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PROLONGED COPULATION IN *PHOTINUS MACDERMOTTI* WITH COMPARATIVE NOTES ON *PHOTINUS COLLUSTRANS* (COLEOPTERA: LAMPYRIDAE)

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ABSTRACT

Photinus macdermotti Lloyd fireflies engage in copulations that are prolonged in comparison to those of *Photinus collustrans* LeConte. Coupling is prolonged to accommodate protracted ejaculate transfer and as a probable male adaptation to reduce sperm competition.

RESUMEN

Las copulaciones de la luciérnaga *Photinus macdermotti* Lloyd, son bastante prolongadas comparadas con las de la *Photinus collustrans* LeConte. Apareamiento es prolongado para acomodar la transferencia de la esperma y como una probable adaptación masculina para reducir la competencia de esperma.

INTRODUCTION

Previous studies of *Photinus macdermotti* Lloyd fireflies have mostly focused on flash communications (Lloyd 1966, 1979, 1981, 1984). This study begins at the behavioral point where those ended: with pair formation. Characteristics of *macdermotti* copulations are here documented and compared with those of *Photinus collustrans* LeConte matings. *P. collustrans* provide a base-line case for comparative studies of firefly sex, because almost all females are monogamous, and males typically invest minimal time and material in mating (Wing 1982, 1984). By contrast, *macdermotti* copulations are shown here to be prolonged. Explanations for this extended coupling are sought through studies of other *macdermotti* characteristics, including reproductive structures, the nature of the ejaculate, and female multiple mating.

METHODS AND MATERIALS

Field studies of *macdermotti* were conducted during 1980-1984 in a blackberry (*Rubus* sp.) thicket north of the Gainesville Regional Airport in Alachua County, Florida. *P. collustrans* were studied in a field nearby. Females were located by their responses to male signals or to male-like penlight flashes. Some females were collected in numbered vials; others were left undisturbed and allowed to attract males and mate. Some insects were individually marked with a Pentel® White Marker (Wineriter and Walker 1984). One mating pair was marked with Radiant® florescent pigment puffed on with a powder insufflator.

P. macdermotti copulations were timed from the start of the end-to-end position in which male and female face opposite directions while genitally connected. Due to the extended duration of some copulations I observed pair formation at night and returned to the site before dawn to watch the pair separate.[1] (Superscripts refer to appendix.) Males and females of 3 such pairs were marked, showing that the pairs to which I returned involved the same insects I had observed to initiate copulation. Other copulating pairs were collected in vials. Four pairs, including 1 in the field, were observed at 1-h intervals throughout the night to detect the occurrence of mating 'bouts'.

For dissection, the wings were removed from a freshly killed specimen. In a pool of saline, the tergites were removed after cutting along the pleural membrane. Sperm assays on females consisted of surgically removing the spermatheca, placing it on a glass slide in a drop of saline, and opening it with pins. Its contents were then examined under a compound microscope, and subsequently stained with Geimsa for storage. For sectioning, 3 copulating *macdermotti* pairs were frozen. Specimens were fixed in paraffin, sectioned with a microtome (10 microns), and stained with Delafield's hematoxylin and eosin.

RESULTS AND DISCUSSION

Prolonged Copulation

Formation of *macdermotti* (and *collustrans*) pairs is typical of the genus, as observed by Lloyd (1966). A flying male locates a perched female by her continued responses to his signals, lands, and reaches her on foot. (*P. macdermotti* females perch on vegetation, whereas *collustrans* females, which are flightless and live in burrows, perch on or near the ground.

Upon reaching the female, the *macdermotti* male climbs upon her dorsum and intromission occurs.[2] About 1 minute after mounting, the male turns end-to-end with the female, and the remainder of copulation occurs in this position.

Copulation durations of 20 *macdermotti* pairs were recorded; 13 in the field and 7 mating pairs were collected and observed in vials. Coupling durations in the field ranged from 1 to 8 h (Table 1). Similarly, 'vial' copulations lasted from 1 to 9 h (Table 1). Intermittent observations (4 pairs) detected no evidence of mating 'bouts' (multiple copulations by the same pair during 1 night).

As compared to the typical *collustrans* coupling duration, which approximates the minimum time required for successful sperm transfer in fireflies, *macdermotti* matings are prolonged. The typical *collustrans* mating lasts about 1 minute (Wing 1984), whereas *macdermotti* copulations lasted from 60 to 540 times longer (1 to 9 h).[3]

Evidence, from histological sections of coupled pairs, indicates that the *macdermotti* male can control the duration of the copulation.[4] The mechanism of this control is the aedeagus. The female abdominal tip is gripped between the inserted median lobe and the 2 lateral lobes, which remain external to the female (Fig. 1).[5] The *collustrans* aedeagus is of similar structure (Green 1956), and it apparently grips the female the

TABLE 1. *Photinus macdermotti* COPULATION DURATIONS. 'VIAL' MATINGS STARTED IN THE FIELD. ALL MATINGS (EXCEPT #14 AND 15 IN MAY) OCCURRED IN JUNE, WHEN SUNRISE WAS AT ABOUT 6:30 A.M.

Mating number	Location	Time start	Time stop	Duration (hours)
1	field	5:19 A.M.	6:22 A.M.	1.0
2	″	10:42 P.M.	6:15 ″ ″	7.5
3	″	10:59 ″ ″	6:22 ″ ″	7.5
4	″	10:43 ″ ″	6:14 ″ ″	7.5
5	″	10:08 ″ ″	6:18 ″ ″	8.0
6	″	10:21 ″ ″	4:27 ″ ″	6.0
7	″	10:52 ″ ″	6:10 ″ ″	7.5
8	″	10:43 ″ ″	12:01 ″ ″	1.5
9	″	11:05 ″ ″	6:22 ″ ″	7.5
10	″	10:48 ″ ″	6:16 ″ ″	7.5
11	″	10:33 ″ ″	6:00 ″ ″	7.5
12	″	9:25 ″ ″	11:00 P.M.	1.5
13	″	10:00 ″ ″	11:25 ″ ″	1.5
14	vial	8:32 ″ ″	9:41 ″ ″	1.0
15	″	9:01 ″ ″	10:36 ″ ″	1.5
16	″	9:58 ″ ″	11:04 ″ ″	1.0
17	″	11:00 ″ ″	8:12 A.M.	9.0
18	″	11:33 ″ ″	2:00 ″ ″	2.5
19	″	10:17 ″ ″	7:30 ″ ″	9.0
20	″	11:20 ″ ″	12:20 ″ ″	1.0

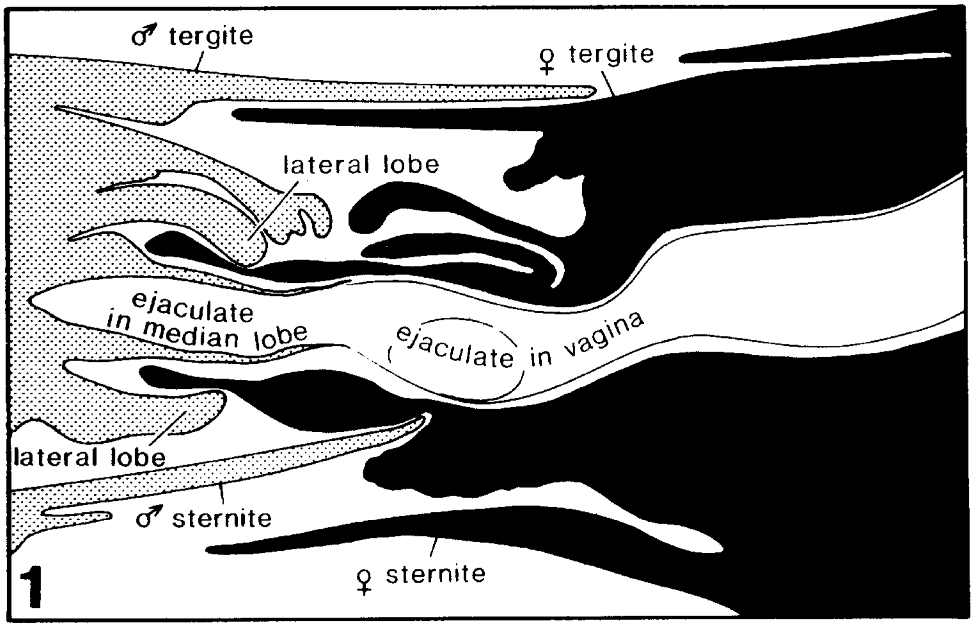

Fig. 1. *Photinus macdermotti* coupling structures: A schematic drawing of a logitudinal section from a pair copulating in the end-to-end position. The region shown is from the posterior abdominal segments of the male (facing left) and female (facing right). The aedeagus is comprised of the median and lateral lobes.

same way. In *Photinus* the grip probably secures the connection with the female during sperm transfer and secondarily allows male control of copulation duration. The strength of the hold is indicated by observations of *macdermotti* males hanging unsupported except by the genital connection, and of *collustrans* females dragging coupled males backwards to and into their burrows (Wing 1984).

Why Are *macdermotti* Copulations Prolonged?

1. Sequential transfer of male products

One reason *macdermotti* copulations last longer than those of *collustrans* is that *macdermotti* ejaculates take longer to transfer. Five *macdermotti* pairs were interrupted after 20-30 minutes of copulation. Three of the 5 pairs (interrupted by freezing) were sectioned, and sperm assays were performed on the females of the other 2 pairs (interrupted by separation). In none of the 5 cases had sperm transfer been started in the 20-30 minutes of copulation. By contrast, *collustrans* commonly complete sperm transfer within 1 minute.

A clue as to why *macdermotti* sperm transfer takes longer than that of its congener *collustrans*, is provided by the male accessory gland structures of the 2 species (Fig. 2 and 3). While *collustrans* males apparently possess few accessory glands (perhaps 1 small pair), *macdermotti* males are endowed with at least 4 prominent pairs.[6] This difference suggests that *macdermotti* males transfer additional substances in the ejaculate, which is confirmed by other evidence. Sections of each of 3 coupled *macdermotti* pairs (mentioned above) show that a secretion from the spiral-shaped male accessory glands was being transferred after about 25 min of copulation, and that sperm had not yet been passed. Thus *macdermotti* males transfer something other than sperm during the first 0.5 hour of copulation. Sperm assays of mated females of the 2 species also seem to indicate that *macdermotti* males transfer additional materials. Spermathecae of mated *collustrans* females appear to contain only semen, while those of mated *macdermotti* females often also contain a gelatinous substance.[7]

These observations, then, indicate that *macdermotti* mating involves sequential transfer of materials, which include at least the secretion from the spiral glands and sperm, and possibly other glandular products as well. The duration of *macdermotti* copulations is prolonged to accommodate the transfer of this ejaculate.[8] As noted above, the durations of timed *macdermotti* matings ranged down to 1 h. This suggests that insemination requires about 1 h to complete. Further prolongation of mating by *macdermotti* males probably serves a different function.

2. Mate guarding

Prolonged copulation enforced by *macdermotti* males may prevent females from re-mating that night. Such a mate-guarding model presumes female multiple mating, and several lines of evidence suggest that *macdermotti* females do mate repeatedly.

Mated and virgin females are found in the same places in the field where courting males search. Thus *macdermotti* females have the opportunity to mate repeatedly. This is not true of *collustrans* females, which typically mate only once.[9] Usually only unmated *collustrans* females are found in the area where mating occurs (Wing 1984).

Mated and virgin *macdermotti* females respond to male signals and male-like penlight signals in the field. Of 9 females that repeatedly answered such signals in the field, 6 were virgins and 3 had already mated. Of 10 *macdermotti* females all, except 1 that died the day after testing, answered male-like signals in the laboratory. Of the 9 responsive females, 6 were mated and 3 were virgins (Wing 1982). Responses by *macdermotti* females are only known to occur in the context of mate attraction, and mated, responsive

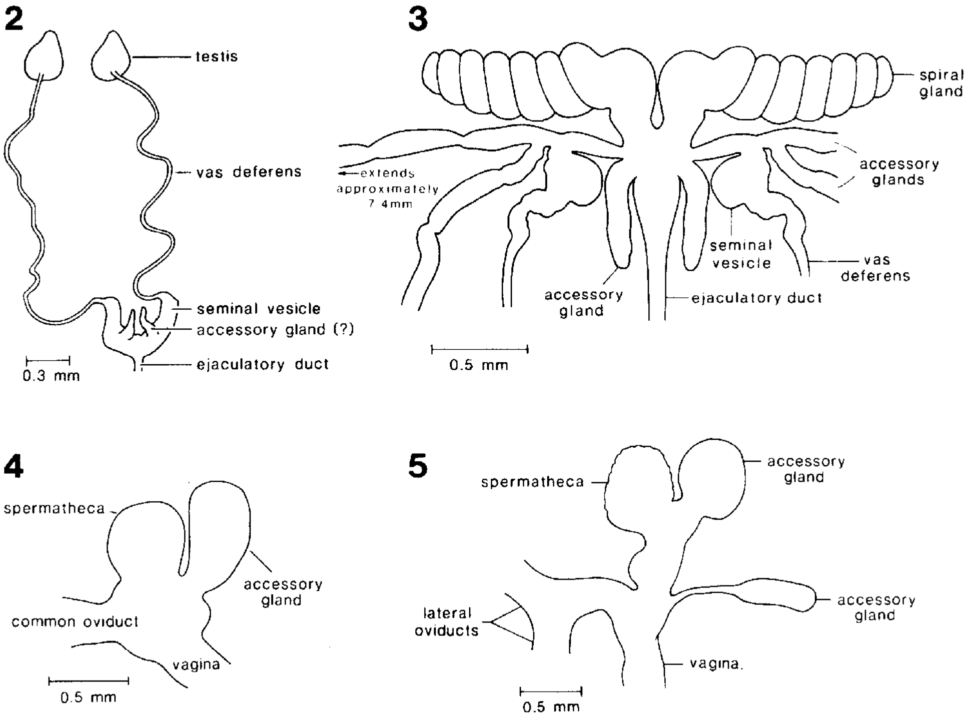

Fig. 2-5. 2. *Photinus collustrans* male reproductive structures. 3. *Photinus macdermotti* male reproductive structures with glands spread. 4. *Photinus collustrans* female reproductive structures. 5. *Photinus macdermotti* female reproductive structures.

females did attract males in the field. Sperm were found in the spermathecae of 2 responsive females that had attracted males in the field, but that were collected before copulation could begin.

Lastly, *macdermotti* females were marked so that they could be identified as individuals in the field. Despite difficulty in relocating marked females in the blackberry thicket on subsequent nights, I observed 1 female to mate on 3 successive nights. (The first 2 matings were with the same marked male and lasted until dawn; the 3rd broke up before dawn.)

Mated *macdermotti* females, then, were found to occur in places where males search, to respond to male-like signals, and to attract males. Furthermore, repeated mating by a *macdermotti* female in the field was observed. Thus I believe that multiple mating by *macdermotti* females does occur.

Although it is not known exactly how long *macdermotti* ejaculate transfer takes, it appears that some males complete it in about 1 hour.[10] The time spent in copula by *macdermotti* pairs after complete insemination is probably a male tactic that prevents other males from gaining access to the female, as is the case when *collustrans* copulations are prolonged (Wing 1984; also see Parker 1970).[11] In some cases *macdermotti* males occupy females until the male flight period for that night is over. Depening on temperature, the courtship flights of males may last until dawn. Males flew until dawn during the period when copulations were observed in the field, and 9 of the 13 matings observed lasted until dawn (Table 1).[12] Why some copulations end earlier is not yet known, but may be related to an early termination of male activity or to previous mating by the female.

CONCLUSION

P. macdermotti and *collustrans* differ remarkably in reproductive characteristics including copulation duration and male accessory gland structures. These differences appear to be related to the difference in likelihood of multiple mating by females of the 2 species.

Due in part to the preponderance of mating only once among *collustrans* females, *collustrans* male reproductive success is probably limited mainly by the number of females they can locate and inseminate. Thus the ability to complete sperm transfer rapidly is advantageous.[13] Typically, *collustrans* copulations occur totaly in the male-above position, thus saving the time that would be required to turn end-to-end. Furthermore, the ejaculate is apparently devoid of all but the constituents essential for successful fertilization. These attributes allow a *collustrans* male to mate quickly and return to searching for females.

By contrast, the reproductive success of a *macdermotti* male probably depends on more than insemination of females because sperm of other males may compete with his own for fertilizations. Prolonged copulation may reduce this sperm competition by restricting access to the female. However, the role of the additional component(s) of *macdermotti* ejaculate remains an enigma.

APPENDIX

[1] Durations could not be determined for several pairs that were mating when I left but that terminated copulation before my return, nor for any matings that were initiated during my absence. For this reason, copulations of intermediate duration may be underrepresented in the data reported here.

[2] Extensive observations of this male-above phase of *Photinus* copulation, along with other studies including some similar to those reported here, have been pursued by Sara M. Lewis, Harvard University. Her studies were independent of and simultaneous with this study.

[3] *P. collustrans* copulations typically occur entirely in the male-above position, but when rival males disturb the mating pair coupling is prolonged until the female re-enters her burrow. The longest recorded mating lasted 24 minutes. In such cases copulation proceeds in an end-to-end position (Wing 1984).

[4] Female musculature around the region of the vagina, where the tip of the inserted aedeagus is located during copulation, may help to seal the connection. It appears unlikely that this mechanism would allow a female to hold a male against his will and thereby prolong coupling.

[5] In the area of the aedeagus where the female terminalia are squeezed, tooth-like projections (visible with scanning electron or light microscopy) may give the grip more 'bite' (Wing 1982 and unpublished). For a different type of female-holding by male fireflies see Wing et al. (1983).

[6] Female *macdermotti* have an additional accessory gland as compared to *collustrans* females (Fig. 4 and 5). This may be related to additional *macdermotti* male secretions.

[7] Sperm are found imbedded in this material, suggesting an intriguing possibility. Males may inject a substance that solidifies in the spermatheca, embedding and immobilizing any sperm left there by previous males, and then transfer their own sperm. For other possible functions see Leopold (1976) and Wing (1982).

[8] Prolonged copulation is associated with numerous male accessory glands and complex ejaculates in some other insects also (Gerber et al. 1971). However, rodent species with longer copulation durations have reduced complements of accessory glands (Dewsbury 1985).

[9] Of 108 *collustrans* females whose complete sexual histories were recorded, only 4 mated more than once (Wing 1984).

[10] Sperm assays were performed on 3 females from pairs that copulated about 1 hour. One of the 3 pairs was separated after 1 hour of mating, and the other 2 pairs separated spontaneously. All 3 females had sperm in their spermathecae. But this is not conclusive evidence that sperm are transferred within 1 hour because the sperm might have come from a previous mating.

[11] Prolonged copulation by insects has also evolved in contexts other than sperm competition (see Sivinski 1983 and Thornhill 1984). Although not detected in this study, there may be additional or alternative causes of prolonged mating in *macdermotti*.

[12] Even longer firefly copulations have been reported (Lloyd 1972, Buschman 1984). These continue past dawn and into or throughout the daylight hours. Buschman found that the mean duration for *Pyractomena lucifera* copulation was 33 hours, and he concluded that mated females are unlikely to mate a 2nd time. The adaptive significance of prolonged copulation in this case is not clear, raising further questions about the activities of these fireflies.

[13] Male *collustrans* have a lifespan of about 6 days (Buschman 1977), and the nightly sexual activity period lasts about 20 minutes (Lloyd 1966). During a *collustrans* male's lifetime the total time available in which to locate females is on the order of 2 hours. According to Lloyd's (1979) study, this is approximately the same amount of time required (on the average) for a male to locate a female. A male that expends unnecessary time during a 1st mating reduces his already limited chance to locate a 2nd female.

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THE FORM AND FUNCTION OF ACOUSTIC COURTSHIP SIGNALS OF THE PAPAYA FRUIT FLY, *TOXOTRYPANA CURVICAUDA* (TEPHRITIDAE)

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ABSTRACT

The male papaya fruit fly, *Toxotrypana curvicauda* Gerstäcker, makes two acoustic signals during courtship: (1) the approach song consists of one to five pulse trains (sound bursts) and is directed at females prior to mounting; (2) the precopulatory song consists of one to seven pulse trains produced immediately after mounting the female. Unlike the somewhat similar "calling song" of the Caribbean fruit fly, the papaya fruit fly's approach song is monotonic, a character which may be due to an absence of selection for escaping vegetation-filtering. Virgin females, but not males, become less active in the presence of broadcast approach sounds, suggesting that the proximate effect of the song is to depress locomotion in the female. Large males pair more often than small and the sound pressure level of large male approach songs is louder. Females may detect the size/vigour of a singer by his song, and such sounds may have evolved as sexually selected male advertisements rather than a means of species isolation.

RESUMEN

El macho de la mosca de la papaya, *Toxotrypana curvicauda* Gerstacker, hace dos señales acústicas durante el cortejo: (1) el canto de acercamiento consiste de una a cinco pulsaciones (estallidos de sonido) y es dirijido a las hembras antes de montarlas; (2) el canto pre-copulatorio consiste de una a siete pulsaciones producidas inmediatamente