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The influence of male ejaculates on female mate search behaviour, oviposition and longevity in crickets

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In animals with internal fertilization, sperm are transferred in ejaculates, which include water and proteins produced by male accessory glands. These proteins help to protect and facilitate sperm passage, and in some species have been identified as having an influence on female behaviour and life history traits. They may increase oviposition rate, reduce sexual receptivity and decrease female life span. Virgin female field crickets *Gryllus bimaculatus* orient and move towards calling song produced by males. However, phonotaxis is greatly reduced after mating. We tested the hypothesis that female phonotaxis, oviposition and longevity are influenced by compounds in male ejaculates. We divided females into two groups: one injected with seminal proteins extracted from spermatophores from which sperm had been removed, and one injected with Ringer's solution. We measured female egg laying and phonotaxis before and after treatment, and recorded female longevity. We did not detect an effect of treatment on either egg laying or phonotaxis. However, females treated with seminal proteins moved less overall and died sooner than females in the control group. We therefore failed to find any evidence that postmating reductions in phonotaxis are due to effects of male seminal proteins. However, the reduction in female movement after treatment with seminal proteins could reduce their likelihood of subsequent matings.

The evolution of differences in gamete size between the sexes (anisogamy) is the basis of a fundamental dichotomy in reproductive investment between males and females. Females are typically the limiting sex, producing relatively few 'expensive' gametes, whereas males often produce very large numbers of sperm. The resulting competition for fertilizations underpins sexual selection and can also drive antagonistic coevolution between the sexes when male adaptations to intrasexual competition have detrimental effects on the reproductive success of their mates (Parker 2006). One source of such conflicts is situations where males attempt to manipulate the behaviour of their mate away from the optimum for her reproductive success. Such manipulation may include direct intervention by males to affect female behaviour. For instance, males of *Gryllus bimaculatus* prevent immediate female remating by mate guarding (Wynn & Vahed 2004), while male scorpions *Vaejovis punctatus* prevent female remating by using mating plugs (Contreras-Garduno et al. 2006). Males may also engage in less obvious manipulations by introducing compounds in seminal fluids, produced in the accessory glands, that influence female behaviour (Gillott 2003).

In *Drosophila melanogaster*, substances transferred in male ejaculate, accessory gland proteins (Acps), have been shown to reduce female sexual receptivity and longevity, and enhance egg production, ovulation and sperm storage (Wolfner 2002). Many of these Acps have targets within the reproductive tract; however, some enter the haemolymph and target other receptors (Ottiger et al. 2000). To date, most work concerning seminal products has been focused on detailed identification and functional analysis of those of *D. melanogaster*. However, if the evolution of manipulative compounds is driven by postmating selection, it is possible that they could be present in any species in which the female mates multiply. Females of the field cricket *G. bimaculatus* mate with multiple males over their reproductive life (Bretman & Tregenza 2005). Hence there is ample opportunity for postmating sexual selection. Additionally, Andres et al. (2006) found that, like seminal proteins transferred by male *D. melanogaster*, the seminal proteins of some gryllid species are also rapidly evolving and positively selected. Existing studies of field crickets suggest that in at least some species, male ejaculates may influence female oviposition; Destephano & Brady (1977) found a role for prostaglandins in egg production in the house cricket, *Acheta domesticus*. Furthermore,

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Loher et al. (1981) found that males of *Teleogryllus commodus* transfer a prostaglandin synthesizing complex to females in their ejaculate. This complex synthesizes the production of PGE_2 from the precursor arachidonic acid, which subsequently stimulates ovipositional behaviour. There have been fewer studies concerning the effects of seminal proteins on sexual receptivity. Fleischman & Sakaluk (2004) found no evidence of an effect of spermatophore contents on sexual receptivity in *A. domesticus*. However, spermatophylaces given as nuptial gifts by male decorated crickets, *Gryllodes sigillatus*, and consumed by their mates, appear to contain substances that inhibit their sexual receptivity. Sakaluk et al. (2006) found that female *A. domesticus*, a non-nuptial gift-giving species, had reduced sexual receptivity after consuming the nuptial gift of *G. sigillatus*, whereas females of *G. sigillatus* had no such reduction. They concluded that conspecific females had evolved resistance to the manipulative compound, but that to maintain positive selection for its production, there must be variation in this resistance within populations of females.

In many species of orthopteran, male ejaculates provide nutritional benefits to females generally through nuptial gifts (Vahed 1998). However, ejaculates may also be harmful to females. If ejaculate components have a positive effect on male reproductive success through increasing the proportion of a female's investment that goes into their offspring, they will be favoured by selection even if they reduce the female's overall reproduction (interlocus sexual conflict, Tregenza et al. 2006; Wedell et al. 2006). There is strong evidence for such a conflict leading to a reduction in life span in female *D. melanogaster* (Chapman et al. 1995), but in field crickets, where the spermatophore is not accompanied by a nutritional gift, potential direct costs or benefits of ejaculates have received limited attention. Wagner et al. (2001) found that in *Gryllus lineaticeps,* females mating repeatedly lived longer than females mating only once. In contrast, in *G. bimaculatus*, Bateman et al. (2006) found that contact with males reduced female longevity but that there was no difference in life span between females where the spermatophore was removed immediately after mating and those where it was retained, suggesting a lack of any direct effects of ejaculates on longevity in this species.

Field cricket mate search behaviour is characterized by calling males and silent, phonotactic females. Females typically become sexually receptive and phonotactic a few days after adult eclosion. However, after mating, female attraction to male song is reduced; this effect was found in both *T. commodus* (Loher et al. 1981) and *Gryllus integer* (Lickman et al. 1998). In *G. bimaculatus*, phonotaxis is reduced after mating, but there is no reduction when spermatophores are removed before transferral of spermatophore contents, and furthermore, phonotaxis is restored when the ventral nerve cord is severed (Loher et al. 1993). It therefore appears that reduction in female phonotaxis is either triggered by some sort of mechanical filling of the spermatheca or perhaps under chemical control from substances present in male ejaculate. This reduction in mate search behaviour could therefore be controlled by the female, or alternatively, it could be induced by male manipulation. In this study we aimed to determine whether substances transferred by males in the spermatophore, hereafter referred to as seminal proteins (SPs), influence female phonotaxis, longevity and egg production in *G. bimaculatus*.

METHODS

Study Species

We used sixth-generation descendants from 30 nonvirgin female *G. bimaculatus* collected near Valencia, Spain. The crickets were fed standard rodent diet and raised in plastic boxes (25×16 cm and 14 cm high) at 28 ± 1 °C. To ensure that the females were virgin, we separated male and female nymphs at the penultimate instar. From hatching to the experimental period, we reared females in complete physical isolation from adult males. Upon adult eclosion, we placed females in individual containers (6×6 cm and 5 cm high) and supplied them with standard rodent diet and fresh water.

SP Experiment

We selected 100 females from the population and allocated them equally to one of two treatment groups: SP group: treated with SPs; Ringer's group: control treated with phosphate buffer solution (PBS, Ringer's solution).

SP Preparation

We collected 200 fully formed and hardened (sperm-provisioned) spermatophores from a group of approximately 50 males over a period of 7 days. We harvested spermatophores by gently squeezing the male's abdomen and removing the emerging spermatophore with a pair of forceps. We suspended the spermatophores in 100 µl of PBS and homogenized them using a pestle. We then spun the SPs in a centrifuge for 5 min at 3300 rpm and removed the supernatant. Spermatophores contain approximately 0.7 µl of water (mean of five spermatophores, fresh weight − weight after drying overnight in a drying oven at 35 °C), so 2 µl of suspended compound contained the spermatophore secretions of approximately 1.7 males. This ensured that females were receiving a dose of SPs expected to be adequate to elicit a response, allowing for a proportion of the SPs being bound to and therefore discarded with the sperm. We confirmed the presence of proteins in the spermatophore secretion by running a sample on a 12% SDS-PAGE. The sample was prepared using spermatophores from eight individuals and to the same concentration as the SPs used in the experiment.

Manipulations

We carried out manipulations when females were 10 days postadult eclosion. We performed the injections using a Narishige IM-6 microinjector (Narishige International, London, UK.) fitted with a µTIP 0.5 µm capillary. In *D. melanogaster*, some Acps targeting female receptivity and longevity enter the haemolymph and have targets outside of the reproductive tract (Ottiger et al. 2000). Many studies concerning the target and effect of Acps on female behaviour in *D. melanogaster* have involved injecting the compounds directly into the female abdominal cavity (reviewed in Kaufman & Lomas 1996). The targets of male ejaculates we wished to examine are the neurological regions responsible for phonotaxis, all of which are located outside the reproductive tract (Atkins & Stout 1994). We therefore injected females directly into the haemocoel, between the seventh and eighth abdominal sclerites. Prior to treatment, we anaesthetized females by placing them on ice for 5 min. We then carried out the following actions: SP group: each female was injected with 2 µl of suspended SPs; Ringer's group: each female was injected with 2 µl of PBS (Ringer's solution).

Female Response

Oviposition and longevity

All females used in the experiment were virgin; however, virgin females still regularly oviposit. Prostaglandins in *T. commodus* (Loher et al. 1981) typically elicit an ovipositional response within 2–24 h of mating; hence we measured female oviposition for 25