Sexual Conflict Over Spermatophore Attachment in a Nuptially Feeding Cricket

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Abstract

Sexual conflict is implicated in the evolution of nuptial feeding. One function of nuptial gifts lies in mating effort, where the female's eating of the gift reduces her likelihood of prematurely terminating sperm transfer. We test several ideas regarding sexual conflict in the nuptially feeding wood cricket *Nemobius sylvestris*. In this cricket, males pass two kinds of spermatophores to females: spermless microspermatophores and larger sperm-filled macrospermatophores. Females may palpate the males' forewing secretions as a possible additional form of nuptial feeding. We manipulated male mobility and female feeding regime to examine the effects on spermatophore transfer, macrospermatophore attachment duration, and palpations of the males' forewings. Neither male confinement nor female feeding regime affected the occurrence of macrospermatophore transfer. Males transferred the macrospermatophore sooner to low-food females than to high-food females. Males that were freely mobile (unconfined and sham treatments) had longer macrospermatophore attachment durations than confined males, while female feeding regime did not affect attachment duration. The overall occurrence of female palpation was not significantly affected by female feeding regime. However, high-food females were more likely to perform short palpations before microspermatophore transfer, while low-food females were more likely to palpate after macrospermatophore transfer. Sexual conflict is evident in that males appear to guard against premature removal of the sperm-filled macrospermatophore. Low-food females appear to facilitate the early transfer of the macrospermatophore, while being more likely to perform relatively long post-insemination palpations than better-fed females.

Introduction

Sexual conflict is thought to shape the evolution of nuptial feeding (Thornhill 1976; Sakaluk 2000; Vahed 1998, 2007a; Arnqvist & Rowe 2005; Gwynne 2008; Warwick et al. 2009). Conflict underlies the male's decisions about which female to provide nuptial feeding, the amount of feeding provided, and the composition of the donation (Gwynne 1993; Gwynne & Simmons 1990; Vahed 2007b).

While females often reap nutritional benefits through nuptial feeding (Gwynne 1984, 1988; Simmons 1990; Voigt et al. 2005; Engqvist 2007; Immonen et al. 2009), research has drawn attention to the effects of male contributions on female receptivity and mating rates, such as inducing mating refractory periods in females, suggesting that nuptial gifts may contain substances that manipulate the female's reproductive behavior to the donating male's benefit (Chapman et al. 1995; Wolfner 1997; Arnqvist &

Nilsson 2000; Andrés & Arnqvist 2001; Gillott 2003; Sakaluk et al. 2006; Vahed 2006, 2007a). Selection is therefore expected to favor males that increase their reproductive success through some combination of boosting female reproductive output and maximizing their own fertilization success through nuptial feeding. Females, in turn, are expected to counteract male manipulation, which includes counteracting manipulative substances through biochemical reactions or by reducing the amount of substances received (Arnqvist & Rowe 2005; Eberhard 1996; Simmons 2001; Bussiere et al. 2006; Sakaluk et al. 2006).

In many insects, particularly orthopterans, the male transfers a spermatophore, often consisting of a sperm-filled ampulla and a proteinaceous spermatophylax, which is attached to the female's genital opening (Alexander & Otte 1967; Thornhill & Alcock 1983; Vahed 1998). Female removal of the spermatophore may interrupt or prevent the transfer of sperm and manipulative substances (Simmons 1986; Bussiere et al. 2006; Vahed 2007a; Gershman 2009; Hall et al. 2010). For the male, prolonging spermatophore attachment typically has fertilization benefits, as male paternity has been found to increase with spermatophore attachment duration (e.g., Sakaluk 1984; García-González & Simmons 2005; Bussiere et al. 2006). In some orthopteran insects, the large spermatophylax is thought to play a role in increasing sperm transfer by delaying ampulla removal through the female's consumption of the spermatophylax (reviewed in Vahed 1998, 2007a). In nuptially feeding species with a relatively simple spermatophore, such as an ampulla without a large spermatophylax, male strategies to prevent early spermatophore removal include behavioral guarding or active pursuit of the female (Alexander 1961; Bidochka & Snedden 1985; Hockham & Vahed 1997).

Previous research on crickets with relatively simple, externally attached spermatophores reveals considerable variation in spermatophore attachment duration (Hockham & Vahed 1997; Bussiere et al. 2006; Prokop & Maxwell 2008). This variation can be influenced by male quality, resulting in longer attachment when the female mates with an attractive male (Bussiere et al. 2006; Hall et al. 2010; see also Eberhard 1996). Certain male behaviours, such as mate guarding or spermatophore guarding, may also influence the duration of spermatophore attachment (Loher & Rence 1978; Hockham & Vahed 1997; Hall et al. 2008). The influence of male behavior on spermatophore attachment duration has received little experimental examination in species with relatively simple spermatophores (Hockham & Vahed 1997; Bussiere et al. 2006; Hall et al. 2008). Here, we provide the first such investigation by manipulating male mobility after spermatophore transmission in the nuptially feeding wood cricket *Nemobius sylvestris* (Bosc).

The wood cricket is remarkable in that males provide multiple forms of nuptial gifts to the females: two kinds of edible spermatophores (a spermless microspermatophore and one or more sperm-filled macrospermatophores) and forewing secretions (Dombrowski & Dambach 1994). In a typical mating encounter, the female may palpate the courting male's right forewing. The male then transfers the spermless microspermatophore to the female, who may eat it (Gabbutt 1954; Prokop & Maxwell 2008). The male then transfers the larger sperm-filled macrospermatophore, which attaches to the exterior of the female's genital opening. The macrospermatophore is relatively simple, lacking a large spermatophylax. Once the macrospermatophore has been attached, the male aggressively pursues the female, often knocking against her body, possibly to guard against premature removal of the macrospermatophore (e.g., Bidochka & Snedden 1985; Hockham & Vahed 1997). After a period of attachment, the female removes the macrospermatophore, consumes it, and may further palpate the male's forewing.

In this study, we primarily focus on sexual conflict by examining the effect of male mobility on macrospermatophore attachment duration. Because male wood crickets may continue to interact with females after macrospermatophore attachment in the form of pursuit, we examine the effect of confining males on attachment duration. Furthermore, given the multiple forms of nuptial feeding in this system, we include the possible effect of female feeding regime on mating interactions. A female's nutritional status may influence her acceptance of an edible spermatophore as well as her likelihood of palpating the male's forewings, as suggested by manipulations of female feeding regime in other nuptially feeding arthropods (e.g., Thornhill 1984; Steele 1986a,b; Johnson et al. 1999; Takakura 2004; Prokop & Maxwell 2009; Piascik et al. 2010). Thus, we examine whether food-limited females are more likely to accept the edible macrospermatophore, and are more likely to palpate, than are better-fed females. We additionally ask whether food-limited females remove the macrospermatophore sooner than satiated females, possibly reflecting a greater motivation to consume the gift. This last question, the effect of female feeding regime on spermatophore attachment

duration, has received little attention in nuptially feeding species.

Methods

Rearing and Mating Trial Protocol

In late June 2008, subadult wood crickets were collected from leaf litter in mixed oak–pine woodland near Trnava, Slovakia (N 48°37′, E 17°58′). Crickets were briefly anaesthetized with CO_2, sexed, and group-reared in single-sex containers housed at room temperature (c. 20 °C) and exposed to natural photoperiod. Subadult crickets were fed ad libitum with crushed dogfood, oat flakes and fresh fruit (Prunus sp.). Each rearing container contained pieces of paper to serve as concealment, and several water reservoirs consisting of wet cotton were placed in Petri dishes. Crickets were checked daily for adult molting. New adults were identified as belonging to subweekly cohorts by means of white paint marked on the legs. Paint patterns changed every day, so adult age was known to 1 d. New adults were maintained in the single-sex containers.

In total, 149 females and 151 males molted into adulthood in early July 2008. All males were fed ad libitum throughout the experiment. Each female was fed ad libitum until the final molt. After final molting, the females were randomly assigned to two feeding regimes: high-food (n = 64) and low-food (n = 70). Low-food females were fed only fresh fruit (Prunus sp.), following Andrade & Mason (2000), and high-food females were fed ad libitum with the food described above.

Virgin adults were used in mating trials at 8 d post-molting. Mating trials were conducted between July 9 and 18, 2008. Each mating trial started between 08:30 and 14:30 and lasted approximately 2 h. Each mating trial consisted of a male and female, paired in a glass mating arena (15 × 8 × 15 cm) with a circular opening at the top covered with fine mesh. Each arena contained fresh paper on the bottom and fresh moist cotton wool. Mating pairs were observed continuously by an experimenter (PP) for 2 h, and their behavior was recorded. If no interaction between male and female cricket occurred within this period, observation was aborted.

In all, 134 mating trials were conducted. Ten mating trials were typically conducted simultaneously, with the experimenter observing the trials and recording the occurrence (to 1 min) of the following behaviors: female palpation of males' forewings,

production, transfer, attachment, and consumption of micro- and macrospermatophores. In contrast to gland-feeding crickets (e.g., Bidochka & Snedden 1985; Brown 1997; Fedorka & Mousseau 2002), palpations by female N. sylvestris typically last for <1 min (Gabbutt 1954; Mays 1971; Prokop & Maxwell 2008). However, because low-food females would mate opportunistically, longer palpations were expected. We therefore recorded the number of separate palpation bouts within a trial (i.e., palpation bout = female palpating the male's body) and the categorical duration of each palpation bout (i.e., palpation by a female ≤1 min, or >1 min). Simultaneous trials were visually isolated from each other by the placement of paper partitions between the mating arenas. At the conclusion of each trial, all contents were removed from each mating arena, crickets were returned to their housing containers, and the arena was cleaned with water. After experiments finished, all experimental animals were returned to their natural habitat. We calculated latency to perform certain behavior (e.g., latency to begin courtship song, latency to transfer the microspermatophore, etc.) as the time elapsed from the start of the trial to the first occurrence of the behavior.

Mating Treatments: Male Confinement and Female Feeding Regime

Before each trial, we weighed the virgin male and female (to 0.001 g), anaesthetized them with CO_2, and measured their pronotum width with digital callipers (to 0.01 mm). Each male and female was then randomly paired to generate one of six treatment combinations: female feeding regime (i.e., females maintained on high-food or low-food, described above) and male confinement (three treatments). Male confinement involved three manipulations: normal (male was not manipulated), confined (male was captured in a clear plastic tube after macrospermatophore transfer, thereby preventing the male from further physical contact with the female), and sham that served as a control for introducing the tube (i.e., the plastic tube was placed close to the male after macrospermatophore transfer, and then removed). The plastic tube was transparent, 10 cm long, 1.5 cm in diameter, and with six openings (3 mm in diameter) to prevent physical contact between the male and female while still allowing for visual, auditory, and olfactory contact. Sample size per treatment is given in Table 1.

Data Analysis

Pronotum width, a body part of fixed measurement, was used to quantify adult size. There was significant correlation between body mass and pronotum width for both males ($r = 0.75$, $p < 0.001$, $n = 134$) and females ($r = 0.57$, $p < 0.001$, $n = 134$). Body condition on the day of a mating trial was calculated as the residuals of regression of body mass on pronotum width; residuals for both male and female conform to the assumption of homoscedasticity. Male condition was similar across six treatments, but female condition did significantly differ (ANOVA for males: $F_{5,128} = 1.65$, $p = 0.15$, ANOVA for females: $F_{5,128} = 3.64$, $p = 0.004$, Table 1). Overall, high-food females were in higher condition than low-food females (unpaired t-test, $t_{132} = 2.63$, $p = 0.009$ mean $\pm$ SE condition of high food $= 0.002 \pm 0.001$, $n = 64$; mean $\pm$ SE condition of low food $= -0.002 \pm 0.001$, $n = 70$). Throughout, mean values are reported with standard errors (SE).

To test effects of treatment on mating behavior, analysis of covariance (ANCOVA) or multivariate analysis of covariance (MANCOVA) was used. Male confinement and female feeding regime were analyzed as categorical independent variables. We included male confinement in analyses of behaviors that occurred before macrospermatophore transmission to test our assumption that basic male behaviors (e.g., latency to begin courtship song) were standardized among the male treatments. Male and female body condition were analyzed as covariates to control for their effects on dependent variables. Mating behaviors, such as latencies to perform behavior, micro- and macrospermatophore transfer, and macrospermatophore attachment duration, were analyzed as dependent variables. When the dependent variable was a binary response, logistic regression was performed. Statistics were performed with the software STATISTICA (version 6; StatSoft 2001, Tulsa, OK, USA, http://www.statsoft.com).

Results

General Mating Behavior, Spermatophore Transfer, and Macrospermatophore Attachment

Early courtship behavior, such as the male's calling song, antennation of the male by the female, and mounting by the female (Gabbutt 1954; Campan & Demai 1983; Prokop & Maxwell 2008), occurred in all 134 trials. Males typically began the courtship song within a few minutes after the start of the trial (overall mean $\pm$ SE latency to begin song: 8 ± 1 min, $n = 134$). Latency to produce the courtship song did not strongly differ across treatments (ANCOVA: female feeding regime $F_{1,104} = 0.001$, $p > 0.9$, male confinement $F_{2,104} = 2.87$, $p = 0.06$, interaction term $F_{2,104} = 1.79$, $p > 0.1$, male body condition, $F_{1,104} = 1.95$, $p > 0.1$), suggesting that males in all treatments were equally ready for mating. There was a tendency for normal males (i.e., not confined or sham) to begin the courtship song later than the other treatments.

Successful microspermatophore transfer occurred in 129 of the 134 trials. Exactly one microspermatophore was transferred in 126 trials (successful macrospermatophore transfer occurred in 111 of these trials), with two microspermatophores being transferred in three trials (only one of these three trials resulted in successful macrospermatophore transfer). Subsequent analyses are restricted to the 111 trials in which all females received one microspermatophore and one macrospermatophore. Neither male confinement nor female feeding regime affected macrospermatophore transfer success (multiple logistic regression: male treatment Wald's $\chi^2 = 7.7$, $p > 0.05$, female feeding regime Wald's $\chi^2 = 0.1$, $p > 0.7$, male body condition Wald's $\chi^2 = 1.3$, $p > 0.2$). Latencies to the transfer of the microspermatophore and to the production and transfer of the macrospermatophore differed across treatments.

Table 1: Female body measurements on the day of the mating trial (mean $\pm$ SE)

Treatment	n	Pronotum Width (mm)	Body Mass (g)	Body Condition
L × N (low food, normal male)	25	2.86 ± 0.02[ab]	0.082 ± 0.002[ab]	−0.001 ± 0.002[a]
L × C (low food, confined male)	22	2.89 ± 0.03[a]	0.084 ± 0.002[ab]	−0.002 ± 0.002[a]
L × S (low food, sham male)	23	2.89 ± 0.03[ab]	0.083 ± 0.002[ab]	−0.003 ± 0.002[a]
H × N (high food, normal male)	25	2.83 ± 0.02[ab]	0.084 ± 0.002[ab]	0.002 ± 0.002[a]
H × C (high food, confined male)	20	2.79 ± 0.03[b]	0.078 ± 0.002[a]	−0.002 ± 0.002[a]
H × S (high food, sham male)	19	2.80 ± 0.03[ab]	0.088 ± 0.003[b]	0.007 ± 0.002[b]

For each body measurement, treatments with different letters denote significant differences between means Tukey *post hoc* test, $\alpha = 0.05$.

Analyzing these three latencies through MANCOVA, we found an effect of female feeding regime (MANCOVA: Wilks's $\lambda = 0.86$, $F_{3,102} = 5.58$, $p < 0.001$), but no effect of male confinement, and no effect of the interaction between female feeding regime and male confinement (MANCOVA: Wilks's $\lambda = 0.91$ and 1.35, $F_{6,204} = 1.59$ and 1.35, $p > 0.1$ and $p > 0.2$, respectively), and no effect of male body condition (MANCOVA: Wilks's $\lambda = 0.96$, $F_{3,102} = 1.27$, $p > 0.2$). *Post hoc* comparisons reveal that males in trials with low-food females transferred microspermatophores sooner, produced macrospermatophores sooner, and transferred macrospermatophores sooner than males in trials with high-food females (Fig. 1).

With regard to macrospermatophore attachment duration, male confinement had a significant effect, whereas female feeding regime did not (ANCOVA: male confinement $F_{2,104} = 7.18$, $p < 0.001$, female feeding regime $F_{1,104} = 0.08$, interaction term $F_{2,104} = 0.34$, $p > 0.7$, male body condition, $F_{1,104} = 0.87$, $p > 0.3$). Trials in which males were confined after transferring the macrospermatophore had shorter attachment durations than trials with normal and sham males (Fig. 2). In all 111 trials, the female ate the macrospermatophore once she removed it.

We analyzed male and female behavior after the transfer of the first macrospermatophore. We restricted our analysis to trials in which one microspermatophore and one macrospermatophore were transferred, and in which the male and female could make physical contact (i.e., normal male and sham
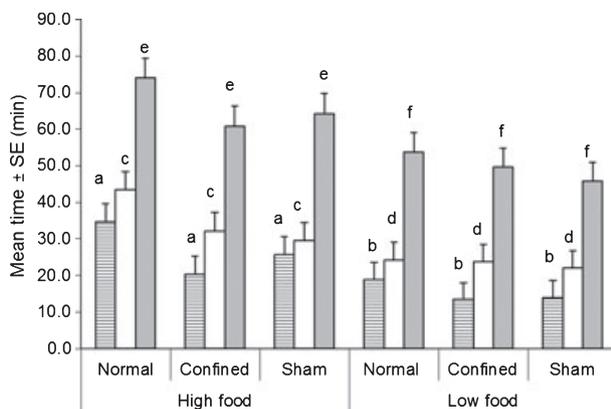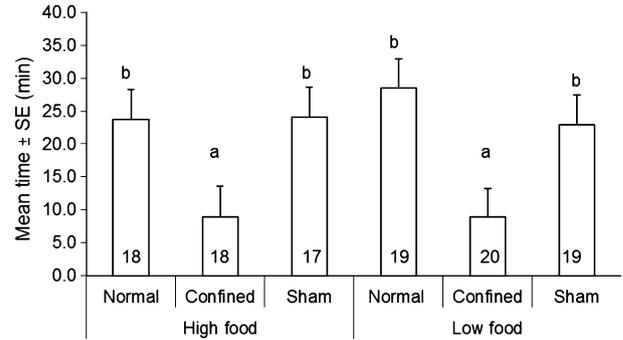

Fig. 2: Differences in macrospermatophore attachment between treatments (n = 111 trials). Different letters denote significant differences based on Tukey *post hoc* tests for the effect of confinement (a vs. b, p < 0.01). Numbers in bars are sample sizes.

male treatments, n = 74 trials). After transferring the macrospermatophore, the male aggressively pursued the female in 46 of 74 trials. Male aggressive behavior involved pursuing the female, with the male knocking his head against her body, presumably to prevent her from prematurely removing the macrospermatophore. The occurrence of this aggressive behavior was not significantly affected by male treatment (normal or sham) or female feeding regime (multiple logistic regression: male treatment Wald's $\chi^2 = 2.4$, $p > 0.1$, female feeding regime Wald's $\chi^2 = 0.1$, $p > 0.7$, male body condition Wald's $\chi^2 = 3.2$, $p = 0.07$, all interaction terms $p > 0.3$). Body condition of aggressive males tended to be higher than non-aggressive males (aggressive males: mean $\pm$ SE = 0.0009 $\pm$ 0.001 residual value, n = 46; non-aggressive males: mean $\pm$ SE -0.003 ± 0.001 residual value, n = 28). The occurrence of male aggression did not significantly affect attachment duration of the macrospermatophore (ANCOVA: male aggression $F_{1,65} = 0.48$, $p > 0.4$, male treatment $F_{1,65} = 0.20$, $p > 0.6$, female feeding regime $F_{1,65} = 0.04$, $p > 0.8$, male body condition, $F_{1,65} = 0.50$, $p > 0.4$, all interaction terms $p > 0.3$).

Female Palpation

We observed at least one female palpation of the male's forewings in 46 of the 74 trials (Table 2). The occurrence of female palpation was not significantly affected by male treatment (normal or sham) or female feeding regime (multiple logistic regression: male treatment Wald's $\chi^2 = 0.0$, $p > 0.9$, female feeding regime Wald's $\chi^2 = 1.6$, $p > 0.2$, male body condition Wald's $\chi^2 = 0.9$, $p > 0.3$, interaction term Wald's $\chi^2 = 2.8$, $p > 0.05$). Females palpated males'

Fig. 1: Latency time to microspermatophore transfer (striped bars) (min), macrospermatophore production (open bars) (min) and macrospermatophore transfer (gray bars) (min) (111 trials). Different letters denote significant differences based on Tukey *post hoc* tests for the effect of feeding regime on three dependent variables (a vs. b and c vs. d, p < 0.01, e vs. f, p < 0.001). Sample size: n = 17–20 trials per treatment.

Table 2: Occurrence of palpation by females with respect to the transfer of microspermatophore and macrospermatophore (normal males and sham males trials combined, n = 74 trials)

Palpation before microspermatophore transfer	Palpation after macrospermatophore transfer	High food females	Low food females
Yes	Yes	5	7
Yes	No	11	4
No	Yes	4	15
No	No	16	12

forewings before microspermatophore transfer (n = 15 females), after macrospermatophore transfer (n = 19 females), or both before and after transfer (n = 12 females). For these three categories (before transfer, after transfer, both before and after), high-food females were more likely to palpate before microspermatophore transfer, and low-food females were more likely to palpate after macrospermatophore transfer (Chi-square: $\chi_2^2 = 9.3$, p < 0.01). This result remains when we restrict analysis to include only those females that palpated either before microspermatophore or after macrospermatophore transfer (Table 2; chi-square with continuity correction: $\chi_1^2 = 7.3$, p < 0.01).

The duration of a palpation bout was categorically expressed as either ≤1 min ('short'), or >1 min ('long'). To control for possible non-independence of data among the 46 females that palpated, we analyzed palpation duration for each female's first palpation of the trial, as 18 females palpated more than once (mean ± SE number of palpations per female = 1.70 ± 0.14, n = 46 females). For the two categories of palpation duration (short and long), high-food females (n = 20) were more likely to do a short palpation before microspermatophore transfer (16/20 high-food females; chi-square: $\chi_4^2 = 46.0$, p < 0.001), whereas low-food females (n = 26) were more evenly split between a short palpation before microspermatophore transfer (11/26 low-food females, all short palpations) and a palpation after macrospermatophore transfer (15/26 low-food females; three short palpations and 12 long palpations). All palpations before microspermatophore transfer (n = 27) were short (i.e., ≤1 min): 16 by high-food females and 11 by low-food females.

Discussion

The present study provides evidence for sexual conflict regarding attachment duration of the sperm-filled macrospermatophore. Trials in which males

were confined after the transfer of the macrospermatophore had shorter attachment durations than trials with normal or sham males, regardless of female feeding regime. Given that spermatophore attachment duration positively correlates with the number of sperm transferred to females in many gryllid species (e.g., Sakaluk 1984; Fleischman & Sakaluk 2004; García-González & Simmons 2005; Bussiere et al. 2006), its premature removal is likely to reduce male fertilization success. Because trials with freely mobile males (normal and sham males) had longer macrospermatophore attachment durations, one can posit that the aggressive pursuit of the female by the male, observed in the present study and previously (Prokop & Maxwell 2008), might prevent the female from prematurely removing the macrospermatophore. This result adds support to previous suggestions that male pursuit and behavioral guarding may prolong spermatophore attachment in crickets (e.g., Loher & Rence 1978; Evans 1988; Hockham & Vahed 1997; Bateman & MacFadyen 1999; Bateman et al. 2001; Bussiere et al. 2006). In the present study, however, the occurrence of aggressive pursuit by freely mobile males did not significantly affect attachment duration of the macrospermatophore. Perhaps the male's mere presence and movements, or acoustic or chemical signals, are involved in prolonging macrospermatophore attachment. Given that such aggressive pursuit occurs after spermatophore transfer in other ground crickets, such as *Allonemobius fasciatus* (Bidochka & Snedden 1985), the function of this behavior warrants further investigation.

We did not find effects of female feeding regime on macrospermatophore transfer success or attachment duration. Curiously, when presented with low-food females, males transferred the spermless microspermatophores sooner, produced the sperm-filled macrospermatophores sooner, and transferred macrospermatophores sooner than males presented with high-food females. It is not clear as to why males should transfer spermatophores earlier to low-food females. We caution that spermatophore transfer might not reflect male mating preferences, as males were equally likely to transfer the macro-spermatophore to low-food and high-food females.

Female reproductive interests might be implicated in the earlier spermatophore transmission to low-food females. These females may have facilitated the production and transfer of spermatophores through subtle responses to the males' sexual signals. Considering that male readiness to mate appeared to be equal between the treatments, as indicated by latency to begin the courtship song, we suggest that

hungry females enticed males to mate sooner. Given that females always consume the macrospermatophore, low-food females may stand to gain more nutritional benefits by eating the macrospermatophore than do high-food females. Therefore, it may be in the interests of food-limited females to prompt the male to transfer the macrospermatophore quickly. This notion agrees with studies that show that food-limited females of nuptially feeding insects are more likely to copulate (e.g., Steele 1986a,b; Johnson et al. 1999; Takakura 2004).

The present study helps shed light on the function of female palpations of the males' wing secretions (Mays 1971; Bidochka & Snedden 1985; Choe 1995; Brown 1997; Fedorka & Mousseau 2002; Prokop & Maxwell 2008). Although the overall occurrence of female palpation was not significantly affected by female feeding regime, high-food females were more likely to palpate before microspermatophore transfer, while low-food females were more likely to palpate after macrospermatophore transfer. Palpations before microspermatophore transfer were categorically shorter than those after macrospermatophore transfer. Two hypotheses have been proposed for the function of forewing palpations: (1) nourishment to females and (2) a mechanism for females to assess male quality (Prokop & Maxwell 2008). If the palpations are performed by females to assess male quality, then one would expect all females to palpate before microspermatophore transfer, not just the high-food females. While it is possible that low-food females might be less choosy than high-food females regarding mates (Brown 1997; Edvardsson 2007; Ursprung et al. 2009), it does not necessarily follow that low-food females would abandon mate assessment altogether. If, on the other hand, forewing palpations provide nourishment to females, then one can expect low-food females to be more likely to palpate, or to palpate for longer periods, than high-food females (Brown 1997). This study provides evidence for low-food females palpating longer after macrospermatophore transfer. The effects of forewing palpation on female fitness components, such as adult longevity and fecundity, remain unknown. These important fitness consequences require examination to draw firm conclusions about the function of forewing palpation.

The multiple forms of nuptial feeding in the wood cricket – dual spermatophores and forewing secretions – highlight the different arenas in which sexual conflict can occur within species. Before spermatophore transfer, females may palpate the males' forewing secretions, whereas the short palpations by well-fed females suggest an assessment function. Food-limited females may facilitate the hastened transfer of the edible sperm-filled macrospermatophore, while males appear to guard against its premature removal. After macrospermatophore transfer, food-limited females perform relatively long palpations of the males' forewings, suggesting a nutritional function at this stage. Further experimentation is required to test these possibilities.

Acknowledgements

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