simply a male trait dictated by how good males are at removing one another's sperm, producing large ejaculates and other related adaptations.

Our aim was to examine the repeatability of success in sperm competition and the potential heritability of this trait in males and females in the red flour beetle *Tribolium castaneum.* This species has been widely exploited as a model system for studying sexual selection and multiple mating (Fedina and Lewis 2008). Females are highly polyandrous but evidence regarding benefits to females of this behaviour is mixed. Lewis and Austad (1994) found increased fecundity with multiple mating when matings took place over several days whereas Pai and Yan (2003) found no effects when females mated differing numbers of times over a 24-h period and subsequent studies have found variation among populations in benefits to females of polyandry (Pai et al. 2007). Lewis and Austad (1990) examined variation in sperm competitive success in *T. castaneum* by placing females with a single male for 24 h and then with a second male for a further 24 h. They found significant variation among males in the number of offspring they sired following these mating periods. However, their design included variation in competition among ejaculates once the female had sperm from two males as well as variation due to the number of matings males achieved and due to whether or not these matings resulted in sperm transfer (although all females had offspring from both males, the relative fertilisation success of males could have been due to differences in the number of matings they achieved). Hence, it is not clear to which of these factors the differences among males and male × female genotype interactions observed in that study were due.

T. castaneum lends itself to studies of sperm competition because visible single locus genetic markers are available that allow rapid determination of paternity in large numbers of individuals. It is straightforward to measure repeatability of success in sperm competition in males by mating the same pair of males to a series of different females. An equivalent approach in females is much more difficult because sperm storage by females means that a single female cannot host independent sequential sperm competitions. Hence, we aimed to measure heritability of P1 and P2 as potentially expressed in females despite not being able to determine repeatability beforehand. The ideal approach to measuring genetic variance is to use a full-sib: half-sib design which allows for partitioning of additive and non-additive variance and non-genetic maternal effects. However, a major constraint on this type of study in *T. castaneum* is that a large proportion of matings fail to result in sperm transfer; hence, large numbers of matings are needed to provide a sufficient number of successful double matings, a problem that is multiplied when the experimental design requires data from several siblings all of whom must be doubly inseminated. We therefore designed our study to maximise our power to detect potentially heritable effects (at the cost of being able to partition variance) by choosing to measure P1 and P2 in sons and fathers and mothers and daughters.

Methods

All experiments were conducted using two strains of *T. castaneum* Ga1 (wild type) and Rd (reindeer antennae) supplied by R. Beeman, US grain marketing and production research center. Rd is a strain derived from Ga1 but carrying a single locus dominant marker that causes a pronounced swelling of the antenna. Beetles were maintained at population sizes of hundreds of individuals in a mixture of 95% flour and 5% baker's yeast in a dark incubator at 30°C and approximately 65% Rh. Pupae of both strains were collected, sexed and isolated to ensure virginity, and kept individually in cells of 5 × 5 cell plastic boxes containing flour. Eclosion dates were recorded for each beetle and adult virgin beetles of 7–13 days post-eclosion at the beginning of the experiment were used. To conduct matings, a male and female were placed together in a 2 × 2 cm cell, the bottom of which was covered with filter paper to provide traction. Pairs were separated after a single copulation occurred (typically within a few minutes). The time to mating and the duration of the mating were recorded. Only copulations lasting longer than 40 s were included in subsequent analyses, as shorter interactions may not allow the opportunity for sperm transfer to occur. In both studies described below, a virgin Ga1 female was first mated with a male carrying one marker (Ga1 or Rd) and then to a male carrying the other maker 24 h after the first copulation. After each double mating, the female was placed in a 100-ml pot containing 30 ml fine organic flour for 7 days at 30°C and 65% Rh to lay eggs. Forty days after the last day of oviposition, offspring were counted (providing ample time for all fertile eggs to hatch and reach adulthood). Where no offspring were sired by one of the males, we assume that this is because there was a complete failure to transfer an ejaculate by this male. This assumption is based on previous observations that rates of complete failure to sire offspring by one or both males in double mated females fitted with those you would expect from rates of complete fertilisation failure in single mated females (Attia 2004). Additionally, since the mean number of offspring scored for paternity in our study was 259 (standard error=9.6), the likelihood that very poor performance of an ejaculate in sperm competition could be confused with failure to transfer sperm is very small. Males were killed by freezing immediately after mating, females were killed after oviposition and the size of both sexes was measured using a microscope with graticule.

Repeatability of male success in sperm competition

Twelve pairs of a Ga1 and an Rd male were designated. Each male mated once every 24 h throughout the study. For each pair of males, a virgin Ga1 female (female number 1 for this male pair) mated to a Ga1 male on the first day, while the Rd male of the same male pair mated to another virgin Ga1 female (female number 2). The same male pair mated for a second time 24 h later, when the Ga1 male mated to female 2 and the Rd male mated to female 1. The females were then placed in individual 100-ml pots containing 30 ml fine organic flour at 30°C and 65% Rh to lay eggs. After 7 days of oviposition, each female was removed from her pot and the eggs were left to develop. Offspring of each female were counted 45 days after the female had been removed. The mating procedure was repeated at 24-h intervals until each male pair had mated to 16 females with alternating mating orders to produce eight independent measures of P_2 for each male. Pairs were observed until the end of a single copulation, when male and female separate. Time to mating and duration of mating for each mating were recorded.

Determining the heritability of P2

Determination of repeatabilities of male success in sperm competition does not represent a definitive test for presence or absence of a genetic basis for this trait; our parent offspring comparison provides independent data relating to this question. More importantly, even if male P2 is not heritable, there might still be heritable female influences on the relative success in sperm competition of her first and second mates, which can only be examined by measuring resemblance between mothers and daughters.

We performed 200 trials in which 200 Ga1 females were mated to 100 pairs of a Ga1 and an Rd male. Each male pair was mated to two different Ga1 females at 24-h intervals over a period of 72 h. On the first day, a Ga1 female (female 1) was mated to a Ga1 male; 24 h later, female 1 was mated to an Rd male while the Ga1 male of the same male pair was mated to another Ga1 female (female 2). On the third day, female 2 was mated to the Rd male of the same male pair. Mating duration was recorded. After double mating, each female was placed singly in a 100-ml pot containing 30 ml flour for 7 days in a dark incubator at 30°C and 65% Rh to lay eggs. Every week, each female was transferred to a new pot with fresh flour. Forty days after the last day of oviposition (ample time for all fertile eggs to hatch and reach adulthood), offspring were counted and paternity assigned via their visible genotype. This process was repeated for 4 weeks.

To provide the second generation, six male and six female pupae were collected at random from each pot containing offspring from both Ga1 and Rd males and were placed singly in separate cells of a 5×5 cell box. After eclosion, all Rd females were discarded. Second generation Ga1 females and both Ga1 and Rd males were used to measure sperm precedence in offspring: Each Ga1 female was mated first to a Ga1 male followed by an Rd male 24 h later and the duration of both matings was recorded. Matings or competition between siblings was completely avoided. After double mating, females were kept singly and paternity assessed in exactly the same way as in the previous generation. All P2 values were arcsine square-root transformed to normalise their distribution.

Of the 200 females that we mated to two males, 101 produced offspring from both males. Offspring were collected from each of these 101 females. To ensure at least one female offspring was successfully mated to two males, we mated up to three Ga1 marker strain daughters from each female to two males as described above. Data were used from one daughter of each female that had offspring from two males (chosen at random when there was more than one). This gave us 58 daughters of unique mothers that produced offspring from both males. Hence, we had P1 and P2 values for 58 independent mother–daughter pairs and 58 independent father–son pairs from the same data.

Results

Sperm competition repeatability

There were significant differences among Ga1 males in whether or not they were successful in sperm transfer (chi-square test of the distribution of success and failures among

Fig. 1 Number of matings out of 16 on consecutive days in which each male was successful in transferring sperm to the female. Mating order was alternated on each mating

males; $\chi^2=77.2$, $df=11$ and $P<0.001$), while differences among males were not significant in the Rd strain ($\chi^2=9.4$, $df=11$ and $P=0.58$; data in Fig. 1). Overall, Rd males were more successful in transferring sperm (165/192 matings successful, mean of 13.8/16, compared with Ga1 males 137/192 matings successful; mean=11.4/16). However, since males vary, the appropriate comparison is among male pairs, which reveals no difference in the relative success of Rd and Ga1 males (Wilcoxon matched pairs test $n=12$, $W_+=27.5$, $P=0.42$). However, there was a significant difference in sperm transfer success between first and second matings (2×2 chi-square test of mating order vs. success or failure to transfer sperm: $\chi^2=45.6$, $df=23$, $P<0.05$) with the second male to mate more frequently successful in sperm transfer. Since 100 pairs of males mated to two different females, we can also examine repeatability of success in sperm transfer in our sperm competition heritability experiment. This reveals significant repeatability of the sperm transfer success of the second male to mate (which was always an Rd male) (Spearman correlation, $n=100$, $r=0.332$, $P=0.001$), but no significant repeatability of the first male to mate (which was always a Ga1 male) ($n=100$, $r=0.07$, $P=0.49$).

There was no difference between male pairs in their mean P_2 (two-way ANOVA, factors=male pair and strain of first male to mate, $F_{10,94}=0.98$, $P=0.47$); no effect of strain of the first male on P_2 ($F_{1,94}=0.015$, $P=0.90$) and no interaction between male pair and the order of males by strain ($F_{10,94}=0.68$, $P=0.74$). Similarly, we found no differences between males pairs in the number of offspring that females produced over the next 7 days ($F_{10,94}=0.29$, $P=0.98$) and mating order of males and the interaction between male pairs and the order of males by strain also had no effect on offspring production ($F_{1,94}=0.89$, $P=0.35$ and $F_{10,94}=0.46$, $P=0.91$). We also examined repeatability of sperm precedence in our second experiment where we had 29 pairs of males that mated to two different females. Again, this failed to provide any evidence of consistent differences between males in their sperm competitive ability (mean P1/P2 over 4 weeks, $F_{1,28}=1.42$, $P=0.18$, repeatability, $r_i=0.015$; repeatability was calculated as the fraction of the summed variance that is among groups using our ANOVA variance components; Whitlock and Schluter 2009). Since we collected offspring from females at weekly intervals, we were able to examine the repeatability of P2 across weeks for each female mated to two males (excluding situations where only one male had any offspring). This confirmed that within a particular mating, the relative success of two males maintains the same pattern over the 4 weeks following the mating ($n=168$ matings, repeatability, $r=0.44$, $P<0.001$).

Heritability of sperm precedence

Parent offspring regression, using data from the 58 independent mother–daughter pairs (see 'Methods' section), failed to find any relationship between the P2 of a mother and that of her daughter (Fig. 2). The narrow sense heritability (h^2) calculated as twice the slope of the mother–daughter regression (Falconer and Mackay 1996) was very low and negative $h^2=-0.08$ (S.E.=0.15). Including female size, mating duration and numbers of offspring into the regression model failed to produce any significant increase in fit (all adjusted $R^2<0.01$). Similarly, regression of paternal P2 on son P2 from our 57 father–son pairs failed to find a significant relationship between the two (Fig. 3) $h^2=-0.07$ (S.E.=0.5), and inclusion of male size mating duration and numbers of offspring into the regression model again failed to produce any significant improvement in fit. P2 was significantly higher in the first generation

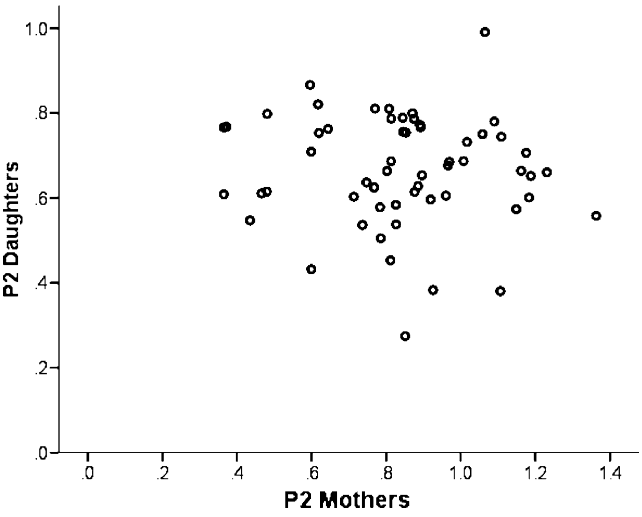

Fig. 2 The relationship between proportion of offspring sired by the second male to mate with a female (P2) (arcsine square-root transformed), in mothers and their daughter. The P2 found in mothers does not predict that found in daughters ($R^2=0.005$, $b=-0.04$, $F_{1,56}=0.25$, $P=0.62$; estimate of $h^2=-0.07$ (S.E.=0.15))

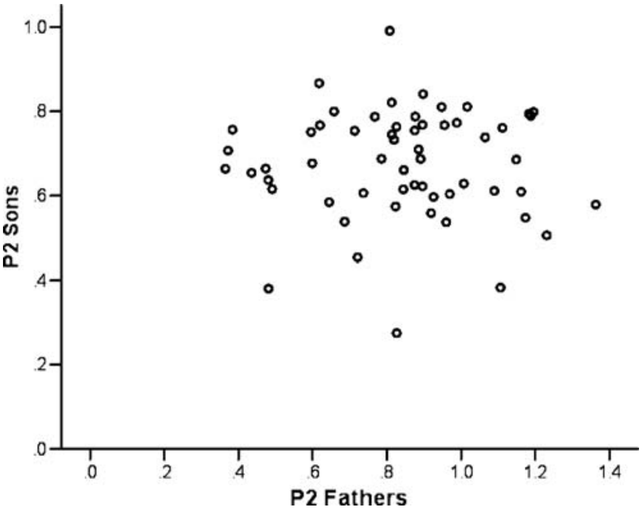

Fig. 3 The relationship between proportion of offspring sired by a male in the role of second mate (P2) (arcsine square-root transformed), and the same trait in their son. The P2 found in fathers does not predict that found in sons (R^2=0.018 $F_{1,56}$=0.02, b=−0.03, P=0.89; estimate of h^2=−0.07 (S.E.=0.5))

(mean=0.64, S.E.=0.03) than in the second generation (mean=0.45, S.E.=0.03) (t test of arcsine square-root transformed P2 values, t=4.68, P<0.001). There was also more variance in P2 in the parental generation (Levene's variance ratio test, $F_{1,114}$=16.1, P<0.001).

Discussion

We found that success or failure in sperm transfer did show some repeatability within males—when pairs of males mated to 16 different females, there were significant differences among males in their sperm transfer success, albeit only in the Ga1 strain. Additionally, in our sperm competition experiment, we found strong repeatability of sperm transfer success in the second male to mate. Because this experiment was not designed to examine this trait, mating order is confounded with strain since the second male was always from the Rd strain. The fact that Rd males did not show differences in sperm transfer success in our first experiment but there was an effect of mating order suggests that the difference in repeatability in our second experiment is more likely to be a mating order effect than a strain effect, although this would merit further investigation. Despite these limitations, our study provides two independent sources of evidence for repeatable differences among males in their ability to successfully transfer sperm to their mates. We did not find any evidence that male success in sperm competition (once a female has sperm from two males in her spermatheca) is heritable or even repeatable within males. We also could not find any evidence for a heritable female trait that could influence the proportion of offspring fertilised by the second male to mate with a female.

Our findings are somewhat unexpected given that the heritability of variation in male sperm competitive ability is an assumption of numerous studies of post-copulatory sexual selection. However, our findings are less surprising given the fact that there is currently very little evidence for repeatability or heritability of success in sperm competition in any species. A number of studies have found significant heritability of traits that may be associated with success in sperm competition (such as testes or ejaculate size and sperm traits) (reviewed by Simmons 2005) and an artificial evolution study in dung flies (Hosken et al. 2001) revealed an effect of enforced monogamy over several generations on success in sperm competition, indicating heritability. There have also been studies revealing differences in success in sperm competition among conspecific and heterospecific males (reviewed by Howard 1999) and among marker strains. For instance, Edwards (1955) found differences in fertilisation success among inbred lines of mice when their sperm were mixed and artificially inseminated into oestrus females, Lanier et al. (1979) found differences in paternity success among inbred rat strains and Dewsbury and Baumgardner (1981) found similar differences between wild-type and marker strains of deer mice (*Peromyscus maniculatus*). However, very few studies have directly examined repeatability or heritability of sperm competitive success within populations. In *Drosophila melanogaster*, chromosome replacement lines have been used to identify significant heritable effects of both males and females on patterns of sperm precedence (Civetta and Clark 2000), but quantitative genetic studies reveal that the additive component of this variation is slight (Hughes 1997). In field crickets, substantial repeatability of sperm number within males has been demonstrated in three species (Schaus and Sakaluk 2002). A previous study in *T. castaneum* (Lewis and Austad 1990) found significant differences among males in their fertilisation success when they were given 24 h to mate with a female either before or after another male spent 24 h with the female. Our results suggest that the differences observed in that study might stem more from variation in males' ability to solicit matings from females and to transfer sperm once such matings were achieved, rather than their ability to out compete the ejaculate of a rival male in a situation where the female receives a single ejaculate from each male.

Our finding of reduced mean P2 and reduced variance in the offspring generation compared with the parental generation highlights the possibility that the lack of heritability observed in our study might be the result of selection in favour of high P1 imposed during our study. Our experiment imposed stronger selection on high P1 than on high P2 (whether mediated by males or females) (only

101 of the original 200 females produced offspring from both males and of these, only 58 produced offspring from both the males they mated to). Since P2 was >0.5 in both generations, females would have been more likely to fail to produce offspring from both males where the first male had poor P1 or the female tended to reduce P1 than where P2 tended to be low (since mean P1 was low already and hence closer to zero). It is conceivable that this might reduce genetic variance in P2 sufficiently that we could no longer detect it, although this seems unlikely in just two generations of selection given that there will be directional selection on P1 and P2 acting in the base population at all times. Also, as discussed above, the number of offspring produced by each female suggests that even males with low success in the role of first mate would produce at least some offspring and so would not be selected against in our regime.

The best evidence for heritable differences in sperm competitive ability among males comes from studies by Radwan (1998) and Konior et al. (2005) who found significant heritability and repeatability of sperm competition success in the bulb mite *Rhizoglyphus robini.* Simmons et al. (2003) failed to find any evidence for repeatability of male success in sperm competition in the field cricket *Teleogryllus oceanicus* in an experiment in which four males mated to six different females. A separate study of heritability of P2 in the same species (Simmons 2003) found non-significant narrow sense heritabilities, but significant broad sense heritability of male P2, leading Simmons to suggest that the trait might be maternally inherited in that species whilst acknowledging that non-genetic maternal effects could not be ruled out. Wilson et al. (1997) found significant but very weak repeatability of P2 when males mated to random females in one strain of bruchid beetles (*Callosobruchus maculatus*) but no repeatability in a second strain. House and Simmons (2005, 2006) found substantial repeatability of both P1 and P2 in the dung beetle *Onthophagus taurus.* Neither of these beetle studies attempted to measure heritability.

Female effects on sperm competitive success have previously been described in bruchid beetles (Wilson et al. 1997), where full sibling females had repeatable patterns of sperm precedence when mated to the same pair of males, despite only limited evidence for a male effect on the same trait. Our study is not designed to determine whether there are genotype-by-genotype interactions between the sexes affecting the outcome of sperm competition; however, previous studies crossing genotypes in *D. melanogaster* (Civetta and Clark 2000; Clark et al. 1999) and *T. castaneum* (Nilsson et al. 2003) have revealed such interactions affecting the outcome of sperm competition. However, the overall picture is one of only limited evidence for additive genetic variation in male sperm competitive ability and even less for female variation influencing this trait independent of the male she mates with. This is a reflection mainly of a paucity of studies specifically designed to examine these traits. Given that repeatability and heritability of sperm competitiveness are key assumptions of the sexually selected sperm hypothesis (Keller and Reeve 1995), and are implicit assumptions of numerous studies seeking to explain mating patterns (Simmons and Kotiaho 2007; e.g. Wedell and Tregenza 1999), it is rather surprising that this question has been so neglected. Within studies that have been carried out, the evidence for heritability of sperm competitiveness is weak, with really strong evidence only in one species, the bulb mite.

Our study provides a further example of a system in which multiple mating is common, and in which sperm competitiveness does not appear to be available for selection. An obvious potential explanation is that our laboratory strain lacks genetic variability in general and is not representative of ‘natural’ populations. We cannot rule out this possibility. However, our population is derived from the Ga1 laboratory strain which has been maintained at population sizes of hundreds of individuals since being established from the wild, and we have kept it at large population sizes throughout. Also, the nature of the very controlled rearing environment, where food is provided in excess, would be expected to reduce environmental variation to a minimum. Numerous examples of laboratory strains of *T. castaneum* harbouring genetic variation for a wide range of traits are described by Sokoloff (1977), and subsequent studies using laboratory strains of *Tribolium* have revealed substantial additive genetic variation for behavioural traits such as cannibalism tendency (Stevens 1994) and proportion of homosexual mountings (Castro et al. 1996) as well as variation in male insemination capacity and female egg to adult viability (Pai and Yan 2002).

Our findings suggest that the very high mating rate observed in this species is unlikely to be the result of females selecting for fathers with high post-copulatory fertilisation success in competition in order to produce sons with the same traits. Rather, the explanation for polyandry is more likely to lie either in some other form of genetic benefit to offspring (Tregenza and Wedell 1998) or grand offspring (Cornell and Tregenza 2007) or in the high frequency of ejaculate transfer failures. The repeatability of variation among Ga1 males and between strains in their ability to transfer ejaculates to females suggests that this could be a larger target for selection in this species than success in classical sperm competition (where two ejaculates are present in the same female). Why so many matings fail to result in sperm transfer, despite what is expected to be strong selection for successful matings, remains a major question in this species (and probably many others). Female *T. castaneum* have been shown to exercise post-copulatory choice in relation to male olfactory attractiveness by restricting sperm transfer (Fedina and Lewis 2007). It is possible that the very high mating rates seen in *T. castaneum* mean that mating opportunities themselves are not at a premium and hence males may be exercising mate choice by failing to transfer sperm on some apparent mating attempts. However, our observation of a greater frequency of sperm transfer failures when females had not mated previously is difficult to understand in this context as virgin females might be expected to be more attractive to males since ejaculates transferred to them do not need to compete with those from previous mates.

Acknowledgements TT was supported by a Royal Society research fellowship. Rolando Rodríguez-Muñoz made valuable comments on experimental design. Sara Lewis and an anonymous referee made numerous insightful comments on earlier drafts of the manuscript.

References

Attia FA (2004) Costs and benefits of multiple mating in the red flour beetle *Tribolium castaneum*. Leeds University Press, Leeds

Birkhead TR, Møller AP (1998) Sperm competition and sexual selection. Academic, London

Castro L, Toro MA, López-Fanjul C (1996) The genetic properties of homosexual copulation behaviour in *Tribolium castaneum*: artificial selection. Gen Sel Evol 26:361–368

Civetta A, Clark AG (2000) Chromosomal effects on male and female components of sperm precedence in *Drosophila*. Genet Res 75:143–151

Clark AG, Begun DJ, Prout T (1999) Female X male interactions in *Drosophila* sperm competition. Science 283:217–220

Cornell SJ, Tregenza T (2007) A new theory for the evolution of polyandry as a means of inbreeding avoidance. Proc Roy Soc Lond B 274:2873–2879

Dewsbury DA, Baumgardner DJ (1981) Studies of sperm competition in 2 species of muroid rodents. Behav Ecol Sociobiol 9:121–133

Edwards RG (1955) Selective fertilization following the use of sperm mixtures in the mouse. Nature 175:215–223

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edition. Longman, New York

Fedina TY, Lewis SM (2007) Female mate choice across mating stages and between sequential mates in flour beetles. J Evol Biol 20:2138–2143

Fedina TY, Lewis SM (2008) An integrative view of sexual selection in *Tribolium* flour beetles. Biol Revs 83:151–171

Hosken DJ, Garner TWJ, Ward PI (2001) Sexual conflict selects for male and female reproductive characters. Curr Biol 11:489–493

House CM, Simmons LW (2005) Relative influence of male and female genital morphology on paternity in the dung beetle *Onthophagus taurus*. Behav Ecol 16:889–897

House CM, Simmons LW (2006) Offensive and defensive sperm competition roles in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). Behav Ecol Sociobiol 60:131–136

Howard DJ (1999) Conspecific sperm and pollen precedence and speciation. Ann Rev Ecol Syst 30:109–132

Hughes KA (1997) Quantitative genetics of sperm precedence in *Drosophila melanogaster*. Genetics 145:139–151

Keller L, Reeve HK (1995) Why do females mate with multiple males—the sexually selected sperm hypothesis. Adv Stud Behav 24: 291–315

Konior M, Keller L, Radwan J (2005) Effect of inbreeding and heritability of sperm competition success in the bulb mite *Rhizoglyphus robini*. Heredity 94:577–581

Lanier DL, Estep DQ, Dewsbury DA (1979) Role of prolonged copulatory-behavior in facilitating reproductive success in a competitive mating situation in laboratory rats. J Comp Physiol Psych 93:781–792

Lewis SM, Austad SN (1990) Sources of intraspecific variation in sperm precedence in red flour beetles. Am Nat 135:351–359

Lewis SM, Austad SN (1994) Sexual selection in flour beetles: the relationship between sperm precedence and male olfactory attractiveness. Behav Ecol 5:219–224

Nilsson T, Fricke C, Arnqvist G (2003) The effects of male and female genotype on variance in male fertilization success in the red flour beetle (*Tribolium castaneum*). Behav Ecol Sociobiol 53:227–233

Pai AT, Yan GY (2002) Polyandry produces sexy sons at the cost of daughters in red flour beetles. Proc Roy Soc Lond B 269:361–368

Pai A, Yan GY (2003) Rapid female multiple mating in red flour beetles (*Tribolium castaneum*). Canad J Zool 81:888–896

Pai A, Feil S, Yan G (2007) Variation in polyandry and its fitness consequences among populations of the red flour beetle, *Tribolium castaneum*. Evol Ecol 21:687–702

Radwan J (1998) Heritability of sperm competition success in the bulb mite, *Rhizoglyphus robini*. J Evol Biol 11:321–327

Schaus JM, Sakaluk SK (2002) Repeatability of sperm number across multiple matings in three cricket species, *Gryllodes sigillatus*, *Gryllus veletis*, and *Gryllus texensis* (Orthoptera: Gryllidae). Can J Zool 80:582–585

Simmons LW (2001) Sperm competition and its evolutionary consequences in the insects. Princeton University Press, Princeton

Simmons LW (2003) The evolution of polyandry: patterns of genotypic variation in female mating frequency, male fertilization success and a test of the sexy-sperm hypothesis. J Evol Biol 16:624–634

Simmons LW (2005) The evolution of polyandry: sperm competition, sperm selection, and offspring viability. Ann Rev Ecol Evol Syst 36:125–146

Simmons LW, Kotiaho JS (2007) Quantitative genetic correlation between trait and preference supports a sexually selected sperm process. Proc Natl Acad Sci USA 104:16604–16608

Simmons LW, Wernham J, Garcia-Gonzalez F, Kamien D (2003) Variation in paternity in the field cricket *Teleogryllus oceanicus*: no detectable influence of sperm numbers or sperm length. Behav Ecol 14:539–545

Siva-Jothy MT (2000) The young sperm gambit. Ecol Lett 3:172–174

Sokoloff A (1977) The biology of *Tribolium:* with special emphasis on genetic aspects. Vol. 3.. Clarendon Press, Oxford

Stevens L (1994) Genetic analysis of cannibalism behavior in *Tribolium* flour beetles. In: Boake CRB (ed) Quantitative genetic studies of behavioral evolution. University of Chicago Press, Chicago, pp 206–227

Tregenza T, Wedell N (1998) Benefits of multiple mates in the cricket *Gryllus bimaculatus*. Evolution 52:1726–1730

Wedell N, Tregenza T (1999) Successful fathers sire successful sons. Evolution 53:620–625

Whitlock MC, Schluter D (2009) The analysis of biological data. Roberts, Colorado

Wilson N, Tubman SC, Eady PE, Robertson GW (1997) Female genotype affects male success in sperm competition. Proc Roy Soc Lond B 264:1491–1495