

Spermatophore Transfer and Ejection in the Beetle *Pseudoxychila tarsalis* (Coleoptera: Cicindelidae)

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ABSTRACT: Spermatophore transfer in the beetle *Pseudoxychila tarsalis* occurred in association with one of the four movements of the body and aedeagus that males performed during copulation. Some copulations did not result in spermatophore transfer, and females ejected some of the spermatophores they received. This suggests females may be capable of exerting cryptic female choice. Data on spermatophore transfer do not fit with a previous hypothesis regarding the "phases" of *P. tarsalis* copulation.

Matings of tiger beetles (Coleoptera: Cicindelidae) have been described as consisting of three phases: an initial intromission in which the flagellum (a sclerite of the male aedeagus) prepares the duct of the female spermatheca for sperm transfer, followed by retraction of the aedeagus, and another intromission in which semen transfer occurs, usually in a spermatophore (Freitag et al., 1980). This was based on three kinds of evidence: that the flagellum of tiger beetles does not connect with the ejaculatory pore, that the shape of the flagellum matches that of the spermathecal duct, and three behavioral phases of mating described for *Pseudoxychila tarsalis* Bates (Palmer, 1976) and five *Cicindela* species (Freitag et al., 1980).

Palmer's (1976) description of the mating behavior of *P. tarsalis* was incomplete (Rodríguez, 1998). Instead of three phases, copulations had 1-20 intromissions in which the male performed up to four different movements with his body and aedeagus: prying, thrusts, small thrusts, and pulling. Prying lasted several seconds and consisted of a deep penetration of the aedeagus into the female and a change in its inclination; thrusts were short pushes of the aedeagus into the female; small thrusts were vibrations at the base of the aedeagus; and in pulling the male partially retracted the aedeagus. Between intromissions, the male courted the female by rubbing her body with his middle legs. There was geographic variation in *P. tarsalis* male copulatory behavior, and stronger differences in comparison to *P. bipustulata*, suggesting sexual selection on male courtship behavior. The female often struggled before and during copulation, and sometimes ejected one or two spermatophores during or after copulation.

The present paper analyzes the copulatory movements of *P. tarsalis* males in relation to spermatophore transfer, presenting observations on spermatophore ejection, the organization of sperm within the spermatheca, and measurements of the male flagellum and the female spermathecal duct. The data of spermatophore transfer and ejection suggest female *P. tarsalis* may be capable of cryptic female choice, in accordance with the hypothesis that male copulatory courtship behavior evolves by sexual selection (Eberhard, 1994, 1996).

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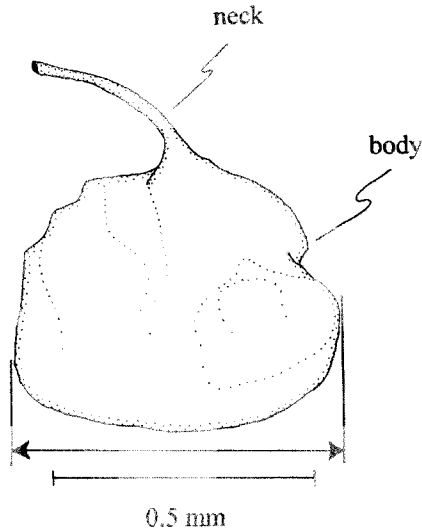


Fig. 1. Schematic drawing of a *P. tarsalis* spermatophore. Arrow: width measured.

Materials and Methods

Beetles were captured throughout the rainy season at El Zurquí de Moravia (el. 1600 m), San José Province, Costa Rica. They were kept in the lab at room temperature, each in a black photographic film cartridge containing leaves or wet napkins to provide hiding places. Beetles that spent several days in captivity were fed prey such as med flies and termites, or pieces of canned meat.

Matings were observed under natural or fluorescent light. Couples were placed in round metal or plastic dishes approx. 15 cm wide and 3 cm high. Verbal descriptions of matings were dictated to a tape recorder. Although beetles were collected throughout the rainy season, copulations probably did not involve very old or very young individuals. Old beetles looked dry, ragged, and were slow (old males were unable to catch females to copulate); and I never found young adults whose exo-skeleton had not yet hardened.

The following measurements were made with an optical grid attached to a dissecting microscope: distance between external margins of the eyes, pronotum width (males and females), aedeagus length (males), for 43 pairs that mated; width of the lumen of the spermathecal duct of females; length of the flagellum and its width at the tip; and width of spermatophores found inside females.

Spermatophores found inside females were classified as "newly transferred" or "old", based on a preliminary sample: some spermatophores ($n = 10$) had a well defined globular body and neck (Fig. 1) and an average width of 0.5 ± 0.1 mm (range = 0.4–0.6 mm). Other spermatophores ($n = 10$) were fuzzy around the edges, approx. 0.2–0.3 mm wide, their neck was missing, broken, or flattened, and they were apparently in the process of degradation. Thus, spermatophores with a well defined globular shape, neck in good condition, and more than approx. 0.4 mm wide were classified as "newly transferred"; and spermatophores less than 0.3 mm wide, with a fuzzy appearance, and a broken or flattened neck were classified as "old".

Table 1. Sperm content of spermatophores found inside females and of ejected spermatophores.

	Spermatophores inside females	Ejected spermatophores
Spermatophore empty or with a few individual sperm	21	1
Spermatophore less than half full	2	0
Spermatophore full of sperm	3	7

$G = 13.51$, calculated adding 1 to all categories, $df = 2$, $P < 0.005$.

The sperm content of spermatophores ejected by females or found inside females frozen two hours after capture in the field was examined under the microscope by crushing on a slide in saline solution.

To study spermatophore transfer and ejection, the following groups of females were dissected in 70% ethanol to examine the spermatophores inside them:

1) females frozen at -5°C in a refrigerator two hours after capture in the field ($n = 20$); 2) females allowed one complete mating the day of capture, observed for 30 min after mating to check for spermatophore ejection, and then frozen ($n = 6$); 3) females allowed four complete matings during 4–5 days, observed for 30 minutes after each mating, and frozen after the fourth mating ($n = 10$).

To study the relationship between male copulatory movements and spermatophore transfer, 29 females were isolated for five days. Each was then allowed a single intromission with a male, and it was noted whether the male dismounted or attempted another intromission (and the couple was separated). The females were frozen after the intromission and dissected. Four control females were isolated for five days and dissected.

The organization of sperm within the spermatheca was observed in preparations under a compound microscope with Nomarski interference contrast optics.

Averages are given \pm the standard error (SE). Except where otherwise indicated, two-tailed Mann-Whitney U tests were used. Voucher specimens were deposited at the Museo de Insectos of the Universidad de Costa Rica, and at INBio (Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica).

Results

SPERMATOPHORE TRANSFER AND EJECTION: Sperm content in spermatophores found inside females from the field was lower than in spermatophores ejected by females after copulation (Table 1). This suggests spermatophores ejected by females after copulation had been transferred by the last male to copulate.

Of 20 females frozen two hours after capture, only 2 had “newly transferred” spermatophores (one each), and the rest had 1–7 “old” spermatophores. When only one spermatophore was present (9 of 20 females), its neck was inside the spermathecal duct, and its body rested on the sclerite associated with the duct (Fig. 2). When more than one spermatophore was present (11 of 20 females), they were clumped on the sclerite associated with the duct, and none or only one had its neck in the spermathecal duct.

Of 6 females allowed one complete mating, only 2 got “new” spermatophores (1 received 1 spermatophore which she ejected, the other received 2 and ejected 1). The

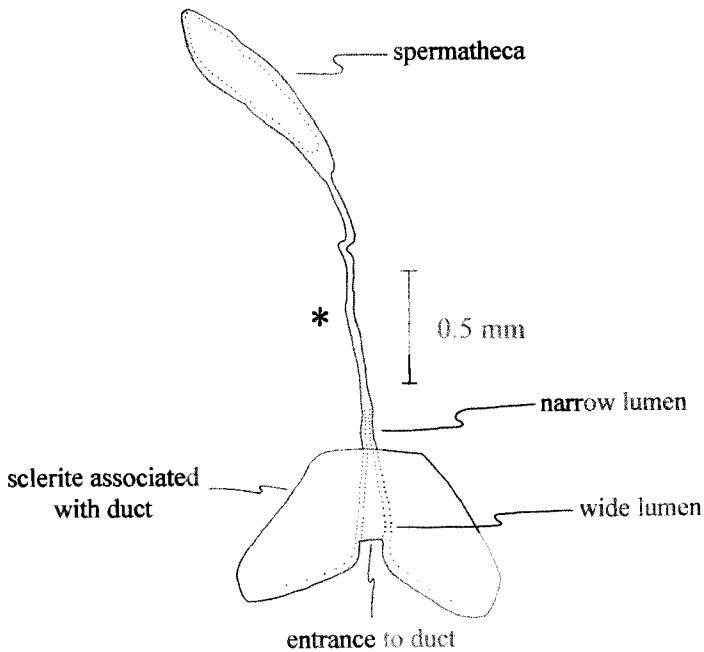


Fig. 2. Schematic drawing of a *P. tarsalis* spermatheca, spermathecal duct, and sclerite associated with the spermathecal duct. The dotted line within the spermatheca marks the region occupied by sperm. Asterisk: approximate point to which the male flagellum might penetrate.

4 matings (67%) in which spermatophore transfer failed to occur consisted of a single intromission, while the other 2 had 4 intromissions each. So, at least 4 of 12 intromissions (33%) failed to transfer spermatophores.

Two of 10 females that copulated four times had no "new" spermatophores upon dissection. Thus, 2 of all 40 copulations (5%) or 2 of 10 final copulations (20%) failed in spermatophore transfer. Eight females got 19 "new" spermatophores, and ejected 7 (37%).

Females that mated four times had fewer "old" spermatophores inside (10 of 29 spermatophores in 10 females) than females from the field (28 of 30 spermatophores in 14 females). Thus, the females may have ejected spermatophores for longer than the 30 min after each mating during which they were observed. Ten females that copulated and did not eject spermatophores after 30 min were placed in individual Petri dishes, and 24 hours later there were no spermatophores on the substrate, but they may dry up and become difficult to recognize with time. One female ate a spermatophore she had just ejected.

In short, 20–67% of complete matings failed to transfer spermatophores, and females ejected at least 37–67% of the spermatophores they received.

MALE COPULATORY MOVEMENTS AND SPERMATOPHORE TRANSFER: None of the control females isolated for five days had "newly transferred" spermatophores (one female did not have any spermatophores, the others had 1–3 "old" ones). Thus, spermatophore degradation within the female seems to take several days.

Of 29 females isolated for five days and allowed one intromission, only 2 had "new" spermatophores (1 had only the "new" spermatophore, the other had that

Table 2. Spermatophore transfer and male copulatory movements in single intromissions. Prying occurred in significant association with spermatophore transfer ($P = (2/29)^2 = 0.0048$).

Spermatophores in females	Occurrence of							
	Prying		Thrusts		Small thrusts		Pulling	
	Yes	No	Yes	No	Yes	No	Yes	No
New (1)	2	0	2	0	2	0	2	0
Old (1–4)	0	18	17	1	14	4	14	4
None	0	9	7	2	5	4	6	3

and 2 “old” ones). The rest had 1–4 “old” spermatophores ($n = 18$) or none at all ($n = 9$).

The two intromissions that resulted in spermatophore transfer were the only ones in which the male performed the prying movement (Table 2). They were also the only intromissions after which the male did not attempt another intromission, but dismounted, and they lasted 258 and 158 s, significantly longer than the other intromissions (86 ± 7 s, $P = 0.028$). Intromissions and copulations in which prying occurred were also significantly longer in a sample of 153 complete matings, and the male movement of rubbing the female occurred more frequently than expected during copulations in which prying occurred (Rodríguez, 1998). Thus, spermatophore transfer seemed to occur in association with prying. But a female that mated for at least 59 min, with at least 15 intromissions in at least one of which prying occurred, had no spermatophores inside.

MEASUREMENTS OF MALES AND FEMALES: The body and genital measurements of males, and the difference between the male and female measurements of pairs, were compared between single intromissions that transferred spermatophores ($n = 2$) or failed to do so ($n = 27$). They were also compared between complete matings ($n = 43$) that did or did not include prying and rubbing, and between complete matings in which females did or did not eject spermatophores. There were no significant differences in any of these comparisons (in all cases $P > 0.30$).

Male body and genital measurements were not significantly correlated with the length of complete copulations, nor with the proportion of intromissions per copulation in which prying or rubbing occurred (in all cases, $r_s < 0.30$, $n = 43$, $P > 0.05$). There were also no such correlations with the difference in the measurements of the male and female of each pair (in all cases, $r_s < 0.31$, $n = 43$, $P > 0.10$).

FLAGELLUM AND SPERMATHECAL DUCT: The tip of the flagellum has a deep groove which joins basally with a blind, inner tube (Fig. 3). The measurements taken (Table 3) suggest it could penetrate the spermathecal duct (Fig. 2). The maximum length of the flagellum that might penetrate was 0.75–0.83 mm. (Fig. 3), about half the length of the spermathecal duct (Fig. 2, asterisk).

ORGANIZATION OF SPERM WITHIN THE SPERMATHECA: Sperm appeared to be highly organized within the spermatheca (Fig. 4). The walls of the spermatheca have folds which suggest it could expand longitudinally (Fig. 4A, B). But the length of spermathecae that looked very folded (0.77–1.11 mm, $n = 7$) was not significantly different from spermathecae that appeared distended (0.97, 1.13 mm, $n = 2$, $P = 0.66$).

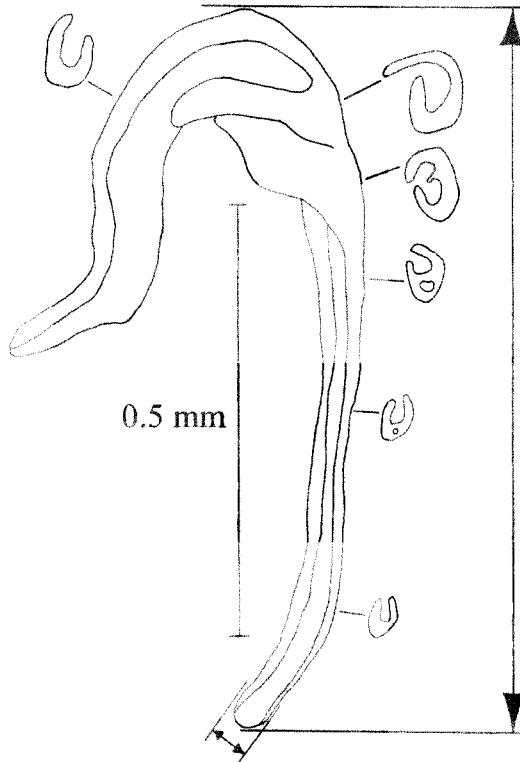


Fig. 3. Schematic drawing of the flagellum of a *P. tarsalis* aedeagus. Small arrow: width of flagellum on its tip. Big arrow: maximum length of flagellum that might penetrate spermathecal duct. This portion has an outer groove and an inner blind tube that join basally.

Discussion

Palmer (1976) proposed that semen transfer in *P. tarsalis* occurred in "phase 3" of copulation, in which there were "leg twitching" and "body shaking", a deep thrust of the aedeagus into the female, and occasional loss of the grasp of the male's mandibles on the female. Although these movements may correspond to the prying movement, other observations presented here are not in complete agreement with this idea. Spermatophore transfer in *P. tarsalis* apparently occurred during relatively long intromissions in which the male performed the prying movement. But *P. tarsalis*

Table 3. Measurements of the female spermathecal duct and male flagellum (in mm).

	Lumen of spermathecal duct (Fig. 2)		Width of flagellum at tip (Fig. 3)
	Widest part	Narrow part	
Av \pm SE	0.055 \pm 0.006	0.027 \pm 0.001	0.025 \pm 0.005
Range	0.038–0.072	0.021–0.032	0.015–0.035
n	6	10	4

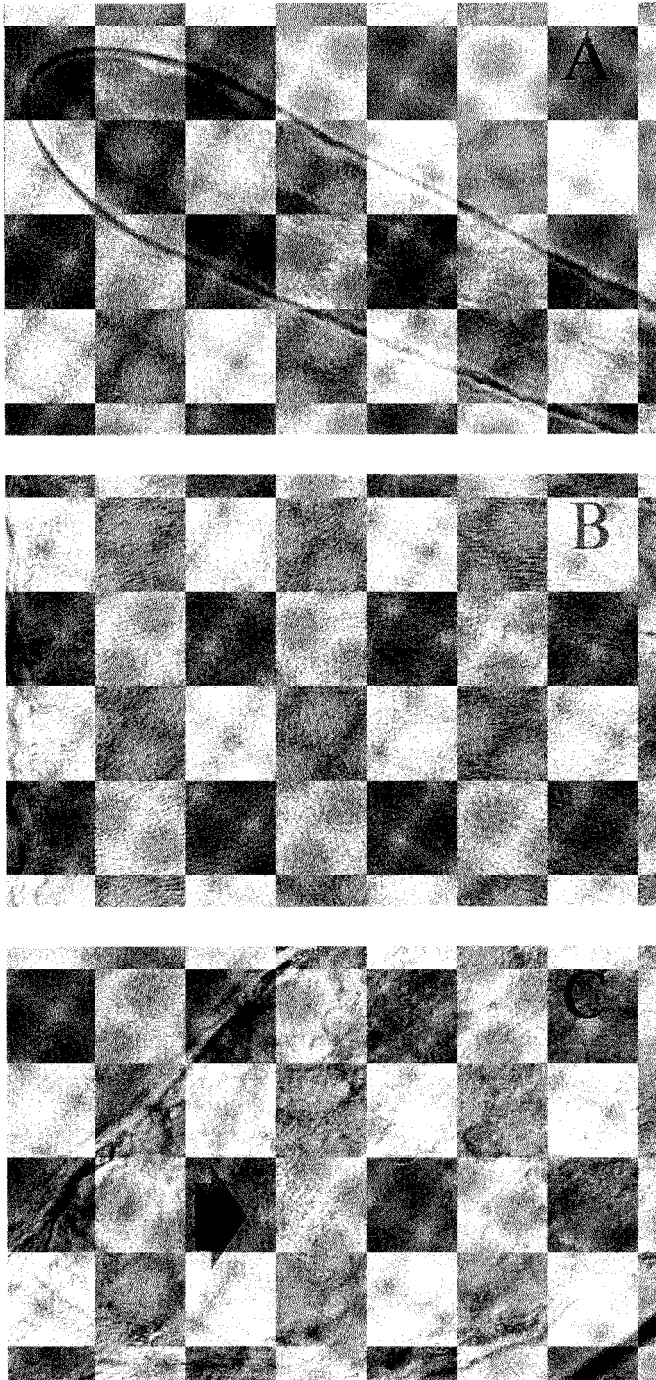


Fig. 4. Photograph of the organization of sperm in a *P. tarsalis* spermatheca. **A:** Sperm in a central bundle and two lateral masses perpendicular to it. The organization seemed less strict in the distal and basal regions. **B:** Detail of the central bundle and lateral masses of sperm in the middle of the spermatheca. There was a sperm free region between the wall of the spermatheca and the central mass of sperm (left). The walls of the spermatheca have folds (A, B left). **C:** Between the entrance to the spermatheca and the spermathecal duct the mass of sperm took an "S" shape (arrow).

copulations involved 1–20 intromissions, and prying might occur in any of them. Also, spermatophores were occasionally transferred during a single intromission, or not at all. These differences are probably not due solely to geographic variation between the site of the present study and Palmer's. In a study on the mating behavior of *P. tarsalis* that included a site very near Palmer's (Rodríguez, 1998), all matings conformed to the basic pattern described here.

There was a high failure rate in spermatophore transfer. Only 2 of 29 single intromissions resulted in spermatophore transfer. Even with several intromissions in complete copulations, it is estimated that 20–67% of copulations, or at least 33% of the intromissions of those copulations, failed to transfer spermatophores. Successful single copulations transferred 1–2 spermatophores, but females ejected at least 37–67% of the spermatophores they received. Thus, a *P. tarsalis* female could reduce the likelihood that a given male would inseminate her and fertilize her eggs in at least six contexts: 1) prevent copulation (Rodríguez, 1998); 2) prevent complete intromission (Rodríguez, 1998); 3) interrupt copulation prematurely; 4) remate; females mated up to twice in a single day, and up to four times in several days; the presence of up to seven spermatophores inside recently captured females suggests they may copulate frequently in the field; 5) prevent sperm transfer; some copulations did not result in spermatophore transfer, which might be because the female prevented it, or because the male failed in the attempt; 6) eject spermatophore prematurely. All but the first context correspond to mechanisms of cryptic female choice (Eberhard, 1996). Other possibilities, such as to manipulate the sperm of different males, or vary physiological responses to seminal products of different males (Eberhard and Cordero, 1995; Eberhard, 1996) were not analyzed. But the highly organized arrangement of sperm within the spermatheca suggests there may be further ways in which sperm of different males may have differential success in fertilizing the female's eggs.

One likely function of male courtship performed during copulation, as occurs in *P. tarsalis* (Rodríguez, 1998), is to affect female cryptic choice (Eberhard, 1994, 1996). The association between the male copulatory courtship movement (rubbing) and the genitalic movement associated with spermatophore transfer (prying) (Rodríguez, 1998) suggests that male *P. tarsalis* may try to induce females to, at the very least of possible contexts, allow the transfer of spermatophores, or not to eject them. Although *P. tarsalis* females frequently struggled before and during copulation, and some males could not even catch them or remain mounted on them, there was no evidence that *P. tarsalis* females favored spermatophores from "strong" males (e.g. Pearson, 1988), since there were no significant differences in the body-size measurements of males in relation to spermatophore transfer, copulation length, or the frequency of the genitalic movement associated with spermatophore transfer.

The measurements of the flagellum and the lumen of the spermathecal duct of *P. tarsalis* show that the flagellum may penetrate deeply into the duct, as in the tiger beetle *Cicindela tranquebarica* (Schincariol and Freitag, 1986) and "prepare" it for sperm transfer. Alternatively, the flagellum might guide the formation of the neck of the spermatophore into the duct of the spermatheca. The groove in the flagellum might serve this purpose, but it could also simply be an "economical" form to construct the flagellum (i.e. no material wasted on filling the inside, B. Huber, pers. comm.).

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