Eur. J. Entomol. **111**(2): 189–197, 2014
doi: 10.14411/eje.2014.019
ISSN 1210-5759 (print), 1802-8829 (online)

Comparison of the mating behaviour of a bush cricket in the laboratory and the field: Calling activity and mating frequency of a long-winged species, *Phaneroptera falcata* (Ensifera: Tettigoniidae)

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Key words. Ensifera, Tettigoniidae, *Phaneroptera falcata*, multiple mating, nuptial gift, polyandry, reproductive behaviour, sperm competition

Abstract. Bush crickets are a model group for testing hypotheses in sexual selection, but much of the information is based on laboratory observations on wingless or short-winged species, which may restrict their generality. Here we describe aspects of the mating behaviour of the long-winged European bush cricket *Phaneroptera falcata* (Poda, 1761). Both in the laboratory and the field, diel calling followed a normal, though slightly left-skewed distribution, peaking about three hours after sunset or lights-off. Under bright greenhouse conditions, when the light was suddenly switched off, calling occurred only after the onset of darkness. Decreasing light intensity may trigger the start of calling activity. In the field, calling decreased from midnight onwards, which may be related to a decrease in temperature. The sequence of events during copulation was identical in the laboratory and the field. However, in two of 14 copulations documented in the field, a pre-copulatory behaviour was observed that resembled the putative removal and ingestion of rival sperm. Previous suggestions that *P. falcata* (Poda) is monogamous are rejected on the basis of both laboratory and field results. In the laboratory males and females mated every 2.3 and 3.6 days, respectively. We introduce a simple way to calculate the average frequency of mating in the field, based on the observation that at any one time 3% of all the individuals are recorded mating and copulation lasts 15 min. We estimate that on average *P. falcata* (Poda) mates once per day. More generally, our results show it is important for evolutionary conclusions to measure behaviourial data in the field.

INTRODUCTION

Bush crickets, or katydids, are model species in sexual selection research (Gwynne, 1997, 2008; Brown, 1999; Vahed, 2006, 2007; Lehmann, 2012; McCartney et al., 2012) because their mating behaviour, sex roles and reproductive investment can be relatively easily manipulated (e.g. Simmons et al., 1993; Simmons & Kvarnemo, 1997; Kvarnemo & Simmons, 1999; Reinhold, 1999; Lehmann, 2012). This wealth of information has resulted in bush crickets being used as model species in comparative studies (Vahed & Gilbert, 1996; del Castillo & Gwynne, 2007; Vahed 2006, 2007; Gwynne, 2008; Vahed et al., 2011a, b; Lehmann, 2012; McCartney et al., 2012).

The majority of studies, however, are on species that are wingless or possess only rudimentary wings (but see e.g., Tauber et al., 2001; Villarreal & Gilbert, 2013), possibly because they are easier to handle than the long-winged species, which can fly. This may be a significant disadvantage in comparative analyses if trade-offs in wing and muscle development with reproductive traits alter investment into searching behaviour, communication and reproduction in winged species. For example, male bush crickets secrete a proteinaceous spermatophylax around the spermatophore. Spermatophylax and spermatophore are attached to the female and the sperm enter the female reproductive tract. Females first consume the spermatophylax and then the spermatophore. Females may benefit from consuming the spermatophore (Vahed, 1998; Voigt et al., 2006, 2008), in which case the intensity of sexual selection might be differently altered in winged than in wingless species (Vahed, 1998; McCartney et al., 2012). Alternatively, the spermatophylax may protect the ejaculate (Vahed, 1998) whereby sperm in the spermatophore cannot be consumed by the female until she has finished consuming the surrounding spermatophylax. Spermatophylaces may also be a paternal investment (Reinhold, 1999). Regardless of its function, the production of a spermatophylax is costly (Vahed, 2007; Gwynne, 2008; Lehmann, 2012) and so any investment by males in wings and, therefore, possibly in mate searching, may lead to reduced investment in the spermatophylax or sperm number. For example, females of several species of the phaneropterine bush cricket *Poecilimon*, which are short-winged, mate several times during their lifetime (Heller & von Helversen, 1991; Lehmann 2012). By contrast, females of its sister group, the long-winged species *Phaneroptera falcata* (Poda) have been observed to mate only once (Gerhardt, 1913, 1919). Similarly, several details of

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this species' mating behaviour have been reported to differ from that of a closely related short-winged species (Gerhardt, 1913, 1919). Finally, females of winged *P. falcata* (Poda) were recorded to either not to eat the spermatophore after transfer or not immediately (Gerhardt, 1913). By contrast, Grassé (1924) mentions females partially consuming the spermatophore and those of *Phaneroptera nana* Fieber consuming it completely (Pener & Ayal, unpubl., cited in Tauber et al., 2001). Thus, there seems to be some uncertainty about these traits in winged species. If the behaviour of winged species in general differs from that of wingless species, then the expectation is that there will be substantial differences in their reproductive strategies.

A second characteristic of bush cricket studies is that while many studies were carried out in the field (Heller, 1992; Hockham et al., 2004; Simmons et al., 2007; Gwynne & Lorch, 2013; Kanuch et al., 2013), most of the manipulative studies were carried out in the laboratory. The data from laboratory studies are often used in comparative studies but it is not always clear how much the artificial conditions experienced in a laboratory reflect the situation in the field. Studies that aimed to address this problem seem to agree that the same behaviours occur in the laboratory and in the field but that quantitative aspects of the behaviour may differ substantially. For example, using molecular markers Simmons et al. (2007) found that females of *Requena verticalis* Walker mate less frequently in the field than in the laboratory and the pattern of sperm precedence differed, too. Similarly, Hockham et al. (2004) recorded a more random use of sperm by female *Ephippiger ephippiger* (Fiebig) in the field than expected from laboratory results. These studies point to the possibility that the frequency of mating in the field may sometimes differ from that recorded in the laboratory.

The present paper presents the results of a study on the mating behaviour of a winged, highly mobile phaneropterine species of bush cricket, *Phaneroptera falcata* (Poda). We studied three important aspects of its reproductive behaviour in the laboratory and the field, general mating behaviour, diel pattern of calling activity and frequency of mating. During the course of this study we recorded a peculiar behaviour that could be attributed to the removal and subsequent ingestion of sperm from a previous mating. As this has only been described in two other species (both bush crickets) we document this behaviour in detail. Finally, we propose a simple method for estimating the average frequency of mating in the field.

MATERIAL AND METHODS

Study site and mating behaviour in the field

Field observations were carried out in xerothermic limestone grassland (Mesobrometum) in the Leutratal nature reserve near Jena/Germany (Samietz, 1994; Samietz & Berger, 1997) between August and October 1994. Within 2 days of the start of adult emergence, 70 males and 79 females of *P. falcata* (Poda) were marked individually by writing a number on their tegmina (Fig. 1, B1). Each individual was additionally labelled with a small piece of self-adhesive reflective tape for easier location at night using a head light torch (Heller & von Helversen, 1990; for the study species: Samietz & Berger, 1997), with the percentage of relocating any marked individual between 82 and 100% per night. Eighteen nocturnal surveys, carried out at 4- to 5-day intervals until the last animal died, resulted in 912 resightings of marked individuals. The highest number of resightings per individual was 16.

On four nights (August 10/11, 15/16, 16/17 and 19/20, 1994) data on mating behaviour were collected in the field. Fourteen pairs that were about to start mating were videotaped. The behavioural sequence was classified according to von Helversen & von Helversen (1991).

Calling activity in the field

The diel pattern in calling activity in the field was recorded on three days in August 1994 on which the conditions were calm, warm and sunny (August 10/11, 15/16, and 16/17, 1994). Each day, the observations began at 08:30 h (local time) and were continued until the next morning. The local time was adjusted for Jena (50.56°N, 11.35°E) and represents Central European time (CET) minus 15 min. During each survey, the calls of individual *Phaneroptera falcata* (Poda) males were recorded for 10 min by means of a bat detector (Skye instruments SBR 2100). The detector was placed in the centre of the study plot. The radius sampled was approximately 15 m (based on a preliminary test at habitat edge). Hourly temperatures and periods of sunshine were obtained from the local weather station (Jena).

Calling activity in the laboratory

The calling pattern of *Phaneroptera falcata* (Poda) was investigated in a greenhouse between August 20 and 25, 1998. Thirty adult males and thirty adult females collected from the field were kept in pairs in cages. Each cage was provided with shrubs and herbaceous plants as perches and food. In addition to natural light, artificial light was provided in a 16L : 8D cycle in the greenhouse, between 5:00 and 21:00 h local time. Cages were monitored for calling activity once every half-hour or hour between 20:00 and 5:00 (this time interval was chosen because previous observation suggested very little activity outside this period). We used the percentage of males calling each hour as a measurement of general calling activity.

Mating frequency in the field

We used two methods to estimate the frequency of mating in the field. First, for every individual that was relocated we recorded whether or not it was mating.

Second, we calculated the probability of detecting an individual mating at any time given a certain frequency and duration of mating. Consider a species that mates once per day, copulates for one hour (d) and is equally likely to copulate at any time of day. One would expect to find the following proportion of individuals in copula (p): p = d [h] / 24 h, if individuals mate once per day. This may be adjusted for shorter periods of observation, say 6 h (then p = d [h] / 6 h) or even for unequal mating probabilities during the day (mating peaks – Reinhardt et al., 2001).

Mating frequency in the laboratory

Using males and females collected in the field on 19 August, 1998, we monitored the frequency of mating in the laboratory. Sixteen males were each placed in a cage with a female, which was replaced after the male had mated. Sixteen females were also each placed in a cage with a male, which was similarly replaced after the female had mated. Replacement animals came from cages containing sexually isolated animals. This is a standard method used to determine the potential or physiologically determined frequency of mating (as opposed to the ecologically realised frequency of mating). Test animals were placed together in the cages between 20:00 h and 6:00 h local time. Mating was recorded at

hourly intervals based on direct observation of copulations or the presence of attached spermatophores.

Mating effort and sperm number

Based on the time between matings we tried to predict when a mating was likely to occur so that males could be weighed before the expected event. If mating occurred on the night predicted, which it did for 15 of the 16 males studied, males were weighed after mating. Males were weighed to the nearest mg (Sartorius portable PT 120). The difference in weight before and after mating was used to estimate spermatophore weight (preliminary investigations indicated that weight loss without spermatophore transfer was negligible, or not measurable).

Numbers of sperm transferred were not systematically recorded. However, because there is only one previous measurement (312,000 – Vahed & Gilbert, 1996) we merely wanted to confirm the order of magnitude of this number. One female collected from the field, was mated three weeks later and examined in the laboratory one week after she mated. Sperm counts were done using a modified version of the method used by Reinhardt et al. (1999). Briefly, the female was dissected and her spermatheca removed, placed in 0.2 ml of locust saline and slightly ruptured. The saline and its contents were lightly drawn up and down a pipette five times to distribute the sperm evenly. One drop of the solution was studied to confirm the presence of sperm bundles. The remainder was drawn up and down five times more forcefully to break up the bundles and homogenize the sperm cells. Then four separate samples were taken and sperm counted at a magnification of × 400 in a haematocytometer. The four counts were used to estimate the total number of sperm and the variation in the numbers in the four samples.

RESULTS

Mating behaviour in the field

In the field, males of *Phaneroptera falcata* (Poda) emitted their call from perches on herbaceous plants or small bushes a few decimetres in height. The call consisted of a series of short verses each followed by a short pause. Females responded by single clicks produced by moving the upper edges of their elytra against each other. Males moved towards responding females. If no female responded, males kept their position and continued calling or moved to another perch. They moved by climbing through the vegetation or, occasionally, by flying distances of between several decimetres to a few metres. Seemingly aggressive interactions occurred between males during encounters in which the males touched each other with their antennae and emitted a sharp, scratching sound. After a few seconds and without any further interactions one of the males left the perch by climbing or flying away.

A male that had located a responding female made contact with her using his antennae. During the subsequent courtship or mating interaction, both partners palpated each other with their antennae, seemingly aiming to maintain contact with one another (Fig. 1, A.1). There were several distinct phases in the mating behaviour (Table 1). The usual courtship (phase-II courtship) lasted between 70 and 660 s (mean = 247 s, Table 1). When the male was close to the female, he reached the female's sub-genital plate from underneath and attached himself to the female by turning around his abdomen (Fig. 1, A.2). The male seems to initiate mating. While coupled, he seems to force the female into a position that is right for the transfer of the spermatophore (Fig. 1, A.3 and A4).

During actual copulation (phase-II coupling, Table 1) the male usually hung backwards holding on to the surrounding vegetation by his tarsi (Fig. 1, A.4 and A.5, B.4 and B.5). Phase-II coupling lasted between 230 and 430 s (mean = 282 s, Table 1). The first part of the spermatophore became visible about 20 to 30 s after coupling (Fig. 1, A.3). The male released the female immediately after spermatophore transfer (Fig. 1, A.6).

Phase-II-courtship and phase-II-coupling together took between 350 and 940 s (mean = 561 s, Table 1). The duration of courtship was not significantly correlated with the

Table 1. Duration of the different phases of mating in *Phaneroptera falcata* recorded in the field. Nomenclature follows von Helversen & von Helversen (1991).

Pair no.	Phase-I courtship [s]	Phase-I coupling [s]	Genitalia rooming (male) [s]	Phase-II courtship [s]	Phase-II coupling [s]	Total duration [s]
1				70	280	350
2				100	250	350
3				130	240	370
4				170	230	400
5				160	280	440
6				200	250	450
7				240	260	500
8				220	310	530
9				260	280	540
10	80	20	20	190	320	630
11				290	430	720
12				460	300	760
13	270	30	20	310	240	870
14				660	280	940
Mean				247	282	561
SD				154	51	195

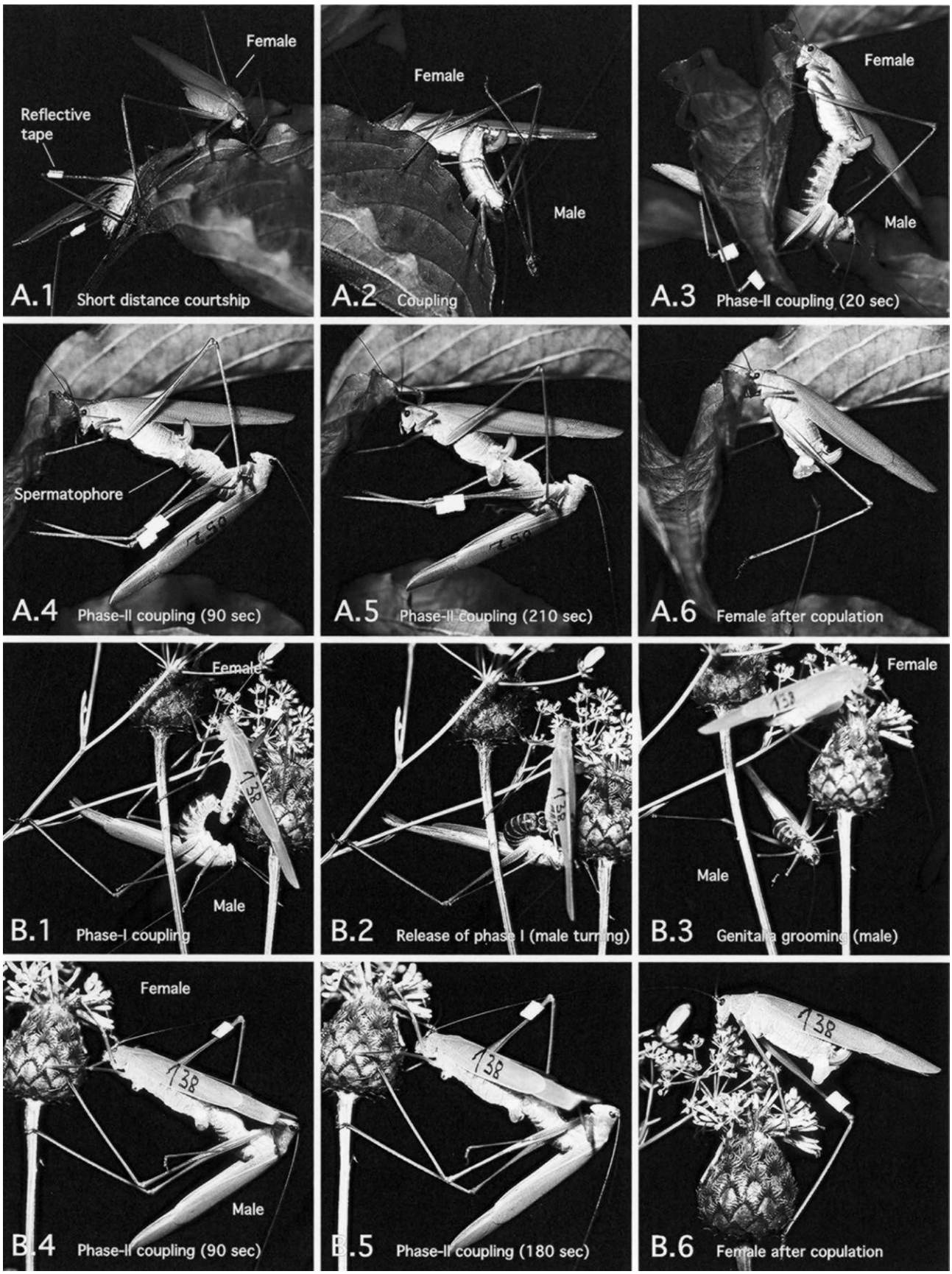

Fig. 1. Mating sequences of two couples of *Phaneroptera falcata* recorded in the field. Panel A shows usual mating procedure (just phase-II coupling) (pair No. 9 in Table 1). B shows a mating involving phase-I coupling, presumed sperm removal, genitalia grooming by the male and subsequent phase-II coupling (pair No. 10 in Table 1).

length of phase-II coupling (Spearman, R_S = 0.298, P = 0.293). In the field none of the females started eating the spermatophore immediately after copulation. However, feeding on herbaceous plants or pollen by the females was observed after all but two matings. The males moved away and did not associate further with the female (Fig. 1, A.6 and B.6) and in particular no post-copulatory mate guarding was observed.

Two of the 14 complete mating sequences recorded in the field included a pre-mating coupling (phase-I coupling): When the male approached the female after the initial courtship he united his abdomens with that of the female as in a usual coupling (Fig. 1, B.1). Twenty to 30 s after the insertion of the genital-like titillators (see Vahed et al. 2011b), the male exhibited a fast turning movement with his body (Table 1, pair 10 and 13) and then released the female (Fig. 1, B.2). Immediately after this movement and separation from the females' genitalia, the male groomed his genitalia by using his palps and mandibles (Fig. 1, B.3). This took about 20 s. The female remained within about 3 cm of the male, still within the reach of the antennae of both partners. In both cases there was only one phase-I coupling. After grooming, the male approached closer to the female, apparently courting her by antennation (190 and 310 s) (Table 1, pair 10 and 13) and the mating continued with a secondary (phase-II) coupling and spermatophore transfer as described above. Phase-II coupling lasted 240 and 320 s.

Mating behaviour in the laboratory

The mating behaviour in the laboratory was very similar to that observed by Gerhardt (1913) and in the field. However, there was a notable exception: in the laboratory at least two females fed or nibbled on the spermatophylax. This behaviour occurred infrequently but was not quantified and the amount of material eaten was not measured. On four occasions, however, the attached spermatophylax was not eaten. Most females fed on herbaceous plants in the cage, as recorded in the field.

Calling activity in the field and the laboratory

In the field, *Phaneroptera falcata* (Poda) males started calling in the afternoon on sunny days (August 15/16 and 16/17) and an hour earlier when it was partly cloudy (August 10/11). Calling activity peaked 3 h (August 15/16 and 16/17) and 4 h after sunset (August 10/11) (Fig. 2). The largest peak was recorded on August 10/11, when temperatures were about 10°C higher than during the other nights. Calling activity dropped to zero after 01:30 h a.m.

In the greenhouse, males started calling shortly after 21:00 h local time when the artificial light was switched off (Fig. 2, bottom diagram). Calling increased towards midnight and decreased thereafter. Males were, on average, recorded singing at every third monitoring (Mean = 31.3%, *SD* = 20.7%, range 0 to 70%).

Mating frequency in the field

Of all the individuals resighted 28 were mating. This represents 3% of all resightings, and 18.8% of all resighted individuals. Three females (3.8%) and two males (2.9%) were observed mating a second time, between 10 days and seven weeks after their first mating.

Frequency of mating in the laboratory and mating effort

All sixteen males mated twice and eight three times. The time between the first and second mating was, on average, 2.3 ± 1.2 (s.d.) days. All sixteen females mated twice and the second mating occurred on average 3.6 days ± 2.1 (s.d.) after the first. For those males that mated a third time, the time between the first and second mating was not correlated with the time between the second and third mating (Spearman rank correlation, r = 0.24, p = 0.564). Males weighed on average 187.0 ± 23.2 mg (N = 15) before mating and lost, on average, 13.0 ± 0.05% of their body weight during mating. Neither proportional weight loss (Spearman rank correlation, r = 0.27, p = 0.528) nor the absolute weight loss (Spearman rank correlation, r = 0.15, p = 0.723) during mating was correlated with the mean time between mating.

Number of sperm stored

The spermatheca consisted of two round chambers, each approximately 1 mm in diameter. The sperm were in bundles attached by their heads to a cap about 10 µm thick. The total length of each sperm was about 100 µm, with the head, which is very slightly curved, making up 36% of the total length (38 µm), the tail 5% (57 µm) and the cap the remaining 1% (10 µm). One homogenised sample contained 270,000 ± 45,500 sperm cells.

DISCUSSION

This comparison of the mating behaviour of *P. falcata* (Poda) revealed that there were distinct peaks in calling activity both in the field and laboratory. In addition, males and females mated more than once and frequency of mating is probably not associated with the size of the spermatophore. Below we discuss how well laboratory studies reflect the situation in the field and present some observations on the reproductive biology of this species that will be useful for comparative analyses. Specifically, we show that females do not consume the spermatophore immediately after mating, can store a considerable amount of sperm and that males may be able to remove and possibly ingest the sperm of a previous mating.

Calling activity

The calling activity of *P. falcata* (Poda) males was similar in the laboratory and the field, approximately normally distributed (with a slight left skew) and peaked around three hours after sunset or when the lights were switched off. A difference was that no male was observed calling in the bright artificial lighting conditions that prevailed during the day whereas in the field calling started in the afternoon. It is possible that decreasing light intensity towards and during sunset triggers calling activity.

We found that the peak in calling was more flat in the greenhouse where there was not a sharp a drop in temperature as in the field. It is possible that the higher absolute

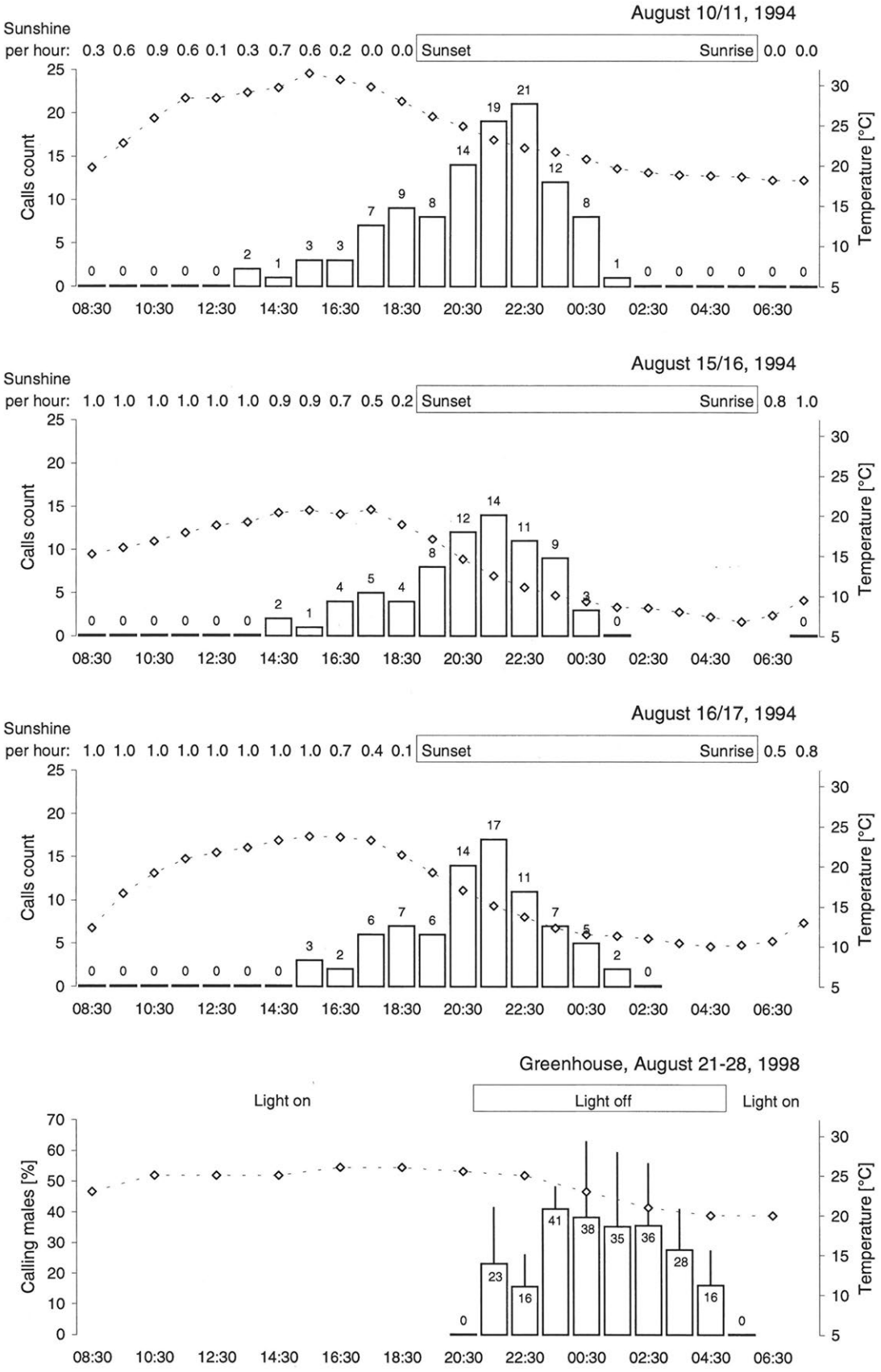

Fig. 2. Diel calling pattern of *Phaneroptera falcata* recorded in the field (upper three panels) and the greenhouse (lower panel). Field data: Calls per 10 min recorded within a 15 m radius each hour. Greenhouse: Percentage of males recorded singing (Mean and *SD*) over a period of four days. 0 means no event recorded, missing numbers: no data recorded. Field temperatures and periods of sunshine periods were recorded at the local weather station.

temperatures, or smaller declines after sunset, are additional factors explaining calling activity during the hours of darkness (Fig. 2).

In *P. falcata* (Poda), as in other phaneropterid species (e.g. Harz, 1957), females responded to the song of males by making a clicking sound and the males moved towards responding females (Heller, 1992; Heller & von Helversen 1993). These findings and our observations on *P. falcata* (Poda) agree with the proposed communication system of the Phaneropterinae (Heller & von Helversen, 1993) in which the females respond to male song and initiate male phonotaxis and the males also call outside the hours of darkness. In species with mute females, male song is restricted to the hours of darkness.

In the related, but short-winged, species *Poecilimon veluchianus* Ramme males also moved towards females and their greater mobility was associated with high mortality (Heller, 1992). At our study site, the males of *P. falcata* (Poda) were slightly more mobile than the females (Samietz & Berger, 1997) but their mortality did not differ (Samietz, 1994). Because during mating *Poecilimon veluchianus* Ramme moves across open ground and not through vegetation like *P. falcata* (Poda) the males of the latter species may suffer less predation during movement, or females of the former species suffer more.

Mating frequency

In katytids, the frequency of mating by males and females is related to the size of the nuptial gift (Vahed, 2006). For example, in *Requena verticalis* Walker, *Ephippiger ephippiger* (Fiebig) and *Poecilimon veluchianus* Ramme the weight of the spermatophylax is about 20% of the male's body weight (Heller & von Helversen, 1991; Vahed & Gilbert, 1996) and male sexual activity starts 4–7 days after the previous mating (Simmons, 1993; Hockham et al., 2004; Vahed, 2006). By contrast, in *Poecilimon affinis* Frivaldsky the weight of the spermatophylax is 15% of the male's body weight and males remate after 2 days (Heller & von Helversen, 1991). Close to the latter data, in the case of *P. falcata* (Poda) the spermatophore (spermatophylax plus sperm ampulla) represented 13% of the body weight, which might indicate a short interval between mating. In the laboratory, for males it was 2.3 days, for females (3.6 days). By contrast, early literature suggests that both male and female *P. falcata* (Poda) die shortly after copulation (Harz, 1957), which would imply they are monogamous, or that females mate only once (Gerhardt, 1913, 1919). We can refute both suggestions for field conditions because multiple mating was recorded between marked males and females. Although this was confirmed for only five individuals it is likely that a more detailed study would result in an increase in the number of multiple matings recorded, especially if the time between surveys were smaller than the average time between mating (2.3 and 3.6 days, respectively).

In addition we used a novel and indirect approach. As stated in the methods, if mating occurs with equal probability at any time of the day and lasts for 15 min (d), i.e. 0.25 h (see Results), one would expect to observe 0.25 / 24, or 1%, of the individuals mating at any one time, if individuals mate once per day. However, mating did not occur throughout the day but only between 18:00 and 0:00 (Results), i.e. only over a period of six hours each day. The expectation, therefore, is that $p = 0.25/6 = 0.04$, or 4% of the individuals will be mating at any one time, if individuals mate once per day. As 3% of the individuals during resightings were mating, this corresponds to approximately one mating per day. Although our method provides only an estimate, our data are clearly inconsistent with the suggestion that *P. falcata* (Poda) is monogamous. We suggest that this simple method can easily be used for other species in the field.

Mating behaviour

Our observations agree with the general description published by Gerhardt (1913) and Grassé (1924). However, in two of 14 cases we observed the insertion of the titillators that was not followed by the attachment of a spermatophore, but the grooming of these structures and then the attachment of the spermatophore. The insertion of the titillators and grooming took 30 s. This has previously been described for *Metaplastes ornatus* (Ramme), where it took ca. 4 min (von Helversen & von Helversen, 1991) and the tree cricket *Truljalia hibinonis* (Matsumura) (Ono et al., 1989), where it took on average 65 s. In both of the latter species, this behaviour was associated with sperm removal. It is, therefore, possible that males of *P. falcata* (Poda) may also be able to remove and ingest sperm in females from a previous mating. However, this needs to be confirmed.

One male can transfer 312,000 sperm (Vahed & Gilbert, 1996). We can confirm the magnitude of this figure as the number of sperm in a female examined a week after mating was similar (15% lower). However, a single value is not enough to predict the number of times they mate based on the number of sperm recorded in randomly collected females in the field (as demonstrated by Reinhardt et al., 2007).

Most comparative studies and reviews (Vahed & Gilbert, 1996; Vahed, 2007; Gwynne, 2008) conclude that the spermatophore protects a male's ejaculate. In an intra-specific experiment Wedell (1998) was able to confirm this and in a species of *Poecilimon* the spermatophore additionally represented paternal investment (Reinhold, 1999). This argument is based on the fact that in many katytids, the female consumes the spermatophylax immediately after it has been atatched. In two laboratory studies *P. falcata* (Poda) either did not or not immediately, eat the spermatophore (Gerhardt, 1913, 1919), whereas its sister species, *P. nana* Fieber did (Pener & Ayal, unpubl., cited in Tauber et al., 2001). Grassé (1924) mentions that before females partially eat the spermatophylax they scrunch it. We can confirm that both in the field and the laboratory female *P. falcata* (Poda) do not consume the spermatophore immediately after transfer and then not entirely. Thus, it is unlikely that male ejaculate protection via "digestive distraction" occurs in *P. falcata* (Poda).

Finally, as the spermatophylax makes up 13% of a male's body weight in *P. falcata* (Poda) it is unlikely that winged species invest fewer resources in reproduction because they invest more in wings.

ACKNOWLEDGMENTS. This study was funded by Deutsche Forschungsgemeinschaft (DFG) grants Sa 770/1-1 and Ko1494/1-1. G. Köhler supervised the work. His and K.-G. Heller's stringent and helpful comments on an early draft substantially improved the manuscript.

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Received August 23, 2013; revised and accepted December 2, 2013
Prepublished online February 24, 2014