Leaping Locomotion in *Mycetophila cingulum* (Diptera: Mycetophilidae): Prepupation Dispersal Mechanism[1]

SCOTT CAMAZINE

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ABSTRACT The legless larvae of the fungus gnat *Mycetophila cingulum* Meigen leap distances up to 20-fold their body length. Leaping occurs during the prepupal stage when larvae disperse from the host fungus, *Polyporus squamosus* Micheli ex Fr., to pupate in the substrate. A larva curls into a tight loop and snaps forward, catapulting itself as much as 15 cm. The jump appears to involve a series of cuticular pegs not previously described. Structural adaptations and mechanics of the jump mechanism are examined. Mature larvae leave the fungus in fairly close synchrony, between 1700 and 2200 hours. Larvae within the fungus that do not disperse during this period wait until subsequent days, when they disperse at approximately the same time. Such gated prepupation circadian behaviors have been described for other insect larvae and appear to be under hormone control in those insects.

LEGLESS AND wormlike, many dipteran larvae are limited in their manner of locomotion. However, some dipteran larvae have evolved specialized anatomical structures and behaviors enabling them to jump and move rapidly over uneven substrates. In a few families (Piophilidae, Clusiidae, Trypetidae, Cecidomyidae, Mycetophilidae [Madwar 1937, Richards and Davies 1957, Curran 1965, Chapman 1982]), larvae can rapidly jump distances many times their own body length. However, the details of these jumping mechanisms and their adaptive signficance have received little attention.

In the prepupal stage, the larva of *Mycetophila cingulum* Meigen disperses by jumping from its fungus host. This paper describes the larva behaviors observed during the prepupal dispersal stage and the structural adaptations for leaping.

According to Madwar (1937), the larva of *M. cingulum* lives exclusively in the fungus *Polyporus squamosus* Micheli ex Fr. The female lays her eggs on the underside of the fungus and the larva passes through four instars within the fungus before pupating in a cocoon in the soil. The larvae are ca. 8 mm long, smooth, soft, roughly cylindrical, and whitish with a strongly sclerotized dark head.

Voucher specimens of the larvae and adults have been placed in the U.S. National Museum of Natural History; in the collections of the Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario; and in the Cornell University Collections (Lot no. 1140).

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Materials and Methods

Behavioral Observations. I studied *M. cingulum* in Ithaca, N.Y., during September and early October 1980–1984, when its fungus host, *P. squamosus*, was fruiting. In some instances fungi containing larvae were observed indoors (20°C) under natural light through a window facing the west. All observations after sunset were made with an incandescent light covered with a red filter. The detailed sequence of events involved in jumping was recorded on motion picture film (super-8) at 54 frames per s and analyzed frame by frame on a film editor. Orientation data was analyzed using the *V* test (Batschelet 1972).

Anatomical Studies. Living and preserved specimens were examined at 6–50×. To examine surface details of the cuticle at higher magnifications (100–1,000×), larvae were soaked overnight in 10% KOH, and flattened by gently extruding the macerated body contents through the anus. The specimens were then examined under phase-contrast light microscopy.

Scanning electron micrographs (SEM) were made by dropping live larvae, crawling or in the act of jumping, into liquid freon chilled to −195°C in liquid nitrogen (Eisner et al. 1976). The larvae, frozen in position, were critical-point dried, coated with gold-palladium, and examined with a scanning electron microscope (Amray 1000).

Results

Prepupation Behavior. Larvae leave their fungus host to pupate in the ground, almost always around sunset (Fig. 1). The larvae began leaving the fungus in great numbers at 1730 hours on day

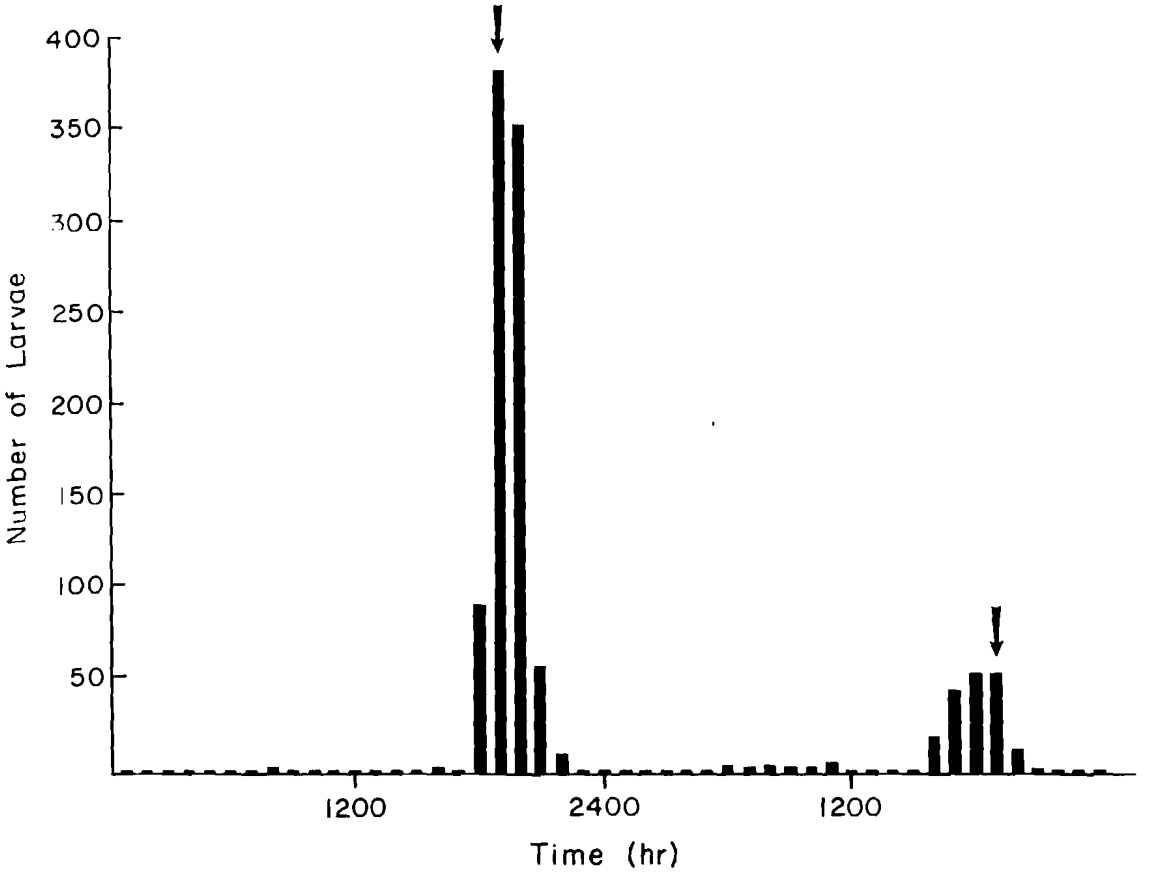

Fig. 1. Number of *M. cingulum* larvae leaving the fungus each hour over the 2-day period from 26 September–27 September 1984. Arrows indicate time of sunset (1900 hours). Baseline value on the graph represents zero.

1, peaking by 1900 hours and ending by 2200 hours. Most larvae that did not emerge during the first peak emerged ca. 24 h later. This pattern of emergence was noted in both laboratory and field observations of larvae-infested fungi.

The larvae display stereotyped behaviors during the prepupal stage. They emerge from holes chewed in the upper or lower surface of the fungus and crawl upwards and towards the sunlight. When a larva reaches the edge of the fungus, it probes into the air with the anterior portion of its body while maintaining a grip on the substrate with its terminal locomotory pads. The larva then curls back upon itself and apposes the dorsa of its anterior and posterior body segments, forming itself into a tight loop (Fig. 2A). After maintaining this position momentarily, the larva suddenly snaps open and catapults itself off the fungus onto the ground. The crawling and jumping behavior continues on the ground so that, over the course of several minutes, larvae can disperse up to a meter or more from the fungus in a direction within 45° of the sun azimuth.

Two experiments demonstrated the positively phototactic and negatively geotactic nature of the larval locomotion. In the first experiment, three groups of larvae (35, 31, and 30 larvae per group) that had recently emerged from a fungus were placed in a cylindrical glass tank (20 cm diam, 25 cm high) and covered for 15 min to exclude sunlight. The tank was then uncovered for 15 min and the location of the larvae was noted. The mean percentage of larvae on the sunlit side of the container was 84.3 ± 9.6 (mean ± SD). At the same time the mean percentage of larvae that had crawled to the upper half of the container was 83.6 ± 5.5. In all three replicates, the larvae showed significant orientation towards the sunlit and upper portions of the tank ($P < 0.01$; χ^2 test).

In the second experiment, 17 recently emerged larvae were randomly dropped onto a sheet of paper illuminated through a sunlit window. Their initial position was marked. They were allowed to crawl 3 cm; then the angle of their path with respect to the sun azimuth was measured to the nearest degree. As shown in Fig. 3, the larvae showed a significant orientation ($P < 0.0001$; V test) (Batschelet 1972) in the direction of the sun.

The positive phototaxis and negative geotaxis of larvae in glass containers containing soil lasted ca. 2 h. At the end of this period the larvae were 1 or 2 cm below the surface of the soil. Over the next

the upper portion of its body becomes slightly wider and shorter. This may be due to the contraction of the longitudinal muscles but was not experimentally verified. This phase, from the formation of the loop until just before takeoff, lasted an average of 0.40 ± 0.08 s (mean ± SD). The larva then rapidly snaps open the loop, catapulting itself into the air. An average jump is 9.5 ± 2.8 cm long (mean ± SD) and is taken at a mean angle of 69.3 ± 8.5° to the horizontal.

The initial (takeoff) velocity of the jump was determined by two methods. In measurements taken from films at 54 frames per s, the takeoff speed was estimated from the distance moved by the center of gravity of the larva in the first two frames of the jump. For two jumps this was calculated to be 89 and 110 cm/s. These measurements are probably underestimates of the actual velocities that the larvae generally attain because of the slowing that occurs towards the peak of the jump when the vertical component of the larva's velocity approaches zero. In addition, these velocities were based upon short jumps (3.1 and 4.0 cm, respectively), chosen because their entire trajectories fit into the field of view of the camera.

The takeoff velocity of the larva's trajectory was also calculated from an equation for the motion of a projectile (Evans 1972):

$$v = \frac{\sqrt{2gh}}{\sin A} \quad (1)$$

where v is initial, takeoff velocity (cm/s), h is range of jump (cm), A is angle of takeoff with respect to the horizontal, and g is acceleration due to gravity (980 cm/s).

Assuming no air resistance, the distances given above, and an average takeoff angle of 69°, the initial velocity of the jumps was between 81–150 cm/s, which agrees well with the measured values. Calculated velocities are also likely to be slight underestimates because air resistance is not considered. A more reasonable assumption is that air resistance for a 1-cm animal that jumps 10 cm is about one-tenth gravitational acceleration (Bennet-Clark 1977), yielding takeoff velocities of between 85–158 cm/s.

The jumps were too quick to follow by eye. From films of short jumps, it was determined that the time from takeoff to landing was <0.15 s.

Estimates were also made of the acceleration required to attain these initial velocities. If one assumes a constant acceleration (a) occurring over a length (l) as the larva exerts a force against the substrate, acceleration can be calculated from the following expression (Evans 1972):

$$a = \frac{v^2}{2l} \quad (2)$$

The acceleration distance, l, is the distance the center of gravity moves from the coiled set position to its takeoff. Conservative estimates are that the center of gravity is at least 1 mm off the ground in the set position and no more than 4 mm off the ground at takeoff, giving a maximal acceleration distance of 3 mm, and an acceleration of 23,400 cm/s. This represents an impressive acceleration of 24 × g.

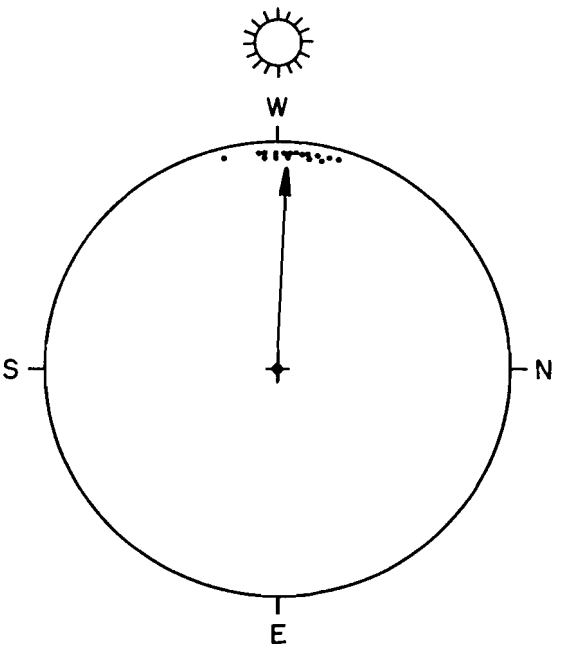

Fig. 3. Positively phototactic crawling orientation of *M. cingulum* larvae. The mean vector bearing points 7° north of the sun.

Discussion

As prepupae, many insects locate a safe environment to build a structure within which to pupate. During their prepupal stage, the larvae of *M. cingulum* suddenly leave their food source, become positively phototactic and negatively geotactic, disperse via a new locomotory pattern (leaping), locate a suitable pupation site, and build a silken cocoon. The prepupation dispersal behavior follows a circadian rhythm. Similar gated prepupation circadian behaviors have been described for other insect larvae such as the tomato hornworm, *Manduca sexta* (L.) (Truman 1972, Dominick and Truman 1984); fleshflies, *Sarcophaga bullata* (Parker) (Roberts 1984); and mosquitoes, *Aedes taeniorhynchus* (Wiedemann) (Brady 1974), and appear to be under hormone control.

Several advantages to dispersal before pupation are likely. First, the host fungus is an ephemeral food source that rots and completely collapses several days after the larvae disperse. It would offer little physical protection for the delicate pupae. Second, if all larvae were to pupate within the fungus, they would be an attractive, concentrated food source for any predator that might find the rotting fungus.

Lacking legs with which to disperse, *M. cingulum* has evolved an unusual leaping mechanism

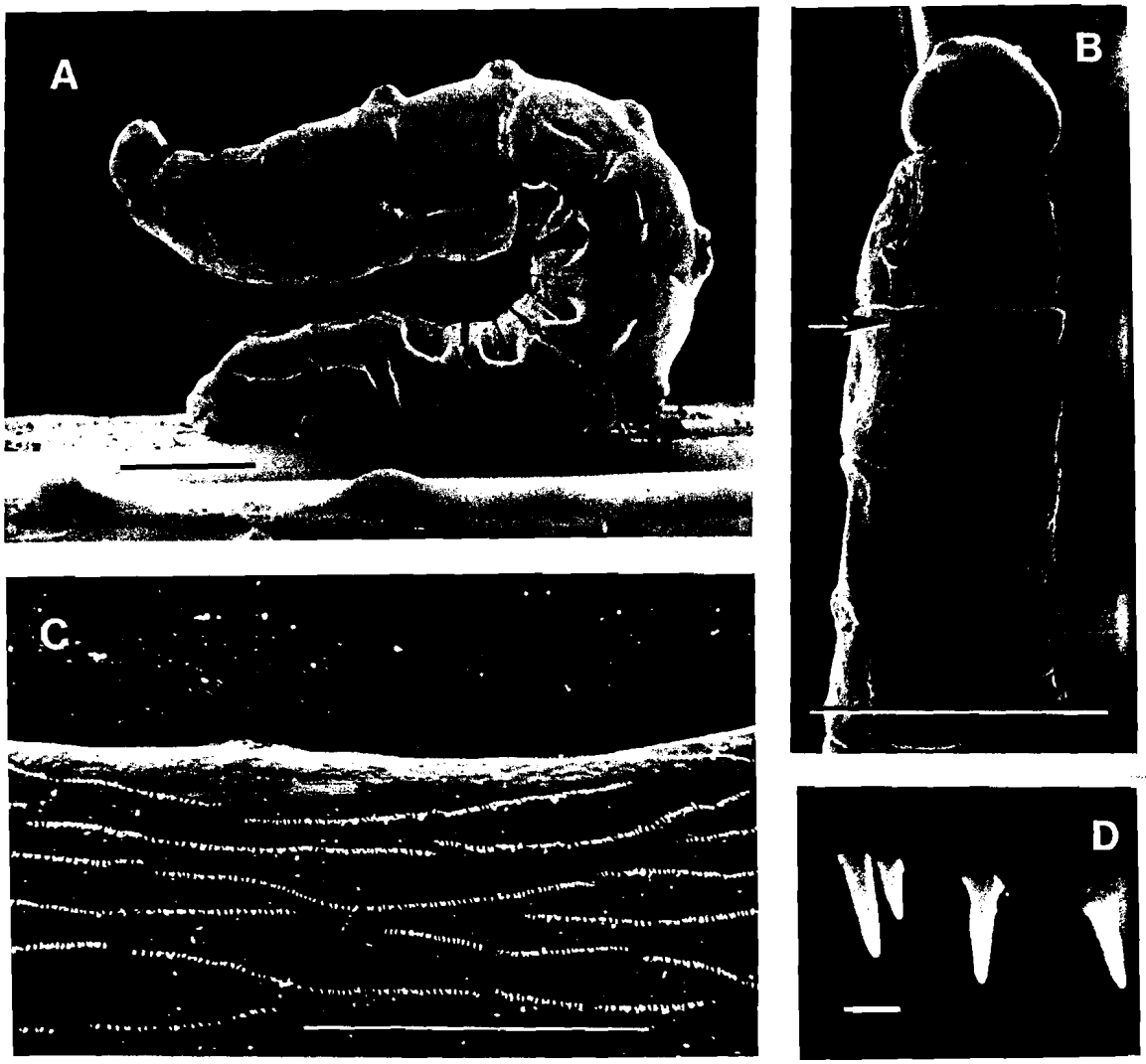

Fig. 2. Scanning electron micrographs of *M. cingulum*. (A) Larva in the initial stage of jumping just after the release of contact between the anterior and posterior dorsal surfaces. (B) Dorsal surface of the head and first four body segments showing rows of pegs (arrow) seen only faintly under low magnification on the cephalad edge of the second body segment. (C) Higher magnification of the cephalad edge of the second body segment showing rows of pegs pointing caudally. (D) Single pegs. Reference bars: (A and B) 1 mm; (C) 100 μm; (D) 1 μm.

few hours, larvae exhibited rolling movements, thus forming in the soil a small cavity slightly larger than a single individual. After ca. 6 h from the time of leaving the fungus, the larva began constructing a coarse silken meshwork that prevented debris from filling this cavity. Then a more finely woven, nearly cylindrical silken cocoon was constructed in which the larva pupated. This double-layered cocoon was finished 12–18 h after emergence from the fungus.

Structural Adaptations for Jumping. Scanning electron microscopy of the mature larvae confirmed most of the anatomical details described by earlier workers (Johannsen 1910, Madwar 1937). In addition, rows of minute conical pegs were found anterodorsally on the second and third body segments (Fig. 2 B–D); these pegs have not been previously described.

The 9–12 transverse rows of pegs point caudally. Their distribution was best seen in the flattened KOH preparations, because SEMs often fail to show the initial rows hidden in the intersegmental folds by the normal curvature of the larva (Fig. 2C). Estimates of the total number of pegs on each segment range from 5,200–9,300, based upon the finding of 9–12 rows of pegs on each segment, containing between 575–775 pegs per row. The pegs measure 1.64 ± 0.23 μm (mean ± SD) in length and 0.65 ± 0.08 μm wide at the base (Fig. 2D). A row of pegs is 1.0 mm long.

The Jump Mechanism. Just before jumping, when the larva curls itself into a loop (Fig. 2A),

within the constraints of a soft-bodied legless form. Because the minute prolegs are inadequate as lever arms over which a muscular force may be applied to effect a jump, the entire body of *M. cingulum* has been recruited to function as a single long lever; the act of the larva curling into a loop can be compared to a locust flexing its long legs before a jump.

This catapulting jumping mechanism has several requirements: 1) muscular contraction before the jump to provide sufficient energy, 2) a means of storing the energy, and 3) a catch mechanism that can be rapidly disengaged to release the stored energy and power the jump. The jump of *M. cingulum* appears to fulfill these requirements. There is a brief delay before jumping during which energy is presumably stored. There are pegs that may act as part of a catch mechanism suddenly disengaged to trigger the catapult. These details require further study. The dorsal surface of the larva is smooth except for the pegs. No anatomical specializations were found on the dorsum of the terminal body segments to which the pegs could attach. Presumably the pegs engage within the soft integument of the larva where adequate friction or perhaps surface tension are developed to maintain the larva in its looped set position. Surface tension forces have been invoked to explain the tension developed during the jump of certain nematodes (Reed and Wallace 1965).

The action of the jump is extremely rapid and is obscured by the tightly apposed dorsal surfaces of the larva. It is possible that a slight relaxation of the contracted anterior longitudinal muscles allows the pegs to disengage. In addition, the energy storage mechanism of the larva has not been identified. Perhaps the elasticity of the cuticle under increased hydrostatic pressure accounts for the energy of the jump.

Among the insects, the detailed anatomical mechanisms and energetics of jumping have been documented in a few cases, for example, fleas (Bennet-Clark and Lucey 1967), click beetles (Evans 1972, 1973), locusts (Heitler 1974, 1977, Bennet-Clark 1975), springtails (Manton 1972, Christian 1978, Eisenbeis and Ulmer 1978), and bristletails (Manton 1972, Evans 1975). The values for the various parameters of the *Mycetophila* larva jumps are similar to those obtained in these other studies of small insects. Catapulting locomotion also appears to operate in certain nematodes (Reed and Wallace 1965) and other jumping dipteran larvae, although the mechanics of the jump have not been studied in detail.

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