
Sperm selection and genetic incompatibility: does relatedness of mates affect male success in sperm competition?

P. Stockley*

Population Biology Research Group, School of Biological Sciences, Nicholson Building, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK

Sperm selection may be said to occur if females influence the relative success of ejaculates competing to fertilize their ova. Most evidence that female animals or their ova are capable of sperm selection relates to male genetic incompatibility, although relatively few studies focus on competition between conspecific males. Here I look for evidence of sperm selection with respect to relatedness of mates. Reduced fitness or inbreeding effects in offspring resulting from copulations between close relatives are well documented. If females are capable of sperm selection, they might therefore be expected to discriminate against the sperm of sibling males during sperm competition. I describe an experimental protocol designed to test for evidence of sperm selection while controlling for inbreeding effects. Using decorated field crickets (*Gryllobates supplicans*), I found that sibling males achieved lower fertilization success in competition with a male unrelated to the female than in competition with another sibling more frequently than expected by chance, although the mean paternity values did not differ significantly between treatments. The tendency for sibling males to achieve relatively lower fertilization success in competition with males unrelated to the female could not be explained by the effects of increased ejaculate allocation, female control of sperm transfer or inbreeding. This study therefore provides some evidence in support of the idea that female insects (or their ova) may be capable of selection against sperm on the basis of genetic similarity of conspecific males.

Keywords: crickets; cryptic female choice; fertilization success; *Gryllobates supplicans*; inbreeding

1. INTRODUCTION

The question of whether female animals can influence the relative fertilization success of competing ejaculates within their reproductive tract has recently begun to attract broad interest among evolutionary biologists (e.g. Eberhard 1996; Olsson *et al.* 1996, 1997; Wedekind *et al.* 1996; Stockley 1997; Wirtz 1997; Birkhead 1998; Clark *et al.* 1999). Female ability for sperm selection has important implications in the field of sexual selection, particularly with respect to predicting the outcome of sperm competition (competition between ejaculates; Parker 1970, 1998). Moreover, it has recently been argued that, by copulating with more than one male to fertilize a single batch of ova, females may promote post-copulatory mechanisms such as sperm selection to reduce investment in offspring sired by genetically incompatible males (Zeh & Zeh 1996, 1997). Detailed investigation of female potential for sperm selection is therefore important both in the context of understanding sperm competition outcomes and in explaining the adaptive value of female promiscuity itself.

Most evidence that female animals may be capable of sperm selection relates to genetic incompatibility

(reviewed in Birkhead 1998). At an interspecific level, the sperm of heterospecific males are often disadvantaged in competition with those of conspecific males (e.g. Hewitt *et al.* 1989; Gregory & Howard 1994; Howard *et al.* 1998). Price (1997), for example, found that when *Drosophila* females mate with both a conspecific and a heterospecific male, the conspecific sperm fertilize most of the eggs, regardless of mating order. Relatively few studies have reported female discrimination between the sperm of competing conspecific males. The best evidence comes from studies of the hermaphroditic sessile compound ascidian *Diplosoma listerianum*, which has a mating system analogous to those of flowering plants (Bishop 1996; Bishop *et al.* 1996). Investigations of the potential for sperm selection in other animal taxa have so far produced varying results. Olsson *et al.* (1996, 1997) found that when female sand lizards (*Lacerta agilis*) produce litters sired by males of varying relatedness to themselves, less genetically similar males father proportionately more offspring. However, no comparable effect was found in a similar analysis for female common shrews (*Sorex araneus*), which have very similar mating patterns to *L. agilis* (Stockley 1997). Among insects, Wilson *et al.* (1997) found that female genotype strongly influences the outcome of sperm competition in cowpea weevils (*Callosobruchus maculatus*), with male success apparently determined by genetic compatibility with the female. In *Drosophila melanogaster*,

*Author for correspondence and present address: Animal Behaviour Group, Faculty of Veterinary Science, University of Liverpool, Leahurst, South Wirral CH64 7TE (stockley@liv.ac.uk).

the success of particular male genotypes in displacing sperm is dependent on the genotype of the female mated (Clark & Begun 1998; Clark *et al.* 1999). Potential evidence of sperm selection was also suggested by the recent study of Tregenza & Wedell (1998), in which female crickets (*Gryllus bimaculatus*) mated with multiple males achieved higher reproductive success than those mated repeatedly with a single male. As yet, however, the mechanism controlling these patterns of non-random paternity and the relevant genetic factors involved are unknown.

Genetic similarity of mating partners is a useful aspect of genetic incompatibility on which to focus investigation of sperm selection, because relatedness of mates can be readily manipulated in the laboratory. Moreover, reductions in the fitness of offspring resulting from copulations between genetically similar individuals are widely documented (reviews in Charlesworth & Charlesworth 1987; Falconer 1989; Keller 1998). If females (or their ova) are capable of sperm selection on the basis of male genetic incompatibility, they should therefore discriminate against sperm from genetically similar males. Evidence of sperm selection is difficult to demonstrate because patterns of non-random paternity can be generated by a variety of different mechanisms, including sperm competition and alternative means of female control (e.g. see Simmons *et al.* 1996; Olsson *et al.* 1997; Stockley 1997; Birkhead 1998). To demonstrate evidence of sperm selection with respect to genetic similarity of mates, it is necessary to control for or eliminate as alternative explanations the effects of sperm competition, alternative mechanisms of female control of paternity and any additional inbreeding effects.

The decorated field cricket *Grylodes supplicans* (Orthoptera: Gryllidae) is an ideal subject for the investigation of sperm selection because mechanisms of sperm competition and female control of ejaculate transfer can each be accounted for in this species. After a brief copulation of ca. 2–3 min, sperm is transferred to storage in the female's spermatheca via an externally attached spermatophore. *Grylodes* females remate readily with expansion of the spermatheca to accommodate additional ejaculates. Random mixing of sperm in storage results in numerical sperm competition and male fertilization success increases in relation to the number of sperm transferred (Sakaluk 1986). The spermatophore consists of two parts: a sperm-containing ampulla and a large gelatinous spermatophylax, which is a nuptial gift consumed by the female after copulation. Sperm transfer is terminated when the female removes the ampulla after consuming the spermatophylax and the provision of a nuptial gift probably functions to deter removal before completion of sperm transfer (Sakaluk 1984). The time taken to consume the spermatophylax increases linearly with its size and larger males produce larger spermatophylaxes (Sakaluk 1988). Male success in sperm competition is therefore related to body size because providing a large spermatophylax increases the duration of ampulla attachment and, hence, the number of sperm transferred (Sakaluk 1984, 1986, 1988).

Here, I look for evidence of non-random paternity with respect to the relatedness of mates in the decorated field cricket *G. supplicans*. I also aim to distinguish between three potential explanations for non-random paternity: (i) variation in ejaculate size, (ii) female control of sperm

transfer and (iii) differential sperm use, while controlling for inbreeding effects.

2. MATERIAL AND METHODS

(a) *Rearing crickets*

Grylodes supplicans has a worldwide distribution in tropical and subtropical regions. The crickets used in this study were first-generation descendants of a genetically diverse population collected from the University of Western Australia and surrounding areas. All individuals were maintained at 29 ± 2 °C under a reversed 12 L:12 D photoperiod. Laboratory rodent pellets were provided *ad libitum* and supplemented weekly with fresh carrot. Moistened cotton-wool pads were used as a source of water and as sites for oviposition, with additional humidity provided by regular light spraying. Cardboard egg cartons and paper provided shelter and increased the surface area within enclosures. The stock population was housed in large ventilated plastic bins and the experimental population in ventilated plastic cake boxes (30 cm × 30 cm × 16 cm) under identical conditions. Virgins were isolated from mixed-sex stock populations as late instars and maintained in single-sex groups until maturity before being assigned randomly to male–female pairs. Oviposition pads provided for each pair were sprayed daily to prevent desiccation of the eggs until the natural death of the female. The offspring of each pair were separated into single-sex groups at a late instar stage and maintained as above until sexual maturity.

(b) *Variation in paternity*

Inbreeding effects are an important consideration in investigations of sperm selection with respect to genetic similarity, as loss of embryos at an early stage of development can influence the conclusions about paternity and differential sperm use by females (e.g. Olsson *et al.* 1997). The sterile male technique is commonly used to assign paternity in studies of insect sperm competition. Females receive sequential copulations from a normal and an irradiated male and, since the offspring of irradiated males fail to develop, the relative paternity achieved by each male can be calculated by counting the number of eggs that hatch (e.g. Boorman & Parker 1976). Where the sterile male technique is employed to investigate sperm selection with respect to relatedness of mates, inbreeding could result in misleading conclusions about paternity, since it may be difficult to distinguish reduced hatching success associated with sperm irradiation from inbreeding effects. In the present investigation, this problem is addressed by comparing the paternity of the first male to mate (P_1) for sibling males within families. Two sibling males were each mated to sibling females. The two females were then mated a second time, either with another sibling male or with an unrelated male. The paternity outcomes of the first males to mate (P_1) were then compared (see figure 1). Comparing the P_1 values of males that are both full siblings of the females mated means that inbreeding effects are balanced and, hence, effectively cancelled out. Similarly, the sequence of irradiated versus normal male copulations was varied between but not within families. Within families, comparisons of P_1 were made only between pairs of sibling males which were both either irradiated or normal, such that any effects of irradiation on sperm competitiveness are also cancelled out in the comparison. If sperm selection operates against males on the basis of their relatedness to the female, the P_1 values should be consistently lower for sibling males in competition with a male unrelated to the female than for sibling males in competition with another sibling.

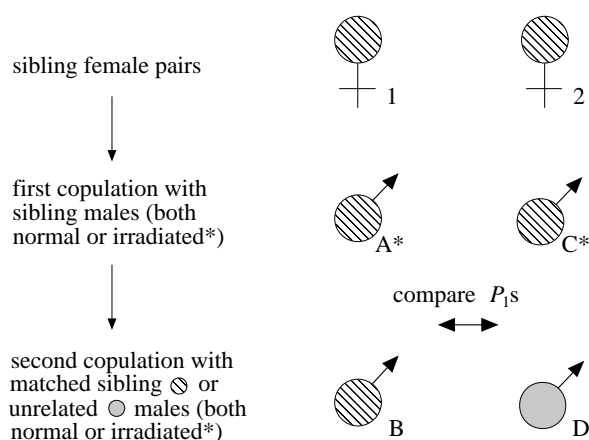


Figure 1. Experimental design used to look for evidence of non-random paternity with respect to the relatedness of mates. Females were matched as sibling pairs, each from different families. All females received a copulation from one normal and one irradiated male, with half of the female pairs receiving copulations first from normal and half from irradiated males. Within each female pair, both first copulations were with sibling males. Second copulations were then either with a third sibling or an unrelated male. Males mated to each female pair were matched for body size, age and rearing density. The paternities of the resulting offspring achieved by sibling males that copulated first (P_1 ; see the text) were then compared to test whether siblings achieve consistently lower paternities in competition with males that are unrelated to the female.

To minimize the variation in paternity due to the number of sperm transferred, male pairs selected for the experiment were matched for body size (± 25 mg) and age (\pm approximately one week). Some variation also occurred between families with respect to rearing density, which can influence sperm production in insects (Gage 1995) and this was controlled for in selecting unrelated male pairs. Half the males were irradiated with a cobalt gamma-ray source at a dosage of 20 krad ($0.5 \text{ krad min}^{-1}$). All experimental males were housed individually in ventilated glass jars with food and water provided *ad libitum* between matings. Irradiated males were maintained overnight before their first copulation. Copulations were arranged during the dark phase of the light cycle in the same constant-temperature room where the crickets were housed. Virgin females were transferred to and subsequently maintained in individual ventilated plastic containers ($20 \text{ cm} \times 20 \text{ cm} \times 20 \text{ cm}$), into which males were introduced for copulations. Males were left in the enclosure following copulation until females had removed the ampulla. Females received only one copulation per day and were offered the opportunity to remate with the second allocated male 24 h later and on subsequent days thereafter until a second copulation was achieved. In a few cases the same sibling male was used to provide a first copulation to both females in a pair when a matched sibling male died before copulating. When this occurred, males were not allowed to copulate more than once within 24 h. Control females received copulations with only irradiated or normal (sibling or unrelated) males. The interval between copulations was recorded for all females. Following their second copulation, females were provided with a moist cotton-wool pad for oviposition (prior to the second copulation, moisture was provided by spraying to prevent oviposition). Containers were checked daily for the

presence of eggs and nymphs and the oviposition pads sprayed lightly with water. Females were removed approximately 14 days after their first oviposition bout. All nymphs and unhatched eggs were counted approximately four to five weeks after the first nymphs appeared, to allow ample time for all viable eggs to hatch.

(c) Variation in ampulla attachment

In species with external spermatophores, females can potentially influence the number of sperm transferred to storage by manipulating the duration of ampulla attachment (review in Eberhard 1996). The ampulla attachment duration in *G. supplicans* is related to male body size, as large males provide large nuptial gifts which take longer for the female to consume (Sakaluk 1985). Competing males in the present experiment were therefore matched for body size and all durations of ampulla attachment were recorded to permit investigation of potential behavioural manipulation of sperm transfer by females.

(d) Variation in sperm numbers

Non-random paternity can also be generated by variation in the number of sperm transferred at mating. As already noted, sperm competition in *G. supplicans* operates like a raffle, with mixing of sperm in storage (Sakaluk 1986). In the present study, matching the body size of competing male pairs was intended to control variation in the number of sperm transferred (see above).

It is also possible that males might adjust the size of their ejaculate adaptively in relation to perceived local conditions at the time of mating. There is evidence, for example, that males of various species adjust sperm numbers in relation to local sperm-competition risk (Gage 1991; Gage & Barnard 1996) or to female traits such as reproductive status, age or body size (e.g. Wedell 1992; Gage 1998). The ability to vary sperm numbers adaptively has previously been demonstrated in crickets, including *G. supplicans* (Gage & Barnard 1996). A further experiment was therefore performed to investigate the possibility that male *G. supplicans* might vary sperm numbers in relation to perceived relatedness to the female mated. Experimental males each copulated with two virgin females, one of which was a sibling and one of which was unrelated to the subject male. Female pairs were matched approximately for size and age. Copulations were arranged as described above and separated by a 24 h interval. Half of the males copulated first with a sibling and half with an unrelated female. Immediately following copulation, spermatophores were carefully removed from the female and the ampulla transferred to a vial containing 4 ml of distilled water. The ampulla was crushed with watchmakers forceps and the mixture stirred vigorously for 10 min. Three $10 \mu\text{l}$ samples of solution were spread onto a clean microscope slide and air-dried. The total number of sperm heads in each sample was counted under phase contrast at $\times 100$ magnification and mean values per sample for each male were multiplied by 400 to give the total number of sperm in the ampulla.

3. RESULTS

(a) Paternity analyses

A total of 32 females produced egg clutches following sequential copulations with one normal and one irradiated male. Out of these, 26 were matched sibling pairs within which one had received a second copulation from a sibling and one from an unrelated male (see figure 1). Seven matched sibling pairs received a first

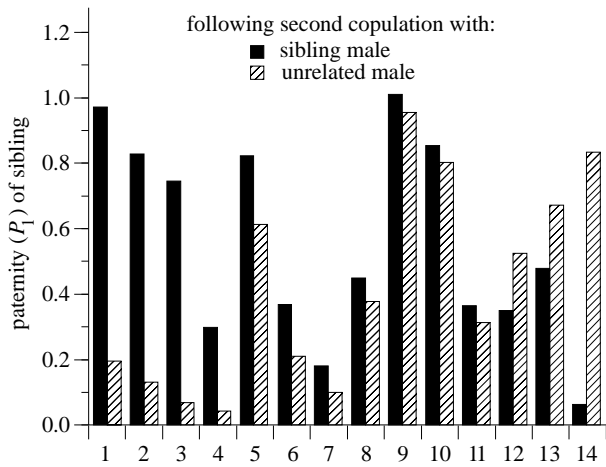


Figure 2. First male paternity values (P_1) of paired sibling females mated first with sibling males and remated with either another sibling or an unrelated male. The data are ranked according to the difference in paired P_1 values. The females in pair number 3 were unrelated (see the text).

copulation from an irradiated male and six from a normal male. Paired siblings of five out of the remaining six females failed to remate and one was excluded from the analyses after producing an unusually small egg clutch. Paternity data from two unrelated females with a matching irradiation sequence and contrasting second copulations (sibling/unrelated) were used to provide an additional P_1 comparison. The remaining four females had each received second copulations from unrelated irradiated males and were not included in the matched-pair analyses. As expected, no eggs resulting from control matings with irradiated males hatched. The paternity of normal males was therefore calculated as $P_N = x/\beta$, where x is the proportion of eggs hatching after double matings and β is the number of eggs hatching after control matings with normal unrelated or sibling males, respectively.

The results of the paternity analysis are shown in figure 2. In 11 out of the 14 paired P_1 comparisons, paternity was lower for sibling males in competition with a male unrelated to the female than for sibling males in competition with another sibling (χ^2 test $p=0.03$). However, the paternity values were highly variable (table 1 and figure 2) and the mean arcsine-transformed P_1 values did not differ significantly with respect to the relatedness of the second male to the female or irradiation sequence (table 2; although with exclusion of comparison 14 as shown in figure 2, $t_{12}=2.37$ and $p<0.04$). The mean overall P_1 was 0.495 ± 0.06 (range 0.04–1.00) with means of 0.50 ± 0.09 and 0.49 ± 0.07 for normal and irradiated males, respectively. The mean total clutch size for all females was 290 ± 22 (range 96–624) and there was no significant difference in the total number of eggs laid by females remated with sibling and unrelated males (paired t -test $t_{13}=0.37$ and $p>0.70$).

(b) Duration of ampulla attachment and remating interval

There was no significant difference in the duration of ampulla attachment for first and second copulations (paired t -test: $t_{31}=1.20$, $p>0.20$). The mean ampulla attachment time was 46.3 ± 4.0 min (range 19.0–115.3;

table 1). For second copulations, the duration of ampulla attachment did not differ significantly with respect to the relatedness of mates or irradiation sequence (table 2). Similarly, there was no significant difference in the remating interval of females receiving second copulations with sibling and unrelated males of varying irradiation sequence (table 2). The mean overall delay between copulations was 2.1 ± 0.2 days (range 1–4; table 1).

(c) Sperm numbers

There was no difference in the number of sperm transferred by males to sibling and unrelated females. Mean (\pm s.e.) total sperm numbers of $37\,960 \pm 5953$ (range 7600–61200) were transferred to siblings and $40\,920 \pm 3701$ (19600–56400) to unrelated females (paired t -test $t_9=0.53$ and $p>0.60$).

4. DISCUSSION

The results of the present investigation provide some evidence in support of the hypothesis that females (or their ova) are capable of sperm selection on the basis of conspecific male genetic similarity. Sibling males achieved lower fertilization success in competition with a male unrelated to the female than in competition with another sibling in 11 out of 14 paired P_1 comparisons. No significant difference was found in the mean first male paternity values for females remated with sibling and unrelated males. However, given the observed significant trend for directional differences in P_1 variation within pairs, it appears unsafe to conclude that the data reveal no evidence of sperm selection. As is typical in studies of relative paternity in insects (Lewis & Austad 1990; Simmons & Siva-Jothy 1998), the P_1 values in the present study were extremely variable, increasing the possibility of type II error. Although it is not possible to assert that the present study provides conclusive evidence of sperm selection, the results are certainly suggestive of such an effect.

No evidence of behavioural discrimination by females with respect to the relatedness of males was found. Females copulated readily with siblings and there was no difference in the mean duration of ampulla attachment or remating interval for those remated with sibling and unrelated males. Hence, the pattern of paternity described above cannot be explained in terms of differences in the number of sperm transferred to storage under female control or the differential effects of sperm ageing and/or loss from storage. This lack of female behavioural discrimination with respect to the relatedness of mates contrasts with the results of previous similar investigations in crickets (e.g. *G. bimaculatus*; Simmons 1989, 1991). Species and population differences in mating preferences may arise due to variation in the strength of selection to avoid copulations with relatives. Patterns of dispersal have not been studied in the natural populations of crickets from which these study populations were derived, although there is no reason to expect that high levels of inbreeding should occur under natural conditions. It seems probable though that selection pressure to avoid inbreeding would increase in laboratory cultures over several generations. This would not apply in the case of the wild-type study population used here.

Table 1. Mean (\pm s.e.) values of first male paternity (P_1), second copulation ampulla attachment duration and remating interval for doubly mated females copulating first with a sibling male and second with either another sibling or an unrelated male

(The copulation sequence was varied in relation to the irradiation treatment of males, with half the females in each treatment group copulating first with an irradiated male (RN) and half with a normal male (NR). The sample sizes are different due to variation in remating success and egg production (see the text).)

irradiation sequence	second copulation with					
	sibling male			unrelated male		
	RN ($n=8$)	NR ($n=6$)	combined ($n=14$)	RN ($n=10$)	NR ($n=8$)	combined ($n=18$)
first male paternity (P_1)	0.57 (± 0.11)	0.51 (± 0.13)	0.55 (± 0.08)	0.43 (± 0.10)	0.49 (± 0.13)	0.46 (± 0.08)
ampulla attachment (min)	42.22 (± 6.28)	42.92 (± 9.17)	42.52 (± 5.10)	39.23 (± 4.35)	61.77 (± 11.25)	49.25 (± 6.00)
remating interval (days)	2.12 (± 0.40)	2.33 (± 0.42)	2.21 (± 0.28)	1.90 (± 0.35)	2.12 (± 0.44)	2.00 (± 0.27)

Table 2. ANOVA of arcsine-transformed first male paternity (P_1) values, second copulation ampulla attachment durations and remating intervals for matched female pairs

(The sources of variation examined are the irradiation sequence of males mated (RN or NR) between pairs and the relatedness of the second male mated (sibling or unrelated) within pairs. MS, mean squares.)

dependent variable	source of variation	MS	d.f.	F	p
paternity P_1	between pairs				
	irradiation sequence	0.003	1	0.019	0.893
	error	0.182	12	—	—
	within pairs				
	relatedness of second mate	0.236	1	2.029	0.180
	interaction	0.006	1	0.054	0.820
ampulla attachment	error	0.116	12	—	—
	between pairs				
	irradiation sequence	64.5×10^5	1	3.310	0.094
	error	19.4×10^5	12	—	—
	within pairs				
	relatedness of second mate	5.2×10^5	1	0.521	0.484
remating interval	interaction	19.0×10^5	1	1.870	0.197
	error	10.1×10^5	12	—	—
	between pairs				
	irradiation sequence	0.146	1	0.118	0.737
	error	1.238	12	—	—
	within pairs				
relatedness of second mate	0.003	1	0.003	0.959	
interaction	0.146	1	0.136	0.719	
error	1.071	12	—	—	

If the population in the present study had not previously been subject to strong selection for avoidance of inbreeding, the trend towards non-random paternity observed here may reflect a more general ability in insects for sperm selection on the basis of male genetic incompatibility. That is, selection may favour discrimination by females against males with incompatible gene combinations, which will probably occur with increased probability in close relatives. Although the basis of selection for genetic compatibility of mates has not yet been investigated in insects, analogous results in vertebrates have been linked to genes which are important in parasite–host interactions. There is evidence, for example, of non-random fertilization with respect to major histocompatibility complex (MHC) loci among inbred mouse strains, with virus-infected mice producing more MHC heterozygous embryos (Wedekind *et al.* 1996; Rüllicke *et al.* 1998).

There was no difference in the number of sperm transferred by males to sibling and unrelated females. Reduced ejaculate expenditure is predicted theoretically for cases of sperm competition between related males (Parker 1999). Where mating with a sister will probably correlate with increased probability of competing with a brother, a male should allocate less sperm when mating with a sister than with an unrelated female. This effect could result in lowered paternity by a sibling of the mated female when competing with an unrelated male. Moreover, if females discriminate against the sperm of close relatives, siblings may be considered to be mating in a disfavoured role with respect to sperm competition (*sensu* Parker 1990*a,b*), which may also affect male allocation patterns (Parker 1998; but see also Mesterton-Gibbons 1999). The fact that males in the present study did not adjust sperm numbers in relation to female relatedness suggests that they may be

unable to recognize kin or that it is not adaptive for them to do so. Gage & Barnard (1996) found that male *G. supplicans* varied the number of sperm in their ejaculates adaptively in response to sperm competition risk but not in response to female size. Hence, although there is evidence that male *G. supplicans* are able to adjust sperm numbers adaptively under certain conditions, they apparently do not respond to female characteristics. In the context of the present study, the finding that male *G. supplicans* did not vary sperm numbers in response to female relatedness rules out differential sperm allocation as a potential explanation for the observed trend in non-random paternity.

An average P_2 of around 0.50 with approximately normal distribution was found for doubly mated *G. supplicans* females. In agreement with previous studies of sperm competition in this species (Sakaluk 1986), this result suggests that there is mixing of sperm from consecutive mates in storage. Under natural conditions, however, it is probable that there will be an advantage for the first male to mate. Females in the present study were prevented from ovipositing prior to the second copulation, but first males would gain exclusive access to any eggs oviposited before remating occurred under natural conditions (see also Calos & Sakaluk 1998).

If, as suggested by the results of the present study, females (or their ova) are capable of sperm selection on the basis of male genetic incompatibility, this may also help to explain the function of female multiple-mating behaviour. The adaptive value of female promiscuity is unclear in many taxa where females regularly incur costs of remating while gaining no obvious increase in fertility or fecundity. The function of multiple mating with more than one male can be explained, however, if sperm selection or some other post-copulatory mechanism allows females to concentrate their investment in offspring sired by genetically compatible males (Zeh & Zeh 1996, 1997). Where females gain direct fitness benefits from multiple mating, such as the nutritional benefits associated with the provision of a nuptial gift, sperm selection would also allow them to accumulate benefits from indiscriminate copulations while minimizing indirect costs such as potential inbreeding depression.

In summary, in agreement with several recent studies (Wilson *et al.* 1997; Clark & Begun 1998; Tregenza & Wedell 1998; Clark *et al.* 1999), the results of this study hint at a widespread ability of female insects for sperm selection on the basis of male genetic incompatibility. These results are particularly interesting because the study population had not previously been subject to inbreeding. Given the broad potential significance of sperm selection in evolutionary biology, this represents a particularly promising area for further detailed investigation.

This research was funded by UK Natural Environment Research Council grant number GR9/3143. I am particularly grateful to Matt Gage for providing crickets and to staff at the Royal Liverpool Hospital Department of Immunology for access to irradiation facilities. Nicola Seal and Ted Morrow helped in rearing the experimental populations and Geoff Parker, Matt Gage, Leigh Simmons, Jane Hurst, Dave Thomson, Tom Tregenza and John Lycett provided much helpful advice and comments on the manuscript.

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