Postcopulatory inbreeding avoidance by female crickets only revealed by molecular markers

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Abstract

Multiple mating is thought to provide an opportunity for females to avoid the costs of genetic incompatibility by postcopulatory selection of compatible sperm haplotypes. Few studies have tested the genetic incompatibility hypothesis directly. Here we experimentally manipulated the compatibility of females with their mates using the gryllid cricket *Teleogryllus oceanicus*. We recorded the hatching success of eggs laid by females mated with two nonsibling males, two siblings, or one nonsibling male and one sibling. In contrast with two previous studies on crickets that have adopted this approach, the hatching success of eggs did not differ between females mated with two full siblings and females mated with two unrelated males, indicating that embryo viability was not a cost of inbreeding in this species. We assigned paternity to off-spring produced by females mated to both a sibling and a nonsibling male using microsatellite markers. As in previous studies of this species, we were unable to detect any difference in the proportion of offspring sired by the 1st and the 2nd male to mate with a female when females were unrelated to their mates. However, in our experimental matings the proportion of off-spring sired by the nonsibling male depended on his sequence position. Paternity was biased toward the nonsibling male when he mated first. Our data show that molecular analyses of paternity are essential to detect subtle mechanisms of postcopulatory sexual selection.

Keywords: cryptic female choice, genetic incompatibility, inbreeding avoidance, polyandry, *Teleogryllus oceanicus*

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Introduction

Despite the apparent costs associated with mating with more than one male (Rowe 1994; Chapman *et al*. 1995; Crudgington & Siva-Jothy 2000), polyandry is a taxonomically widespread phenomenon. In some species females obtain material benefits from males that may outweigh the costs of polyandry. However, in their meta-analysis of the insect literature Arnqvist & Nilsson (2000) found that polyandry was also associated with an increase in reproductive success among species where males do not transfer any obvious material benefits to females.

A number of genetic benefit hypotheses have been proposed to explain the evolution of polyandry (Zeh & Zeh 1996; Zeh & Zeh 1997; Jennions & Petrie 2000; Tregenza &

Wedell 2000). These models have a common currency in that polyandrous females are assumed to produce off-spring of greater genetic quality than monandrous females, either because their offspring have a higher viability (Zeh & Zeh 1996; Yasui 1997) or because their sons are more successful in obtaining mating partners and/or in gaining fertilizations when subject to sperm competition (Curtsinger 1991; Keller & Reeve 1995). These models fall into two distinct categories, depending upon the mechanism by which parental genes contribute to offspring fitness. The 'sexy sperm' (Keller & Reeve 1995) and 'good sperm' (Yasui 1997) models assume heritable variation in fitness traits so that the benefits of polyandry stem directly from the intrinsic genetic quality of sires. In contrast, the genetic incompatibility hypothesis (Zeh & Zeh 1996, 1997) proposes that certain combinations of male and female genotypes produce offspring of low viability, so that the quality of an individual male as a potential mate varies among females, depending upon the female's own genotype.

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There are a growing number of empirical studies that have reported increased performance of offspring when females were allowed to mate polyandrously, in fitness traits ranging from embryo viability to adult fecundity (Madsen *et al.* 1992; Tregenza & Wedell 1998; Kozielska *et al.* 2003; Stockley 2003; reviewed in Simmons 2005). Several studies of the effects of polyandry on offspring fitness in insects have attempted to control for the potentially confounding effects of multiple mating, and these studies suggest a small but general positive effect of multiple mates on egg or embryo viability (Simmons 2005). Many of these studies have been taken as evidence in support of the genetic incompatibility hypothesis. However, few experiments have actually provided direct evidence for genetic incompatibility effects on egg viability. Rather, genetic incompatibility has become the default interpretation based on subexperiments or analyses that fail to reveal consistent sire effects on egg viability (Tregenza & Wedell 2000).

Inbreeding results in a general increase in homozygosity which can lead to the phenotypic expression of deleterious recessive mutations that reduce fitness (Pusey & Wolf 1996). Inbreeding avoidance can therefore be viewed as a form of mate selection for genetic compatibility, or the avoidance of genetic incompatibility (Tregenza & Wedell 2000). In their study of polyandry in the field cricket *Gryllus bimaculatus*, Tregenza & Wedell (2002) experimentally manipulated genetic incompatibility by allowing females to mate with either full sibling males or males that were unrelated. Females mated twice to two sibling males had a lower egg viability than females mated twice to two nonsibling males, indicative of a cost of inbreeding. Females mated twice, once to a sibling and once to a nonsibling male, had an egg viability similar to those females mating with two nonsibling males. These data are consistent with the argument that females selectively fertilize their eggs with sperm from unrelated males since random sperm use would predict an egg viability mid-way between that expected from fertilizations by sibling males and that expected by fertilizations by nonsibling males (Tregenza & Wedell 2002). In a similar experiment with the field cricket *Teleogryllus commodus*, Jennions *et al.* (2004) likewise found a cost of inbreeding in terms of reduced egg viability when females had mated with siblings. These authors argued that in this species there was no selective sperm use from unrelated males because the hatching success of females mating with both a sibling and a nonsibling male was the same as that of females mating with two siblings. Indeed, the patterns of hatching success were exactly opposite to those found in *G. bimaculatus*, and if anything, the data for *T. commodus* tend to suggest that females bias fertilization toward sibling males.

Here we report the results of an experiment with the Australian field cricket *Teleogryllus oceanicus* in which we experimentally manipulated genetic compatibility via controlled matings with sibling and nonsibling males, and examined both the patterns of egg viability, and assigned paternity using microsatellite markers. Like *G. bimaculatus* (Tregenza & Wedell 1998), polyandrous female *T. oceanicus* have an increased hatching success compared with monandrous females (Simmons 2001a). We use our data to test the hypothesis that increased hatching success of polyandrous females arises from the postcopulatory avoidance of kin based genetic incompatibility, and show how molecular markers for assigning paternity are essential for drawing conclusions regarding postcopulatory mechanisms of mate selection.

Materials and methods

Crickets used in this study were the offspring of 120 mated females collected from a banana plantation in Carnarvon, Western Australia, in July 2001. Offspring were reared in family groups of approximately 30 individuals per 5 litre plastic container in a constant-temperature (25 °C) room with a 12 : 12 h light : dark cycle. Food (cat chow) and water were available ad libitum and a cardboard egg carton was provided for shelter. The sexes were separated at the final nymphal instar. We generated 29 full sibling family groups by pairing one adult female with one adult male from a different field-derived family group. The offspring from these pairings were reared to adulthood as above and used in our experiments in January 2002.

Mating design

Our experimental design consisted of 29 blocks of four females and four males. Within a block all four females were full siblings, two males were full siblings (S) of the females, and two males were nonsiblings (N) of the females in the block but were themselves full siblings. Thus four sisters from each of our 29 families were assigned to one of four mating treatments. One female was mated twice, once to two full sibling males from a different unrelated family group (NN). The second female was mated to her two brothers (SS). The third female was mated first to one of the nonsibling males and second to one of her brothers (NS), and the forth female was mated first to the other brother and second to the other nonsibling male (SN) (see also Tregenza & Wedell 2002). To ensure experimental males were sexually competent, all were mated once to a random female prior to their experimental pairing.

Matings were conducted in clear plastic boxes (7 cm × 7 cm × 5 cm). The male and female were placed together and the time when the male attached his spermatophore was noted. Sperm transfer proceeds over a period of ~40–50 min. Sperm numbers do not appear to influence paternity in this species (Simmons *et al.* 2003). Nevertheless, we controlled for any potential variation in sperm transfer. Thus, after mating, the pair were observed for 60 min during

which time the male's postcopulatory guarding behaviour prevented females from dislodging the spermatophore (Loher & Rence 1978; Simmons *et al.* 2003). Drained spermatophores were removed with forceps after 60 min of guarding. The first male was then replaced with the second male and the process repeated. Thus, all females should have received 2 full complements of sperm, one from each of their mates. Males were frozen immediately after they had completed their required matings. Following the second mating the female was left in her box, provided with cat chow and a Petri dish containing damp sand in which to oviposit, and left for 10 days before being frozen.

Hatching success and paternity analysis

Eggs were rinsed from the sand, placed onto moist filter paper in a sealed Petri dish and incubated at 25 °C. Eggs were checked every second day until two weeks after the last nymph had emerged. Newly hatched nymphs were counted, placed in family groups in Eppendorf tubes and frozen. The proportion of eggs hatching was calculated for each female and arcsine transformed prior to statistical analysis. All means are presented ± 1 SE.

To examine postcopulatory mechanisms of inbreeding avoidance we assigned paternity to 20 randomly chosen offspring produced by each female that had mated with both a brother and an unrelated male. Paternity assignment was conducted using microsatellite markers. DNA was extracted from the hind leg of the adults and from the whole nymph using a rapid salt-extraction method in which the tissue was placed in 350 µL of DNA extraction buffer (50 mM Tris-HCl pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS and 50 µg proteinase K). Following homogenization the samples were incubated at 65 °C for 15 min, cooled and 150 µL 5 M NaCl added. The samples were vortexed and the debris pelleted by centrifugation for 10 min at 13000 r.p.m. The supernatant was transferred to a clean tube and 500 µL isopropanol added and mixed by inversion. The DNA was then pelleted by centrifugation at 4 °C for 10 min at 13000 r.p.m. The isopropanol was removed and the pellet washed with 70% ethanol, dried and resuspended in 50 µL TE buffer. The DNA was then treated with 0.5 units of Riboshredder (Epicentre) to remove RNA by incubating at 37 °C for 30 min.

The samples were then screened using 6 microsatellite markers developed for *T. oceanicus* (Beveridge & Simmons 2005), 4 markers in one PCR reaction and 2 markers in a second PCR reaction. The first 10 µL PCR reaction contained 1 × PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl) (Invitrogen), 3.5 mM MgCl$_2$ (Invitrogen), 200 µM of each dNTP (Invitrogen), 250 nM of forward primer Totri54 labelled with 6-FAM (Geneworks), 250 nM of forward primer Totri59 labelled with VIC (Applied Biosystems), 250 nM of reverse primers Totri54 and Totri59 (Geneworks),

0.5 units of Platinum Taq polymerase (Invitrogen) and 1–10 ng DNA. The second 10 µL PCR reaction contained 1 × PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 1.5 mM MgCl$_2$, 200 µM of each dNTP, 250 nM of each of the following forward primers: Totri9a labelled with 6-FAM (Geneworks), Totri55a labelled with NED (Applied Biosystems), Totri57 labelled with VIC (Applied Biosystems), Totri78 labelled with VIC (Applied Biosystems), 250 nM of each of the following reverse primers Totri9a, Totri55a, Totri57 and Totri78 (Geneworks), 0.5 units of Platinum Taq polymerase and 1–10 ng DNA. PCR amplification was performed in a PTC-0200 DNA Engine (Geneworks) with cycling conditions as follows: 94 °C for 1 min, then 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, and finally 72 °C for 45 min. The products from both PCR reactions were combined (1 µL of each) and analysed on an ABI3430 sequencer, sized using Genescan-500 LIZ internal size standard and genotyped using GENEMAPPER software (version 3.0).

Paternity assignment was via a maximum likelihood approach using Cervus version 2.0 (Marshall *et al.* 1998). Each family unit was analysed with the female as the known parent and the 2 males with which she had mated as the candidate parents. Parentage was assigned with > 95% confidence in all cases.

Patterns of paternity following random mating

To establish the basic patterns of paternity when two males compete for fertilizations, 20 unrelated females were randomly assigned to 20 pairs of males. Females were unrelated to the males with whom they were paired, and the males were unrelated to each other. Females were allowed to mate once with each of the 2 males in a manner identical to that described in the mating design above. Females were then left with damp sand to lay eggs for a period of 10 days. Each female and her partners were frozen after use. Eggs were incubated at 25 °C and all nymphs frozen on hatching. Paternity was assigned using microsatellite markers as described above.

Results

Hatching success

The mean number of eggs laid by females was 160 ± 5.4 (range 75–342). There was no significant effect of mating treatment on the number of eggs laid ($F_{3,84} = 1.07$, $P = 0.368$) but there was a significant effect of block ($F_{28,84} = 3.10$, $P < 0.0001$). Thus, some female families laid more eggs than others, with family means ranging from 102 ± 23 to 259 ± 23. Hatching success did not differ significantly between mating treatments ($F_{3,84} = 0.47$, $P = 0.705$) or between blocks ($F_{28,84} = 0.98$, $P = 0.509$). The untransformed mean
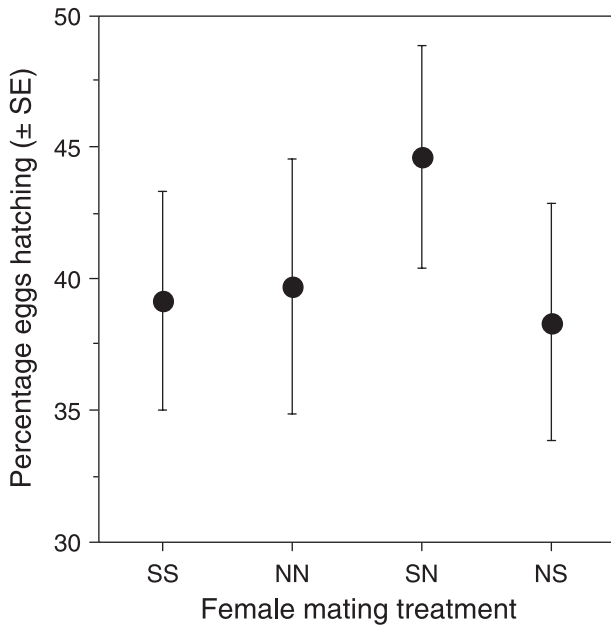
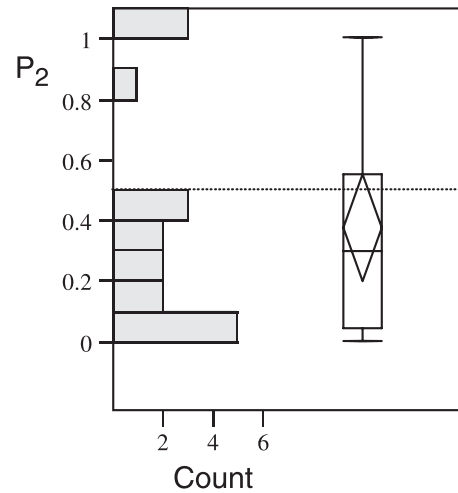**Fig. 1** Mean (± SE) percentage of eggs that hatched after females had mated with two sibling males (SS), two nonsibling males (NN), one sibling followed by one nonsibling (SN), or one nonsibling followed by one sibling (NS).

Fig. 2 Frequency distribution of the proportion of offspring sired by the second male (P_2) for randomly mated females. Box plots delimiting the upper and lower quartiles are shown to the right of the distribution. Within boxes the diamond provides the mean and 95% confidence intervals, and the tails give the range of values. The horizontal line at 0.5 is the expected P_2 under a model of random sperm use.

hatching success of eggs laid by females in the different mating treatments are shown in Fig. 1. Across female mating treatments the mean percentage of eggs hatching was 40.4 ± 2.2%.

Paternity analysis

Of the 20 females that were randomly allocated 2 unrelated males, 18 produced offspring for paternity assignment (23 or 24 offspring per female). There was no significant difference between the numbers of offspring sired by the first and second male to mate (matched pairs $t_{17} = 1.53$, $P = 0.144$). The average proportion of offspring sired by the second male to mate (P_2) was 0.37 ± 0.08. The distribution of P_2 values is shown in Fig. 2. Thus, although there was a tendency toward a first male advantage, the pattern of paternity following double matings did not deviate significantly from random sperm mixing in which both males are expected to obtain an equal proportion of offspring (Wilcoxon Signed-Rank, T = −30, $P = 0.199$). This pattern is consistent with that found in previous studies using both allozyme (Simmons 2001a) and morphological (Simmons *et al.* 2003; García-González & Simmons 2005b) markers for assigning paternity.

There were 20 blocks for which females from both the NS and SN mating treatments had sufficient numbers of hatched offspring for paternity assignment. The proportion of offspring sired by the second male to mate (P_2) was analysed using a Generalized Linear Model with binomial

error distribution and a logit link function in GENSTAT 7.2.0.208 (VSN International). The number of offspring sired by the second male was fitted as the response variable with the total number of offspring genotyped as the binomial denominator. Williams' (1982) procedure was adopted to correct for over dispersion. The main predictor variables were block and relatedness of the second male. There were no significant effects of block ($\chi^2 = 1.06$, d.f. = 19, $P = 0.329$) or relatedness of the second male on P_2 ($\chi^2 = 0.62$, d.f. = 1, $P = 0.432$). Although not significant in this analysis, the trend was for sibling second males to achieve a lower paternity ($P_2 = 0.26 ± 0.08$) than nonsibling second males ($P_2 = 0.33 ± 0.08$). We observed complete first ($P_2 = 0$) or last ($P_2 = 1$) male sperm precedence in 50% of females (Fig. 3). Extreme values of paternity were distributed randomly with respect to male relatedness (9 nonsibling and 11 sibling males achieved 100% paternity; Binomial $P = 0.412$). Some of these extreme values of paternity bias result from insemination failure, rather than biased use of sperm from sibling or nonsibling males (García-González 2004). We therefore re-analysed our data set including only those females that produced broods with mixed paternity, and who were thereby certain to have been successfully inseminated by both males. There was a significant effect of relatedness of the second male on P_2 ($\chi^2 = 6.44$, d.f. = 1, $P = 0.011$) but again no effect of block ($\chi^2 = 1.40$, d.f. = 14, $P = 0.145$). The second male's paternity was lower when he was related to the female (sibling second male, 0.32 ± 0.06; nonsibling second male, 0.52 ± 0.09). When the second male was a female's sibling the average P_2 deviated significantly from
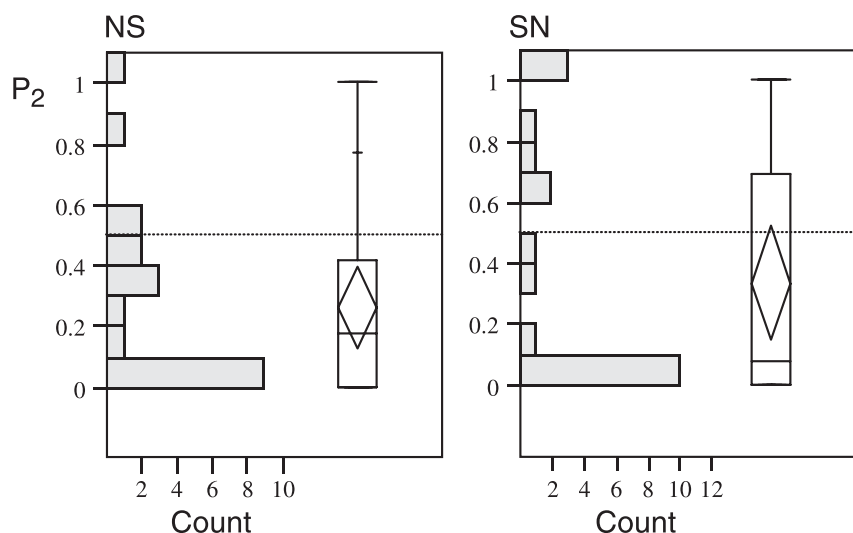
Fig. 3 Frequency distributions of the proportion of offspring sired by second males (P_2) when they were siblings (NS) or nonsiblings (SN) of doubly mated females. Box plots delimiting the upper and lower quartiles are shown to the right of the distribution. Within boxes the diamond provides the mean and 95% confidence intervals, and the tails give the range of values. The horizontal lines at 0.5 are the expected P_2 under a model of random sperm use.

a model of random mixing (Wilcoxon Signed Rank T = –26.0, $P = 0.019$). In contrast, when the second male was a female's nonsibling, the average P_2 did not deviate significantly from a model of random sperm mixing (T = 1.5, $P = 0.891$).

Discussion

Inbreeding depression and genetic incompatibility

Recent experimental studies have concluded that females mate with multiple males to avoid the costs of decreased embryo viability that can arise due to genetic incompatibility between maternal and paternal haplotypes (reviewed in Simmons 2005). Often these studies do not test directly for genetic incompatibility. Rather, they infer the role of incompatibility when analyses of subsets of the data or subsidiary experiments, fail to reveal consistent differences between males in their ability to sire viable offspring across several females.

Two previous studies of gryllid crickets have attempted to test the genetic incompatibility hypothesis directly by experimental manipulation of genetic incompatibility using individuals of known relatedness. Thus, matings between full-siblings have been reported to result in reduced hatching success in both the European field cricket *G. bimaculatus* (Tregenza & Wedell 2002) and the Australian field cricket *T. commodus* (Jennions *et al*. 2004). In *G. bimaculatus*, females mated to both a sibling and a non-sibling male had a hatching success close to that achieved with non-sibling males alone, leading Tregenza & Wedell (2002) to conclude that polyandry allowed females to avoid the costs of inbreeding by postcopulatory selection of compatible sperm. For *T. commodus*, the hatching success of females mated to a sibling and a nonsibling male did not differ from that of females mated to siblings alone, suggest-

ing that in this species females had no postcopulatory mechanism for avoiding the costs of inbreeding (Jennions *et al*. 2004).

Here we provide data from a third species *Teleogryllus oceanicus*. In contrast to Tregenza & Wedell (2002) and Jennions *et al*. (2004), we found no difference between the hatching success of eggs laid by females mating to different combinations of related and unrelated males. Our data indicate that for this species there are no embryo viability costs associated with inbreeding (although we note that inbreeding effects may vary across populations). Although the average hatching success reported in this study seems rather low (40%), it is remarkably consistent with the values reported for *G. bimaculatus* (~48%) (Tregenza & Wedell 2002) and *T. commodus* (~40%) (Jennions *et al*. 2004). In *T. commodus* hatching success is highly repeatable across clutches suggesting strong maternal and/or additive genetic variance (Jennions *et al*. 2004). A previous study of *T. oceanicus* has established that polyandrous females do have a higher hatching success (55%) than monandrous females (48%) (Simmons 2001a). Moreover, García-González & Simmons (2005a) adopted a quantitative genetic approach to test for intrinsic male effects on hatching success, finding that a male's ability to sire viable embryos exhibited significant additive genetic variance, and that this intrinsic male effect did not depend on variation in the genotype of females to which males were mated. Consistent with these findings, the data presented here show that directly manipulating genetic incompatibility using males and females of known relatedness failed to influence hatching success. Thus, the combined data support the conclusion that the increased hatching success of eggs laid by polyandrous female *T. oceanicus* does not result from the avoidance of genetic incompatibility, and that intrinsic sire effects on hatching success are more important in this model system.

Although mating with siblings does not appear to reduce embryo viability in *T. oceanicus*, it would be unwise to conclude that there is a lack of inbreeding depression in this species. Other studies of gryllid crickets have revealed inbreeding depression at later developmental stages. Thus, in *G. firmus* inbreeding depression is manifest as reduced fecundity in adult female offspring (Roff 1998; Roff & DeRose 2001). Likewise in bulb mites *Rhizoglyphus robini*, sib mating results in reduced fecundity in female offspring (Radwan 2003) and reduced sperm competition success of male offspring (Konior *et al*. 2005). Thus, inbreeding avoidance could be adaptive in *T. oceanicus*, even though inbreeding has little consequences for hatching success.

Postcopulatory mechanisms for inbreeding avoidance

If females are to gain genetic benefits via polyandry there must be postcopulatory mechanisms by which fertilization is biased toward those sires offering superior haplotypes, either through superior sperm competitiveness or through sperm selection by females (Zeh & Zeh 1997; Yasui 1998). Previous studies have inferred patterns of fertilization based on observed patterns of hatching success (Tregenza & Wedell 2002; Jennions *et al*. 2004). Using molecular markers, we found evidence to suggest that females might have a postcopulatory mechanism for biasing paternity toward unrelated males. Analysis of data derived from females producing broods of mixed paternity, and thereby known to have been successfully inseminated by both male types, showed that P_2 depended on the relatedness of the second male to mate. Consistent with previous paternity studies of this species (Simmons 2001a; Simmons *et al*. 2003; García-González & Simmons 2005b), our microsatellite markers showed that for randomly mated females, sperm utilization did not deviate significantly from a model of random sperm mixing. However, in contrast to these previous studies, the pattern of sperm utilization following matings with both a nonsibling and a sibling male, depended on the nonsibling male's sequence position. On average there was first male sperm precedence when the nonsibling male was first to mate, but paternity was assigned at random when the nonsibling was second to mate.

Our paternity data are remarkably similar to those reported by Bretman *et al*. (2004) for *G. bimaculatus*. They used microsatellite markers to assign paternity to offspring produced by the female *G. bimaculatus* that had mated with both a sibling and a nonsibling male in Tregenza & Wedell's (2002) original study. They found the expected result of a higher paternity success for unrelated males when the unrelated male was the first to mate with the female. However, there was no effect of male relatedness on paternity when the unrelated male was the second to mate with the female. The patterns of paternity bias were thus inconsistent with the patterns of egg viability which did not depend on

the mating order of related and unrelated males (Tregenza & Wedell 2002). In a recent study of pseudoscorpions, Zeh & Zeh (2006) found that the patterns of embryo mortality when females mated with related and/or unrelated males were consistent with the hypothesis of biased use of sperm from unrelated males. However, when they assigned paternity using microsatellite markers, they found a bias in sperm use toward related males. These observations show that conclusions regarding differential fertilization success based on patterns of hatching success alone are misleading, and that paternity data are an essential component to any study of postcopulatory mechanisms of inbreeding avoidance. Such data are both time consuming and expensive to obtain because of the need to genotype large numbers of offspring within each family in order to obtain an accurate measure of P_2. The irradiated male technique is often used in the study on insect sperm competition because it is fast, inexpensive, and allows P_2 values to be calculated from very large numbers of offspring (Simmons 2001b). However, the irradiated male technique is unlikely to be suitable for studies of postcopulatory inbreeding avoidance, since the disruption to the genome that generates infertility may also disrupt genotype based sperm recognition and subsequent paternity biasing by females.

Subtle biases in paternity toward unrelated males when females mate with both siblings and unrelated males have also been reported from decorated crickets *Gryllodes supplicans* and from *Drosophila melanogaster*. In decorated crickets the mean P_1 value did not differ with mating sequence, although here too P_1 tended to be greater when the unrelated male mated first (Stockley 1999). Unfortunately in Mack *et al*.'s (2002) study of *D. melanogaster*, unrelated competitors were always mated in the position of second male so the influence of sequence position on the strength of paternity bias is unknown. Interestingly however, a female's experience of the preferred sperm haplotype has been shown elsewhere to influence her ability to bias paternity in this species. Childress & Hartl (1972) studied a translocation system in *D. melanogaster* in which paternity was biased toward sperm carrying the Bs +4 haplotype. They found that prior exposure of the female reproductive tract to Bs +4 sperm haplotypes enhanced a female's ability to subsequently discriminate against Bs +4-bearing sperm.

The finding that female crickets tend to show stronger biases toward sperm from unrelated males if they mate with them first could be explained if females treat sperm from related males differently depending on whether they already have sperm in storage (Bretman *et al*. 2004), or if they need to learn to recognize sibling males or their sperm. Female *G. bimaculatus* are better able to recognize their siblings in precopulatory mate choice, if they have had prior exposure to them (Simmons 1989). Indeed, learning preferred phenotypes seems to be just as important in insect mate choice as it is in vertebrate mate choice (Dukas

2005). Thus, like *D. melanogaster*, female crickets may be better able to discriminate against sperm from siblings once their reproductive tracts have been exposed to sperm of the preferred unrelated haplotypes.

Alternatively, observed mating order effects on paternity bias could represent the outcome of female cooperation and conflict with male sperm competition mechanisms. Thus in crickets, when the preferred male is in the role of second male it may be harder for females to bias fertilization toward that male than when he is the first to mate, because her postcopulatory preference is in conflict with a mechanism of sperm competition in which the first male tends to achieve higher paternity. A recent study of seaweed flies *Coelopa frigida* examined postcopulatory mechanisms of female choice for males carrying an inversion system that is known to influence precopulatory mate selection (Blyth & Gilburn 2005). Females of this species prefer to mate with males carrying a karyotype different from their own, because heterokaryotypic offspring have higher egg-adult viability. Although these flies exhibit a mechanism of sperm competition in which the last male to mate sires the majority of offspring, Blyth & Gilburn (2005) found that females could bias paternity toward a male with a compatible (opposite) karyotype when that male was the first to mate, but only managed to do so if the second mating occurred immediately after the first, otherwise the mechanism of second male sperm precedence became established. Likewise across the hybrid zone of the grasshopper *Chorthippus parallelus* in the Pyrenees, fertilization success is generally biased toward sperm carrying the female's own race haplotype. Although the mechanism of sperm competition in this species is also one of last male sperm precedence, here again the observed fertilization outcome seems to be a balance between male and female interests; P_2 is greater when the second male is homogametic than when he is heterogametic (Bella *et al.* 1992).

In conclusion, we found no evidence for mate relatedness genetic incompatibility effects on the viability of eggs laid by female *T. oceanicus*. Nevertheless, despite there being no differences in hatching success across matings with sibling and unrelated males, the patterns of paternity following double matings involving both male types were consistent with previous studies that have shown how females can bias paternity to avoid inbreeding depression. Our data for *T. oceanicus* show us that data on hatching success alone are unlikely to provide reliable insights into postcopulatory mechanisms of fertilization bias.

Acknowledgements

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