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Treatment comparisons

The calculated nitrogen uptake data (Table 2) were used to compare how species differ in their patterns of uptake across treatments. We emphasize that the data cannot be used to compare how species partition total nitrogen uptake within treatments because the total amount of ^{15}N uptake by each species strongly depends on the relationship between lateral rooting distance and the spacing of ^{15}N injections. In this experiment, where ^{15}N injections were spaced in a 7.5-cm grid, *Ledum* took up six times more ^{15}N across all treatments than *Eriophorum* (^{15}N data not shown). In a different experiment designed to determine species' lateral rooting distances, we injected a constant amount of ^{15}N (114 mg as a mixture of 25 mmol l^{-1} each of glycine, ammonium and nitrate) at different treatment radii (5, 10, 20, 40, 60 and 100 cm) around individual plants of *Eriophorum* and *Ledum*. That design allowed ^{15}N uptake data to be spatially integrated across all treatment radii, simulating how ^{15}N would be taken up from injections spaced infinitely close together over an area with a radius of 100 cm. Although those results showed that the primary lateral radius of uptake (about 60% of total uptake) of *Eriophorum* was about half that of *Ledum* (5 versus 10 cm), *Eriophorum* took up almost twice as much ^{15}N as *Ledum* when the data were extrapolated on a community basis (uptake per unit ground area) (R.B.M., unpublished data). The results of both experiments show that widely spaced tracer injections disproportionately label species with larger rooting areas but lower uptake capacities per unit ground area (for example, *Ledum*). In contrast, species' patterns of uptake across treatments (within rows), as presented here, should be relatively robust with respect to the spacing of tracer injections.

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Polyandrous females avoid costs of inbreeding

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Why do females typically mate with more than one male? Female mating patterns have broad implications for sexual selection[1,2], speciation[3] and conflicts of interest between the sexes[4], and yet they are poorly understood. Matings inevitably have costs[5], and for females, the benefits of taking more than one mate are rarely obvious. One possible explanation is that females gain benefits because they can avoid using sperm from genetically incompatible males, or invest less in the offspring of such males[6,7]. It has been shown that mating with more than one male can increase offspring viability[8–12], but we present the first clear demonstration that this occurs because females with several mates avoid the negative effects of genetic incompatibility[13]. We show that in crickets, the eggs of females that mate only with siblings have decreased hatching success. However, if females mate with both a sibling and a non-sibling they avoid altogether the low egg viability associated with sibling matings. If similar effects occur in other species, inbreeding avoidance may be important in understanding the prevalence of multiple mating.

Previous studies of the highly polyandrous[14] (mating with more than one male)[10] field cricket *Gryllus bimaculatus* have shown that polyandry is associated with increased egg hatching success[10]. This benefit appears to stem from males having higher fertilization success when they mate with females with whom they are genetically more compatible. As yet, the source of this incompatibility is unknown. Within natural populations the negative effects of homozygosity for deleterious recessive alleles and at loci with heterozygote advantage[15] mean that mating with a close relative is likely to be a major source of genetic incompatibility. We suggest that females may be able to avoid this threat to their reproductive success through some mechanism that enables them to preferentially fertilize their eggs with sperm from genetically compatible males. To test this hypothesis we conducted a study in which females were allocated matings with males of known relatedness. Our prediction is that females mating with two relatives will have low offspring viability, but that polyandrous females mating with both closely related and unrelated males will have offspring viability comparable with females only mating to unrelated males.

Blocks of four sibling females were assigned to one of four treatments, all of which involved one mating with each of two different males, either with two siblings (SS), two non-siblings (NN) or a sibling and a non-sibling in either order (SN and NS) (see Methods). After mating, the hatching success of eggs was recorded. Randomized block analysis of variance reveals a significant effect of mating treatment on proportional egg hatching success ($F_{3,75} = 6.01$, $P = 0.001$) (Fig. 1). Hatching success does not differ significantly between experimental blocks ($F_{25,75} = 1.48$, $P = 0.10$). Post hoc analysis (Tukey test) of egg hatching indicates that the significant effect of treatment is due to the lower hatching success of females mated to two siblings relative to females mated to at least one non-sibling (Fig. 1) (Tukey test: NN versus SN or NS, minimum $P = 0.68$; SS mating versus other treatments, maximum $P = 0.05$). This suggests that females mating with a sibling and a non-sibling have egg viability similar to that of completely outbreeding females, rather than halfway between completely outbreeding and completely inbreeding females. We can test this explicitly by comparing the hatching success of females within each block using a paired t-test of mean hatching success of NN and SS females versus SN and NS females. This shows there is indeed a significant improvement in egg viability in polyandrous females mating to both related and unrelated males, relative to that which would be expected if sperm from both males were used equally ($t = 2.53$, degrees of freedom, d.f. $= 25$, $P = 0.018$).

There is no significant difference in hatching success according to the order in which females mated to a sibling and a non-sibling (NS versus SN, Tukey test $P = 0.68$), which confirms the lack of any effect of mating order on sperm precedence[16]. The overall mean hatching success across females was 48% (standard error of the mean, s.e.m. = 2.2%). This is similar to the 46% hatching success observed in females from wild populations which were caught as adults (presumably having already mated), and allowed to mate repeatedly with a single male[17]. It is also similar to the 47% hatching success we observed in females mating twice to each of two males in a previous study[10].

There is no effect of mating treatment on the number of eggs laid in the three days following mating (randomized block analysis of variance with female body size as a covariate, $F_{3,74} = 1.25$, $P = 0.30$). There is a difference between blocks ($F_{25,74} = 2.58$, $P = 0.001$) which may be due to variation between families in their egg-laying rate (because each family was used in only one or two blocks) although any such differences cannot be separated from possible differences arising from the fact that blocks were carried out at different times. The lack of effect of mating treatment indicates that females do not refrain from oviposition when mated to siblings, a behaviour observed in two species of *Drosophila*[18,19].

The finding that females mated to a brother and a non-sibling have higher egg viability than would be expected if related and unrelated males had equal fertilization success provides the first direct evidence, to our knowledge, that polyandrous females increase offspring fitness through avoidance of the negative effects of genetic incompatibility. The only plausible way this effect could arise is if there is differential fertilization success of sperm[2] in favour of the ejaculate of unrelated males. The alternative explanation that females mated to an unrelated male increase egg viability by allocating more resources to eggs (increasing their viability), is unconvincing. There is limited scope for differential allocation in this species since the eggs laid in the period we observed are typically already chorionated in four-day-old females and cannot be further provisioned[20]. Additionally, if there are other costs of inbreeding that mean females should avoid investing in eggs fertilized by siblings it would be very much more efficient for them simply to oviposit less when mated to incompatible males. Females of this species have been shown to increase oviposition when given the opportunity to choose a mate rather than having one allocated to them[21], so they are clearly capable of such behaviour.

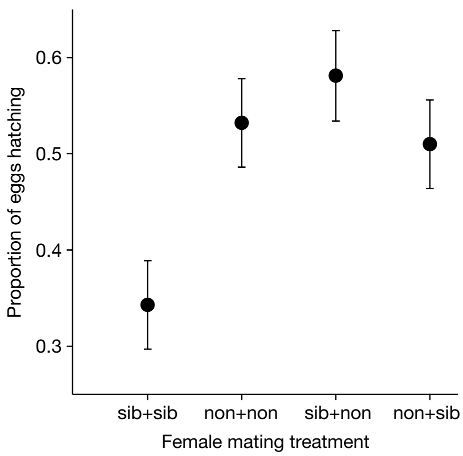

Figure 1 Relative hatching success. Eggs were from female field crickets mated either to two siblings, two non-siblings, a sibling followed by a non-sibling or a non-sibling followed by a sibling (means and standard errors).

The mechanism by which differential fertilization occurs is unknown. One possibility is that males choose to inseminate less sperm into related females. However, this is unlikely because males in our experiment had produced a spermatophore before contact with the female and hence could not manipulate the size of their ejaculate, suggesting that differential male-fertilization success is a female effect. Females from laboratory populations are able to recognize kin using olfaction[22], and have been found to be less willing to mate with full siblings[23]. No precopulatory differences in female behaviour in relation to male relatedness were observed in our study, but females may exercise choice by accepting less sperm from closely related males, or through post-copulatory mechanisms that reduce the fertilization success of sperm from related males. Higher sperm competition success of unrelated males has been described in another species of cricket[24], although the effect was not statistically significant. Analogous differences in postcopulatory fertilization success are known in matings between species or geographic races[25], where sperm from more genetically distant males is frequently less successful. This is clearly a different process to that observed in this study, where more genetically similar individuals are less successful, but the existence of conspecific sperm precedence illustrates the potential for female sperm choice.

Previous studies have found evidence that mating with more than one male is associated with higher egg or offspring viability[26]. Field studies of adders[8] and sand lizards[9] have shown correlations between female promiscuity and offspring viability. Experiments using pseudoscorpions[11], field crickets[10,27] and cuis[12], controlling number of matings and allocating pairings at random to rule out precopulatory choice, have also found viability benefits of polyandry. Because these studies fail to find any evidence for 'viability genes' they provide indirect support for benefits of polyandry due to avoidance of genetic incompatibility. However, none of them attempted to manipulate genetic compatibility and therefore direct evidence for the hypothesis is lacking. The best existing evidence that within-population polyandry may allow females to avoid genetic incompatibility comes from the sand lizard[28], in which there is a negative correlation between male relatedness and success in sperm competition. Although no direct link between differential success in sperm competition and increased offspring fitness has been demonstrated in sand lizards, the existence of inbreeding depression[29] suggests it may occur.

A previous study of the cricket *Teleogryllus oceanicus*[27] attempted to test the genetic incompatibility hypothesis through the prediction of a positive association between hatching success and paternity skew. No such relationship was found in 16 females, each mated to 2 males. However, the prediction that females biasing offspring paternity to a greater extent will have higher offspring viability is much more difficult to test if there is variation in the relative compatibility of mates. If some females are mated to two compatible males they will have high hatching success even if they do not bias paternity, whereas a female mated to two incompatible males could bias paternity completely in favour of the slightly more compatible of the two but still have low hatching success. Male compatibility was not manipulated in this previous study[27], so it is difficult to exclude genetic incompatibility as an explanation for the observed viability benefits of polyandry.

It is not known how common matings between full siblings are in natural populations of crickets, although the large numbers of eggs produced by a single female certainly create the potential for sibling matings. The substantial fitness benefits of polyandry in females exposed to brothers and unrelated males suggests that selection could favour polyandry even if sibling matings are rare, or if polyandrous females can avoid the lesser, but still appreciable costs of inbreeding with more distantly related males. If the ability of female field crickets to avoid using sperm from related males is shared by other species, this form of genetic incompatibility avoidance may be an important factor promoting female promiscuity across taxa. □

Methods

Crickets

All individuals were F_2 descendants of gravid female crickets collected from the wild in Gabarone, Botswana. Offspring of the parental females were kept separately and one virgin F_1 female from each parental female was mated to a single male from another family to create a set of unrelated full-sibling families. It is possible that females in the original collection may include relatives; this would be conservative in relation to our study. Families were reared in 131 plastic cages at 29 °C and 18:6 hours light:dark and freely provided with rodent food pellets and water. Experimental individuals were collected from families as late-instar nymphs to ensure virginity and isolated in separate 9-cm-diameter pots provided with food and water. The experiment was arranged in blocks of four females and four males; six of these were siblings (four females and two males from one family), and two were male siblings from another family.

Mating

All females were virgins; all males had mated once to an unrelated female on the previous day. Only males who had a spermatophore ready for transfer were used: such males produce courtship song as soon as they contact a female[30]. Males were used in one block only. Matings were allowed by adding the male to a 9-cm-diameter pot containing a female. Nearly all pairs mated within 10 minutes of being introduced. If the female did not mate within an hour of being placed with her first mate she was replaced with a sister (5 out of 114 females). After mating the male was allowed to stay with the female for a further 45 min to prevent her from removing the spermatophore; no female removed a spermatophore before this time. An hour after her first mating the female was mated a second time to a different male using the same protocol. In each block, females were allocated to one of four treatments: (1) one mating to each of two of her brothers (SS); (2) one mating to each of two males which were brothers to one another but unrelated to the female (NN); (3) one mating to a sibling male followed by one mating to an unrelated male (SN); (4) one mating to an unrelated male followed by one mating to a sibling male (NS). Therefore, all females mated twice and all males mated twice during the experiment and once previously. Twenty-eight blocks were carried out, with 15 families each used in a maximum of two blocks. In two blocks a female died before laying eggs. These blocks were excluded from the analysis.

Eggs

After mating, the female was placed in a 9-cm-diameter pot and provided with food and fine wet sand for oviposition. Sand was kept moist at all times, and after three days was sieved to remove eggs. Eggs were counted and placed on a wet cotton wool pad in a petri dish and maintained under the same conditions as the adults. Eggs were checked daily for hatching until seven days after the last emergence, by which time eggs that have not hatched have begun to break down. To check for changes in hatching success over time, females laying less than 100 eggs were given a further three days for oviposition, repeated for up to 12 days or until the female had laid 100 eggs. Paired t-tests of the proportional hatching of eggs laid in the first three days versus those laid in the second three days, or versus all subsequent eggs, indicated no change in hatching success over time ($t = 0.4$, $P = 0.6$, d.f. $= 31$, in both cases). The cube of the length of the hind femur of all females was used as a body size measure, it did not affect egg hatching success ($F_{1,102} = 0.005$, $P = 0.94$), but did have an effect on number of eggs laid ($F_{1,102} = 6.67$, $P = 0.01$), and is included in the analysis of eggs laid as a covariate. In all analyses, proportions were arcsine transformed and numbers of eggs laid were square-root transformed to normalize their distribution. All P values are two-tailed.

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The optic tectum controls visually guided adaptive plasticity in the owl's auditory space map

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The midbrain contains an auditory map of space that is shaped by visual experience[1–3]. When barn owls are raised wearing spectacles that horizontally displace the visual field, the auditory space map in the external nucleus of the inferior colliculus (ICX) shifts according to the optical displacement of the prisms[4]. Topographic visual activity in the optic tectum could serve as the template that instructs the auditory space map[5]. We studied the effects of a restricted, unilateral lesion in the portion of the optic tectum that

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