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# No postcopulatory response to inbreeding by male crickets

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**Previous studies of the cricket *Teleogryllus oceanicus* have shown a paternity bias towards non-sibling males. Although non-kin-biased paternity could represent a mechanism of postcopulatory inbreeding avoidance by females, evolutionarily stable strategy (ESS) models of ejaculate evolution also predict that males should reduce their expenditure on the ejaculate when mating with their sisters. Here we provide a test of these models, finding that male crickets invest equally in matings with full-siblings, half-siblings and non-sibling females. The data suggest that in this species, males and females differ in their response to inbreeding.**

**Keywords:** strategic ejaculation; ejaculate quality; inbreeding; cryptic female choice

## 1. INTRODUCTION

Inbreeding increases homozygosity and allows the expression of deleterious recessive mutations, generating a reduction in fitness known as inbreeding depression (Keller & Waller 2002; Roff 2002). It is often thought, therefore, that inbreeding avoidance should be an adaptive strategy in mate choice with individuals selected to avoid breeding with close relatives (Pusey & Wolf 1996). However, breeding with close relatives can provide inclusive fitness benefits. For example, a male can increase his mating success, and therefore inclusive fitness, by mating with his sister if there are no opportunity costs associated with the mating. Similarly, a female will gain fitness benefits via mating with her brother, directly through her own offspring and indirectly by improving her brother's mating success (Parker 1979; Lehmann & Perrin 2003; Kokko & Ots 2006; Parker 2006). Whether selection should in fact favour inbreeding or its avoidance will depend on both the costs associated with inbreeding depression and the inclusive benefits of mating with kin. Theory suggests that males and females may have different tolerance thresholds to inbreeding: males should mate with their sisters if inbreeding depression is less than two-thirds, while females should avoid mating with their brothers if inbreeding depression is greater than one-third, generating a conflict zone within which male and female interests differ (Parker 1979, 2006). Sexual conflict over inbreeding is therefore expected to generate selection for divergent male and female reproductive strategies.

The avoidance of inbreeding via precopulatory mate choice has been documented in many taxa

(Pusey & Wolf 1996). Fewer studies have documented preferences for related individuals as mates (Cohen & Dearborn 2004), although this may be due to the overwhelming preconception that mating with close relatives is bad (Kokko & Ots 2006). Consistent with the theoretical expectation, recent work with red jungle fowl has revealed counteracting sex-specific responses to inbreeding (Pizzari *et al.* 2004). Male jungle fowl are equally likely to mate with their sisters as with non-sibling females, but ejaculate more sperm when mating with their sisters. Females counteract male responses that might otherwise promote inbreeding, by retaining fewer sperm when mated by a brother.

Several recent studies have suggested that postcopulatory mechanisms may be important avenues for inbreeding avoidance by females. Studies of crickets (Stockley 1999; Bretman *et al.* 2004; Simmons *et al.* 2006) and house mice (Firman & Simmons 2008) suggest that, when inseminated by both a brother and a non-sibling male, paternity is biased towards the non-sibling (although see Denk *et al.* 2005). These data suggest that sperm–female interactions facilitate the preferential fertilization of ova for the production of outbred offspring. Ball & Parker (2003) examined an evolutionarily stable strategy (ESS) model of the effects of such postcopulatory female choice on male ejaculate expenditure, finding that when male roles are assigned randomly, as would be the case with kin-biased postcopulatory female choice, males in the disfavoured role (siblings) should expend less on their ejaculate than males in the favoured role (non-siblings; Parker 2006). Thus, observations of paternity bias towards non-sibling males may arise owing to adaptive plasticity in male ejaculation strategies. However, there have been few attempts to test these ESS models.

In this study we examined male pre- and postcopulatory responses to inbreeding in the field cricket *Teleogryllus oceanicus*. Previous studies of this species have revealed that when females mate with both a sibling and a non-sibling male, paternity is biased towards the non-sibling (Simmons *et al.* 2006). However, the mechanism underlying paternity bias is unclear. We test the hypothesis that strategic ejaculation by males is a contributing factor (Ball & Parker 2003; Parker 2006). Strategic ejaculation consistent with other ESS models (Parker 1998; Engvist & Reinhold 2006) has been demonstrated in this species: male *T. oceanicus* increase their expenditure on the ejaculate when the risk of sperm competition is increased and decrease their expenditure as the intensity of sperm competition is increased (Simmons *et al.* 2007; Thomas & Simmons 2007). In *T. oceanicus*, success in competitive fertilization depends more on the quality of a male's ejaculate (García-González & Simmons 2005) than on the number of sperm transferred (Simmons *et al.* 2003); accordingly, phenotypic plasticity in ejaculate expenditure is manifested as changes in ejaculate quality, rather than the numbers of sperm (Thomas & Simmons 2007). Thus, we ask whether males adjust ejaculate quality when mating with females of differing degrees of relatedness.

## 2. MATERIAL AND METHODS

We used a half-sibling breeding design, with animals collected from Carnarvon, northwestern Australia. Twenty-five adult males were

each housed for one week with two unmated females that were not related to each other, or to the male. Females were then housed individually with damp cotton wool for oviposition. Thirty newly hatched nymphs were kept in full-sibling groups in 51 containers and reared at 25°C under a 12 L : 12 D cycle. Sexes were separated prior to the adult moult. After adult eclosion, individuals were kept in individual boxes (7×7×5 cm) until used in the experiment (13±3 days after adult eclosion).

We used a randomized block design. Blocks consisted of three full-sibling males, each assigned to one of three different mating treatments: mating to a full-sibling female (FS); mating to a half-sibling female (HS); or mating to a non-sibling female (NS). Males and females were used only once. All females were previously unmated and all males had mated once to a non-sibling unmated female immediately before being used in experiments. This initial non-experimental mating ensured that males were sexually mature, and that the ejaculate allocated to the spermatophore in the presence of the experimental female reflected the male's response to the degree of relatedness perceived during ejaculation and spermatophore production. Twenty-four blocks were used, with each block represented by males from one family.

Once males had mated with the experimental female, the spermatophore was removed from the female and the viability of the sperm contained within the ejaculate analysed. The spermatophore is a discrete vessel containing sperm, and remains attached outside the female's body after mating. Sperm viability was analysed using the live/dead sperm viability assay (García-González & Simmons 2005). This assay stains live sperm green with SYBR-14 and dead sperm red with propidium iodide. The first 500 sperm per sample were scored on a fluorescence microscope at 200× magnification. Sperm scores were performed blind to the experimental treatment and family block, and the proportion of live sperm arcsine transformed for statistical analyses.

In other species, including another species of gryllid cricket, *Acheta domestica* (Gage & Barnard 1996), female size is known to influence ejaculate allocation (Wedell *et al.* 2002). To examine potential female size effects, we weighed females to an accuracy of 0.1 g. It was also of interest to determine whether males and/or females displayed any behavioural differences between treatments that may reflect kin-biased mating preferences. For this reason we also measured the time males took to produce a spermatophore and offer it to the female (courtship latency) and the duration of courtship females required before mating (courtship duration). However, in some cases the onset of courtship or mating was not observed, resulting in missing data for some treatments. We therefore restricted our analysis to family blocks in which data were not missing ( $N=10$  for courtship latency and  $N=9$  for courtship duration).

### 3. RESULTS

Male crickets did not vary their expenditure on the ejaculate in response to the relatedness of their female partner. Analysis of variance revealed no effect of mating treatment on the viability of sperm transferred to females (figure 1;  $F_{2,45}=0.457$ ,  $p=0.636$ ), and sperm viability did not differ significantly between family blocks ( $F_{23,45}=0.750$ ,  $p=0.769$ ). The effect of the covariate female weight was not significant ( $F_{1,45}=1.372$ ,  $p=0.248$ ). The effect sizes of relatedness on sperm viability (Hedge's  $d$  {95%CI}) were 0.21 {−0.36, 0.77} for the FS–NS contrast and −0.07 {−0.63, 0.50} for the HS–NS contrast. These effect sizes are of very different magnitude and range from those reported for risk when mating with previously unmated females (0.75 {−0.01, 1.51}), and the effect of female mating frequency (−1.42 {−1.95, −0.88}) calculated from data in Simmons *et al.* (2007).

We found no behavioural differences between females in their willingness to mate with full-sibling, half-sibling or non-sibling males. The duration of courtship required to elicit a female to mount did not differ significantly between treatment groups ( $F_{2,16}=0.510$ ,  $p=0.609$ ) and did not differ significantly between family blocks ( $F_{8,16}=1.140$ ,  $p=0.389$ ).

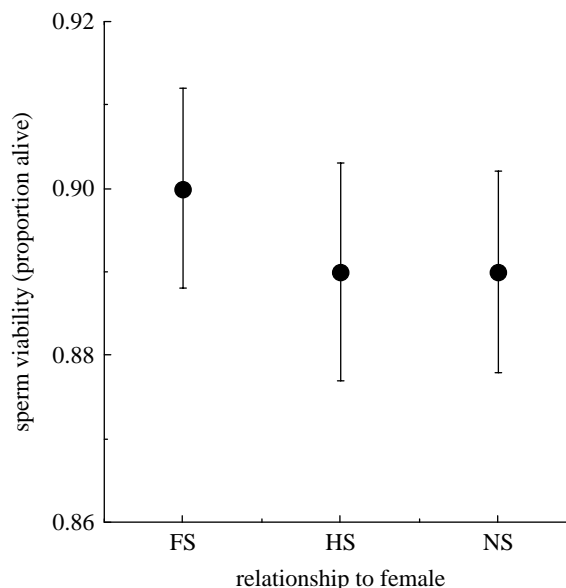


Figure 1. Quality of ejaculates (mean±s.e. proportion of sperm alive) transferred to full-sibling (FS), half-sibling (HS) and non-sibling (NS) females by male *T. oceanicus*.

Similarly, males showed no variation in the time required to produce a spermatophore and begin courtship ( $F_{2,20}=0.626$ ,  $p=0.545$ ), and this latency to court did not vary among families ( $F_{10,20}=0.544$ ,  $p=0.839$ ).

### 4. DISCUSSION

We were unable to detect any inbreeding avoidance by male *T. oceanicus*. Males were equally persistent in precopulatory courtship and transferred spermatophores as quickly to their sisters as they did to half-siblings and non-sibling females. Moreover, they did not reduce their expenditure on the ejaculate when mating with their sisters. Previous studies of inbreeding avoidance by female *T. oceanicus* showed a paternity bias towards non-sibling males (Simmons *et al.* 2006). Paternity bias could arise from sperm–female interactions, and/or from reduced investment in the ejaculate when males mate with their sisters (see also Parker 2000; Ball & Parker 2003). Based on this study, it seems more likely that female–sperm interactions are involved in the observed paternity bias towards non-siblings.

Collectively, the data suggest that male and female *T. oceanicus* differ in their postcopulatory responses to inbreeding: males invest equally in matings with siblings and non-siblings, while females exercise postcopulatory avoidance of inbreeding (Simmons *et al.* 2006). Sexual differences in inbreeding tolerance are expected from theory. Although estimates can be conservative, particularly if, as is the case in crickets, mate choice is sequential (Kokko & Ots 2006), females should be selected to avoid inbreeding when the costs exceed one-third, but males selected to avoid inbreeding only when the costs exceed two-thirds (Parker 1979, 2006).

In natural populations of animals, inbreeding depression typically lies somewhere between 15 and 25% (Keller & Waller 2002). Inbreeding costs are currently not well documented for crickets, although studies of related species allow us to speculate on the



parameter space that might be biologically realistic for *T. oceanicus*. Based on full-sibling matings, Roff (1998) reported average inbreeding depression for morphological traits of 0.4% (ranging from 0 to 1.15%) and for life-history traits of 5.3% (ranging from 0 to 16%) in the cricket *Gryllus firmus*. The corresponding value for life-history traits in the congeneric *Teleogryllus commodus* is 8.6% (Drayton *et al.* 2007). These values might be expected to generate tolerance of inbreeding by both males and females. However, it is becoming clear that traits important in sexual selection might have significantly higher inbreeding depression than morphological and life-history traits (Oosterhout & Trigg 2003). In *T. commodus*, fine-scale parameters of male calling song were influenced by inbreeding depression by as much as 350% (Drayton *et al.* 2007). Depending on how these signalling parameters contribute to net fitness, the costs of inbreeding could be considerably higher than the data on morphological and life-history variables would suggest, negating any inclusive fitness benefits of mating with siblings, and favouring inbreeding avoidance. Clearly, we need more studies on inbreeding depression in net fitness, and from natural populations, in order to assess the general fit of theory to observations of male and female responses to inbreeding.

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