

Growth and Development of *Pseudacteon* Phorid Fly Maggots (Diptera: Phoridae) in the Heads of *Solenopsis* Fire Ant Workers (Hymenoptera: Formicidae)

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ABSTRACT We studied the growth and development of the parasitic phorid fly *Pseudacteon littoralis* Borgmeier in the fire ant, *Solenopsis invicta* Buren. The fly apparently lays one egg in or on the thorax of a major worker. The newly hatched maggot migrates into the head of the ant where it develops through its last two instars in ≈ 3 wk (23°C). Just before pupariation, an enzyme is apparently released that begins dissolving the cuticular membranes of the ant. The maggot then consumes the entire contents of the ant head, a process that usually results in decapitation of its living host. After pushing the mouth parts aside, the maggot pupariates within the empty head capsule, positioned so that the anterior three segments precisely fill the oral cavity. The pupal stage lasts ≈ 3 wk. Results of this study indicate that it should be possible to rear this fly for release as a natural biological control agent of imported fire ants in the United States.

KEY WORDS biocontrol, parasite, Brazil, South America, larva, pupa

PHORID FLIES in the genus *Pseudacteon* Coquillett appear to be promising biocontrol agents for imported fire ants in the United States because of their effect on fire ant foraging (Orr et al. 1995; Feener & Brown 1992) and their specificity to ants in the genus *Solenopsis* Westwood (Porter et al. 1995, Disney 1994). The attack behavior of *Pseudacteon* flies and the dramatic defensive responses of fire ants to these attacks (unpublished data, Feener & Brown 1992) leave little doubt that these flies are actual fire ant parasites; nevertheless, the immature stages of this genus are completely unknown (Williams et al. 1973).

Pseudacteon maggots probably develop internally in their hosts, like the maggots of other parasitic phorid genera (Disney 1994, Brown & Feener 1993, Feener & Brown 1993, Brown et al. 1991, Brown & Feener 1991, Wojcik et al. 1987, Goidanich 1928, Pergande 1901). However, it is also possible that these flies could shift from fire ant workers to fire ant brood (Williams & Whitcomb 1974), or even to another host at some stage in their development. The objectives of this study were first to confirm that *Pseudacteon* flies are parasites of fire ants and second to study larval growth and development in their presumptive host. Information provided by this study will be vital for fu-

ture efforts to rear *Pseudacteon* flies for possible release as natural biological control agents of imported fire ants in North America.

Materials and Methods

We collected two fire ant colonies on the campus of São Paulo State University at Rio Claro (UNESP-Rio Claro) in February 1994. The first colony was *Solenopsis saevissima* (F. Smith) and the second was *Solenopsis invicta* Buren. Both colonies were separated from the soil using drip flotation and placed in Fluon-coated white trays (30 by 40 cm) each with a 10-cm petri dish nest (Banks et al. 1981). Use of laboratory colonies to study phorids is a useful technique because ants in the trays cannot easily hide from phorid attacks, and attacks are easily observed without fear of being stung. Use of laboratory colonies also allows repeated field sampling without the need to find and repeatedly disturb local fire ant mounds. Over the course of 3 d, both colonies were set out six times at four sites just north of state road SP-191 between Rio Claro and Araras, SP, Brazil. We collected as many workers as possible after they were attacked by the phorids. Collections were made in the mornings (0800-1000 hours) and the evenings (1700-1900 hours). Attacked workers appeared stunned, usually standing immobile while rising up on their legs as if they were on stilts. After an attack, workers were picked up with light-weight forceps and aspirated into a holding vial. They were

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then taken back to the lab and divided into groups of 1–3 and placed into disposable culture tubes (13 by 100 mm). Each tube had three wet cotton wads stuffed into the bottom, with a dry cotton wad plugging the top. Test workers were offered sugar water (1 M) once a week for the first 4 wk. Beginning with day 4, we dissected a few workers every couple of days to study larval development. Test workers were maintained at 23°C throughout the study. Voucher specimens have been retained by the authors and also deposited in the Museu de Zoologia, Universidade de São Paulo, Brazil.

Results

Oviposition. Our two colonies attracted several species of *Pseudacteon* phorids including *Pseudacteon litoralis* Borgmeier, *Pseudacteon wasmanni* (Schmitz), and *Pseudacteon pradei* Borgmeier. *P. litoralis* predominated because of the time of day that collections were made (unpublished data). Workers attacked by *P. litoralis* were preferentially collected because *P. litoralis* attacked the largest-sized workers. We preferred larger workers because they were easier to collect and tend to live longer than smaller ones (Calabi & Porter 1959). Oviposition of *P. litoralis* was very rapid (<1 s) and it appeared to occur in or on the posterior part of the thorax, perhaps around the coxae (compare Feener 1987, Williams & Banks 1987). The fly was more or less oriented in parallel with the ant, facing in the same direction.

From the *S. saevissima* colony, we collected 66 fire ant workers that had been attacked by phorids and 34 that had not been attacked. Almost all workers in both groups were dead within 1 wk. The reason for this poor survival is unknown, but we also had similar problems with workers from three other fire ant colonies that had been collected 1–2 wk before. We collected 171 workers from the *S. invicta* colony after they had been attacked by phorids, plus 33 that had not been attacked. The mean time to 50% mortality in these groups was \approx 3 wk.

Larval Growth and Development. Ten of the oviposited workers from the *S. saevissima* colony were dissected and one very small (0.2 mm) 4-d-old second-instar maggot was found. We were much more successful with the *S. invicta* colony. Phorid maggots were found in 35% of the workers that had been attacked (59/171). None of the medium-sized workers contained a maggot, but 38% of the large workers contained a maggot (0/16 versus 59/155; Fisher's exact test, $P < 0.001$). Only one worker contained more than one maggot.

We were able to identify the last two instars (Fig. 1). The maggot probably progressed through one additional instar before we began dissections on day 4 because phorids usually have only three instars (Disney 1994). The second instar (Fig. 1A) is vermiform and appears to have 12 segments. It ranged in size from 0.2 to \approx 0.9 mm. The cephal-

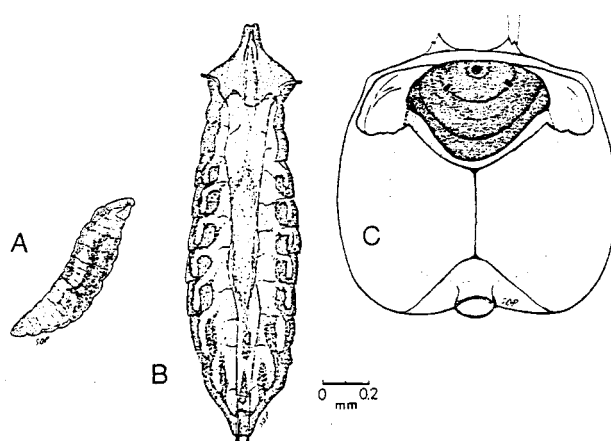


Fig. 1. (A) Lateral view of the second instar of the phorid fly *P. litoralis*. This figure was drawn with dark-field illumination so areas that appear dark are actually clear. (B) Dorsal view of the third instar, also drawn with dark-field illumination. The white areas are fat bodies. Traces of the digestive tract are indicated along with the tracheal system. (C) Ventral view of the head capsule of an *S. invicta* major worker with a *Pseudacteon* puparium in the area previously occupied by the maxillo-labial mouth parts of the ant. The exposed parts of the puparium are sclerotized as indicated by dark stippling; note the remains of the larval mouth and the two anterior spiracles. The dotted area inside the ant head capsule indicates the approximate extent of the puparium.

opharyngeal skeleton is brownish black. Most of the rest of the maggot is whitish or transparent with a number of fine silvery tracheal tubes. The maggots did not appear to be attached to anything and were usually found between the brain of the ant and its mandibular muscles.

The living final-instar maggot is flattened and shaped somewhat like a planarian worm (Fig. 1B). This instar ranged in size from 1.0 to 1.8 mm. Assuming the two primary tracheal trunks are dorsal (Snodgrass 1935), this instar usually curls dorsally into a horseshoe shape. The mouth and the anterior and posterior spiracles are frequently extended (as illustrated) and retracted as the maggot moves and twists. The four exterior extensions of the spiracular tubes are orange-brown in color. The cephalopharyngeal skeleton is also orange-brown. The live maggot, with its ladder-like collection of lateral fat bodies, looks somewhat like a plastic bag of wet cotton balls twisting back and forth. The central gut area is a light greenish yellow. Thoracic segments lack the lateral projections of fat body. On at least four occasions, we found the cast skin of the second instar floating in ant hemolymph.

Almost all of the maggots were recovered from the heads of the ants; consequently, the maggots must move from the thorax, where the eggs are laid, into the head in the first 3–4 d. We found three or four maggots in the thorax, but most were dead and none of them had succeeded in developing past the second instar. The poor development of maggots in the thorax is not surprising

because it does not contain enough room for the final instar to mature.

Pupariation. Ant workers with maggots in their heads generally do not exhibit pathological signs until just before the phorid begins to pupariate. At this stage the maggot begins to slither around in the head and consumes all available tissue, including the brain, muscles, and mandibular glands until nothing is left but cuticle. The maggot appears to release or somehow stimulate the ant to release enzymes that begin to dissolve the cuticular membranes. At some point in the process, the ant head usually falls off, and the decapitated body is left standing motionless in an upright posture. The ant legs and sting apparatus are often still twitching, but the membranes between the first pair of coxae are so weak that the legs usually fall off with slight pressure. Sometimes the other coxae and even the petiolar segments are also affected.

Eventually, the maggot cuts away the ant maxillo-labial plate and pushes aside one or two of the mandibles (usually the left one). The maggot then positions itself in the opened head capsule as illustrated (Fig. 1C). Other ants can be hostile to the pupariating maggot because at least one was cut out of the head capsule and killed. The average head width of fire ant workers with puparia or pupariating maggots was 1.19 ± 0.08 mm (SD; $n = 11$). By comparison, the median size of workers in the colony was ≈ 0.7 mm (estimate, see Porter & Tschinkel 1985).

Upon pupariation, the first three segments of the maggot contract and sclerotize in < 1 d into a dark reddish brown plate that is almost the same color as the ant head capsule. The sclerotized area does not continue around the portions of the first three segments that are covered by the ant clypeus, but a little sclerotization does continue laterally along the cavities where the mandibles insert. The sclerotization pattern is not dependent on exposure to air because one maggot pupariated crosswise in the head capsule and the sclerotization pattern was essentially the same as those that pupariated normally.

Emergence of the Adult. The puparium begins to darken internally ≈ 3 –4 d before the fly emerges. On emergence, the fly pops the sclerotized portion of the puparium open like a lid and crawls out. Initially the body is almost entirely white and distended with liquid. The fly extends its wings and darkens to its normal black color within 6–12 h. A total of four adult phorids were reared from 10 puparium including three female *P. litoralis* and one unidentified male (presumably also *P. litoralis*). The flies lived only 1–2 d after emergence. Flies collected from the field usually die within 1–4 d. Three puparia were apparently killed by fungus, which quickly engulfed head capsules that were in direct contact with moist sand or cotton. One puparium failed to emerge because it was improperly oriented and another failed to sclerotize.

At 23°C, the duration of the second instar, the final instar, and the puparium are ≈ 9 , 9, and 24 d, respectively. The total developmental time from oviposition to adult was 47 ± 2 d (SD; $n = 4$).

A large number of the maggots in this study appeared to develop abnormally slow. Eight second-instar maggots were found > 30 d after oviposition. Several of these maggots appeared to have been dead for some time before the ants were dissected. Another 10 last-instar maggots died after they began to consume the contents of the ant head but before they were able to open its mouth to pupariate. The fact that earlier attempts at pupariation were more successful (7/10) than the later attempts (3/10) suggests that slower developing maggots were not as healthy, although the difference was not significant (Fisher's exact test, $P = 0.10$).

Discussion

Pupariation. This study clearly demonstrated that *P. litoralis* is a parasite of fire ant workers. The somewhat grotesque practice of this fly in decapitating its living host, while consuming the contents of its head and then pupariating in the empty head capsule, is particularly intriguing. Pergande (1901) reported a similar *ant-decapitating* behavior for a maggot of the phorid *Apocephalus pergandei* Coquillett with the carpenter ant *Camponotus pennsylvanicus* (DeGeer). The larva of this or a similar parasite is also apparently capable of pushing the decapitated head of its host along like a miniature snail for 50 cm or more (Fox 1887). The fact that host decapitation occurs in both *Apocephalus* and *Pseudacteon* suggests that it may occur in other genera as well. Certainly, it is likely that other species of *Pseudacteon* also decapitate their hosts and pupariate in the empty head capsule.

The puparium of *P. litoralis* is different from all other known phorids in that the anterior three segments are flattened and sclerotized to form a slightly convex plate (Fig. 1C). The precise fit of this plate in the host head capsule is probably a specialized adaptation that helps the puparium avoid detection by hostile ants. The remainder of the puparium inside the ant head capsule remains unsclerotized. Unlike most other phorids (Disney 1994), *P. litoralis* does not appear to have dorsal eclosion plates; only the sclerotized anterior three segments pop off when the adult emerges. No respiratory horns were observed in this species. The phorid puparium that Wojcik et al. (1987) recovered from the abdomen of a winged fire ant queen is also largely unsclerotized, but it is clearly not *Pseudacteon* because it is too large. It also lacks the flattened plate, and the eye of the developing fly inside only contains 16 or so ommatidia, rather than the hundreds present in *Pseudacteon* flies.

Larvae and Development. The third instar of *P. litoralis* (Fig. 1B) differs from maggots of the phorids *Apocephalus paraponerae* Borgmeier (Brown & Foener 1991), *Apocephalus schmitzi* Mi-

nozzi (Goidanich 1928) and *Trucidophora camponoti* (Brown) (Brown et al. 1991) in that it lacks any posterior external structures other than two small spiracular tubes. Also, the maggot is relatively broader in width and flattened dorsoventrally. The anterior spiracles and general body shape of *P. litoralis* are somewhat similar to the maggot of *Rhyncophoromyia maculinea* Borgmeier, a solitary phorid parasite found in the abdomens of *Camponotus* workers (Brown & Feener 1993). The *Rhyncophoromyia* maggot also appears to bend dorsally like the *Pseudacteon* maggot. The four phorid species mentioned in this paragraph all exit their ant host and pupariate in the ground. As might be expected, the puparia of these species are all completely sclerotized and the anterior segments are not flattened into a plate.

Developmental rates of 22 d for *P. litoralis* maggots plus 24 d for puparia were slower than rates reported for most other phorids (Brown & Feener 1991). These slow developmental rates are partially a result of a relatively cool rearing temperature (23°C). During the summer, temperatures in fire ant mounds often average 28°C or more (Porter & Tschinkel 1993). Phorid developmental rates at 28°C would, of course, be much faster, perhaps more than twice as fast (if the effect of temperature on fire ant brood development is any indication, Porter 1988).

Oviposition. As might be expected for one of the largest species of *Pseudacteon*, *P. litoralis* was highly selective in the size of fire ant workers it parasitized. It selected major workers with head widths of ≈ 1.2 mm, even though the median size of workers was only ≈ 0.7 mm. Feener (1987) reported that *Pseudacteon crawfordi* Coquillett preferred larger-than-average *Solenopsis xyloni* McCook workers (0.69-versus 0.60-mm head width) and also larger-than-average *Solenopsis geminata* (F.) workers (0.74 versus 0.70 mm). Campiolo et al. (1995) reported that *Pseudacteon tricuspis* Borgmeier preferred larger fire ants than *Pseudacteon curvatus* Borgmeier and that there was almost no overlap in the sizes of workers preferred.

Approximately 35% of the workers attacked by *P. litoralis* flies developed phorid maggots. The reason why the other 65% of the workers did not develop a maggot is unknown, but several possibilities exist. The first is that the phorids were not always able to lay an egg during the attack. Oviposition attempts are extremely rapid, and it seems likely that flies are not always able to align their ovipositors properly or that an egg is not always able to be ejected. On a number of occasions, we observed a fly strike an ant and then circle back and strike it again as if it had been unsuccessful on the first attempt. A second possibility is that flies may be selective about which workers actually receive eggs. For example, flies may assess the size or age of a worker during an oviposition attempt and then choose not to lay an egg if the cues are inappropriate. This may explain why none of the

medium-sized workers that were attacked contained a maggot. The third possibility is that some of the oviposited eggs may not have developed because of infertility or various ant defenses.

Rearing Phorids. We successfully reared *Pseudacteon* flies through all stages, from eggs to adults. This accomplishment is encouraging because it suggests that it will be feasible to rear these parasites in the laboratory for future release in North America. An important point is that the puparia apparently do not require a diapause or any special handling. A number of technical problems remain, but none of them appear insurmountable. For example, the attack behavior of *Pseudacteon* flies has already been observed to occur under laboratory conditions (Williams & Banks 1987, unpublished data) and mating appears to occur when both male and females are attracted to fire ant colonies (Feener 1987). One concern is that adults are apparently very short-lived; thus, rearing attempts will probably require fairly large numbers of flies to ensure that both males and females emerge on the same day for mating. A second concern is that we reared only $\approx 10\%$ of the undissected larvae through to the adult stage. Some of the failed larval development may be normal mortality or the natural result of ant defenses. A second possibility is that the last-instar maggots are not receiving some kind of triggering cue like temperature or light changes and, therefore, are delayed until the success of pupation is greatly reduced. However, the most likely possibility is that the low success rate is simply the result of food stresses associated with keeping the ants isolated in small groups of 1–3 with limited access to food. Presumably, more normal conditions for the ants would also improve the developmental success of the parasites.

Several interesting questions remain to be answered. For example, how do parasitized workers behave in the colony? Does the growing maggot inhibit its host worker from foraging or alter its performance of other colony tasks? Where are the parasitized workers when the maggots begin to pupariate? If pupariation occurs inside the colony, are the head capsule and puparium retained in the colony for a while, or are they immediately hauled out to a refuse pile? Once on the soil surface, what keeps the puparium from dying at high soil temperatures or molding when the soil is moist? What percentage of colony workers are normally parasitized under field conditions? Additional information is also needed about the adult flies. We suspect that they are short-lived, but we know little about their feeding behavior, mating, or what they do when they are not attacking fire ants.

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