

SHORT COMMUNICATION

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Female bushcrickets mated with parasitized males show rapid remating and reduced fecundity (Orthoptera: Phaneropteridae: *Poecilimon mariannae*)

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Abstract Following mating, female bushcrickets undergo a refractory period during which they are sexually unreceptive. The length of the refractory period correlates with the size of the spermatophylax. However, the size of the nuptial gift of acoustically signalling bushcrickets is often reduced as a result of infections by parasitoid flies. We examined the effect of male parasitoid infection on the induction of the refractory period and fecundity of females. We found a drastically reduced refractory period in females if the first mating partner was infected. During this shortened period fewer eggs were deposited, as an effect of the shorter refractory period, whereas the daily egg-laying rate remained the same regardless of whether the females were mated with a parasitized or an unparasitized male.

Introduction

Males of a variety of insect orders provide their mates with a nutrient gift during or prior to copulation (Thornhill and Alcock 1983; Zeh and Smith 1985). Male bushcrickets (Tettigonioidae) feed their mates with products of the accessory gland, the spermatophylax, which is attached to the sperm containing ampulla and transferred to the female during copulation (Boldyrev 1915; Gwynne 1997). The ejaculate of bushcrickets comprises sperm and seminal fluid as well as accessory secretions associated with the complex spermatophore. The spermatophylax and ampulla constitute a

single spermatophore, which may attain 30% of the male's body mass (reviews Vahed and Gilbert 1996; Wedell 1993a, 1994). After copulation the female feeds on the spermatophylax while sperm are transferred to her spermatheca. In most bushcrickets, once the spermatophylax is eaten the female consumes the ampulla. The spermatophylax is thus considered to have evolved as a sperm protection device to prevent ampulla removal by the female before sperm have been transferred (Sakaluk 1984; Reinhold and Heller 1993; Wedell 1993a,b). Any reduction in spermatophylax size reduces the time for ejaculate transfer and increases the intensity of sperm competition with the sperm of previous males, because fewer sperm are transferred to the female.

Females are unreceptive to males throughout the postmating refractory period, when eggs are laid. This phenomenon has been demonstrated in virtually all bushcrickets tested (Simmons and Gwynne 1991; Wedell 1993a; Simmons 1995). The length of the refractory period is variable and positively associated with the amount of ejaculate transferred, both within (Gwynne 1986; Simmons and Gwynne 1991) and between species (Wedell 1993b). A reduced spermatophylax size will shorten the time for ejaculate transfer and thereby decrease the duration of the induced refractory period. Thus selection for increased spermatophylax size, independent of sperm number, may arise through the effect of increased ejaculate size on reducing the risk of future sperm competition. Males that transfer larger spermatophylaxes to females may benefit in two ways: (1) The spermatophylax consumption may increase a female's output, because females are better nourished, as found in two Australian bushcricket species (Gwynne 1984; Simmons 1990); (2) The larger amount of ejaculate transferred increases the timespan until remating (Gwynne 1986; Simmons and Gwynne 1991), giving females the chance to lay more eggs.

Acoustically signalling orthopterans suffer from parasitism by tachinid flies of the tribus Ormiini. *Therobia leonidei* (Mesnil, 1965) is the only representative of or-

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miin flies in Europe (Leonide 1969) and has been found to attack members of three different families of bushcrickets. In Greek populations of the phaneropterine bushcricket *Poecilimon mariannae* Willemse and Heller, 1992, a large proportion of the males are parasitized (Lakes-Harlan and Heller 1992; Lehmann and Heller 1998). We showed that the male nuptial gift size in this species is affected by parasitism. Spermatophylax size is reduced in newly parasitized males compared with unparasitized males, whereas sperm number and ampulla size remains the same (Lehmann and Lehmann 2000). The transfer of a spermatophylax of reduced size due to infection may decrease a male's potential reproductive success. In this study we measured female remating interval and fecundity when mated with either parasitized or unparasitized males.

Methods

Animals for this experiment were collected from a grassy ridge bordered by farmland and a road near Vrissia (22°19'E, 39°15'N, Nomos Lárissa). Individuals were collected as penultimate instar nymphs separated by sex and reared to adulthood. The bushcrickets were provided with unlimited access to fresh green leaves and flowers of local plant species. Adults were placed individually in 200 ml plastic jars. Individuals used in the experiment moulted to adult within 3 days, leading to little variation in age.

To induce parasitism, 50 adult males were released into an field area of 50 m² (Kanalia 21°59'E, 38°53'N, Nomos Fthiótida) from 20 June to 10 July, recaptured nightly and checked for parasitism. The fly larvae produce a breathing funnel in the host abdomen, which is visible as a brown dot 4 days after infection (Lehmann and Heller 1998). In the field, females were removed from the study area, preventing all males from mating. We demarcated the experimental area with wooden rods and surrounded it with a gauze fence 80 cm high. Prior to the experiment, all individuals were removed from the surrounding area and during the experiment we checked the area for invading individuals. Unparasitized males were housed separately until they were randomly chosen for the experiment. Therefore we presume that mating history did not differ between parasitized and unparasitized males.

Experimental manipulations

The spermatophylax mass is reduced in parasitized males by 19.3% compared with unparasitized males, whereas sperm number and ampullae weight remains the same (Lehmann and Lehmann 2000). We determined whether the occurrence of parasitism in males and the consequent reduction of spermatophylax mass affected the female refractory period. Additionally the fecundity of females was measured during and following the refractory period.

Two groups of adult females were established randomly that were either mated with an unparasitized ($n=12$) or a parasitized male hosting one parasitoid larva ($n=12$). To obtain matings, individual pairs were placed into 500 ml plastic boxes and observed until copulation took place. During copulation a large spermatophore is transferred from the male to the female. Successfully mated females were allowed to feed on the spermatophore freely. In addition, they were provided with food ad libitum and fine dry sand as oviposition substrate. Females were provided with a virgin unparasitized male in the consecutive evenings after their initial mating. The females were observed for the first 6 h of the dark cycle, afterwards the males were removed for that night. Immediately after remating the egg-laying substrate was replaced by new sand. Following the second mating all females were allowed

to lay eggs for 5 subsequent days. The number of eggs was counted by sieving the sand and opening the eggpods deposited by individual females.

Results

Females initially mated with unparasitized males had a mean refractory period of 6.9 (± 1.8 SE) days, ranging from 5 to 9 days. This is possibly an underestimate of the true refractory period since two of the 12 females did not remate during the whole observation period of 20 days. In contrast, females mated initially with a parasitized male had a reduced refractory period of 1.8 (± 1.4 SE, range 0–4) days and all 12 females remated. The difference in the remating interval was highly significant with respect to the status of the first mating partner (t -test: $t=8.6$, $df=20$, $P<0.01$).

Female fecundity also differed in response to the treatment. Females mated with unparasitized males laid a median of 10.5 eggs before they remated. In contrast the corresponding number for females which had mated initially with a parasitized male was zero (Fig. 1), as eight out of the 12 females (66.7%) laid no eggs prior to remating. Thus, the difference between female treatments was significant (Mann-Whitney U -Test: $U=21.5$, $P_2<0.01$). However, the number of eggs laid within 5 days following remating did not differ significantly between females initially mated with a parasitized or an unparasitized male (U -Test: $U=45.0$, $P_2=0.32$ NS).

The difference in fecundity prior to remating may simply be a result of the differences found in the refractory period. Therefore, we calculated the daily egg-laying rate (Fig. 2). This rate was significantly influenced by remating (repeated measures ANOVA within subjects 'remating': $F_{(1, 20)}=8.03$, $P<0.01$). Regardless of the parasitic status of their first mate the females laid twice as many eggs per day after remating (2.8 ± 0.4 SE), than in the refractory period between initial mating and remating (1.3 ± 0.3 SE). However, the daily egg-laying rate was not significantly affected by the parasitic status of the first mating partner (repeated measures ANOVA between subjects: parasitism of first male: $F_{(1, 20)}=3.46$, $P=0.08$ NS) and there was no sig-

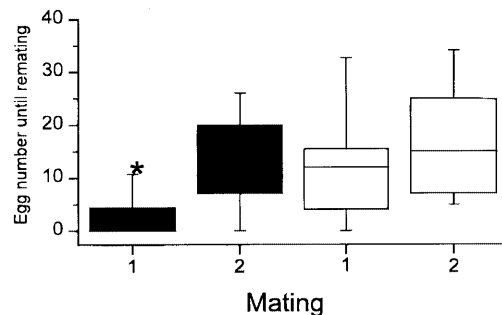


Fig. 1. Box-plot of the egg number laid (1) prior to and (2) after remating when the females initially mated with a parasitized (black, $n=12$) or an unparasitized (white, $n=10$) *Poecilimon mariannae* male. (Pairwise U -test: * $P<0.05$)

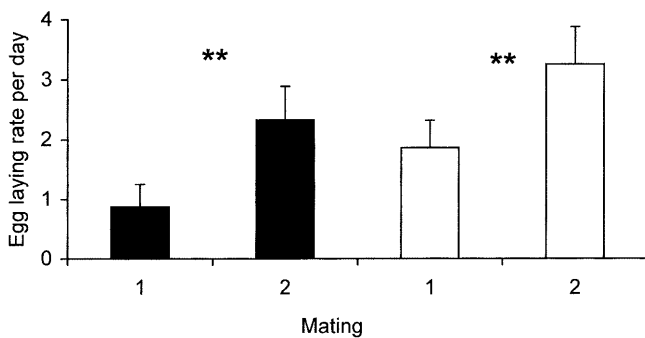


Fig. 2. Daily egg laying rate (1) prior to and (2) after remating when the females initially mated with a parasitized (black, $n=12$) or an unparasitized (white, $n=10$) *Poecilimon mariannae* male. (Repeated measures ANOVA: $**P<0.01$)

nificant interaction between parasitism and remating (within subjects: $F_{(1, 20)}=0.01$, $P=0.95$ NS).

Discussion

In this study of *Poecilimon mariannae* we show that parasitized males induce shorter refractory periods in females compared with unparasitized males. Males transfer about 20% of their bodyweight as spermatophore during mating (Lehmann and Lehmann 2000). A reduced spermatophylax size should lead to a reduction in ampullae attachment period. As a consequence the time for ejaculate transfer may be reduced, leading to a smaller amount of sperm and seminal fluids transferred. It is therefore not surprising that the refractory period induced by parasitized males is reduced in response to the smaller spermatophylax size. In other species of bushcrickets the length of the female refractory period is correlated with the time of ampulla attachment (Gwynne 1986; Wedell and Arak 1989) which in turn is correlated with spermatophylax size (see Gwynne 1997 for a review). Further, there is comparative evidence that the refractory period is positively correlated with spermatophylax size (Wedell 1993b). Due to different handling (cage/field experiment) other factors than parasitism may have added variation in size of the spermatophore in our experiment. However, we assume that this influence on remating interval of the females is negligible.

Despite this, the 75% reduction in the duration of the postmating refractory period is surprising. This confirms that in unparasitized males selection for increased spermatophylax size occurs to ensure complete sperm transfer and establishment of a full refractory period. Thus any reduction in spermatophylax size leads to a faster remating of females, as found in our study. Males unable to produce a sufficiently large spermatophylax will be selected against due to the production of fewer offspring. The selection pressure on males to produce a large spermatophylax size may be independent of the intensity of sperm competition within the sperm stor-

age organs of the females but may arise via the risk of future sperm competition by a more rapid remating of the females. In addition to the shorter refractory period in females mated initially to a parasitized male the number of eggs deposited was significantly reduced. Given that in the related bushcricket *Poecilimon veluchianus* the last male fathers 80% of the offspring produced (Achmann et al. 1992), it must be assumed that the reproductive success of parasitized males was lowered to a quarter of that of healthy males. The reproductive success might be further reduced for parasitized males, if the smaller spermatophylax leads to a reduced proportion of offspring, as demonstrated in the bushcricket *Decticus verrucivorus* (Wedell 1991). The negative influence of parasitoid infection on spermatophylax size is enhanced in the second copulation after infection and with an increased number of fly larvae (Lehmann and Lehmann 2000) which might further decrease the reproductive success of parasitized males.

If we correct the egg number for the refractory period, the difference between the two groups is no longer significant. Therefore we cannot detect any influence of the first mating partner on the reproductive output. The parental investment function of the spermatophylax cannot simply be negated by the fact that the rate of egg production is not significantly influenced by the size of the spermatophylax. However, at this stage the parental investment theory is not supported by our results. An increase in the egg-laying rate after the second mating led to a doubling in the number of eggs laid per day. Three explanations are possible: (1) The increase may reflect male investment where the number of spermatophores received by a female increases the number of eggs laid (Gwynne 1984, 1988); (2) In contrast, this increase may simply reflect an increase in the egg-laying rate with female age, as found in the closely related bushcricket *P. veluchianus* (K. Reinhold, personal communication); (3) A third explanation could be that the higher egg-laying rate is due to the receipt of more hormonal substances in the ejaculate which stimulate oviposition (Stanley-Samuelson and Loher 1986). It is difficult to distinguish between these alternative explanations, as a control experiment for our study species has not yet been conducted.

We conclude that parasitized males are handicapped in comparison with non-parasitized males with regard to their potential reproductive success.

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