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## MATING BEHAVIOR OF TWO *PSEUDOXYCHILA* BEETLES (COLEOPTERA: CICINDELIDAE)

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### Abstract

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Copulations of *Pseudoxychila tarsalis* Bates and *Pseudoxychila bipustulata* Latreille consisted of 1–20 intromissions. The male performed four different genitalic movements: prying, thrusts, small thrusts, and pulls. The male also courted the female during copulation by rubbing her with his middle legs. The observed male behavior does not fit previous hypotheses concerning the phases of copulation in tiger beetles. The female sometimes ejected 1 or 2 spermatophores during or after copulation. There were geographic differences in quantitative aspects of *P. tarsalis* male copulatory behavior, and more pronounced differences between *P. tarsalis* and *P. bipustulata*. This variation could result from sexual selection by cryptic female choice on male copulatory behavior.

Rodríguez S., R.L. 1998. Comportement d'accouplement chez deux espèces de *Pseudoxychila* (Coleoptera : Cicindelidae). *The Canadian Entomologist* 130 : 735–750.

### Résumé

Chez *Pseudoxychila tarsalis* Bates et *P. bipustulata* Latreille, l'accouplement se fait par 1–20 intromissions. Le mâle fait quatre mouvements différents avec ses genitalia: exploration, poussées, petites poussées et retraits. Pendant l'accouplement, le mâle courtise la femelle en la frottant de ses pattes médianes. Ce comportement chez des mâles ne correspond pas aux hypothèses déjà avancées sur les phases de l'accouplement des cicindèles. La femelle projette parfois un ou deux spermatophores pendant ou après l'accouplement. Il existe des différences géographiques dans les aspects quantitatifs du comportement de copulation chez les mâles de *P. tarsalis* et des différences encore plus importantes entre les comportements de copulation des mâles de *P. tarsalis* et de *P. bipustulata*. Il est possible que cette variation résulte d'une sélection sexuelle affectant le comportement des mâles en réaction à des choix non apparents des femelles.

[Traduit par la Rédaction]

### Introduction

Male courtship during copulation is very common in insects and spiders (Eberhard 1991, 1994, 1996). It probably functions to affect cryptic female choice,

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which occurs after copulation has begun by processes inside the female that affect the chances that the male's sperm will be used to fertilize her eggs (Thornhill 1983; Eberhard 1991, 1994, 1996). This hypothesis places male copulatory courtship under sexual selection and predicts it should often differ between closely related species (Eberhard 1991, 1994, 1996). This prediction has been met in all 27 genera for which data are available (Simpson 1975; Cowan 1986; Eberhard 1992, 1993a, 1993b, 1994, 1996; Lachmann 1997; Rodríguez 1994; Rodríguez S. and Eberhard 1994; Fielding and Knisley 1995; Rodríguez S.<sup>2</sup>). Intraspecific variation in male copulatory courtship has been studied less often, but has been observed in 10 of 11 species studied (Jaenson 1979; Cowan 1986; Wcislo et al. 1992; Huber 1993; Eberhard 1994; Fielding and Knisley 1995; Wcislo and Buchmann 1995; Hunter et al. 1996). Male courtship performed after copulation has been shown to indirectly affect sperm storage and usage by the female in the fly *Dryomyza anilis* Fall (Otronen and Siva-Jothy 1991; Otronen 1997).

This paper records the first systematic attempt to correlate intraspecific differences in male copulatory behavior with geographic distribution. It documents intraspecific and interspecific variation in the male copulatory behavior of the tiger beetles *Pseudoxychila tarsalis* Bates and *Pseudoxychila bipustulata* Latreille (Coleoptera: Cicindelidae). *Pseudoxychila tarsalis* is found in Costa Rica, Panama, and possibly Honduras, and *P. bipustulata* occurs in South America but not Panama (Anonymous 1996). Several aspects of the natural history of *P. tarsalis* were studied in San Vito, Puntarenas, Costa Rica, by Palmer (1976a, 1976b, 1982). Palmer (1976a) described three phases in matings by *P. tarsalis*: (1) aedeagus insertion, of about 1 min; (2) aedeagus retraction, of about 30 s; and (3) reinsertion and sperm transfer, of about 2 min, during which there is "leg twitching and body shaking" by the male and the aedeagus is thrust deeply into the female. Phases 1 and 2 may be repeated several times before phase 3.

## Materials and Methods

*Pseudoxychila tarsalis* was collected in Costa Rica and Panama and *P. bipustulata* in Colombia (Fig. 1). Las Alturas de Cotón, Costa Rica, is near San Vito de Java, Palmer's (1976a) study site. The beetles were kept at room temperature, each in a black plastic vial about 3 cm high and 2 cm wide. The vials contained leaves or wet napkins that provided hiding places. Beetles that spent several days in captivity were fed prey such as med flies (*Ceratitis capitata* Wiedemann Tephritidae) and termites or pieces of canned meat (Spam).

Matings were observed under natural or fluorescent light. Couples were placed in round metal or plastic dishes approximately 15 cm wide and 3 cm high. One hundred and fifty-three matings by *P. tarsalis* were recorded (15 with an Omnipro video camera with +6 closeup lenses, and 138 with verbal descriptions dictated into a tape recorder). Each copulation of beetles from Monteverde, San Ramón, and Las Alturas involved different individuals, but several copulations of the same individuals were observed with beetles from Moravia (61 copulations of 22 pairs), El Calvario (31 copulations of 25 pairs), and Fortuna (26 copulations of 9 pairs). Thirty-four matings by *P. bipustulata* were recorded (6 on video and 28 with verbal descriptions), all with different individuals. After each of 136 matings by *P. tarsalis* the male was removed from the dish and the female observed for 30 min to check for spermatophore ejection. Spermatophore ejection was also checked for in 28 matings by *P. bipustulata*.

<sup>2</sup>R.L. Rodríguez S. Mating behavior in two *Ozophora* bugs (Heteroptera: Lygaeidae). In preparation.



FIG. 1.—Collecting sites for *Pseudoxychila tarsalis* in Costa Rica and Panama (●) and *Pseudoxychila bipustulata* in Colombia (⊙). Site elevation is as follows: Monteverde (1400–1600 m), San Ramón (Reserva Alberto Brenes, 850 m), Moravia (1500–1600 m), Las Alturas de Cotón (1500–1600 m), Fortuna (1100 m), El Calvario de El Copé (continental divide, 850 m), Saladito (approx. 1600 m), La Planada (approx. 1800 m). The dotted lines mark an elevation of 600 m.

Averages are given  $\pm$  the standard error (SE). Unless otherwise indicated, two-tailed Mann–Whitney  $U$  tests were used. The sequential Bonferroni technique (Rice 1989) was used to avoid spurious significance when several statistical tests were made with the same data set. Voucher specimens have been deposited at the Museo de Insectos of the Universidad de Costa Rica and Instituto Nacional de Biodiversidad (INBio), Santo Domingo de Heredia, Costa Rica.

## Results

**Mating Behavior of *P. tarsalis*.** When placed in the dishes, the beetles walked rapidly and tried to escape. Only once did a beetle fly out of the dish. They calmed down after a few minutes, especially if there was an object that provided a hiding place, like a folded piece of paper or leaf, and then walked slowly or remained still until another beetle walked nearby. Same-sex encounters were brief. When two females met they walked away from one other. Sometimes a male grabbed another by the thorax with his mandibles and apparently attempted to copulate; the other male kicked and turned on his side or back and they separated after a few seconds.

When a male encountered a female he attempted to mount her and grab her thorax with his mandibles without any prior courtship. The female often ran away and the

TABLE 1. Comparison of the mating behavior of *Pseudoxychila tarsalis* and *Pseudoxychila bipustulata*

No. of intromissions per copulation	No. of copulations	
	<i>P. tarsalis</i> ( <i>N</i> = 152)	<i>P. bipustulata</i> ( <i>N</i> = 31)
1	16 (11%)	15 (48%)
2	43 (28%)	3 (10%)
3	36 (24%)	1 (3%)
4	22 (14%)	3 (10%)
5	5 (3%)	2 (6%)
6 or more	30 (20%)	7 (23%)

NOTE: The number of intromissions per copulation was significantly different between *P. tarsalis* and *P. bipustulata* ( $G^2 = 29.56$ ,  $df = 5$ ,  $p < 0.001$ ).

male chased her for 2–3 cm. If he did not catch her within a few seconds, he appeared to walk aimlessly until he ran into her again, resumed walking slowly, or became still.

Once a male grabbed a female by the thorax he tried to introduce his aedeagus into her genital opening. Sometimes the female resisted by walking quickly, pushing the male's abdomen upwards with her hind legs, pushing his head backwards with her middle legs, turning sideways or backwards, or pressing the tip of her abdomen to the substrate. Sometimes the female removed the male and the pair separated. The female also struggled after intromission began. When a female struggled the male used his front and middle legs to hold on to the ventral side of her thorax or to hinder her gait; when a female walked quickly he usually raised his front legs and rode on her back.

In 67% of 149 matings the male introduced his aedeagus into the female less than 1 s after mounting her, but sometimes ( $N = 39$ ) there was a delay of  $18 \pm 6$  s (range 6–132 s). Delays were significantly more common when the female struggled at the beginning of copulation (26 of 44 copulations) than when she did not (23 of 105 copulations;  $\chi^2 = 19.43$ ,  $df = 1$ ,  $p < 0.001$ ).

Copulations lasted  $12.9 \pm 1.5$  min (range 0.3–93.3 min,  $N = 153$ ). They consisted of 1–20 intromissions (Table 1), and each intromission lasted about 80 s (Fig. 11). Between intromissions the male withdrew his aedeagus completely from the female. Longer copulations had more intromissions (Fig. 2) and longer pauses between intromissions (Fig. 3) but did not involve longer intromissions ( $r_s = 0.09$ ,  $p > 0.10$ ,  $N = 151$ ).

During intromission the male sometimes remained still or performed up to four different genitalic movements: prying, thrusts, small thrusts, and pulls (Fig. 4; Table 2). Except for prying, individual movements lasted less than 1 s. The movements usually occurred in series (Table 3), except for pulls, which occurred singly in between series of other movements or periods or stillness.

Each prying movement (Fig. 4a) was held for  $14 \pm 1$  s (range 3–29 s,  $N = 40$  movements). Prying seemed to be a big effort to the male, and the force was exerted at least partially by a ventral flexion of his head. In 11 of the 125 copulations in which prying occurred the male's mandibles lost their grasp on the female's thorax during the movement, but the male grabbed the female again and continued the movement. In another case the female walked quickly when the male lost his grasp, his aedeagus came out of the female, and the copulation ended. Intromissions and copulations in which prying occurred ( $114 \pm 6$  s and  $14.4 \pm 1.7$  min, respectively) were significantly longer than intromissions and copulations without prying ( $60 \pm 6$  s and  $4.3 \pm 1.1$  min, respectively;  $p < 0.0001$  in both cases). Prying was the only genitalic movement that occurred

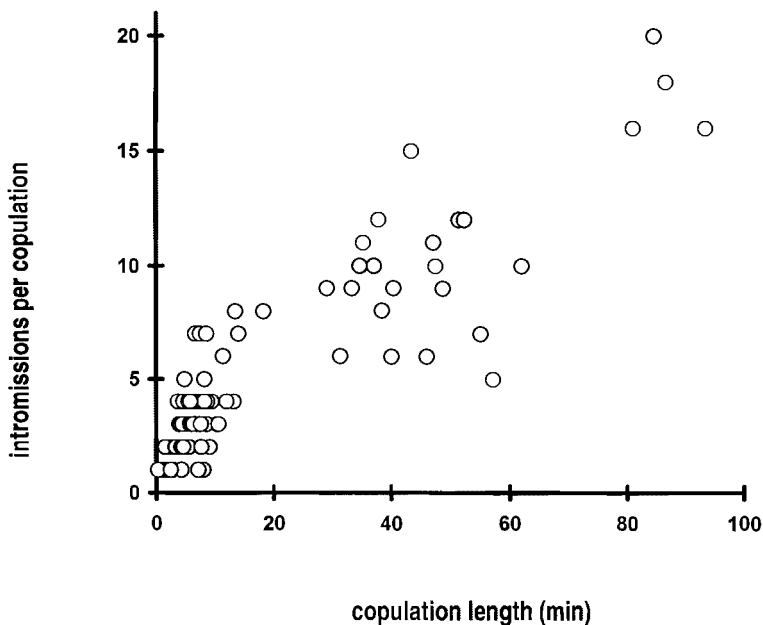


FIG. 2. In *Pseudoxychila tarsalis* the number of intrusions per copulation was significantly correlated with copulation length ( $r_s = 0.84$ ,  $p < 0.001$ ,  $N = 152$ , significant after the Bonferroni correction). The correlation was not linear; a logarithmic transformation changed the standard error of the estimate of the regression from 1.49 to 0.15.

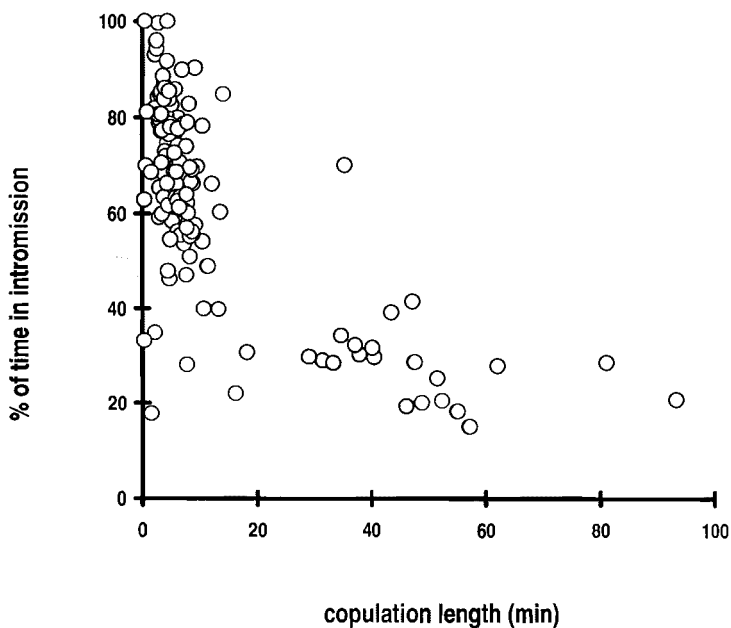


FIG. 3. In *Pseudoxychila tarsalis* the percentage of time spent in intrusion was inversely correlated with copulation length ( $r_s = -0.62$ ,  $p < 0.001$ ,  $N = 146$ , significant after the Bonferroni correction). The correlation was not linear; a logarithmic transformation changed the standard error of the estimate of the regression from 14.82 to 0.14.

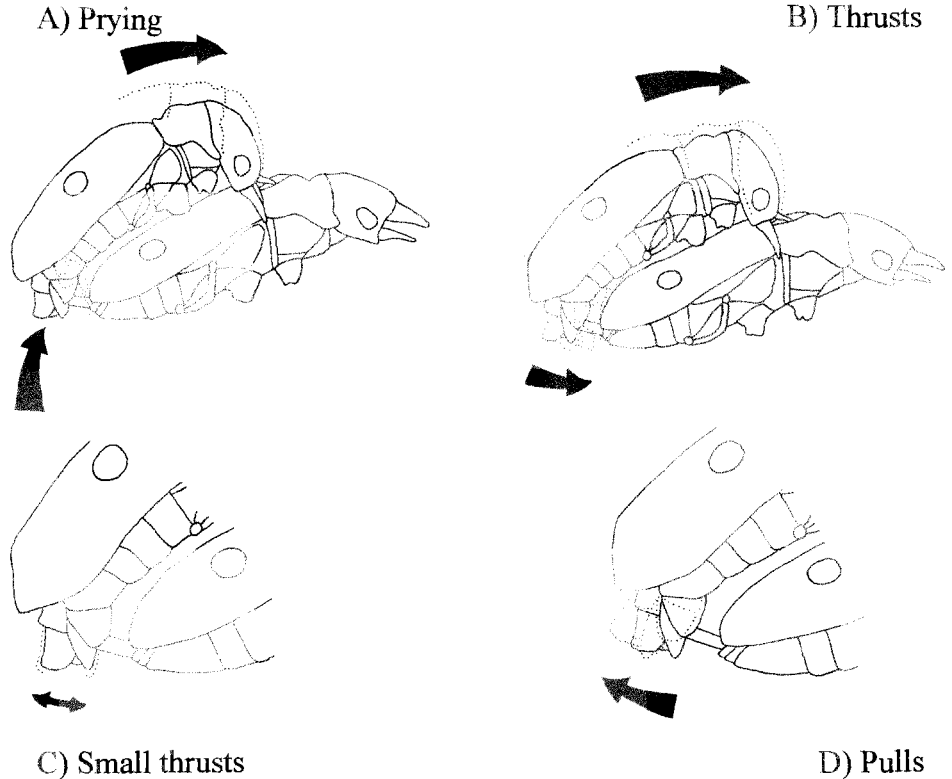


FIG. 4. Schematic drawing of the genitalic movements male *Pseudoxychila tarsalis* performed during intromission. Solid lines show the initial "resting position," and dotted lines the extreme positions of the movements. Drawings were copied from video images. (A) Prying. The aedeagus was introduced deeply into the female and the male's head and body moved forwards and upwards, moving the female's abdomen upwards. Time lapsed between positions = 0.2 s. (B) Thrusts. The aedeagus was introduced deeply into the female and the male's head and body moved forwards but not upwards. Time lapsed = 0.2 s. (C) Small thrusts. The tip of the male's abdomen moved slightly back and forth, and his body and aedeagus remained still. (D) Pulls. The aedeagus was withdrawn almost completely (dotted lines), then introduced deeply (not shown). Time lapsed = 0.3 s.

in intromissions in which spermatophore transfer occurred and did not occur in intromissions in which spermatophore transfer did not occur (Rodríguez S. 1998).

Sometimes a male attempting an intromission seemed unable to penetrate a constriction at the entrance of the female genital opening, withdrew after about 1 s, and tried again. There were at least seven such "failures" in at least five copulations in which there were other intromissions. Some short intromissions (for example, one of 1 s) may also not have fully penetrated the female.

The male's parameres never touched the female during copulation, as occurred in the ground beetle *Pasimachus punctulatus* Haldemann (Alexander 1959).

The male sometimes used his middle legs to rub the female on her posterior sternites, the dorsal, lateral, and posterior sides of her elytra, and her hind legs (Fig. 5; Table 2). One male rubbed with his front legs and another with his hind legs, but both also used their middle legs. Rubbing occurred just before the end of an intromission or between intromissions. Bouts of rubbing lasted  $14 \pm 2$  s (range 3–51 s,  $N = 27$  bouts). Copulations with rubbing ( $17.5 \pm 2.4$  min,  $N = 73$ ) were significantly longer than those without it ( $5.0 \pm 0.5$  min,  $N = 73$ ,  $p < 0.0001$ ). Rubbing was associated with prying: it

TABLE 2. Frequency of *Pseudoxychila tarsalis* and *Pseudoxychila bipustulata* male copulatory movements

Copulatory movement	Frequency of copulations in which movement occurred (%)		Comparison between species*	Proportion of intromissions per copulation in which movement occurred ( <i>P. tarsalis</i> )
	<i>P. bipustulata</i> (N = 34)	<i>P. tarsalis</i> (N = 153)		
Prying	91 (31 of 34)	83 (125 of 152)	$\chi^2 = 1.64, df = 1, p > 0.10, ns$	0.36 ± 0.02
Thrusts	41 (14 of 34)	91 (138 of 152)	$\chi^2 = 45.73, df = 1, p < 0.001$	0.74 ± 0.03
Small thrusts	76 (25 of 33)	93 (141 of 152)	$\chi^2 = 8.50, df = 1, p < 0.05$	0.79 ± 0.02
Pulls	50 (17 of 34)	77 (117 of 152)	$\chi^2 = 10.03, df = 1, p < 0.05$	0.40 ± 0.02
Rubbing	34 (11 of 32)	53 (81 of 152)	$\chi^2 = 3.78, df = 1, p > 0.05, ns$	0.26 ± 0.02

NOTE: The number of copulations in which thrusts, small thrusts, and pulls occurred was significantly different between both species. Each comparison involved different movements, so the Bonferroni technique was not applied.

\*ns, not significant.

TABLE 3. Series of male genitalic movements in *Pseudoxychila tarsalis* and *Pseudoxychila bipustulata*

	No. of movements per series		Series length (s)		
	Prying	Thrusts	Prying	Thrusts	Small thrusts
<i>P. tarsalis</i>	2.4 ± 0.1 (N = 25)	4.2 ± 0.4 (N = 20)	30 ± 3 (N = 20)	7 ± 1 (N = 51)	11 ± 1 (N = 48)
<i>P. bipustulata</i>	2, 3, 7	6.3 ± 1.1 (N = 8)	33, 66, 90	21 ± 9 (N = 8)	41 ± 11 (N = 9)
Comparison between species	—	—	—	p = 0.03	p = 0.0003

NOTE: Sample sizes vary because not all series could be observed equally well.

occurred in 74 of 126 copulations in which prying occurred but only in 5 of 26 copulations in which prying did not occur ( $\chi^2 = 13.47, df = 1, p < 0.001$ ).

In 18% of 144 copulations the male dismounted the female immediately after the last intromission, but in most cases there was a delay of about 30 s between aedeagus withdrawal and dismounting (Fig. 12).

Part of one mating of a pair found in copulation was observed in the field at Moravia. There were 15 intromissions in 59 min and all male genitalic movements observed in captivity occurred. The female walked quickly during the first 30 min and then remained still beneath a rock. When she walked the male rode on her back with his front legs raised. During and after two intromissions she pushed his head with her middle legs and his abdomen with her hind legs. After the three next-to-last intromissions the male dismounted but then grabbed the female again. After the last intromission he dismounted and walked away.

**Spermatophore ejection.** The female ejected one or two spermatophores (Fig. 6) in 35% of 136 copulations. She ejected one spermatophore in 39 copulations (after copulation in 35 cases, between intromissions in four cases). She ejected two spermatophores in eight copulations (both after copulation in one case, both between intromissions in one case, and one between intromissions and the other after copulation in six cases).

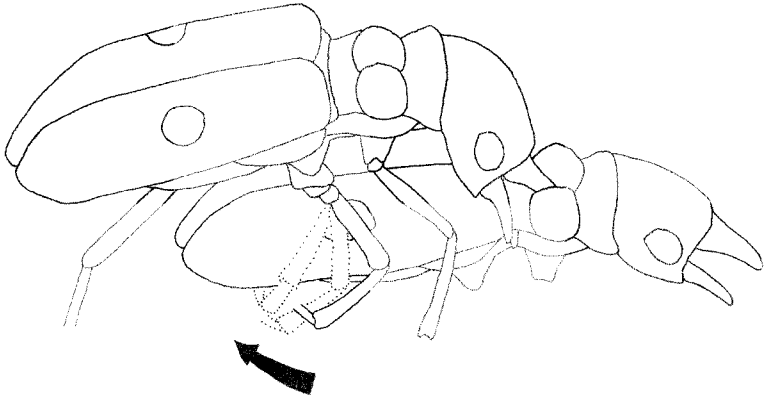


FIG. 5. Schematic drawing of the movement of rubbing the female in *Pseudoxychila tarsalis*. Time lapsed between starting and final positions = 0.06 s. The drawing was copied from video images.

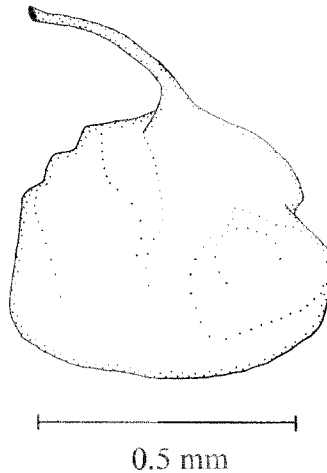


FIG. 6. Schematic drawing of a spermatophore ejected after copulation by a *Pseudoxychila tarsalis* female.

Spermatophore ejection did not occur after the first intromission of copulations or after any of 14 single-intromission copulations. During copulation it often occurred a few seconds after an intromission. After copulation it occurred  $2.2 \pm 0.9$  min (range 0.5–9 min,  $N = 9$ ) after the male dismounted, but females may eject spermatophores up to a few days after copulation (Rodríguez S. 1998).

To eject a spermatophore the female pressed the tip of her abdomen to the substrate and walked forwards. This movement was distinct from oviposition in the field, during which the female stood still, introduced her ovipositor in the chamber she dug, and did not walk (personal observation). All but one of the 47 females that ejected spermatophores left them on the substrate. The other turned around, picked it up with her mouthparts, and presumably ate it.

Most ejected spermatophores had a globular form and were pearly white. Seven of these were checked under the microscope and all were full of live sperm. One white spermatophore was ejected in pieces and four were ejected crumpled and brownish in color (one of those contained only a few sperm).

**Female struggle.** Female struggle during copulation was classified in three categories: (1) female remained still (no struggle, in 43% of all 153 copulations); (2) female

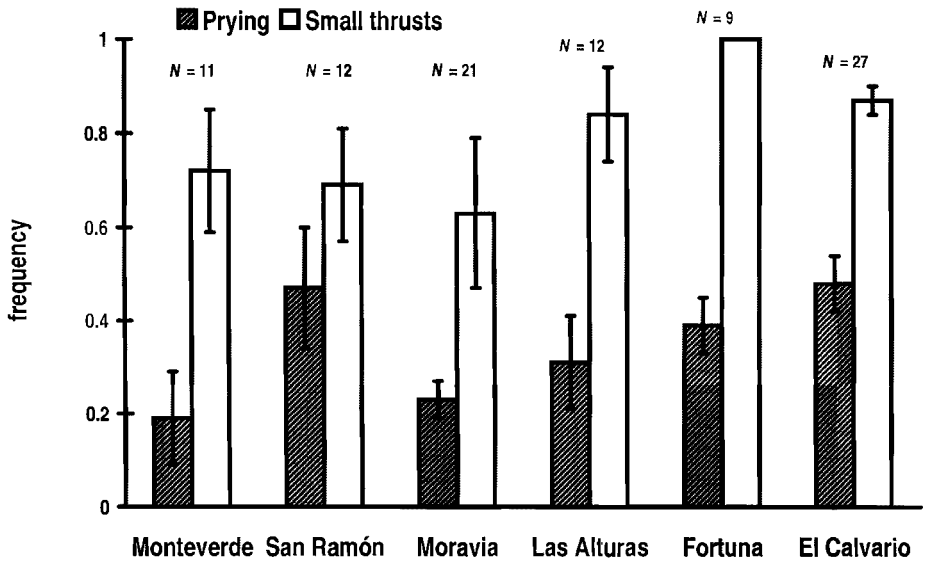


FIG. 7. Geographic variation in the frequency (mean  $\pm$  SE) of prying and small thrusts in *Pseudoxychila tarsalis*. Kruskal-Wallis  $p = 0.017$  and  $0.016$ , respectively. The order of the sites from left to right is from northwest to southeast.

struggled weakly, usually at the beginning (29%); and (3) female struggled and walked quickly during most or all of the copulation (28%).

There were no significant differences when copulation length, the proportion of intromissions in which prying occurred, the proportion of intromissions after which rubbing occurred, and the occurrence of spermatophore ejection were compared between categories of female struggle (in all cases, Kruskal-Wallis  $p > 0.10$ ; for spermatophore ejection  $\chi^2 = 1.37$ ,  $df = 2$ ,  $p > 0.10$ ).

In 37% of 145 copulations the male dismounted when the female was struggling. But the delay between aedeagus withdrawal and dismounting was not significantly associated with female struggle: the female was struggling when the male dismounted in 10 of 24 copulations in which there was a delay and in 42 of 117 copulations in which there was no delay ( $\chi^2 = 0.29$ ,  $df = 1$ ,  $p > 0.10$ ).

**Geographic variation.** Copulations of repeated individuals were excluded from comparisons between sites. There was significant variation between sites in the frequency of prying and small thrusts, copulation length, and the number and length of intromissions per copulation (Figs. 7–10). Different comparisons involved different movements or data sets, so the Bonferroni technique was not applied except for copulation length and the number of intromissions per copulation, which were correlated (Fig. 2). The frequency of thrusts, pulls, and rubbing did not vary significantly between sites (Kruskal-Wallis  $p = 0.09$ ,  $0.75$ , and  $0.18$ , respectively). There also was significant variation between sites in the frequency of spermatophore ejection (Table 4).

**Mating Behavior of *P. bipustulata* and Comparison with *P. tarsalis*.** Copulations of *P. bipustulata* consisted of 1–16 intromissions. Single-intromission copulations were significantly more common in *P. bipustulata* than in *P. tarsalis* (Table 1). Intromission length was more than three times higher in *P. bipustulata* (Fig. 11).

In *P. bipustulata* the male sometimes performed similar genitalic movements as in *P. tarsalis* (prying, thrusts, small thrusts, and pulls). Prying was held for  $19 \pm 5$  s (range

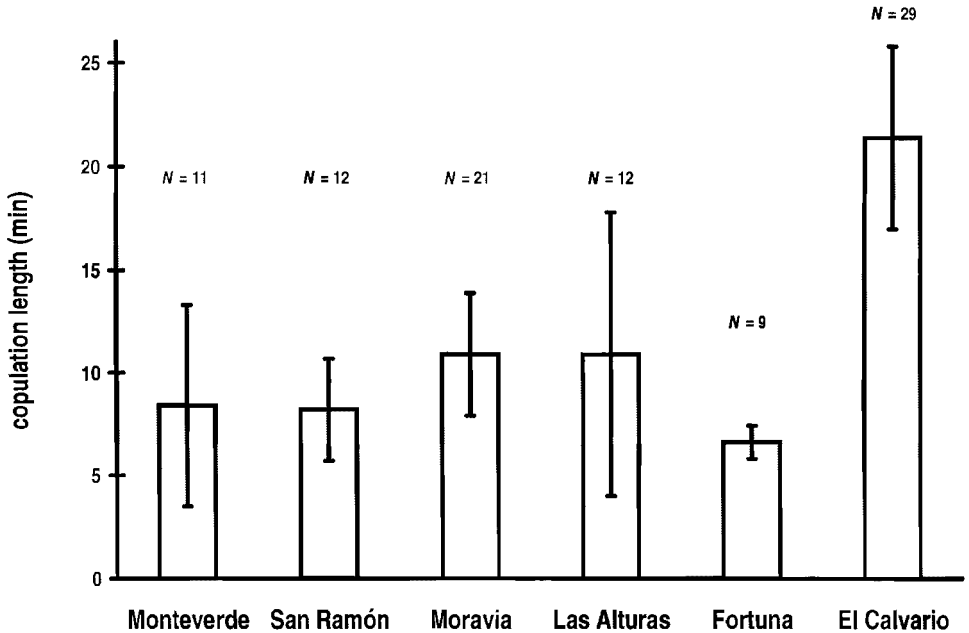


FIG. 8. Geographic variation in copulation length (mean  $\pm$  SE) in *Pseudoxychila tarsalis*. Kruskal-Wallis  $p = 0.0016$ , significant after the Bonferroni correction. The order of the sites from left to right is from northwest to southeast.

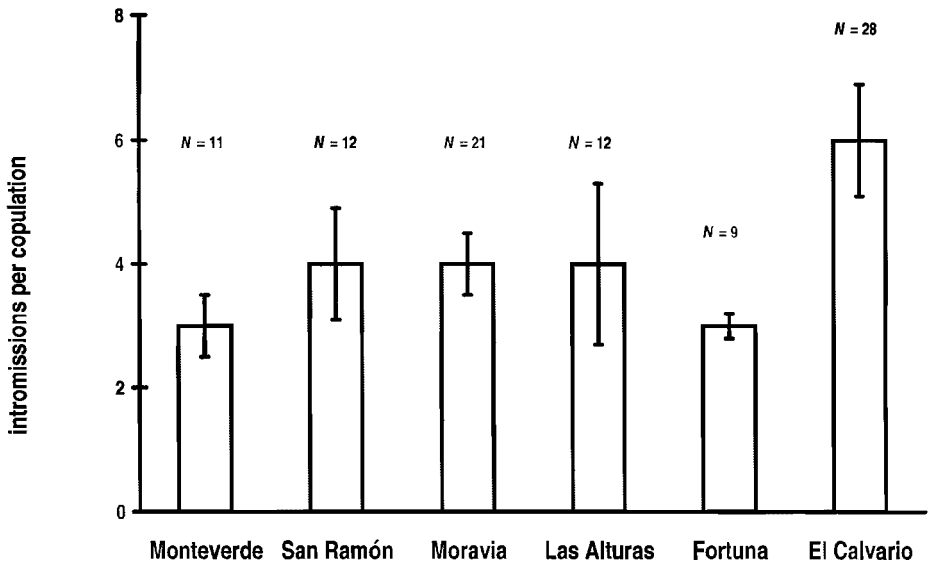


FIG. 9. Geographic variation in the number of intrusions per copulation (mean  $\pm$  SE) in *Pseudoxychila tarsalis*. Kruskal-Wallis  $p = 0.012$ , significant after the Bonferroni correction. The order of the sites from left to right is from northwest to southeast.

3–52 s,  $N = 9$ ). The other movements lasted less than 1 s each and occurred in series (Table 3), except pulls, which occurred singly.

Thrusts, small thrusts, and pulls occurred in significantly more copulations in *P. tarsalis* than in *P. bipustulata* (Table 2). Series of thrusts and small thrusts were significantly longer in *P. bipustulata* (Table 3).

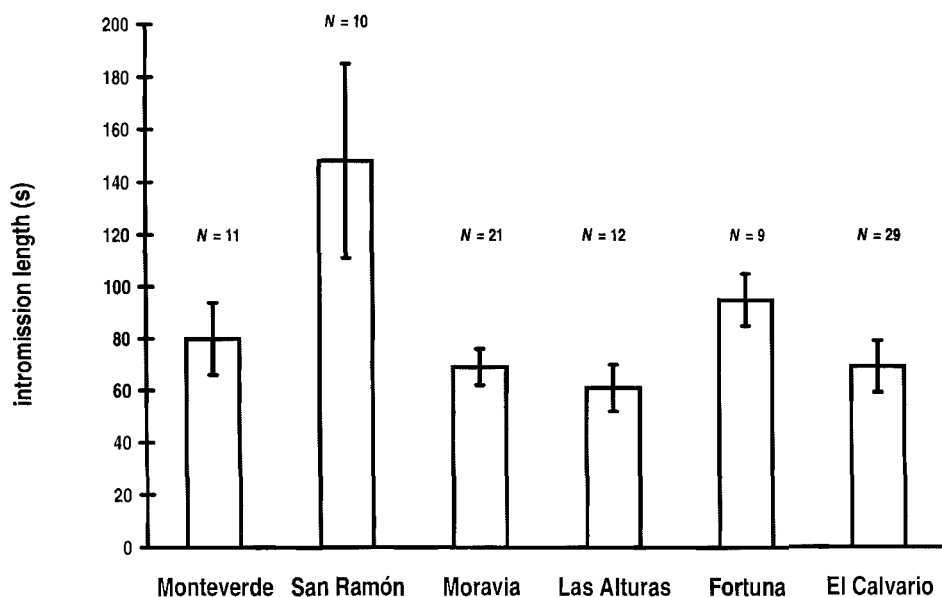


FIG. 10. Geographic variation in the length of intromission (mean  $\pm$  SE) in *Pseudoxychila tarsalis*. For the comparison, averages per copulation were obtained and used as data points. Kruskal-Wallis  $p = 0.05$ . The order of the sites from left to right is from northwest to southeast.

TABLE 4. Geographic variation in the frequency of spermatophore ejection in *Pseudoxychila tarsalis*

	Female ejected	Female did not eject
Costa Rica		
Monteverde	2	7
Moravia	3	24
Las Alturas	0	11
Panama		
Fortuna	1	8
El Calvario	22	7

NOTE: San Ramón was not included because copulations there were observed before noting that females sometimes ejected spermatophores. The frequency of spermatophore ejection did not differ significantly between Monteverde, Moravia, Las Alturas, and Fortuna ( $G^2 = 1.63$ , calculated adding 1 to all categories,  $df = 3$ ,  $p > 0.10$ ), but those sites combined had a significantly lower frequency of spermatophore ejection than El Calvario ( $\chi^2 = 36.73$ ,  $df = 1$ ,  $p < 0.001$ , significant after the Bonferroni correction).

Between intromissions the male sometimes rubbed the female with his middle legs (Table 2). In *P. bipustulata* the male only touched the tip of the female's abdomen, whereas in *P. tarsalis* he rubbed her abdomen, elytra, and hind legs.

In 37% of 30 copulations the male dismounted the female less than 1 s after the last intromission, but in 19 cases there was a delay between aedeagus withdrawal and dismounting. It was more than four times longer than that in *P. tarsalis* (Fig. 12). Another male remained on the female for 90 min after the last intromission until they were separated (the copulation was observed under cold rain and the value was excluded from statistical comparisons). Delays between aedeagus withdrawal and dismounting

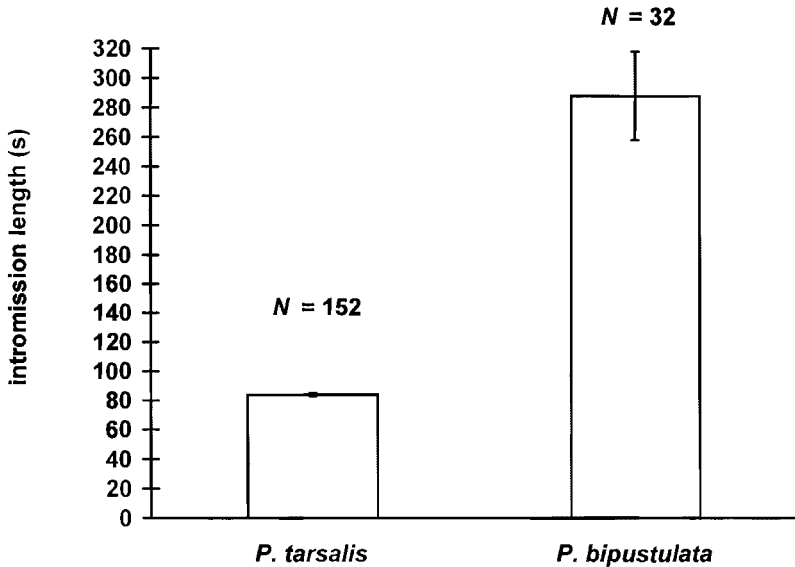


FIG. 11. Intromission length (mean  $\pm$  SE) was significantly different between *Pseudoxychila tarsalis* and *Pseudoxychila bipustulata* ( $p < 0.0001$ ). For the comparison, averages per copulation were obtained and used as data points. For *P. bipustulata* data from Saladito and La Planada were pooled because they were not significantly different ( $p = 0.63$ ).

were more frequent in *P. tarsalis* (delay in 117 of 144 copulations) than in *P. bipustulata* (19 of 30 copulations,  $\chi^2 = 4.67$ ,  $df = 1$ ,  $p < 0.05$ ). They were not associated significantly with female struggle: the female was struggling when the male dismounted in 10 of 16 copulations in which there was a delay and in 6 of 12 copulations in which there was no delay ( $\chi^2 = 0.44$ ,  $df = 1$ ,  $p > 0.10$ ).

The female ejected 1 or 2 spermatophores in 36% of 28 copulations. She ejected one spermatophore in nine copulations (after single-intromission copulations in three cases, after a several-intromission copulation in one case, between intromissions in five cases). She ejected two spermatophores in one copulation, one between intromissions and one after copulation. Eight of the 11 ejected spermatophores had sperm, and in three of the eight the sperm were alive. Five of the 11 spermatophores ejected by *P. bipustulata* females were ejected after the first or single intromission of a copulation.

Spermatophore ejection was not associated with female struggle: the female ejected a spermatophore in eight of 16 copulations in which she was struggling when the male dismounted and in two of 12 copulations in which she was not struggling when he dismounted ( $G^2 = 3.52$ ,  $df = 1$ ,  $p > 0.05$ ).

## Discussion

The description given here of the mating behavior of *P. tarsalis* differs from that given by Palmer (1976a) in several aspects. Three phases of copulation were not apparent, and behavior was much more variable, even within sites, than Palmer's description suggests. There were 1–20 intromissions and four different male genitalic movements. The movements of Palmer's phase 3 ("leg twitching and body shaking," a deep thrust of the aedeagus into the female, and the occasional loss of the grasp of the male's mandibles) might correspond to the prying described here. But thrusts and pulls also involved a "thrust" of the aedeagus, and prying sometimes occurred from the first intromission or not at all. Palmer did not mention spermatophore ejection, and there were no

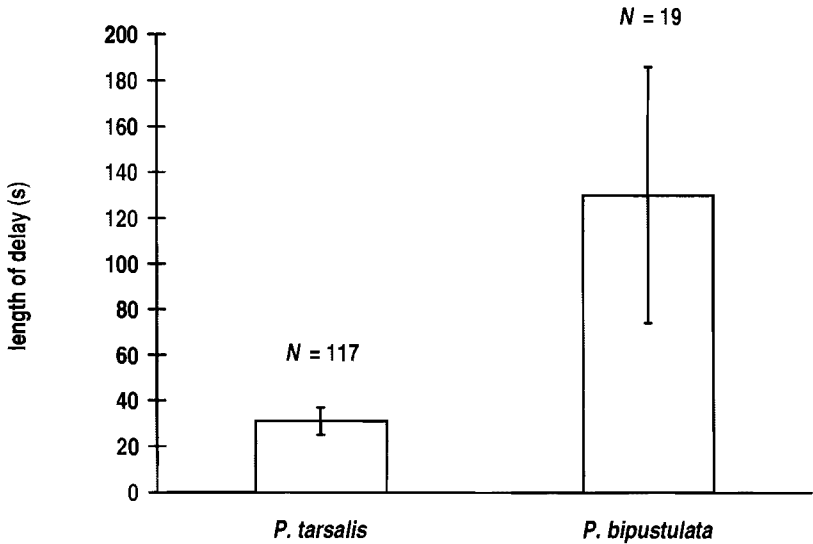


FIG. 12. The length of the delay (mean  $\pm$  SE) between aedeagus withdrawal and dismounting at the end of copulation was significantly different between *Pseudoxychila tarsalis* and *Pseudoxychila bipustulata* ( $p = 0.00002$ ).

three phases when spermatophore transfer was studied in *P. tarsalis* (Rodríguez S. 1998). Thus, the “phases of copulation” of *Pseudoxychila* should not be used to evaluate hypotheses of functional sexual morphology (i.e., Freitag et al. 1980) or phylogeny (i.e., Fielding and Knisley 1995).

There was geographic variation within *P. tarsalis* in the frequency of prying and small thrusts, copulation length, the number and length of intromissions, and the frequency of spermatophore ejection. Different populations varied in different traits. *Pseudoxychila tarsalis* lives at about 600–2000 m elevation in Costa Rica (Palmer 1991), and the collecting sites were along the side of mountain ranges that do not seem to offer physical barriers to the distribution of the beetles (Fig. 1). However, the effective size of a subpopulation can in theory be smaller than that of a continuous population if dispersal capacity is low (Kawata 1995), and there can be divergence within continuous populations (Lande 1982; Wu 1985). The flight of *P. tarsalis* is slow and heavy (Palmer 1976a; personal observation), but they may be dispersed by wind. The present abundance of habitats for the beetles (dirt trails, pastures, and other sites with hard mud walls) is relatively recent, so the collecting sites were probably more isolated or uninhabitable not long ago.

Differences between *P. tarsalis* and *P. bipustulata* were greater than the variation within *P. tarsalis* for the number and length of intromissions; the frequency of thrusts, small thrusts, pulls, and spermatophore ejection; the frequency and length of delays between aedeagus withdrawal and dismounting; and the form of rubbing.

Differences within *P. tarsalis* and between *P. tarsalis* and *P. bipustulata* in male mating behavior are in accord with the hypothesis that copulatory courtship evolves under sexual selection by cryptic female choice (Eberhard 1991, 1994, 1996). There were several contexts in which female choice might occur in *P. tarsalis*, and most occurred after copulation began. Females sometimes prevented copulation or intromission, interrupted copulation, remated, prevented spermatophore transfer, and ejected spermatophores with live sperm (this paper; Rodríguez S. 1998). The latter also occurred in *P. bipustulata*.

The male movement of rubbing the female in *P. tarsalis* and *P. bipustulata* fits the criteria to be considered courtship (Eberhard 1994); it was (1) stereotyped and repeated within and between copulations; (2) appropriate to stimulate the female (some other movements of *P. tarsalis* males were considered incidental and not "designed" to stimulate the female: the maxillary palps of males often vibrated and occasionally contacted the female's thorax; the tarsi of both sexes often vibrated during copulation but did not contact the body of either sex; and the male labrum rubbed the female's thorax during thrusts); and (3) mechanically irrelevant to the male's problems of remaining on the female (rubbing was not observed when the female struggled or walked quickly). Also, rubbing in *P. tarsalis* was associated with prying.

*Pseudoxychila tarsalis* thrusts, small thrusts, and pulls were not directly involved in spermatophore transfer (Rodríguez S. 1998) and fit the criteria used to evaluate male movements as courtship. Some studies of copulation have not considered male genitalic movements within the female as courtship, partly because of lack of data on their function on sperm transfer (Eberhard 1994). But male genitalic movements may function to stimulate the female (Huber and Eberhard 1997).

Another hypothesis that predicts specificity in copulatory courtship is the species-recognition hypothesis, which states that sexual characters that diverge between species allow the sexes to pair with individuals of the correct species (Shapiro and Porter 1989; Andersson 1994). However, the species-recognition hypothesis does not predict intraspecific divergence, such as observed in *P. tarsalis*, and since the ranges of *P. tarsalis* and *P. bipustulata* do not overlap at present (Anonymous 1996) and probably were more restricted in the past, the prediction of interspecific divergence would not apply. But there may be an as yet undescribed species of *Pseudoxychila* in Costa Rica (P. Hanson, personal communication). The species-recognition hypothesis also predicts males should perform pre-copulatory courtship and that copulatory courtship should be concentrated at the beginning of copulation. But there never was any pre-copulatory courtship and the male never rubbed the female until after at least one intromission.

In some tiger beetles the male spends long periods mounted on the female without genital intromission. This has been considered a strategy to prevent female remating (Pearson 1988) in the sense of postinsemination mate guarding (Alcock 1994). For example, males of *Cicindela marutha* Dow spent 98% of copulation after insemination and without genital intromission (Kraus and Lederhouse 1983). Males of *Pseudoxychila* did not appear to defend females in this manner. Periods without intromission were often followed by further intromissions, and spermatophore transfer rarely occurred in the first intromission in *P. tarsalis* (Rodríguez S. 1998). Delays between aedeagus withdrawal and dismounting were short (about 30 s in *P. tarsalis* and 130 s in *P. bipustulata*) in comparison with copulation length (13 min in *P. tarsalis*) or intromission length (288 s in *P. bipustulata*; copulation length was not measured). One female of *P. bipustulata* and several females of *P. tarsalis* were seen ovipositing in the field but never with males present (personal observation). Palmer (1976a) did not mention females of *P. tarsalis* ovipositing with males mounted on them, but did see some males begin copulation with females that were ovipositing. Thus, males of *P. tarsalis* and *P. bipustulata* apparently did not attempt to guard females from other males, even though at least some were probably in control of copulation termination, since female struggle did not seem to affect copulation length.

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## References

- Alcock, J. 1994. Postinsemination associations between males and females in insects: the mate-guarding hypothesis. *Annual Review of Entomology* **39**: 1–21.
- Alexander, R.D. 1959. The courtship and copulation of *Pasimachus punctulatus* Haldemann (Coleoptera: Carabidae). *Annals of the Entomological Society of America* **52**: 485.
- Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ.
- Anonymous. 1996. Checklist of the Western Hemisphere Caraboidea (Coleoptera). Smithsonian Institution Natural History Web, [http://www.nmnhgoph.si.edu/gopher\\_menus/ChecklistoftheWesternHemisphere-CaraboideaColeoptera.html](http://www.nmnhgoph.si.edu/gopher_menus/ChecklistoftheWesternHemisphere-CaraboideaColeoptera.html).
- Cowan, D.P. 1986. Sexual behavior of eumenid wasps (Hymenoptera: Eumenidae). *Proceedings of the Entomological Society of Washington* **88**: 531–541.
- Eberhard, W.G. 1991. Copulatory courtship and cryptic female choice. *Biological Reviews* **66**: 1–31.
- . 1992. Copulatory courtship and notes on the natural history of *Ochtera occidentalis* Clausen (Diptera: Ephydriidae). *The Pan-Pacific Entomologist* **68**: 261–267.
- . 1993a. Copulatory courtship and genital mechanics of three species of *Macrodactylus* (Coleoptera: Scarabaeidae: Melolonthinae). *Ethology, Ecology and Evolution* **5**: 19–63.
- . 1993b. Copulatory courtship and morphology of genitalic coupling in seven *Phyllophaga* species (Coleoptera: Melolonthinae). *Journal of Natural History* **27**: 683–717.
- . 1994. Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. *Evolution* **48**: 711–733.
- . 1996. Female control: sexual selection and cryptic female choice. Princeton University Press, Princeton, NJ.
- Fielding, K., and C.B. Knisley. 1995. Mating behavior in two tiger beetles, *Cicindela dorsalis* and *C. puritana* (Coleoptera: Cicindelidae). *Entomological News* **106**: 61–67.
- Freitag, R., J.E. Olynyk, and B. Barnes. 1980. Mating behaviour and genitalic counterparts in tiger beetles (Carabidae: Cicindelinae). *International Journal of Invertebrate Reproduction* **2**: 131–135.
- Huber, B.A. 1993. Genital mechanics and sexual selection in the spider *Nesticus cellulanus* (Araneae: Nesticidae). *Canadian Journal of Zoology* **71**: 2437–2447.
- Huber, B.A., and W.G. Eberhard. 1997. Courtship, copulation, and genital mechanics in *Physocyclus globosus* (Araneae, Pholcidae). *Canadian Journal of Zoology* **74**: 905–918.
- Hunter, M.S., M.F. Antolin, and M. Rose. 1996. Courtship behavior, reproductive relationships, and allozyme patterns of three North American populations of *Ereimocerus* nr. *californicus* (Hymenoptera: Aphelinidae) parasitizing the whitefly *Bemisia* sp., *tabaci* complex (Homoptera: Aleyrodidae). *Proceedings of the Entomological Society of Washington* **98**: 126–137.
- Jaenson, T.G.T. 1979. Mating behaviour of males of *Glossina pallidipes* Austen (Diptera: Glossinidae). *Bulletin of Entomological Research* **69**: 573–588.
- Kawata, M. 1995. Effective population size in a continuously distributed population. *Evolution* **49**: 1046–1054.
- Kraus, B., and R.C. Lederhouse. 1983. Contact guarding during courtship in the tiger beetle *Cicindela marutha* Dow (Coleoptera: Cicindelidae). *The American Midland Naturalist* **110**: 208–211.
- Lachmann, A. 1997. Sperm transfer during copulation in five *Coproica* species (Diptera: Sphaeroceridae). *European Journal of Entomology* **94**: 271–286.
- Lande, R. 1982. Rapid origin of sexual isolation and character divergence in a cline. *Evolution* **36**: 213–223.
- Otronen, M. 1997. Sperm numbers, their storage and usage in the fly *Dryomyza anilis*. *Proceedings of the Royal Society of London Series B* **264**: 777–782.

- Otronen, M., and M.T. Siva-Jothy. 1991. The effect of postcopulatory male behavior on ejaculate distribution within the female sperm storage organs in the fly *Dryomyza anilis*. *Behavioural Ecology and Sociobiology* **29**: 33–37.
- Palmer, M.K. 1976a. Natural history and behavior of *Pseudoxychila tarsalis* Bates. *Cicindela* **8**: 61–92.
- . 1976b. Notes on the biology of *Pterombrus piceus* Krombein (Hymenoptera: Typhiidae). *Proceedings of the Entomological Society of Washington* **78**: 369–375.
- . 1982. Biology and behavior of two species of *Anthrax* (Diptera: Bombyliidae), parasitoids of the larvae of tiger beetles (Coleoptera: Cicindelidae). *Annals of the Entomological Society of America* **75**: 61–70.
- . 1991. *Pseudoxychila tarsalis* (Abejón tigre, Tiger Beetle). pp. 779–780 in Janzen, D.H. (Ed.), *Historia Natural de Costa Rica*. Universidad de Costa Rica, San José, Costa Rica.
- Pearson, D.L. 1988. Biology of tiger beetles. *Annual Review of Entomology* **33**: 123–147.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- Rodríguez, V. 1994. Sexual behavior in *Omaspides convexicollis* Spaeth and *O. bistriata* Boheman (Coleoptera: Chrysomelidae: Cassidinae), with notes on maternal care of eggs and young. *The Coleopterists Bulletin* **48**: 140–144.
- Rodríguez S, R.L. 1998. Spermatophore transfer and ejection in the beetle *Pseudoxychila tarsalis* (Coleoptera: Cicindelidae). *Journal of the Kansas Entomological Society*. In press.
- Rodríguez S, R.L., and W.G. Eberhard. 1994. Courtship before and during copulation in two species of *Xyonyssius* bugs (Hemiptera, Lygaeidae). *Journal of the Kansas Entomological Society* **67**: 37–45.
- Shapiro, A.M., and A.H. Porter. 1989. The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. *Annual Review of Entomology* **34**: 231–245.
- Simpson, K.W. 1975. Biology and immature stages of three species of nearctic *Ochtera* (Diptera: Ephydriidae). *Proceedings of the Entomological Society of Washington* **77**: 129–155.
- Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *The American Naturalist* **122**: 765–788.
- Wcislo, W.T., and S.L. Buchmann. 1995. Mating behaviour in the bees, *Dieunomia heteropoda* and *Nomia tetrazonata*, with a review of courtship in Nominae (Hymenoptera: Halictidae). *Journal of Natural History* **29**: 1015–1027.
- Wcislo, W.T., R.L. Minkley, and H.C. Spangler. 1992. Precopulatory courtship behavior in a solitary sweat bee *Nomia triangulifera* (Hymenoptera: Halictidae). *Apidologie* **23**: 431–442.
- Wu, C.-I. 1985. A stochastic simulation study on speciation by sexual selection. *Evolution* **39**: 66–82.

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