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Rapid Evacuation of Spermatophore Contents and Male Post-mating Behaviour in Alderflies (Megaloptera: Sialidae)

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Abstract. The members of Megaloptera are separable into three groups: dobsonflies, fishflies and alderflies. In dobsonflies, a large gelatinous spermatophore is produced, and copulation ends shortly (within a few minutes) when attaching the spermatophore externally to the female genitalia. After separation the female eats the gelatinous mass for more than 2 h, during which time the sperm migrates into the female genital cavity. By contrast, in fishflies, there is prolonged copulation following spermatophore transfer (which lasts 2–6 h), and the spermatophore possesses no gelatinous mass. To examine the relationship between spermatophore structure and male mating behaviour of the third group, the alderflies, the mating behaviour of seven species of *Sialis* was observed in the laboratory. They inseminated via the externally attached spermatophore in the same way as dobsonflies and fishflies. However, the spermatophore had no gelatinous mass as in fishflies, and copulation ended shortly after spermatophore transfer (in copula for only 0.5–2 min) as in dobsonflies. The female removed the spermatophore shortly after mating, which was attached for only 7.3–12.7 min. Why do not male alderflies prevent the female from removing the spermatophore? Because the time to empty the spermatophore is very short (6.4–7.3 min when placed in insect Ringer), which time is ensured even though the male does not guard the spermatophore. Rapid evacuation of spermatophore contents may be owing to a thicker and shorter spermatophore tube in alderflies than in the other groups. Thus, three types of male post-mating behaviour are observed in megalopteran species; (1) rapid evacuation of spermatophore contents without post-mating guarding as in alderflies, (2) behavioural protection afforded by direct contact with the mated female as in fishflies, and (3) indirect protection by feeding the mated female with a gelatinous mass as in dobsonflies. These types of mating behaviour also occur in crickets and to some extent in bushcrickets, probably as a result of evolutionary convergence among these insect groups inseminating via the externally attached spermatophore.

Key words: Evolutionary convergence, Megaloptera, Orthoptera, postcopulatory mate guarding, sperm, spermatophylax.

Introduction

The Megaloptera constitute a small worldwide fauna of probably less than 300 species, representing only two families, Corydalidae and Sialidae (New & Theischinger, 1993). The former family is separable into two well-defined subfamilies, Corydalinae and Chauliodinae, called dobsonflies and fishflies, respectively, but the latter (alderflies) is not thus subdivided.

The dobsonflies (*Protohermes grandis* and *P. immaculatus*) produce a large spermatophore at mating (Hayashi, 1992). The male attaches the spermatophore externally to the female genitalia. Following separation, the female bends her body and eats the gelatinous part covering the sperm package (ampulla). After almost all the gelatinous material is consumed, the sperm package is removed and abandoned. This process takes more than 2 h, during which time the sperm move into the female genital duct.

Spermatophores of the fishflies (*Parachauliodes continentalis, P. japonicus* and *Neochauliodes sinensis*) are also externally attached, but include no gelatinous material (Hayashi, 1996). In dobsonflies, copulation ends shortly after spermatophore transfer, whereas in fishflies, there is prolonged copulation following spermatophore transfer (which lasts for 2–6 h). The time needed to empty the spermatophore coincides with

copulation duration, and non-copulating females quickly remove the spermatophore before sperm transfer is complete. Thus, prolonged copulation in fishflies and the large gelatinous material covering the ampulla in dobsonflies are analogous in function, i.e., to ensure complete ejaculate transfer (Hayashi, 1996).

The mating behaviour of alderflies (*Sialis* spp.) is summarized by New & Theischinger (1993) as follows. Matings occur in the daytime and last for only several minutes, during which the simple spermatophore is transferred to the female. The spermatophore is attached externally. The female has been observed to chew on the spermatophore soon after mating, consuming it. This suggests that the spermatophore of alderflies is not gelatinous, and that the male does not prevent the female from removing it.

The question arises as to why male alderflies fail to guard the spermatophore in contrast to dobsonflies or fishflies. The present study examines, in detail, the processes of spermatophore transfer and evacuation of its contents in alderflies. Because lifetime reproductive activity of female alderflies has not been studied, oviposition patterns in the laboratory are also described. Finally, because insemination via an externally attached spermatophore is found in the suborder Ensifera (Orthoptera)(reviewed by Gwynne, 1997; Vahed, 1998), the structure and function of megalopteran spermatophores are discussed in comparison to those of ensiferan Orthoptera.

Materials and Methods

Two species of *Nipponosialis* and seven species of *Sialis* have been recognized from Japan (Hayashi & Suda, 1995, 1997). In this study, two sympatrically distributed species, *S. longidens* and *S. sibirica,* were selected for the main materials. The final-instar larvae of these two species were collected from a small stream in Kita-kashiwagi, Eniwa, Hokkaido, northern Japan, on 29 March 1997 and 4 April 1998. The larvae of *S. sibirica* were also collected from the Yobetsu River, Yobetsu, Shakotan, Hokkaido, on 28 September 1996, and from a small stream in Sakuragi, Chitose, Hokkaido, on 5 October 1996.

Collected larvae were reared at 10±1°C under a 12 h light (L): 12 h dark (D) cycle in winter and at 16±1°C under 14 h L: 10 h D in spring. They were kept individually in glass vessels (4 cm in diameter, 6 cm in height, 1 cm in water depth, with small stones on the bottom) and given live chironomid larvae ad libitum. They pupated in moist peat moss in spring. Adults were kept in individual glass vessels (7 cm in diameter, 9 cm high, with a nylon net on the top and wet filter paper on the bottom) at 23±1°C (14 h L : 10 h D). Adults were given a sugar solution everyday (water and fermented milk, CALPIS, in a volume ratio of 9 : 1).

Matings were conducted at 23±1°C under the light condition by placing a male and a female in a glass vessel (7 cm in diameter, 9 cm high, with a nylon net on the top and wet filter paper on the bottom). The behaviour of each pair was observed for 2 h, measuring time intervals with a stopwatch. After observation, individuals were returned to their respective rearing vessels. The pairs were randomly chosen and allowed to mate only once, although both males and females mate multiply in their lifetime.

Males and females were killed with ether, dissected, and the general morphology of their internal reproductive systems was observed under a binocular microscope. Dissected organs were treated in insect Ringer (0.9 g NaCl, 0.02 g $CaCl_2$, 0.02 g KCl and 0.02 g $NaHCO_3$ in 100 m*l* water). Spermatozoa stored in the seminal vesicles were also observed under a light microscope. Ten spermatozoa per individual were measured for their total length (tip of heads to end of tails) with an ocular micrometer (×100) to the nearest 0.001 mm.

The spermatophore was removed from the female genitalia carefully with forceps immediately after the pair separated, by anesthetizing the female with carbon dioxide. The spermatophore was then placed in insect Ringer at 23±1°C to observe the evacuation of their contents under a binocular microscope, recording the time from pair separation to complete emptying with a stopwatch. After observation, the ampulla length (X mm) and width (Y mm), and the tube length (Z mm) in the ventral side of spermatophores were measured with an ocular micrometer (×10) to the nearest 0.01 mm (see Fig. 9).

Weights of the male body and male internal reproductive system were measured with a microbalance to the nearest 0.01 mg before and immediately after dissection, respectively. Then, the size of the male internal reproductive system was represented as % body weight and compared among the unmated males, those just after mating, and those 3 or 6 days after mating. However, because it was difficult to dissect out the testes and fine-tubed vas deferens, these organs were excluded when weighing the male internal reproductive system.

Mated females were checked everyday for egg laying and survival to assess the time from emergence to the first oviposition, the intervals of ovipositions,

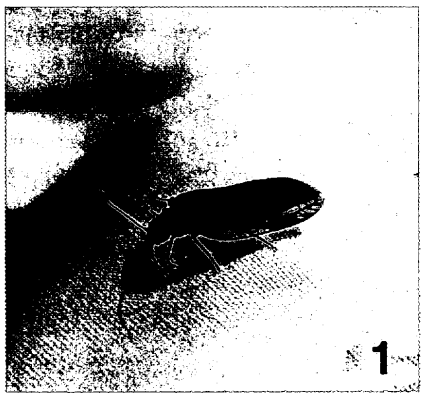
and longevity. In some cases when the egg mass was deposited on the net or the wall of the glass vessels, the number of eggs was counted under a binocular microscope.

In addition to *S. longidens* and *S. sibirica,* the other five species of Japanese *Sialis, S. yamatoensis, S. japonica, S. bifida, S. melania* and *S. sinensis,* were also observed for their mating behaviour, evacuation process of spermatophore contents, sperm length, and/or egg laying patterns. Adults of *S. japonica* were collected around a small pond in Todori, Hachioji, Tokyo, central Japan, on 2 May 1995, and those of *S. bifida* from the banks of the Karasu River, Minamiazumi-mura, Nagano, central Japan, on 1 June 1997. Adult *S. yamatoensis* and *S. sinensis* were raised from the final-instar larvae collected from a small stream in Yokosawa, Itsukaichi, Tokyo, central Japan, on 12 March 1995, and from a small pond in Asato, Amami Island, the Ryukyu Islands, southern Japan, on 20 February 1996, respectively. Male adults of *S. melania* were collected near Anado Fork, a tributary of the Natori River, Miyagi, northern Japan, on 17 May 1997, and also raised from the final-instar larva obtained from a small pond, Minamiosawa, Hachioji, Tokyo, central Japan, on 31 October 1997.

Results

Mating behaviour and oviposition

General patterns of mating behaviour did not differ among species of *Sialis* studied. After being placed in a glass vessel, the male that detected the presence of a female began an approach from behind her. The cue mediating sexual encounters was unknown in the present study, and the male was not observed to vibrate his abdomen or tap his abdomen and wings on the substrate as European *Sialis* do (Rupprecht, 1975). When the female walked, the male pursued her. When the male was able to move underneath the female, he then curled his abdomen dorsally to grasp the end of her abdomen. The male abdomen was curled from either side of right or left wings which were extended (Figs. 1, 2). The pair remained in copula for 0.5–2.0 min for respective species (Table 1) and separated, during which time the spermatophore was transferred from the male to the female. The female began eating the externally attached spermatophore bending her head and abdomen beneath her thorax 7.3–12.7 min after separation (Table 1), which was taken as the duration of spermatophore attachment prior to de-



Figs. 1–2. Non-mating (1) and mating (2) females of *Sialis yamatoensis.* In copula, the male sliding underneath the female curled his abdomen from either side of left or right extended wings to grasp the female abdominal tip.

Table 1. Copulation duration, the time from the end of copulation to the start of spermatophore consumption (duration of spermatophore attachment), and the time from start to finish spermatophore consumption in six species of *Sialis.* The time being taken to evacuate spermatophore contents in insect Ringer is also shown.

Duration (min)	*Sialis* species					
	S. longidens	*S. sibirica*	*S. yamatoensis*	*S. japonica*	*S. bifida*	*S. sinensis*
Copulation	1.3±0.2 (15)	1.4± 0.3 (17)	1– 3 (2)	0.5±0.2 (4)	0.7 (1)	2.0±0.5 (3)
Beginning to eat spermatophore	7.9±1.3 (4)	10.0± 4.7 (6)	1– 5 (2)	7.3±2.6 (4)	—	12.7±4.0 (3)
Finishing to eat spermatophore	14.0±5.6 (4)	15.7±13.6 (6)	8–20 (2)	7.5±3.4 (4)	—	11.7±5.7 (3)
Evacuation in saline	7.3±1.2 (11)	6.4± 0.9 (6)	—	—	6.4 (1)	—

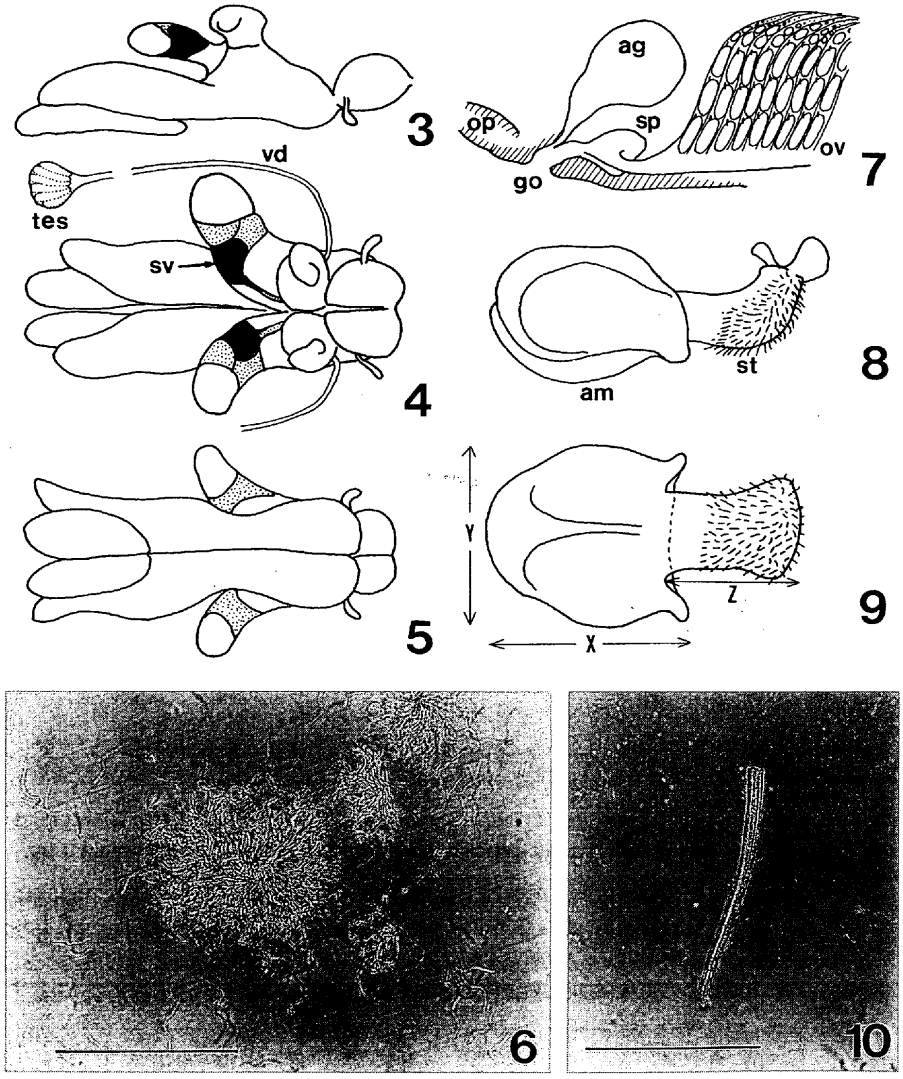
Values are mean±SD or range (the number of observations)

struction by the female. She continuedeatinguntilthe spermatophore was completely removed from her genitalia. This took 7.5–15.7 min (Table 1). The male was less active after mating, and guarding behaviour to prevent the female from eating and removing the spermatophore was not observed.

The female internal reproductive system consisted of a simple storage organ and paired ovaries with many tubed ovarioles (Fig. 7). The storage organ was not distinguished into a bursa copulatrix and a spermatheca as is usually observed in other insect groups.

In *S. longidens,* female longevity was 12.5 days (SD=5.7, range 5–17, N=4). Females laid the first egg mass 4.5 days (SD=1.0, range 4–6, N=4) after emergence, and the second egg mass 7.5 days (range 3–12, N=2) after the first oviposition. The number of eggs was 769 (SD=163, range 625–1000, N=4) in the first egg mass and 243 (range 57–429, N=2) in the second. Dead females had 114 mature eggs in their ovaries (SD=29, range 80–132, N=3).

In *S. sibirica,* female longevity was 10.3 days (SD=4.6, range 4–16, N=7). Females laid the first egg mass 4.7 days (SD=2.4, range 1–8, N=7) after emergence, and the second egg mass after another 4.5 days (SD=1.3, range 3–6, N=4). One female laid the third egg mass 2 days after the second oviposition. The number of eggs was 604 (SD=93, range 515–700, N=3) in the first egg mass and 92 (N=1) in the second. The number of eggs in the third mass was not



Figs. 3–10. Male internal reproductive system (3–5), spermatozoa stored in the seminal vesicles (6), female internal reproductive system (7), spermatophore (8, 9), and a spine on the surface of the spermatophore tube (10) of *Sialis longidens.* 3, 7 and 8, lateral views. 4, dorsal view. 5 and 9, ventral views. Scale bars, 0.1 mm. Abbreviations: ag, accessory gland; am, ampulla; go, genital opening; op, ovipositor; ov, ovary; sp, spermatheca; st, spermatophore tube; sv, seminal vessicle; tes, testis; vd, vas deferens; *X,* length of the ampulla; *Y,* width of the ampulla; *Z,* length of the spermatophore tube.

counted. Ovaries of two females examined after death contained 0 and 10 mature eggs.

In *S. yamatoensis,* female longevity was 10.2 days (SD=1.9, range 8–13, N=5). Females laid the first egg mass 3.8 days (SD=1.6, range 2–5, N=5) after emergence, and the second egg mass 3.0 days (SD=0, N=3) after the first oviposition. One female laid the third egg mass after another 2 days. The number of eggs was 570 (SD=199, range 410–850, N=4) in the first egg mass and 167 (range 56–362, N=3) in the second. The number of eggs in the third mass and those contained in the dead females were not counted.

Spermatophore and male internal reproductive system

The spermatophore was rather hard-walled and consisted of two parts, a spermatophore tube and an ampulla in all *Sialis* examined (Figs. 8, 9). Only the spermatophore tube was inserted into the female genital duct, and it had many spines laterally and ventrally. These rough-surfaced spines (Fig. 10) may serve to prevent the spermatophore from being physically expelled from the female genital duct.

The male internal reproductive system consisted of a pair of testes, each leading to a tublar vas deferens and entering a spherical seminal vesicle, and several pairs of large accessory glands (Figs. 3–5). The seminal vesicles were surrounded by a brown membrane in all species of *Sialis* examined and contained a sperm mass (Fig. 6). Spermatozoa were usually single, but occasionally clumped. Sperm was filamentous with a total length of 0.044 mm (SD=0.001, N=7) in *S. longidens,* 0.042 mm (SD=0.001, N=12) in *S. sibirica,* 0.044 mm (range 0.043–0.044, N=2) in *S. yamatoensis,* 0.043 mm (SD=0.001, N=3) in *S. japonica,* 0.040 mm (SD=0.003, N=5) in *S. bifida,* 0.044 mm (range 0.043–0.044, N=2) in *S. melania,* and 0.051 mm (SD=0.004, N=3) in *S. sinensis.*

Just after copulation, the male internal reproductive system decreased in weight from 7.39% (SD=0.99, N=8) to 2.49% (SD=0.25, N=6) of body weight in *S. longidens,* and from 4.19% (SD=0.66, N=9) to 1.78% (SD=0.36, N=6) in *S. sibirica* (Fig. 11). This suggests that the spermatophores produced in the first mating were 4.90% and 2.41% of male body weight, respectively. Male body weight was 22.26 mg in fresh weight (SD=4.07, N=23) in *S. longidens* and 27.45 mg (SD=4.87, N=28) in *S. sibirica.* The ampulla length of spermatophores was 1.44 mm (SD=0.10, N=10) in *S. longidens* and 1.04 mm (SD=0.12, N=8) in *S. sibirica,* and its width was 1.30 mm (SD=0.07, N=10) and 1.07 mm (SD=0.07, N=8), respectively. The length of the spermatophore tube was 1.22 mm

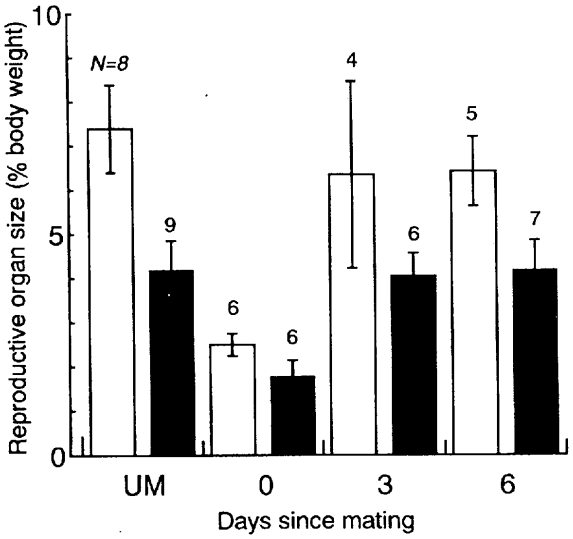


Fig. 11. The size of male internal reproductive system (% of body weight) of unmated individuals (UM) and those just after mating (0) or 3 or 6 days after mating in *Sialis longidens* (open bars) and *S. sibirica* (closed bars). Vertical bars, ±SD.

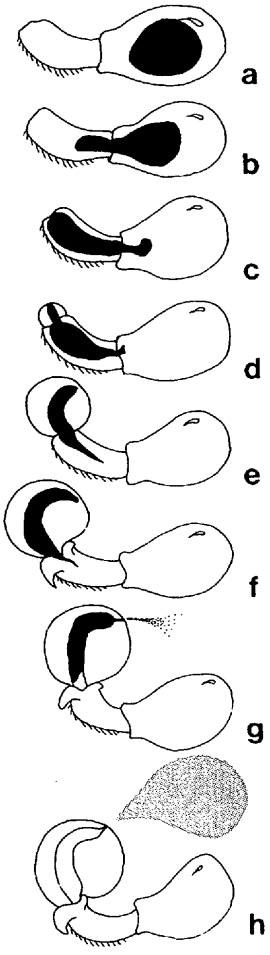


Fig. 12. Emptying process (a to h in sequence) of a spermatophore of *Sialis longidens* in insect Ringer. Black and dotted areas indicate a sperm mass. For detailed description, see text.

(SD=0.21, N=10) in *S. longidens* and 0.90 mm (SD =0.15, N=8) in *S. sibirica*. Thus, *S. longidens* had the larger internal reproductive system and produced larger spermatophores than *S. sibirica*. In both species, the male internal reproductive system seemed to recover nearly to its pre-mating weight 3 days after mating (Fig. 11).

Evacuation of spermatophore contents

In all species examined, spermatophore contents packed in the ampulla were gradually evacuated in insect Ringer as shown in Fig. 12. After the contents reached the tip of the spermatophore tube, a spherical sac extended from the tube (Fig. 12d). The contents were finally released through a narrow pipe in this sac. It took 6.4–7.3 min to complete the evacuation (Table 1). The contents released from the spermatophore include numerous spermatozoa, some of which were clumped as observed in the seminal vesicles (Fig. 6). The spermatozoa were motile.

Discussion

Insemination in alderflies occurs via an externally attached spermatophore which is easily removed by the female, as is also the case in dobsoflies and fishflies (Hayashi, 1992, 1996). The dobsonflies prevent the female from removing the spermatophore before complete sperm transfer by feeding her a large gelatinous mass, and the fishflies prevent spermatophore detachment by prolonged copulation. However, male alderflies do not guard the spermatophore. This is because the time needed to empty the spermatophore is very short in the alderflies; 7.3 min in *S. longidens* and 6.4 min in *S. sibirica* (Table 1), while it takes more than 2 h in dobsonflies and 2–6 h in fishflies (Hayashi, 1992, 1996). This emptying time is ensured in alderflies even though the male does not guard the spermatophore, which was attached for 7.9 min in *S. longidens* and 10.0 min in *S. sibirica* (Table 1). Rapid evacuation of spermatophore contents may be due to the wide and short spermatophore tube of alderflies. The spermatophore tube is much narrower and longer in dobsonflies and fishflies (Hayashi, 1992, 1996).

Thus, there are three types of male mating behaviour in Megaloptera; (1) rapid evacuation of spermatophore contents without post-copulatory guarding as in alderflies, (2) behavioural protection afforded by direct contact with the female as in fishflies, and (3) indirect protection by feeding the female with a large gelatinous mass as in dobsonflies. The dobsonflies with type (3) mating behaviour invest heavily in each spermatophore (up to 20% of body weight in *P. grandis* and up to 10% in *P. immaculatus*; Hayashi, 1993) as compared with fishflies (up to 1.4% body weight in *P. continentalis*, up to 2.9% in *P. japonicus*, and up to 3.1% in *N. sinensis*; Hayashi, 1999) and alderflies (up to 4.9% body weight in *S. longidens* and up to 2.4% in *S. sibirica*).

Although they are hemimetabolous insects, most crickets and bushcrickets (subfamily Ensifera of Orthoptera) inseminate via an externally attached spermatophore which is eaten and removed by the female if unguarded (Gwynne, 1997; Vahed, 1998), as is also observed in Megaloptera (holometabolous insects). According to Dambach & Beck (1990), in the cricket *Cycloptiloides canariensis*, the isolated spermatophore empties within 35 s when placed in insect Ringer, and the female detaches the spermatophore 31 s after transfer. The male of this cricket shows no guarding behaviour. One reason for such rapid emptying is thought to be the unusually thick spermatophore tube which is even shorter than the diameter of the ampulla. This is evidence of the strongly convergent evolutionary pattern also found in alderflies (type 1 mating behaviour).

In other cricket species, however, post-copulatory mate guarding by the male is widespread (reviewed by Loher & Dumbach, 1989; Zuk & Simmons, 1997). After attaching the spermatophore to the female genitalia, male crickets enter a guarding phase during which they attempt to remain in contact with the female (type 2 mating behaviour). This is characterized by mutual antennation and a series of aggressive actions by the male in response to female movement. In *Gryllus bimaculatus*, it takes about 1 h to empty the spermatophore, and the male guards the female for about 1 h (Simmons, 1986). The presence of the guarding male has been found to have a significantly positive effect on the duration of spermatophore attachment in *G. bimaculatus* (Simmons, 1986), *G. campestris* (Huber, 1955), *Teleogryllus commodus* (Loher & Rence, 1978; Evans, 1988), *T. natalensis* (Hockham & Vahed, 1997), and *Balamara gidya* (Evans, 1988), although no such effect of guarding is found in *Acheta domesticus* (Khalifa, 1950; Sakaluk & Cade, 1980).

The spermatophore of the cricket *Gryllodes sigillatus* holds a large gelatinous mass which keeps the female preoccupied while sperm drain from the ampulla into her body (type 3 mating behaviour) (Sakaluk, 1984, 1985). In this as in other crickets, post-copulatory mate guarding occurs. However, rather than serving to prevent the early removal of the sperm ampulla by the female, guarding functions pri-

marily to deter rival males from courting the mated female (Sakaluk, 1991; Frankino & Sakaluk, 1994).

Two types of male mating behaviour occur also in bushcrickets (Tettigoniidae). Many species of bushcrickets produce spermatophores with a large gelatinous mass eaten by the female after mating but before removing the ampulla, and it has been confirmed that this gelatinous mass functions to ensure the complete transfer of spermatophore contents into the female storage organ in *Requena verticalis, Decticus verrucivorus, Kawanaphila nartee* and *Poecilimon veluchianus* (reviewed by Gwynne, 1997; Vahed, 1998). This is the indirect ejaculate protection of type (3). However, spermatophores in the bushcrickets *Meconema meridionale* and *M. thalassinum* have no gelatinous mass, and copulation following spermatophore transfer continues for a longer time than in most other bushcrikets which produce large gelatinous spermatophores (Vahed, 1996). In bushcrickets belonging to the subfamily Ephippigerinae, copulation is also prolonged in *Uromenus rugosicollis,* lasting about 100 min following spermatophore transfer, and the spermatophore is small with little gelatinous mass, representing about 7% of male body weight; however, this stands in contrast to the 15 other species, in which copulation following spermatophore transfer is brief, lasting an average of about 5 min, and the gelatinous mass of spermatophores is large, representing over 20% of male body weight (Vahed, 1997). Moreover, in *Coptaspis* sp. 2, males do not produce a large gelatinous spermatophore that the female can feed on during insemination, but remain attached to the female's genitalia up to 6 h after spermatophore attachment, during which time the spermatozoa are gradually transferred into the female storage organ (Wedell, 1998). The prolonged copulation observed in these bushcrickets serves as the behavioural protection of ejaculates (type 2). At present, however, ejaculate protection of type (1) has not been reported in bushcrickets.

Strongly convergent forms of male mating behaviour associated with spermatophore structure among the three insect groups, Megaloptera, Gryllidae (Ensifera) and Tettigoniidae (Ensifera), suggest that in mating systems in which externally attached spermatophores are provided by males to females, early spermatophore removal by unguarded females may play a role in sexual selection for such male insemination tactics.

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