Interactions Between Multiple Forms of Nuptial Feeding in the Wood Cricket *Nemobius sylvestris* (Bosc): Dual Spermatophores and Male Forewings

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Abstract

Nuptial feeding is widespread in insects, with many species showing one form of feeding. In the wood cricket *Nemobius sylvestris*, the male may provide multiple forms of feeding during an encounter: two kinds of edible spermatophores (microspermatophore and macrospermatophore) and forewing secretions. We examined the roles and interactions of the spermatophores and forewing exposure in the mating sequence of this species. The small microspermatophore was not found to contain sperm, whereas the larger macrospermatophore contained sperm. In mating trials, the microspermatophore may be transferred to the female early in the trial. Transfer of the microspermatophore was not a necessary prerequisite to the subsequent transfer of one or more sperm-filled macrospermatophores. Forewing exposure increased male mating success, as males with exposed forewings were more successful in transferring the macrospermatophore than males with experimentally covered forewings, both in terms of occurrence of successful transfer and the number of macrospermatophores transferred. Male mating success was very low when the male's forewings were covered and when the male did not transfer a microspermatophore. The sperm-filled macrospermatophore may have nutritional value, as females eventually consumed all transferred macrospermatophores, and males consumed all rejected macrospermatophores. Somewhat unexpectedly, this study casts doubt on the role of the forewings in nuptial feeding. Although males with exposed forewings were more successful in macrospermatophore transfer, females actually palpated these males' forewings less. We posit the alternative hypothesis that the forewing secretions play a role in chemical communication to the female (e.g., signaling male quality), possibly instead of female nourishment.

Introduction

Nuptial feeding, a male's provision of nourishment to his mate, occurs in many arthropods (Thornhill & Alcock 1983; Zeh & Smith 1985; Parker & Simmons 1989; Simmons & Parker 1989; Boggs 1995; Vahed 2007). This nourishment has been observed in various forms including food items captured by the male, glandular secretions by the male, edible spermatophores, and even the male's body parts (reviewed in Gwynne 1997; Vahed 1998, 2007). Research over the past few decades points to male mating benefits via nuptial feeding, as well as potential male paternal investment and reproductive benefits and costs to the female (Gwynne 1997; Vahed 1998, 2007).

For many species, males provide a single form of nuptial feeding (Gwynne 1997; Vahed 1998). In some insects, however, males offer multiple modes of nuptial feeding. On the one hand, males may present different gift items to successive females. Male scorpionflies, for example, may offer either dead arthropod prey or salivary secretions to a female (reviewed in Sauer et al. 1998). Alternatively, a male may provide two or more forms of feeding during an encounter with a female. Examples include the sagebrush cricket *Cyphoderris strepitans*, wherein the female may consume the spermatophylax (gelatinous component of the spermatophore) as well as portions of the male's hind wings (Dodson et al. 1983; Eggert & Sakaluk 1994; Gwynne 1997). Similarly, a female of the striped ground cricket *Allonemobius fasciatus* may eat the spermatophore in addition to feeding on a glandular spur on the male's hind tibia (Mays 1971; Bidochka & Snedden 1985).

Multiple forms of nuptial feeding within a species raise intriguing questions. The male faces decisions regarding energy and nutrient allocation between the different forms. For the female, multiple offerings broaden information relevant to mate choice, while simultaneously broadening the arena for sexual conflict regarding male investment and potential manipulation (Arnqvist & Rowe 2005; Vahed 2007). Nevertheless, the interaction between different modes of nuptial feeding has received relatively little attention. In this study, we examine the roles of the forewings and edible spermatophores in the mating behavior of the wood cricket *Nemobius sylvestris*. In *N. sylvestris*, females palpate secretions on the males' forewings (Richards 1952, 1953; Gabbutt 1954). This species is also notable for having two kinds of spermatophores: small 'microspermatophores' and larger 'macrospermatophores.' Such dual spermatophores have been recently studied in other gryllids (e.g., Shaw & Khine 2004; deCarvalho & Shaw 2005).

The mating sequence of *N. sylvestris* is complex, consisting of stridulation by the male, the passage of two kinds of edible spermatophores to the female, and the female's palpations of the male's forewing secretions (Richards 1952, 1953; Gabbutt 1954; Mays 1971; Campan & Demai 1983; Dombrowski & Dambach 1994). After attraction via male stridulation, tactile exchange occurs between the sexes including antennation of the male by the female (Gabbutt 1954; Campan & Demai 1983). If the female is receptive, the male typically passes a small microspermatophore, affixing it to the female's genital opening. The female may eat the microspermatophore, and then palpate liquid secretions on the dorsal surface of the male's right forewing (tegmen), which overlaps and covers the left forewing when the male is not stridulating. This behavior is similar to female palpations of male metanotal secretions in other gryllids (e.g., Ono et al. 1995; Brown 1997; reviewed in Gwynne 1997). After palpations, the female may remount the male, who then passes a larger macrospermatophore to the female's genital opening. The macrospermatophore is approx. 1 mm in diameter, being 3× wider than the microspermatophore (Gabbutt 1954; Campan & Demai 1983). The female typically eats the macrospermatophore after it has been attached to her genital opening for a period. The male may pass multiple macrospermatophores to the female, and the female may perform multiple bouts of palpations on the male's forewing.

Interactions between the transfer of the dual spermatophores and the forewing secretions remain unclear in this species, as well as their influences on male mating success. We address these questions experimentally. First, we examine the roles of the microspermatophore and macrospermatophore. We ask whether both contain sperm, as spermless microspermatophores have been found in the gryllids *Laupala* spp. (Shaw & Khine 2004; deCarvalho & Shaw 2005). Through mating trials, we ask whether successful transfer of the microspermatophore is necessary for successful transfer of the macrospermatophore, as suggested by Gabbutt (1954) and Mays (1971). Second, we examine the influence of exposure of the male's forewings on mating success. We experimentally cover the forewings of males, and ask how this treatment affects mating success.

Methods

Rearing and Mating Trial Protocol

In early July 2006, subadult wood crickets were collected from leaf litter in mixed oak-pine woodland near Trnava, Slovakia (48°37′ N and 17°58′ E). Crickets were briefly anaesthetized with CO_2, sexed, and group-reared in single-sex containers housed at room temperature (approx. 20°C) and exposed to natural photoperiod. Crickets were fed *ad libitum* with crushed dog food, oat flakes, fresh fruit, and dry *Daphnia* sp. Each rearing container contained pieces of paper to serve as concealment, and several water reservoirs consisting of wet cotton placed in Petri dishes. Crickets were checked daily for adult molting. New adults were identified as belonging to

Ethology **114** (2008) 1173–1182

subweekly cohorts by means of white paint marked on the legs. Paint patterns changed every 4–5 d, so adult age is known to 4–5 d. New adults were maintained in the single-sex containers.

Virgin adults were used in mating trials at 8–15 d post-molting. Mating trials were conducted between 19 and 28 July 2006. To control for potential diurnal effects on spermatophore production (e.g., deCarvalho & Shaw 2005), each mating trial started at 10:00 and finished at 16:00. Each mating trial consisted of a male and female, paired in a glass mating arena ($15 \times 8 \times 15$ cm) with a circular opening at the top covered with fine mesh. Each arena contained fresh paper on the bottom, a fresh moist cotton wool and fresh fruit (*Prunus* sp.). Mating pairs were observed continuously by the experimenter (P. Prokop) for 6 h, and their behavior was recorded. Overall, 107 mating trials were conducted: 15 for inspection of spermatophore content and 92 for experimental manipulation of the males' forewings. 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 mounting and palpation of males' forewings, production, transfer, attachment, and consumption of spermatophores. In contrast to gland-feeding crickets (e.g., Bidochka & Snedden 1985; Brown 1997; Fedorka & Mousseau 2002), palpations by female *N. sylvestris* typically last for a few seconds (Gabbutt 1954; Mays 1971). We, therefore, recorded the number of separate palpation bouts within a trial (i.e., palpation bout = mounting by a female and palpating male's body), rather than total time spent palpating. 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.

Spermatophore Content

We checked for the presence of sperm cells in microspermatophores and macrospermatophores in 15 trials. For five trials, a virgin male was paired with a virgin female. At the moment of microspermatophore transfer, the female was anaesthetized with CO_2, and the microspermatophore was removed from the female's genital surface with fine tweezers. The microspermatophore was placed on a glass slide, shredded with a sharp pin, and mixed with a drop of water to create a diluted sperm solution (Laird et al. 2004). The entire glass slide was searched under a compound microscope (400× total magnification) for the presence of sperm cells. The presence of sperm cells was checked for macrospermatophores using similar methods in 10 trials. To get macrospermatophores, males were anaesthetized with CO_2 at the appearance of the trial's first (five trials), second (three trials) or third (two trials) macrospermatophore on the surface of the male's genitals. Each macrospermatophore was removed with fine tweezers and examined under the microscope as described above.

Forewing Manipulations

We conducted 92 trials to examine the effect of covering the forewing secretions on mating behavior. At 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 calipers (to 0.01 mm). Each male was then randomly assigned to one of the three treatments: control ($n = 28$), wing ($n = 35$), and pronotum ($n = 29$). Males in the control treatment were left intact. For males in the wing treatment, we brushed water-based wax (distributed by AV TRADING, Vráble, Slovakia) onto the dorsal surface of the right forewing. The wax is a liquid mixed with water; after application, the water quickly evaporates, leaving a uniform covering on the wing. Males receiving this wax treatment could still stridulate. Males in the pronotum treatment served as a sham treatment: the water wax was brushed onto the pronotum only.

Data Analysis

Pronotum width, a body part of fixed measurement, was used to quantify adult size (cf. Andrade & Mason 2000). There was a significant correlation between body mass and pronotum width for both males ($r = 0.71$, $p < 0.001$, $n = 92$) and females ($r = 0.66$, $p < 0.001$, $n = 92$). Body condition on the day of a mating trial was calculated as the residuals of regression of body mass on pronotum width. Male and female conditions were similar across treatments (ANOVA for males: $F_{2,89} = 0.57$, $p > 0.50$; ANOVA for females: $F_{2,89} = 1.83$, $p > 0.10$). All statistical tests are two-tailed and calculated with STATISTICA (StatSoft, Inc 2001, Version 6; StatSoft, Inc., Tulsa, OK, USA). Mean values are presented with standard errors (SE).

Results

Spermatophore Content

We failed to find sperm cells in any of the five microspermatophores examined. In contrast, we found sperm cells in all 10 macrospermatophores: first transferred in the trial (n = 5), second transferred (n = 3), and third transferred (n = 2).

General Mating Behavior

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), occurred in all 92 experimental trials. Outcomes of the trials are summarized in Fig. 1.

Of the 92 trials, males transferred a microspermatophore to the females in 64 trials. Because of its small size, actual production and consumption of the microspermatophore was difficult to observe consistently, so we only report microspermatophore transfer. No more than one microspermatophore was transferred during a trial, and males did not pursue the females after transfer. Palpation of the males' forewings occurred in 37 trials. With regard to the large macrospermatophores, males produced at least one in 83 trials, and successfully transferred at least one in 55 trials. A total of 73 macrospermatophores were transferred: 40 males transferred exactly one macrospermatophore, 12 males transferred two, and three males transferred three. Females consumed macrospermatophores only after successful transfer and attachment. The females ate all of the 73 macrospermatophores that were transferred and attached to them.

Latencies to produce and transfer spermatophores were similar across treatments. Overall, $\bar{x} \pm$ SE time from beginning of trial to microspermatophore transfer = 58 ± 6 min (n = 64), $\bar{x} \pm$ SE time from beginning of trial to the first macrospermatophore production = 77 ± 8 min (n = 83), $\bar{x} \pm$ SE time from beginning of trial to the first macrospermatophore transfer = 83 ± 6 min (n = 55). Significant differences in these times were not detected across treatments (MANCOVA, with treatments as categorical and male and female conditions as covariates: treatment Wilk's $\lambda = 0.74$, $F_{6,62} = 1.65$, $p > 0.1$; male and female conditions, Wilk's $\lambda = 0.89$ and 0.98, $F_{3,31} = 1.26$ and 0.20, $p > 0.3$ and $p > 0.8$, respectively).

After transferring the first macrospermatophore, the male aggressively pursued the female for several minutes in 23 of 55 trials. In these cases, the females initially crawled away from the males, which might increase the chance of the macrospermatophore becoming dislodged. The males pursued the females, knocking their heads against the females' bodies, presumably to prevent the females from prematurely removing the macrospermatophores (cf. Bidochka & Snedden 1985). In the 28 trials where the male produced a macrospermatophore but did not transfer it to the female, the male always ate it ($\bar{x} \pm$ SE time to macrospermatophore consumption after its production = 43 ± 6 min, n = 28).

Successful transfer of the microspermatophore was not a necessary prerequisite to successful transfer of the macrospermatophore. Of the 64 trials in which a microspermatophore was transferred to the female, one or more macrospermatophores were transferred in 44 trials (69%). Of the 28 trials in which a microspermatophore was not transferred, one or more macrospermatophores were transferred in 11 trials (39%). Similarly, palpation was not a necessary prerequisite to macrospermatophore transfer. Palpation preceded macrospermatophore transfer in 15 of 37 trials (41%). When palpation did not occur, macrospermatophore transfer occurred in 40 of 55 trials (73%).

Forewing Manipulations and Mating Success

Males with exposed forewings (control and pronotum treatments) most commonly transferred a microspermatophore, received no palpation from the female, and transferred one or more macrospermatophores (57.14% and 34.48% of control and pronotum trials, respectively; Fig. 1a, b). On the contrary, the most common outcome for males with covered forewings (wing treatment) was not transferring a microspermatophore, receiving palpation, while not transferring a macrospermatophore (34.28% of wing trials; Fig. 1c). The link between microspermatophore and macrospermatophore transfer was significantly affected by treatment (chi-square test: $\chi^2 = 35.7$, df = 7, $p < 0.001$; Table 1). For control and pronotum males, macrospermatophore transfer occurred with high probability, regardless of microspermatophore transfer (Fisher's exact test, control treatment: p = 0.286; Fisher's exact test, pronotum treatment: p = 0.633; Table 1). In contrast, males with covered forewings that did not pass a microspermatophore were particularly unsuccessful in macrospermatophore transfer (Fisher's exact test: p = 0.015; Table 1).

We examined the influences of the male's forewing treatment, male condition, and female condition on

Ethology 114 (2008) 1173–1182

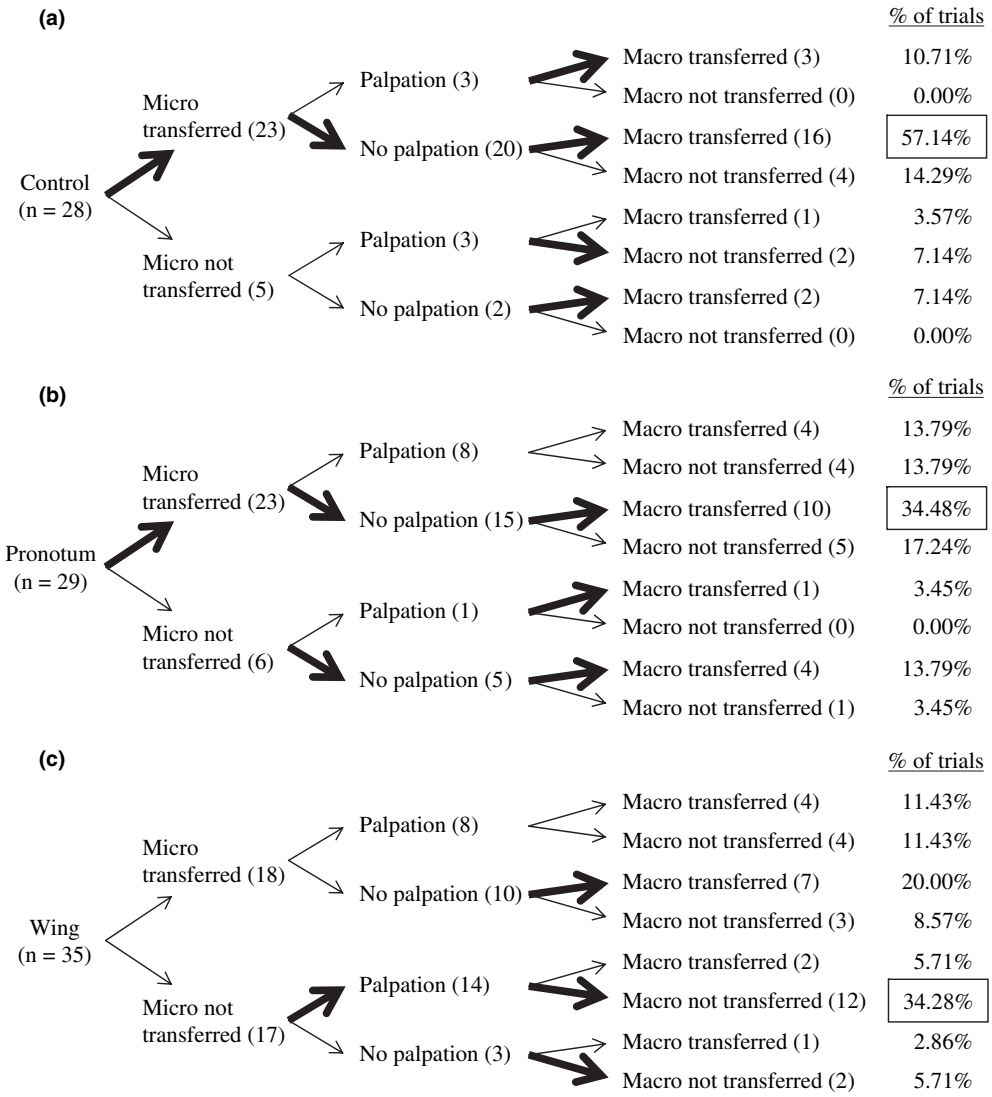

Fig. 1: Transfer of microspermatophore, occurrence of palpation, and transfer of one or more macrospermatophores for the three forewing treatments (92 trials in total). Numbers of trials showing a certain behavior are given in parentheses. Sequential percentages greater than 60% are indicated by heavy arrows. For each treatment, the most common outcome is boxed. (a) Control treatment (28 trials), (b) pronotum treatment (29 trials), and (c) wing treatment (35 trials).

the occurrences of microspermatophore transfer, palpation, macrospermatophore production, and macrospermatophore transfer (Table 2). Forewing treatment showed significant effects on microspermatophore transfer, palpation and macrospermatophore transfer (Table 2). With regard to microspermatophore transfer, males of the wing treatment were significantly less successful than control and pronotum males [wing: 18 of 35 males, control: 23 of 28 males, pronotum: 23 of 29 males; *post hoc* subdivision of contingency table, Zar (1984): χ^2 with Yates' correction = 7.4, df = 1, $p < 0.01$]. Females in better condition were more likely to receive a microspermatophore (Table 2; $\bar{x} \pm$ SE female condition with microspermatophore transfer = 0.001 ± 0.001 residual values, n = 64; $\bar{x} \pm$ SE female condition without microspermatophore transfer = -0.002 ± 0.001 residual values, n = 28). Regarding palpation, males of the wing treatment were significantly more likely to receive palpation than control and pronotum males [wing: 22 of 35 males, control: 6 of 28 males, pronotum: 9 of 29 males; *post hoc* subdivision of contingency table, Zar (1984): χ^2 with Yates' correction = 10.6, df = 1, $p < 0.01$]. Regarding macrospermatophore transfer, males of the wing treatment were significantly less successful than control and pronotum

Table 1: Microspermatophore transfer and macrospermatophore transfer by forewing treatment. Overall, chi-square test for microspermatophore × macrospermatophore × treatment: $\chi^2 = 35.7$, df = 7, $p < 0.001$. Results of the Fisher exact tests appear below each treatment table. Microsperm: presence or absence of microspermatophore transfer, Macrosperm: presence or absence of macrospermatophore transfer

	Control (n = 28)		Pronotum (n = 29)		Wing (n = 35)	
	Macrosperm		Macrosperm		Macrosperm	
Microsperm	Yes	No	Yes	No	Yes	No
Yes	19	4	14	9	11	7
No	3	2	5	1	3	14
	p = 0.286		p = 0.633		p = 0.015	

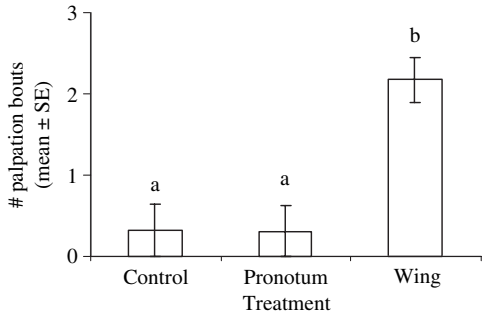

Fig. 2: Number of palpation bouts on male forewings by females. Different letters denote significant differences based on Tukey *post hoc* tests (a vs. b, $p < 0.001$).

males [wing: 14 of 35 males, control: 22 of 28 males, pronotum: 19 of 29 males; *post hoc* subdivision of contingency table, Zar (1984): χ^2 with Yates' correction = 7.9, df = 1, $p < 0.01$].

Analysis of actual number of palpation bouts confirms more palpation with males of the wing treatment (ANCOVA: treatment $F_{2,87} = 14.53$, $p < 0.0001$; male and female condition, $F_{1,87} = 0.005$ and 3.48, $p > 0.9$ and $p = 0.065$, respectively; Fig. 2). Females in relatively poor condition showed a non-significant tendency to perform more palpation ($p = 0.065$); correlation between number of palpation bouts and female condition was weak, negative, and non-significant ($r = -0.12$, $p = 0.25$, $n = 92$). Female condition failed to significantly differ between treatments (see 'Methods'), and ANCOVA interaction between female condition × treatment showed no significant effect ($F_{2,75} = 1.94$, $p > 0.1$). As a subset of these data, we examined trials in which males' forewings were exposed (control and pronotum, n = 57 trials), but failed to detect a difference in female condition between trials with and without palpation ($\bar{x} \pm$ SE female condition with palpation = -0.0006 ± 0.0018 residual value, n = 15; $\bar{x} \pm$ SE female condition without palpation = -0.0006 ± 0.0011 residual value, n = 42; the Mann–Whitney $U = 302.0$, $p > 0.8$). In this subset of 57 control and pronotum trials, the occurrence of palpation by the females was not associated with successful macrospermatophore transfer. When palpation occurred (15 trials), at least one macrospermatophore was transferred in nine trials (60%); when palpation did not occur (42 trials), at least one macrospermatophore was transferred in 32 trials (76%; Fisher's exact test: $p > 0.3$).

Analysis of actual number of macrospermatophores produced and transferred points to a disadvantage for males with covered forewings (wing treatment). The number of macrospermatophores produced and transferred significantly differed between treatments (Fig. 3; MANCOVA, overall effect of treatment: Wilk's $\lambda = 0.82$, $F_{4,172} = 4.58$, $p < 0.01$; effect of treatment on macrospermatophore production: $F_{2,91} = 5.91$, $p < 0.01$; effect of treatment on macrospermatophore transfer: $F_{2,91} = 5.42$, $p < 0.01$). Males in the wing treatment did not necessarily produce fewer macrospermatophores than control males, but they transferred fewer macrospermatophores than control and pronotum males (Fig. 3). Neither male condition (Wilk's $\lambda = 0.99$, $F_{2,86} = 0.52$, $p > 0.6$) nor female condition (Wilk's $\lambda = 0.99$, $F_{4,172} = 0.2$, $p > 0.8$), which were defined as covariates, significantly influenced these

Table 2: Effects of forewing treatment (control, pronotum, and wing), male condition, and female condition on four dependent variables: transfer of microspermatophore (yes/no), occurrence of palpation (yes/no), production of macrospermatophore (yes/no), and transfer of macrospermatophore (yes/no). Four separate multiple logistic regression tests conducted

	Transfer microsperm	Palpation	Produce macrosperm	Transfer macrosperm
Ind. variable	Wald's χ^2, p	Wald's χ^2, p	Wald's χ^2, p	Wald's χ^2, p
Treatment	9.53, $p < 0.01$	12.13, $p < 0.01$	3.04, NS	10.08, $p < 0.01$
Male condition	0.07, NS	0.00, NS	2.53, NS	0.07, NS
Female condition	4.46, $p < 0.05$	1.02, NS	1.18, NS	1.71, NS

NS: $p > 0.05$.

Ethology **114** (2008) 1173–1182

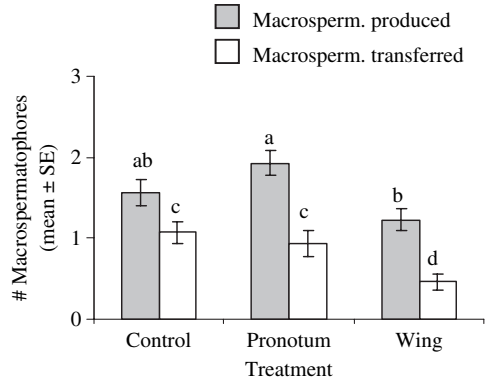

Fig. 3: Number of macrospermatophores produced (dark bars) and number of macrospermatophores transferred (open bars). Different letters denote significant differences based on Tukey *post hoc* tests (a vs. b, $p < 0.01$; c vs. d, $p < 0.05$).

results. For trials in which the males' forewings were exposed (control and pronotum, n = 57 trials), the number of transferred macrospermatophores failed to differ between trials with and without palpation ($\bar{x} \pm$ SE transferred macrospermatophores with palpation = 1.5 ± 0.2, n = 15; $\bar{x} \pm$ SE transferred macrospermatophores without palpation = 1.8 ± 0.1, n = 42; the Mann–Whitney U = 283.5, $p > 0.9$).

For trials with macrospermatophore transfer, overall $\bar{x} \pm$ SE attachment duration of the first macrospermatophore was 25 ± 2 min (n = 55). Attachment duration of the first macrospermatophore failed to differ among treatments (ANCOVA, with treatments as categorical and male and female condition as covariates: treatment $F_{2,50} = 0.07$, $p > 0.9$, male and female condition $F_{1,50} = 0.25$ and 0.43, $p > 0.6$ and $p > 0.5$, respectively). Inclusion of the occurrence of palpation as a categorical variable did not significantly change this result. Looking at trials in which males' forewings were exposed (control and pronotum) and in which a macrospermatophore was transferred (n = 41 trials), attachment duration of the first macrospermatophore failed to differ between trials with and without palpation ($\bar{x} \pm$ SE duration with palpation = 28 ± 6 min, n = 9; $\bar{x} \pm$ SE duration without palpation = 24 ± 3 min, n = 32; the Mann–Whitney U = 109.5, $p > 0.2$). For these 41 trials, attachment duration of the first macrospermatophore failed to significantly differ between trials with and without transfer of the microspermatophore ($\bar{x} \pm$ SE duration with microspermatophore transfer = 24 ± 3 min, n = 33; $\bar{x} \pm$ SE duration without microspermatophore transfer = 28 ± 6 min, n = 8; the Mann–Whitney U = 107.5, $p > 0.4$).

Discussion

This study elucidates several aspects of the mating system of the wood cricket *N. sylvestris*. We found sperm cells in the macrospermatophore, but not in the microspermatophore. Transfer of the microspermatophore was not a necessary precursor to successful transfer of the macrospermatophore. Macrospermatophore transfer occurred in the absence of microspermatophore transfer, whereas microspermatophore transfer was not always followed by macrospermatophore transfer. Exposure of the forewings (control and pronotum treatments) increased male success in terms of microspermatophore and macrospermatophore transfer. An interaction between forewings and spermatophores was detected, as males with covered forewings (wing treatment) were significantly less successful in macrospermatophore transfer when they failed to transfer a microspermatophore.

Role of the Dual Spermatophores: Microspermatophore and Macrospermatophore

Our study is the first to examine the contents of the microspermatophore of *N. sylvestris*. We failed to find sperm in the microspermatophore. We note that Gerhardt (1921) referred to *'pseudospermatophoren'* in this species, although it is not clear whether he equated this with the microspermatophore (Gabbutt 1954). Furthermore, Campan & Demai (1983) assert that the smaller first spermatophore does not contain sperm, but they do not describe sample size or methods of inspecting the spermatophore.

In *N. sylvestris*, the apparently spermless microspermatophore does not appear to be a necessary prerequisite to the transfer of the sperm-filled macrospermatophore. Transfer of the macrospermatophore occurred in the absence of microspermatophore transfer, whereas the transfer of the microspermatophore was not always followed by transfer of the macrospermatophore.

Microspermatophores and macrospermatophores have been recently described in gryllids of the genus *Laupala* (Shaw & Khine 2004; deCarvalho & Shaw 2005; Mendelson & Shaw 2006). The microspermatophores of at least two species, *Laupala cerasina* and *Laupala pacifica*, are similar to those of the wood cricket *N. sylvestris*: the microspermatophore is roughly one-third the diameter of the macrospermatophore, and the microspermatophore does not contain sperm (Shaw & Khine 2004; deCarvalho & Shaw 2005). *Laupala* males transfer multiple microspermatophores, yet their function remains

unclear (Shaw & Khine 2004; deCarvalho & Shaw 2005).

Hypotheses concerning the role of the microspermatophore include male fertilization success and female benefits (Gwynne 1997; Vahed 1998, 2007). The microspermatophore may increase the male's fertilization success by (1) manipulating the female's reproductive behavior, and/or (2) prolonging sperm transfer. Chemical substances in the microspermatophore may make the female more likely to accept the male's sperm, may shorten the latency to lay eggs, or may make the female less likely to mate with subsequent males (Loher & Dambach 1989; Simmons 2001; Vahed 2007). With regard to the female's acceptance of the male, males in the present study generally transferred macrospermatophores regardless of whether they transferred a microspermatophore (Table 1). Males with covered forewings, however, were significantly less likely to transfer a macrospermatophore if they did not transfer a microspermatophore. The present study cannot address the manipulation of female oviposition or remating behavior. In the sagebrush cricket (*Cyphoderris strepitans*), females that feed on the males' hind wings delay remating when compared with females that are prevented from feeding (Johnson et al. 1999). With regard to the notion of prolonging sperm transfer, it seems unlikely that the female's consumption of the microspermatophore prolongs the attachment of the macrospermatophore. In *N. sylvestris*, the microspermatophore is transferred and consumed quickly by the female, averaging 25 min before the transfer of the macrospermatophore. Furthermore, the transfer of the microspermatophore did not affect macrospermatophore attachment duration in the present study.

The female may derive direct benefits from nutrients in the microspermatophore, although we note that the microspermatophore makes a small meal (approx. 0.3 mm diameter). Under this hypothesis, one would expect females in relatively poor condition to be more likely to receive a microspermatophore. The present study, however, does not strongly support this hypothesis, as females in better condition were more likely to receive the microspermatophore. We note that our measure of female feeding condition reflects quantity of food eaten. A stronger test of the nutritional benefit hypothesis would involve experimental manipulation of food quantity and quality, as the microspermatophore may contain certain key nutrients. Female indirect benefits include acquiring information about male quality via the microspermatophore (Kokko et al. 2003; Vahed 2007). The present study does not strongly support this hypothesis for the microspermatophore alone, given that failure to transfer a microspermatophore *per se* did not prevent eventual macrospermatophore transfer by the male.

In *N. sylvestris*, female and male behaviors suggest that the larger sperm-filled macrospermatophore has nutritional value, despite the lack of a large spermatophylax found in other orthopterans (Gwynne 1997). Females consumed every macrospermatophore that they accepted. Importantly, males always consumed macrospermatophores that were rejected by females. Male body condition, however, failed to significantly affect the production of the macrospermatophore, and female body condition failed to affect macrospermatophore transfer. We emphasize that stronger tests of the nutritional value of the macrospermatophore would involve manipulations of male and female feeding regimes, as well as examinations of female lifespan and fecundity.

Forewing Manipulations and Interactions with the Dual Spermatophores

Males with exposed forewings (control and pronotum treatments) were more successful in microspermatophore transfer and macrospermatophore transfer than males with covered forewings (wing treatment). An interaction between forewing treatment and the spermatophores was detected in that male mating success (macrospermatophore transfer) was particularly low for males that had covered forewings and had failed to transfer a microspermatophore. Thus, male mating success increases when the forewings are exposed. Failing that, providing a microspermatophore appears to increase male success. Either the microspermatophore or the forewing secretions may provide direct benefits (e.g., nutrition) or indirect benefits (e.g., mate assessment information) to the female before sperm transfer (Loher & Dambach 1989; Simmons 2001; Kokko et al. 2003; Vahed 2007).

Somewhat unexpectedly, this study casts doubt on the role of the forewings in nuptial feeding. Although males with exposed forewings were more successful in macrospermatophore transfer, females actually palpated these males' forewings less. Palpation by the female was not strongly associated with male acceptance, either in terms of the occurrence of macrospermatophore transfer or the number of macrospermatophores transferred. In trials with males with exposed wings, palpation occurred in only 9 of 41 trials (22%) with macrospermatophore

Ethology **114** (2008) 1173–1182

transfer. Similarly, Gabbutt (1954) reports palpation in 19 of 52 trials (36%) with macrospermatophore transfer. When the males' forewings were covered in the present study, palpation by females was significantly more frequent, and the actual number of palpation bouts during the trials was greater with covered forewings. The higher incidence of female palpation of covered forewings suggests searching behavior by the females, wherein they were attempting to locate or sample the forewing secretions.

Hypotheses concerning the role of the forewings in *N. sylvestris* require a consideration of the proximate behaviors involved. First, we acknowledge that our experimental covering of the forewings might have affected stridulation by the males. Although we did not perform acoustic recordings of the males, we observed stridulation by every male in all treatments. Furthermore, Richards (1952, 1953) and Gabbutt (1954) present data that suggest that close-range stimuli in addition to stridulation are important for mating: a small number of males (four) were able to mate successfully, despite lacking functional forewings. Second, the present study echoes earlier doubts on the role of the forewing secretions as nuptial feeding. The female wood cricket typically brushes the male's secretions with her labial palps, which has lead previous authors to question how much 'feeding' actually occurs (Richards 1952, 1953; Gabbutt 1954; Mays 1971). Further work, such as radiolabeling substances in the forewing secretions, would help to determine ingestion of the secretions by the females.

If female wood crickets ingest the males' forewing secretions, then hypotheses about the secretions' function include male fertilization success as well as direct and indirect benefits to the females. As discussed for microspermatophores, increasing male fertilization success can involve manipulating the female's behavior (e.g., mate acceptance) and prolonging sperm transfer, as in other orthopterans with glandular or wing feeding (Hohorst 1937; Morris et al. 1989; Eggert & Sakaluk 1994; Brown 1997; Johnson et al. 1999; Bussière et al. 2005; reviewed in Gwynne 1997). Regarding mate acceptance, palpation by the female was not strongly associated with the occurrence of macrospermatophore transfer or the number of macrospermatophores transferred. Regarding sperm transfer, attachment duration of the first macrospermatophore failed to differ across treatments, and was not affected by the occurrence of palpation. We note that males with exposed wings transferred significantly more macrospermatophores than males with covered wings, so access to the forewing secretions may lead to more total sperm transferred. Additionally, females accruing direct benefits via ingestion of the secretions remain a possibility, but the present study cannot address the secretions' effects on female lifespan or fecundity.

The female gaining indirect benefits via forewing secretions is a viable hypothesis, regardless of whether she ingests the secretions or merely senses them. By sampling the secretions, the female may gain information about the male's quality. We posit that the female might sample the secretions by means other than palpation, based on two lines of evidence. First, the occurrence of palpation *per se* did not predict mating success in the present study, as discussed above. Second, we observed antennation of the male by the female throughout the mating trial, as noted by previous authors (Gabbutt 1954; Campan & Demai 1983). During these sweeps of the antennae, the female may be sampling the forewing secretions via olfaction. The possible nutritional and informational roles of the forewing secretions and microspermatophore warrant further investigation in this species.

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Ethology **114** (2008) 1173–1182