

COPULATION AND OVIPOSITION BEHAVIOUR OF *PROTOPIOPHILA LITIGATA* (DIPTERA: PIOPHILIDAE)

RUSSELL BONDURIANSKY and RONALD J. BROOKS

Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Abstract

The Canadian Entomologist **130**: 399 – 405 (1998)

Using field and laboratory (stereoscope) observations, we investigated copulation and oviposition behaviours of *Protopiophila litigata* Bonduriansky, which mates and oviposits exclusively on discarded cervid antlers. Typically, a male leapt onto a female, briefly tapped the sides of her abdomen with his legs, then stimulated her abdominal tip with his tarsi, parameres, and gonopods until she extended her genitalia (~4 min), after which the pair established genital lock (~10 min). Meanwhile, the female emigrated to a part of the antler where density of single males was lower. Following sperm transfer (~87 min) and genital separation (~5 min), the female deposited and ingested two droplets containing spermatozoa (~6 min). Then she oviposited (~29 min) into cracks or pores in the antler's surface while the male remained in tandem and pushed away single males with his wings. Males often wrestled for possession of females, but take-overs were rare. Whereas most aspects of the copulation-oviposition cycle of *P. litigata* are characteristic of other necrophagous or scatophagous flies, the manner in which *P. litigata* females expel and ingest ejaculate materials after copulation has not been reported in any other species of Diptera.

Bonduriansky, R., et R.J. Brooks. 1998. Comportements d'accouplement et de ponte chez *Protopiophila litigata* (Diptera : Piophilidae). *The Canadian Entomologist* **130** : 399-405.

Résumé

Des observations sur le terrain et en laboratoire (à la loupe binoculaire) nous ont permis d'étudier les comportements d'accouplement et de ponte chez *Protopiophila litigata* Bonduriansky, qui s'accouple et pond exclusivement sur les bois rejetés des cervidés. De façon caractéristique, le mâle saute sur la femelle, frappe brièvement les côtés de son abdomen avec ses pattes, stimule ensuite le bout de son abdomen avec ses tarsi, ses paramères et ses gonopodes jusqu'à ce qu'elle déploie ses genitalia (~4 min), après quoi leurs genitalia s'unissent (~10 min). Pendant ce temps la femelle émigre vers la partie des bois où la densité des mâles non accouplés est plus faible. Après transfert du sperme (~87 min) et séparation des genitalia (~5 min), la femelle émet et ingère deux gouttelettes contenant des spermatozoïdes (~6 min). Ensuite, elle pond ses oeufs (~29 min) dans des crevasses ou des pores à la surface des bois, alors que le mâle reste sur elle en tandem et repousse les autres mâles avec ses ailes. Les autres mâles se battent parfois pour prendre possession de la femelle, mais réussissent rarement. Bien que la plupart des aspects du cycle accouplement-ponte de *P. litigata* soient caractéristiques des autres diptères nécrophages ou scatophages, l'expulsion et l'ingestion, après l'accouplement, de substances éjaculées par le mâle n'ont jamais été signalées chez d'autres espèces de diptères.

[Traduit par la Rédaction]

Introduction

Behaviours involved in courtship, copulation, and oviposition are sources of tremendous interspecific variability and may be important factors in sexual selection. Although these behaviours have been described in detail for several species of *Drosophila*

(e.g., Spieth 1952, 1984; Steele 1986), and a few other taxa (e.g., Otronen 1984), such knowledge remains sparsely distributed among species of Diptera. Next to nothing has been published on the copulation behaviour of any species of Piophilidae, except for the synanthropic *Piophila casei* Linné (e.g., Jones et al. 1971).

The antler fly *Protopiophila litigata* Bonduriansky (1995) is a small-bodied (1.6–3.1 mm body length) necrophagous acalyptrate that mates and oviposits exclusively on discarded cervid antlers. Single males form dense aggregations on flat, central parts of the upward-facing surfaces of antlers, where some of them defend territories, whereas copulating pairs tend to aggregate on the undersides or peripheries of antlers, where single males rarely defend territories (Bonduriansky 1996). If an antler is flipped over, the flies relocate so as to maintain this pattern. Females visit antlers to feed and mate and, after each copulation, oviposit into cracks or pores in the antler. Final-instar larvae leave the porous interior of the antler and pupate in the leaf litter (Bonduriansky 1995).

Our objective was to describe and time the behaviours involved in the copulation–oviposition cycle of *P. litigata*, between initial male–female contact and final separation. We were particularly interested in behaviours associated with mate choice (e.g., Thornhill 1983; Otronen 1984) and male “nonpromiscuous mating effort” (Gwynne 1984), such as mate guarding (e.g., Parker 1970a) or nutritive gift transfer (e.g., Steele 1986). We also wanted to compare the behaviours of *P. litigata* to those of other Diptera.

Materials and Methods

We collected six discarded moose antlers near the Wildlife Research Station (45°30', 78°40'), Algonquin Park, Ontario, Canada, and set them on 0.8 m high wooden stands in the forest. Using the unaided eye, a hand-held lens, and a stopwatch, we observed and timed copulation and oviposition behaviours of *P. litigata* on these antlers, from initial male–female encounter to separation (1100–1700 hours, 12–17 June 1995; $N = 23$ pairs), and observed >300 pairs for part of the copulation–oviposition cycle (0800–1700 hours, June–August 1994 and 1995). We marked some flies on the thoracic notum with individual codes using the technique of Bonduriansky and Brooks (1997), released them at the antlers, and inspected the antlers periodically to determine whether individual flies mate repeatedly over their lifetimes (see Bonduriansky 1996).

We also collected single (i.e., noncoupled) males and females (by covering them with small jars) from the discarded moose antlers and paired them ($N = 15$ pairs) in an observation container consisting of a perforated piston and a cylinder, identical to the fly measuring–sexing device described in Bonduriansky and Brooks (1997), but with the closed end of the cylinder (covered with a transparent plastic membrane) raised ~1 cm above the piston. We released one male and one female at a time into the cylinder, inserted the piston, placed the container under a stereoscope (dissecting microscope) equipped with a fluorescent light source, observed copulations through the plastic membrane, and timed the distinctive “phases” (see Results). Descriptions of behaviours associated with each phase of copulation are based mainly on these stereoscope observations ($N = 12$ –15 observations for each phase). Deposition and ingestion of droplets (see Results) were observed ~30 times in the laboratory and in the field. Oviposition was observed (in part) ~160 times on antlers. Because phase durations obtained under the stereoscope did not differ significantly from those obtained in the field, we pooled these data to calculate mean phase durations (format: mean duration \pm standard deviation, $N =$ number of observations). However, mean phase durations are based on different sample sizes (see Results) because, for most pairs, we were not

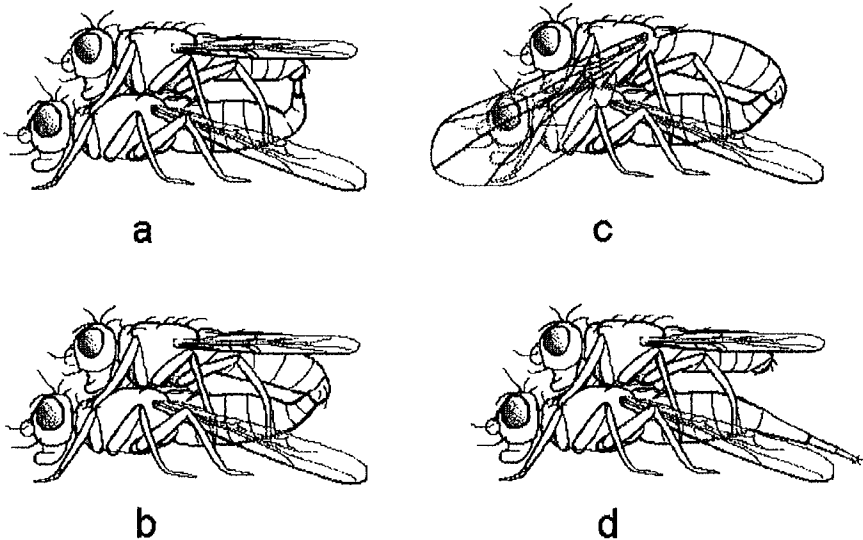


FIG. 1. Coupled pair (male on female) of *Protopiophila litigata*, shown (a) in extended position (phase 2; beginning of phase 4), where male and female abdomens are parallel and the female's abdomen tip is bent dorsally at a 90° angle; (b) in contracted position (end of phase 2; phase 3), where male and female abdomens are joined at an oblique angle; (c) exhibiting wing-lowering behaviour; and (d) exhibiting mate guarding – oviposition behaviour (phases 5, 6). Scale 16:1.

able to determine the duration(s) of one or more phases. We calculated the mean total duration of the copulation–oviposition cycle by pooling complete cycles (see Results) of known total duration (on antlers $N = 13$; under the stereoscope $N = 9$).

To investigate the droplets deposited by females after copulation, we transferred 11 pairs in late stages of copulation into plastic jar lids (depth 4 mm; diameter 16 mm) covered with glass cover slips, and observed them under a stereoscope. When a droplet was deposited on the glass, we removed the cover slip before the female could ingest the droplet, wet-mounted the droplet on a glass slide, and examined it under a compound microscope at 500× magnification ($N = 8$ droplets, each from a different female). We then allowed the wet-mounts to desiccate, and examined the residues.

Results

Phases of the Copulation–Oviposition Cycle. We divided the copulation–oviposition cycle into six phases (below). The mean total duration of cycles that proceeded to oviposition with mate guarding (“complete cycles”) was 137 ± 52 min ($N = 22$ pairs).

Phase 1: coupling (3.71 ± 1.68 min, $N = 12$). The male charged and mounted the female, and tapped her sides with his mid and hind legs. Then he vigorously massaged the tip of her abdomen with his gonopods, parameres, and mid and hind tarsi until she extended her abdomen tip, which he grasped with his gonopods and parameres. He then began to establish genital lock while she walked (carrying him on her back) to the periphery or underside of the antler, where the pair typically remained during phases 2–5. Coupled males used their wings to push away single males whenever these attacked the pair. Coupled males also occasionally exhibited wing-lowering behaviour (Fig. 1c), which appeared to stimulate the female to walk or fly to a new location. Wing-lowering behaviour could be induced by moving a hand near the pair.

Phase 2: locking (10.23 ± 4.06 min, $N = 11$). In extended position (Fig. 1a), the male continuously repeated a cycle consisting of pushing against the female's

abdomen with his hind legs (~1 s) and resting (~5 s). This action probably drove his aedeagus into her genital tract. Finally, he vigorously rubbed the tip of her abdomen with his hind tarsae for several seconds, and shifted to contracted position (Fig. 1*b*). The female usually remained still throughout this phase.

Phase 3: pumping (86.8 ± 31.21 min, N = 10). In contracted position (Fig. 1*b*), the male continuously repeated a cycle consisting of pushing against the female's abdomen with his abdomen (~1 s) and resting (~34 s). Sperm transfer probably occurred during this phase. The female occasionally flexed her abdominal tip or wedged a hind leg against the pair's genitalia.

Phase 4: unlocking (4.75 ± 2.06 min, N = 4). The pair shifted to extended position (Fig. 1*a*). Then the male performed a vigorous "dance" (2–3 s): he partially spread and raised his wings, partially straightened his legs, raising his entire body, and took several small, quick steps back and forth over the female's dorsum. After this, he extracted his aedeagus by pushing against her dorsum with his legs. After termination of genital contact, the male usually remained in tandem, and the female remained still.

Phase 5: preoviposition (5.41 ± 1.43 min, N = 3). Several minutes later, the female extended her ovipositor, deposited a small droplet on the substrate, turned 180°, and ingested this fluid. Nearly always, several minutes later, she deposited and ingested a second droplet. In one case (observed on an antler), the droplet was ingested by a single male before the female could obtain it. Droplets contained water, motile spermatozoa, bacteria (rod shaped), and various small protozoa. One droplet also contained nematode larvae. Desiccation of droplets produced residues of different opacities (degrees of cloudiness), suggesting variation among droplets in concentration of solids.

Phase 6: oviposition (29.13 ± 14.99 min, N = 8). The female returned to the upward-facing surface of the antler, probed its surface with her ovipositor, and deposited eggs in several cracks or pores. The male remained in tandem until she retracted her ovipositor. After separation, the female usually (70–80% of observations) flew off the antler. Some marked females were observed mating more than once, at intervals of 1 d or more. Of the males, ~50% immediately resumed searching for mates on the upward-facing surface of the antler. Other males returned to the underside or peripheries of the antler and remained relatively still for several minutes (occasionally, for several hours) or (rarely) flew off the antler.

Wrestling Bouts and Take-overs. Of 23 cycles observed from initial male–female encounter to separation, 13 (57%) proceeded to oviposition with mate guarding, and 9 (39%) were terminated prematurely by a member of the pair. One additional separation (4%) resulted from an attack by a single male, but no take-over (replacement of the coupled male by the other male) occurred because the female flew away. Take-overs were occasionally observed (1–2% of copulations) during phase 1. Prolonged wrestling bouts (2–30 min duration) occurred (<10% of copulations) when one or more single males mounted a coupled pair. These could result in (i) the grappling flies rolling off the antler (frequently observed); (ii) the female struggling free, sometimes with visible injury to wings or legs, and escaping from the males (less frequently observed); or (iii) take-over (rarely observed).

Discussion

Copulation Duration. In *P. litigata*, a complete copulation–oviposition cycle takes ~2.3 h, of which >1.5 h is spent in copula. This may be a midrange copulation duration in comparison with other Diptera. For example, copulation duration ranges from <1 h in *Lordotus pulcherrimus* Williston (Toft 1989) and *Mydas ventralis* Gerst.

(Alcock 1989) to 56 h in *Plecia nearctica* Hardy (Hieber and Cohen 1983). These interspecific differences may result in part from variation in ecological factors, such as time of day or ambient temperature preferred for copulation. These factors may, in turn, depend on predation risks (Yuval and Bouskila 1993) or physiology (Heinrich and Pantle 1975).

Tapping Behaviours Performed by Males. After mounting the female, the male taps her abdomen with his mid and hind tarsae. Abdominal tapping may enable *P. litigata* males to assess female egg load and reject nongravid females (Bonduriansky 1996). An apparently similar abdominal "probing" behaviour is involved in male mate choice in the necrophagous fly *Dryomyza anilis* (Fall.) (Otronen 1984).

Emigration Behaviour. At the start of copulation, the female carries the male to the periphery or underside of the antler, where the pair remains until the oviposition phase. Emigration benefits both sexes by reducing the frequency of harassment by single males, which tend to be less densely aggregated on the peripheries and underside of the antler than on the upward-facing surface (Bonduriansky 1996). Because take-overs are rare and emigration is accomplished by the female, emigration may have evolved through selection on females for avoidance of injury. Emigration also occurs in *Sepsis cynipsea* Linné, where the female carries the male off the dung pat before copulation (Parker 1972), and in *Scatophaga stercoraria* Linné, where the male flies the female off the dung (Parker 1971).

Mate Guarding. Throughout copulation and oviposition, the male uses his wings to push away single males, a behaviour known as "contact mate guarding" (Alcock 1994). This behaviour benefits the male by reducing likelihood of take-over (Parker 1974). It also suggests high last-male sperm precedence, because degree of last-male sperm precedence determines the strength of selective pressure on males to guard. Mate guarding also benefits the female by reducing the likelihood of prolonged wrestling bouts where she may be injured, and by facilitating successful oviposition. Similar forms of mate guarding occur in *S. stercoraria* (Parker 1970a) and *D. anilis* (Otronen 1984).

Ingestion of Ejaculate Materials. Fluid droplets expelled and ingested by females during phase 5 contain spermatozoa, and may contain additional nutrients, energy, and water that can be used for oogenesis or somatic maintenance. Hence, these droplets may contain a "nuptial gift," nutrients transferred from male to female during copulation (Gwynne 1984). Such nuptial gifts are not likely to enhance the viability of zygotes sired by the male because incorporation of male-donated compounds into eggs generally takes 24–48 h in insects (Simmons and Parker 1989), whereas *P. litigata* females oviposit immediately after copulation. Hence, if droplets contain a nuptial gift, its most likely function is to make the male more attractive to the female (Gwynne 1984; Wickler 1986). Nuptial gifts occur in several *Drosophila* species (e.g., Markow and Ankney 1984; Bownes and Partridge 1987; Markow 1988). Alternatively, the droplets may contain sperm from males "rejected" (i.e., prevented from fertilizing any eggs) by means of cryptic female mate choice (Thornhill 1983), or displaced sperm from previous copulations (see Parker 1970b; Gwynne and Snedden 1995). Although the quantity seems small, these droplets could constitute an important food source for *P. litigata* females. An apparently similar quantity of fluid, donated during courtship by males of *Drosophila subobscura* Collin, increases female fecundity (Steele 1986). Droplets may also contain particular important compounds (Baumann 1974; Bownes and Partridge 1987). Because droplets constitute a direct benefit of

copulation for *P. litigata* females, they may play an important role in mate choice (Bonduriansky 1996).

Oviposition Behaviour. *Protopiophila litigata* females probe cracks and pores in the antler surface with the ovipositor tip, and deposit eggs into several locations. Crack depth, moisture content, or easy access to larval food resources (probably microbes inside antlers) may be used to locate suitable oviposition sites (i.e., sites where eggs will be most likely to survive). As in the closely related *P. casei* (Jones et al. 1971), oviposition may be induced by contact with the type of microbial culture on which larvae feed.

Conclusion

Most elements of the copulation–oviposition cycle of *P. litigata* resemble behaviours of other necrophagous or scatophagous flies, such as *S. cynipsea*, *S. stercoraria*, and *D. anilis*. In contrast, the manner in which *P. litigata* females expel and ingest ejaculate materials after copulation has not, to our knowledge, been reported for any other species of Diptera.

Acknowledgments

We thank the staff of the Wildlife Research Station in Algonquin Park, Ontario. The Natural Sciences and Engineering Research Council of Canada (PGS-A grant to R. Bonduriansky and grant A5990 to R.J. Brooks) and the Ontario Ministry of Natural Resources (Environmental Youth Core) provided financial support.

References

- Alcock, J. 1989. The mating system of *Mydas ventralis* (Diptera: Mydidae). *Psyche* **96**: 167–176.
- 1994. Postinsemination associations between males and females in insects: the mate-guarding hypothesis. *Annual Review of Entomology* **39**: 1–21.
- Baumann, H. 1974. Biological effects of paragonial substances PS-1 and PS-2, in females of *Drosophila funebris*. *Journal of Insect Physiology* **20**: 2347–2362.
- Bonduriansky, R. 1995. A new Nearctic species of *Protopiophila* Duda (Diptera: Piophilidae), with notes on its behaviour and comparison with *P. latipes* (Meigen). *The Canadian Entomologist* **127**: 859–863.
- 1996. Effects of body size on mate choice and fecundity in the antler fly, *Protopiophila litigata* (Diptera: Piophilidae). M.Sc. thesis, University of Guelph, Guelph, ON.
- Bonduriansky, R., and R.J. Brooks. 1997. A technique for measuring and marking live flies. *The Canadian Entomologist* **129**: 827–830.
- Bownes, M., and L. Partridge. 1987. Transfer of molecules from ejaculate to females in *Drosophila melanogaster* and *Drosophila pseudoobscura*. *Journal of Insect Physiology* **33**: 941–947.
- Gwynne, D.T. 1984. Male mating effort, confidence of paternity, and insect sperm competition. pp. 117–149 in Smith, R.L. (Ed.), *Sperm Competition and the Evolution of Animal Mating Systems*. Academic Press, Toronto.
- Gwynne, D.T., and A.W. Snedden. 1995. Paternity and female remating in *Requena verticalis* (Orthoptera: Tettigoniidae). *Ecological Entomology* **20**: 191–194.
- Heinrich, B., and C. Pantle. 1975. Thermoregulation in small flies (*Syrphus* sp.): basking and shivering. *Journal of Experimental Biology* **62**: 599–610.
- Hieber, C.S., and J.A. Cohen. 1983. Sexual selection in the lovebug, *Plecia nearctica*: the role of male choice. *Evolution* **37**: 987–992.
- Jones, B.L., P.P. Graham, and R.F. Kelly. 1971. Microorganisms as inducers of oviposition for the cheese skipper, *Piophilidae casei* (L.) Diptera. *Journal of Milk and Food Technology* **34**: 410–415.
- Markow, T.A. 1988. *Drosophila* males provide a material contribution to offspring sired by other males. *Functional Ecology* **2**: 77–79.
- Markow, T.A., and P.F. Ankney. 1984. *Drosophila* males contribute to oogenesis in a multiple mating species. *Science (Washington, D.C.)* **224**: 302–303.
- Otronen, M. 1984. The effect of differences in body size on the male territorial system of the fly *Dryomyza anilis*. *Animal Behaviour* **32**: 882–890.

- Parker, G.A. 1970a. The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). IV. Epigamic recognition and competition between males for the possession of females. *Behaviour* **37**: 113–139.
- 1970b. The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). V. The female's behaviour at the oviposition site. *Behaviour* **37**: 140–168.
- 1971. The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). VI. The adaptive significance of emigration from the oviposition site during the phase of genital contact. *Journal of Animal Ecology* **40**: 215–233.
- 1972. Reproductive behaviour of *Sepsis cynipsea* (L.) (Diptera: Sepsidae). II. The significance of the precopulatory passive phase and emigration. *Behaviour* **41**: 242–250.
- 1974. Courtship persistence and female-guarding as male time investment strategies. *Behaviour* **48**: 157–184.
- Simmons, L.W., and G.A. Parker. 1989. Nuptial feeding in insects: mating effort versus paternal investment. *Ethology* **81**: 332–343.
- Spieth, H.T. 1952. Mating behavior within the genus *Drosophila* (Diptera). *Bulletin of the American Museum of Natural History* **99**: 399–474.
- 1984. Courtship behaviors of the Hawaiian picture-winged *Drosophila*. *University of California Publications in Entomology* **103**.
- Steele, R.H. 1986. Courtship feeding in *Drosophila subobscura*. I. The nutritional significance of courtship feeding. *Animal Behaviour* **34**: 1087–1098.
- Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *The American Naturalist* **122**: 765–788.
- Toft, C.A. 1989. Population structure and mating system of a desert bee fly (*Lordotus pulcherrimus*; Diptera: Bombyliidae). 2. Female demography, copulations and characteristics of swarm sites. *Oikos* **54**: 359–369.
- Wickler, W. 1986. Mating costs versus parental investment: a reply to Gwynne. *Ethology* **71**: 78–79.
- Yuval, B., and A. Bouskila. 1993. Temporal dynamics of mating and predation in mosquito swarms. *Oecologia* **95**: 65–69.

(Date received: 18 December 1996; date accepted: 15 January 1998)