Trail Marking by Larvae of the Eastern Tent Caterpillar

Abstract. *Eastern tent caterpillars that are successful foragers deposit trails as they return to the tent that are more attractive than the exploratory trails of the unfed larvae. The trails of these fed returning larvae attract unfed tentmates to food finds and are followed preferentially during the colony's next forage en masse. One or more chemical factors account for the attractiveness of these trails.*

Larvae of the eastern tent caterpillar *Malacosoma americanum* (Fabricius) (Lepidoptera: Lasiocampidae) live colonially in a silken tent formed in the branches of cherry or apple trees. The colonies ordinarily consist of 50 to 300 siblings which develop from a single egg mass. The caterpillars feed gregariously on the leaves of the host and lay down persistent silk trails from their tent to distant feeding sites. Host trees are often covered with these trails, yet the caterpillars move efficiently to their most recent foraging sites, ignoring trails that lead to previously defoliated areas. The trails of these caterpillars contain one or more chemical factors and the caterpillars distinguish, presumably on the basis of a chemical cue, between trails used and not used during their last forage (*1*).

Chemical trail substances are commonly employed by certain of the eusocial insects, notably the ants and termites, to recruit foraging workers to food finds (*2*), but they have not been previously reported to be utilized in this context by caterpillars or other non-eusocial insects. I report here that a chemical component of the exploratory trail deposited by the caterpillars as they move from their tent in search of food is essential to the elicitation of the trail-following response. In addition, caterpillars that succeed in locating food deposit trails on their return trip that are more attractive than those of unsuccessful foragers. These trails serve to attract unfed tentmates to food finds and are followed preferentially during the colony's next en masse forage.

Laboratory colonies of 1st- to 5th-instar eastern tent caterpillars were maintained in the open on inverted tripods and allowed to forage on cherry branches placed at the end of a horizontal bridge (Fig. 1). Caterpillars typically moved to the host en masse four times per day at approximately 6-hour intervals, spending 10 to 30 minutes at feeding sites. Outbound foragers deposited continuous strands of silk as they followed each other over the branches of the host in search of food (*3*). After feeding they followed the aggregate silk trail back to the tent. Preliminary studies showed that a soluble component of the exploratory trail is essential to the elicitation of the trail-following response. Silk deposited by third instar larvae moving from their tent to feeding sites was collected on an 8 cm-long glass rod placed between the end of the bridge and the host. While the caterpillars were feeding the rod was washed in methylene chloride for 10 to 15 minutes, then replaced. The treatment removed the soluble components of the silk but had no visually apparent effect on the structure of the trail. The returning caterpillars stopped abruptly upon encountering the extracted section, then turned back to the branch. When a silk extract, prepared by soaking 8 mg of silk in 1 ml of the solvent for 24 hours, was pipetted back onto the half of the trail nearest the branch the larvae moved over this section of the trail, and then turned back (*4*). When the extract was laid out in a curved 8 cm-long line on a glass plate situated between the host and bridge, the caterpillars followed the artificial trail and returned to the tent.

The larvae distinguished between trails of new and aged extract laid down on the arms of Y-mazes drawn on paper cards. Five microliters of the extract were pipetted onto one of the 20 mm-long arms of the maze, then 7 to 11 hours later identical quantities were applied to the opposite arm and stem. Sections of card surrounding the choice point were cut away to sharpen the boundaries between the two trails and to assure that the head of each larva would sweep across both trails. Mazes were used only once. In 17 of 20 tests, first or second instar larvae started at the base of the stem turned onto the new trail when they reached the choice point. Trails prepared by pipetting 5 μl of the extract along 20-mm lines on paper cards still elicited trail-following from eight of ten larvae when the trails had aged indoors at 18°C for 196 hours, but the larvae turned repeatedly and took an average of 187.1 ± 44.3 (standard error) seconds to cross the trails. In comparison, all of ten larvae crossed newly deposited trails in an average of 17.9 ± 3.2 seconds. Solvent extracts of freshly spun tent silk also elicited the trail-following response from the caterpillars, suggesting that the chemical trail factor is a common component of both tent and trail silk.

Table 1. Attraction of unfed *M. americanum* caterpillars to trails of fed returning larvae. A small contingent of caterpillars that had just finished feeding was returned to the colony over one of three newly silked trails formed on the legs of the inverted tripod supporting their tent. Branches were then placed at the end of each trail and the number of unfed caterpillars moving from the tent to each branch recorded.

Test	Number of fed returning larvae	Total number of larvae moving to branches	Number selecting trail of fed returning larvae* (%)
1	2	117	73
2	1	12	91
3	4	52	90
4	7	60	95
5	6	94	60
6	5	86	99
7	7	37	86
8	1	17	94
9	5	76	68
10	2	119	84

*Expected percentage if selection was random, 33 percent.

Colonies enlarged their tents by spinning new silk over the old tent surface just prior to the initiation of foraging. The silk subsequently shrunk and lifted from the subjacent layer, leaving a gap of 10 mm or more. The initial contingent of fed caterpillars to return from the forage often formed a narrow trail over this fresh tent surface to a new or previously used entrance site. These "tent trails" were then followed by successive returning caterpillars. Late-emerging caterpillars, still unfed, became noticeably stimulated when they encountered these trails, and followed them off the tents.

These observations suggested that trails deposited by fed caterpillars as they returned from the forage were more attractive than the exploratory silk trails of outbound foragers, and functioned in a capacity similar to the recruitment trails of social insects. To further explore this possibility colonies were established on tripods and each was allowed to forage for several days on a branch placed at the end of the bridge. At the onset of a subsequent activity period the branch was removed, isolating the caterpillars on the tent and causing them eventually to lay down new exploratory trails over laterally extended rods that had been added to each of the legs of the tripod (Fig. 1). When the caterpillars were regularly moving over these rods, several larvae were let out over the bridge to the host. When they finished feeding they were returned to the tent by joining the branch to the end of a randomly selected rod. Just prior to returning the caterpillars, the colony was quieted by direct-

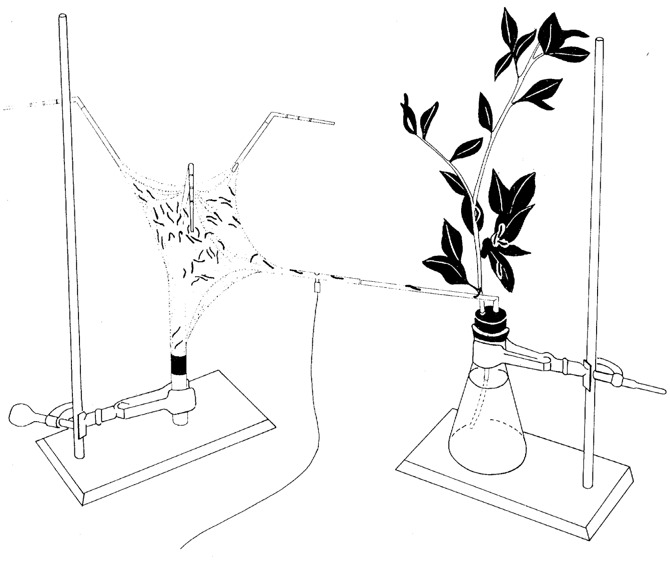

Fig. 1. An inverted tripod supporting a small colony of the fourth instar *M. americanum*. The caterpillars spin silk over the surface of their tent just prior to moving to the host to feed. Following the forage the caterpillars return to the tent to rest. The movement of the caterpillars is recorded as they pass over a phototransistor mounted under the bridge as an aid to anticipating the onset of the next forage and the timing of experiments. Lateral extensions have been added to the legs of the tripod in conjunction with an experiment described in the text. (Scale bar, 1 cm).

ing a light onto the tent, causing the larvae to aggregate in a tight, motionless cluster. The light was removed after the fed, returning larvae reached the tent. Additional branches were then placed at the ends of the other two rods and the number of larvae moving to each of the three branches over a maximum interval of 15 minutes was recorded as a measure of the ability of the fed larvae to attract their tentmates to food finds. In each of ten tests (seven colonies) most of the caterpillars moved to the branch located at the end of the rod used to return the initial contingent of larvae (Table 1). The caterpillars usually detected the new trail left by the fed, returning larvae at a distance from the base of the tripod leg, then followed it off the tent. The tent trails were not marked by the addition of new silk strands since, with few exceptions, the caterpillars that established these trails stopped spinning silk before they reached the edge of the tent. The exceptional attractiveness of these trails could be due either to the independent deposition of a relatively large quantity of a chemical trail factor that also occurs as a component of the exploratory silk trail, or to the deposition of a unique substance. Neither the anatomical source nor the site of secretion of this trail marker was determined.

An additional experiment showed that trails deposited by successful foragers on host branches at sites remote from the tent were also followed preferentially by their tentmates. The end of the long stem of a forked cherry twig was joined to the end of the bridge of a tripod bearing a colony of tent caterpillars. An artifically defoliated cherry branch was placed at the end of one of the arms of the fork and an intact branch at the end of the other. At the onset of a colony's activity period 30 caterpillars were let out onto the stem of the fork, then 15 of these were channeled onto each of the arms. Caterpillars let out onto the intact branch went immediately to feeding sites while those in the other group explored the defoliated branches. The branches were detached from the arms until the caterpillars in the former group had finished feeding. Both groups of larvae were then allowed to return simultaneously. Care was taken to prevent the larvae from moving onto the opposite arm of the fork. The returning larvae were collected individually when they reached the end of the stem of the fork and were not returned to the tent. New foliated branches were then placed at the ends of both arms of the fork and the remainder of the unfed caterpillars let out. In each of five tests (three colonies) the caterpillars strongly preferred the trail deposited by the fed, returning larvae (Table 2, column 1) (5).

Table 2. Number of *M. americanum* caterpillars selecting variously aged trails deposited by fed and unfed tentmates.

Test	Age of trail (hours)					
	0		6		0	6
	Fed	Unfed	Fed	Unfed	Fed	Fed
1	37	0	15	0	17	2
2	96	1	15	0	92	11
3	111	10	15	0	93	1
4	49	5	15	0	134	15
5	57	4	15	0	96	8
Average	70	4	15	0	86	7

The larvae in both initial groups of 15 laid down silk on their outbound and return trips. The caterpillars also deposited the silk at the same time. The ability of the unfed caterpillars to discriminate between the two trails could not, therefore, be due either to differences in silk quantity or trail age, indicating that successful foragers deposited an additional trail component particularly attractive to their tentmates. The first of the fed caterpillars to return usually established a narrower trail over the broad silk pathway laid down by the outbound foragers. The remainder of the returning larvae, both fed and unfed, as well as the newly introduced caterpillars, followed this narrower trail to and from the tent.

The trails laid down by successful foragers are persistent and are followed preferentially by the caterpillars during their next forage en masse. This was established by employing the same experimental procedure just described, but discrimination tests were not conducted until the colony's next activity period. In all of five tests (three colonies) the first 15 larvae let out onto the fork followed the trail deposited by the fed, returning larvae 6 hours previously (Table 2, column 2). These larvae moved onto an artifically defoliated branch placed at the end of the arm. To determine if the attractive factor had aged during this period a second contingent of 15 was then let out onto the stem and channeled onto the disfavored arm by temporarily blocking the other arm. These were collected onto a foliated branch placed at the end of the arm. When these larvae had finished feeding both contingents were allowed to return simultaneously. Foliated branches were then placed at the end of each arm and additional unfed colony members allowed to choose between the arm crossed by fed returnees approximately 6 hours previously and the arm just crossed by the fed returnees. In all cases the caterpillars strongly preferred the latter trail (Table 2, column 3).

This study indicates that the larvae of the eastern tent caterpillar communicate to a degree not previously suspected. Under field conditions the value of discriminative trail marking becomes increasingly apparent as the season progresses. Palatable leaves are initially abundant relative to the needs of the colony and a single feeding site may be repeatedly revisited. Later, as the leaves mature, the larvae feed preferentially on the youngest ones and range more widely in search of these. Eventually whole areas of the tree are defoliated and in the extreme, but not uncommon situation, small trees or trees supporting multiple colonies are completely denuded. In the latter case colonies are occasionally able to establish trails over the ground to distant trees. Increased foraging efficiency and inclusive fitness resulting from the sharing of successful search efforts among siblings may well have been a major impetus for the evolution of aggregative behavior in this species.

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References and Notes

1. T. D. Fitzgerald and E. M. Gallagher, *J. Chem. Ecol.* **2**, 187 (1976).
2. E. O. Wilson, *The Insect Societies* (Belknap, Cambridge, Mass., 1971).
3. This was determined by placing a fine thread in the path of a larva, then lifting it after it had passed. Of 480 larvae (eight colonies) moving from the tent to feeding sites over previously silked trails, 94 percent were spinning. Only 13 percent of these same larvae added silk to the trail as they returned to the tent after feeding. Larvae crossing previously unsilked branches, however, usually spun silk on both the outbound and return trip. Returning larvae rarely continued to spin after reaching the tent or the heavily silked bridge.
4. The silk was obtained by letting small groups of larvae that had not fed for more than 6 hours circle in petri dishes.
5. A separate test was conducted to determine if the choice of larvae in this and other tests recorded in Table 2 was also influenced by the choice of preceding larvae, since each outbound forager reinforced the trail with an additional strand of silk. The first 15 caterpillars let out onto the fork following the return of the initial contingent of 30 were channeled onto the arm crossed by the unfed returning larvae. The choice of larvae in the next group of 15 was then recorded. An average of 92 percent of these larvae (five tests) still selected the trail used by the fed, returning larvae. However, when the trails formed by the initial contingent of 30 larvae were allowed to age for 6 hours before the additional larvae were channeled onto the arm used by the unfed returning larvae, the additional silk deposited by these caterpillars had a marked influence on the choice of the next 15 caterpillars. An average of 46 percent of these larvae (five tests) selected the trail reinforced with the new silk.
6. I want to thank A. Costanzo for his technical assistance.

9 July 1976; revised 13 September 1976

A Blind Fish Can School

Abstract. *Vision is not required in order for fish to school. Five individual saithe,* Pollachius virens, *were able to join schools of 25 normal saithe swimming in an annular tank, while blinded with opaque eye covers. Test fish maintained position within the school indefinitely and responded to short-term movements of individuals within the school, although quantitative differences in reaction time and schooling behavior were noted. Five fish with lateral lines cut at the opercula were unable to school when wearing opaque eye covers. Although it is unlikely that blind saithe could school in the wild, the constraints of the apparatus permitted a demonstration of a role of the lateral line organ in schooling.*

Vision has long been considered a vital sensory component of schooling behavior (*1–3*) not least because fish usually stop schooling at low light intensities (*4*). Individual blind fish sometimes turned toward normal members of their own species (*1–3, 5, 6*) and have occasionally been observed to swim parallel to a control school for a few seconds (*3*) but have never been observed to maintain position for longer. We report here that adult saithe, *Pollachius virens*, wearing opaque eye covers (blinders) were capable of maintaining position indefinitely in a school of normal saithe swimming at approximately two body lengths per second, although their behavior within the school was quantitatively different from that of normal fish.

Underwater motion pictures taken in the wild (C.S.W.) show that saithe swim in polarized and synchronized groups, thus meeting Shaw's (*3*) definition of schooling. The film suggests that saithe are one of the strongest facultative schoolers (*7*) among the gadoid fishes.

Twenty-five newly caught saithe between 25 and 35 cm long were individually freeze-branded and introduced into an annular channel 1.5 m wide in the 10-m diameter gantry tank at the Marine Laboratory, Aberdeen, Scotland. A rectangular pool of light was projected onto the floor of the annular channel from spotlights on the counterclockwise rotating radial gantry; superimposed on it was a random speckled light pattern. The school of saithe were trained during a period of 4 days to swim above this moving background and, thereby, to remain in roughly the same area relative to the gantry (*8*). A continuous record of fish schooling in this area was provided in plan view by a television camera mounted on the gantry and linked to a videotape recorder. A saithe from the trained school was anesthetized and fitted with opaque blinders, which completely covered both eyes. After the fish had partially recovered from the anesthetic, it was returned to the channel, where it soon swam slowly about in a restricted area. (The rest of the school was swimming continuously around the tank.) The fish with blinders avoided swimming into the walls of the channel (*5*). After about an hour it began to respond to the school, turning toward the leading fish as they passed on each circuit of approximately 69 seconds. The test fish soon began to follow and then swim with the school for increasing distances, until after about 3 hours, it was able to school indefinitely (Fig. 1). After experiments lasting an additional 3 to 4 hours, we caught the test fish, removed the eye covers, and returned it to the tank, where it immediately rejoined its fellows and behaved quite normally. Three more fish tested in the same manner were each found capable of schooling while effectively blind.

In order to be certain that these fish were unable to see, we fitted blinders lined with a smooth layer of aluminum foil to another test fish. This saithe took longer to begin schooling again (about 6 hours), but once it had started, its behavior in the school was similar to that seen in the other four blind fish experiments. Further evidence that all the test fish were unable to see objects is that although hand-netting normal saithe from the channel was very difficult, none of the temporarily blinded fish avoided the mouth of nets placed in their path, even in bright light. In addition, the test saithe soon became dark in color (Fig. 1); this response is typical of blind fish, who match their skin color to their visual assessment of the surroundings (*9*).

When the school was startled by one of the experimenters suddenly reaching over the side of the tank, blind fish responded only if their neighbors were less than one body length away (Fig. 2) and only after these adjacent fish had accelerated. For six cases in which the fish with blinders did startle, the average lag from the acceleration of their nearest neighbor was 0.4 second (20 frames on the video record). The average lag for normal fish was 0.045, but this value is not strictly comparable since sighted fish may have responded directly to the stimulus (*10*). When startled, blinded fish tended to bump into the walls of the tank and into other fish, whereas sighted fish rarely did this. On no occasion did we observe a test fish collide with the walls of the tank or other objects when approaching them at normal speed. Very occasionally they swam into others in the school, but only when the latter were executing some rapid maneuver [see also (*5*)].

Fish wearing blinders did not school normally but appeared to change position within the school far more often than their sighted fellows did. In order to test this, we superimposed a square grid on the video image and recorded the frequency with which test fish and controls