Defensive use of a fecal thatch by a beetle larva (*Hemisphaerota cyanea*)

Thomas Eisner* and Maria Eisner

Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

Contributed by Thomas Eisner, January 5, 2000

The larva of the tortoise beetle, *Hemisphaerota cyanea* (Chrysomelidae, Cassidinae), constructs a thatch from long filamentous fecal strands, beneath which it is totally concealed. The thatch is not discarded at molting but is enlarged by addition of strands as the larva grows. Thatch construction begins when the larva hatches from the egg. Pupation occurs beneath the thatch. Two predators, a coccinellid beetle larva (*Cycloneda sanguinea*) and a pentatomid bug (*Stiretrus anchorago*), were shown to be thwarted by the thatch. However, one predator, a carabid beetle (*Calleida viridipennis*), feeds on the larva by either forcing itself beneath the thatch or chewing its way into it. The attack behavior is stereotyped, suggesting that the beetle feeds on *Hemisphaerota* larvae as a matter of routine.

Coleoptera | Chrysomelidae | Carabidae | *Calleida*

The larvae of tortoise beetles (Chrysomelidae, Cassidinae), so called because of the turtle-like appearance of the adults, have an odd habit. Instead of ridding themselves of their feces in conventional fashion, they void these onto the back, where the wastes accumulate to form what in many species takes on the appearance of a shield (Fig. 1 *A* and *B*). The shield does not rest directly on the dorsum of the larva, but on a fork that projects forward from the abdominal tip (Fig. 1*C*) and serves specifically to take up the feces (1–6). By revolving the abdominal tip and thereby rotating the fork, the larva can bring the shield to face in any direction. It can thus deter a number of enemies, including ants (1–5).

The fecal shield varies in appearance and physical consistency in different larvae (1–4). In some species it is rigid, in others pasty, and whereas in some it is wide enough to provide cover for the entire larva, in others it is more narrowly shaped. In addition to the feces, the dorsal fork usually bears the remnants of molted larval skins (1–4).

Most remarkable, perhaps, is the fecal "thatch" of *Hemisphaerota cyanea* (henceforth called *Hemisphaerota*) (7). In the larva of this beetle, the feces are emitted in strands, which, as they build up over the course of larval life, form a loose assemblage that totally hides the larva from view (Fig. 1 *H* and *I*). We here describe how the larva forms this remarkable structure, how the structure serves in defense, and how this defense is breached by a beetle that preys on the larva.

Materials and Methods

Observations were made on the grounds of the Archbold Biological Station, Lake Placid, Highlands County, FL. The habitat at the site is typical Florida "scrub," a unique dry-land ecosystem characterized by sandy ridges and scrubby vegetation (8). *Hemisphaerota* is a common inhabitant of the scrub, where it occurs, at all developmental stages, on two palmetto plants, *Serenoa repens* and *Sabal etonia*. The blue iridescent adult (Fig. 1*D*) and the thatch-covered larva are very conspicuous on these plants. Both larva and adult feed by trenching, that is, by scraping narrow linear grooves into the surface of the palmetto fronds with their mouthparts.

For laboratory study, larvae were transported indoors on a piece of the palmetto frond on which they had been feeding. To keep the palmetto piece fresh, its cut edges were kept wrapped in wet tissue paper. Larvae built normal thatches in confinement and developed normally into adults.

The coccinellid beetle (*Cycloneda sanguinea*), pentatomid bug (*Stiretrus anchorago*), and carabid beetle (*Calleida viridipennis*) used in predation tests were also taken at the Archbold Station (all three are referred to henceforth by their generic designation).

At the end of the experiments, all surviving insects were released close to where they had been collected. One *Calleida* was kept for voucher purposes.

Photographs were taken mostly with a Wild (Heerbrugg, Switzerland) M400 Photomakroscope. For scanning electron microscopy, specimens were critical-point dried and gold coated.

Observations and Results

Life History. The eggs of *Hemisphaerota* are large, ovoid, and laid singly (Fig. 1*E*). They are embedded in a hardened gelatinous matrix, encrusted with fecal pellets [use of excrement in protection of eggs is common in chrysomelids (9, 10)]. The larvae remain thatch-covered throughout development. Pupation occurs on the larval foodplant, beneath the thatch (Fig. 2*D*). A droplet of adhesive, on the ventral surface of the abdomen just anterior to the abdominal tip, fastens the pupa to the plant surface. The adult, upon emergence, remains beneath the thatch until its exoskeleton has hardened.

Thatch Construction. Within minutes of hatching, while still beside the egg, the larva begins feeding. The first fecal strand emerges from the anal turret minutes later (Fig. 1*E*). Subsequent strands follow in quick succession. By the end of 1.6 h, five strands are in place (Fig. 1*F*). After 12 h, the thatch is virtually formed (Fig. 1*G*). Whereas the first two strands are typically short, subsequent ones are longer and coiled. During production of a strand, the larva maintains the anal turret flexed, either to the right or left, with the result that the strands are made to curve around either the right or the left side of the larva (Fig. 2 *A* and *E*). "Right hand" and "left hand" strands are typically produced in regular alternation.

The caudal fork (Fig. 2*B*) is essential to thatch construction, because it is to the fork that the individual strands are fastened upon completion. When a strand has been extruded to its full length, the larva rotates the anal turret upward until the turret contacts the fork. It then emits a droplet of glue from the turret, while at the same time pinching off the strand (by anal constriction?). The glue hardens quickly, with the result that the strand is cemented to the fork (Fig. 2 *H–J*). Consecutive strands are cemented one beside the other on the fork.

As is the case with other cassidine beetle larvae (11), the fork is not shed at molting. Instead, it is retained as a terminal

*To whom reprint requests should be addressed. E-mail: te14@cornell.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.050002197. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.050002197

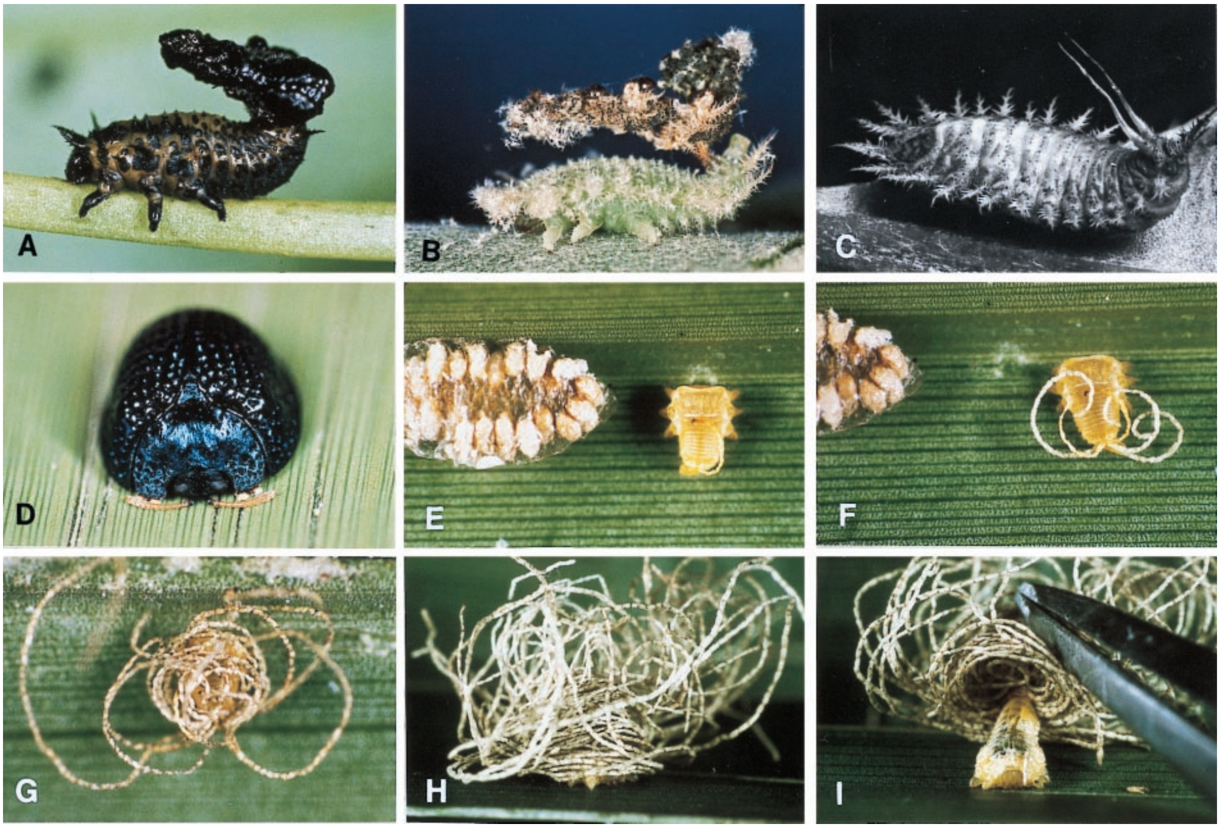

Fig. 1. (*A*) *Chelymorpha cassidea* and (*B*) *Gratiana pallidula*, two cassidine beetle larvae that carry fecal shields above their backs. The shields also contain exuviae, as is apparent in *B*. (*C*) Larva of the cassidine *Cassida rubiginosa*, from which the fecal shield has been removed to expose the caudal fork that ordinarily holds the shield. (*D*) *Hemisphaerota cyanea*, adult. (*E*) Larva of same, beside egg, 40 min after hatching (note single fecal strand). (*F*) Same larva, 1.6 h after hatching (fifth fecal strand is being extruded). (*G*) Same larva, 12 h after hatching (thatch is virtually completed). (*H*) Thatch of a third instar larva. (*I*) Same as preceding, pried up to show the larva. (*A–C,* ×5; *D,* ×7.5; *E–G,* ×10; *H* and *I,* ×4.)

extension of the newly formed fork of the subsequent instar. The prongs of the new fork are never extricated from those of the old, with the result that, at each molt, a distal section is added to the fork (the section carrying the strands from the previous instar), and a new basal portion is provided for attachment of new strands. By the end of larval development, therefore, the fork is a composite structure, consisting of the individual forks of each instar, fitting one over the other like stacked hats (Fig. 2*B*). There is thus a fixed spatial arrangement of strands in the thatch. The oldest and narrowest strands, produced when the larva was youngest, line the domed central portion of the thatch, whereas the thicker strands, produced at later larval stages, provide the outer covering. More strands are produced during larval life than needed for construction of the central portion of the thatch. Late in larval life, therefore, many strands are produced that are "extras." These may be voided by the larva without being curved in any particular fashion (the anal turret may remain undeflected during their production) and, rather than being attached to the fork, may be extruded until they break off spontaneously.

Dissection of the larva reveals the presence of an exceptionally long hindgut, with a terminal straight section that extends nearly the length of the abdomen (Fig. 2*C*). One can expect the fecal strands to be compacted in this section, because the hindgut typically serves for water reabsorption in insects (12). We envision the fecal strands to emerge from the anus semisoft in consistency and to be bent into curvatures determined by the degree of deflection of the abdominal turret.

Microscopic examination revealed the strands to be membrane-coated (Fig. 2*G*). Encased in "skin," like sausages, they can be expected to be less susceptible to breakage. The membrane could be conventional peritrophic membrane, such as is produced by many insects (12).

Thatch Repair. The larva has the ability to repair the thatch if it is damaged. Thus, if the "roof" of the thatch is cut open with scissors (Fig. 3 *A–D*), the larva needs produce only two strands to provide a degree of renewed cover for itself. It first produces a strand on one side, then one on the other, and it brings both into place so their coils come to lie side by side within the orifice. The two strands are initially laid out around the outside of the thatch (Fig. 3*A*). For them to be positioned requires that they be swung inward. The larva does this for each strand by rotating the anal turret, while at the same time lifting the thatch (by deflecting the abdominal tip downward) so as to make room for the strand. Each strand is cemented to the fork after it is in place. Strands subsequently produced provide for the total closure of the orifice. One wonders how the larva "knows" that it is the roof of the thatch that is missing. The increased lighting of its back is apparently not the reason, because we found the larva to repair the roof similarly in darkness. Perhaps the decisive factor is the absence of proprioceptive feedback from the strands ordinarily present over its back.

Damage inflicted to one side of the thatch (Fig. 3 *E* and *F*) is automatically repaired in the course of normal strand production. Because every second strand extruded curves around the damaged side, the thatch on that side is eventually reconstituted. Damage to the front of the thatch is similarly repaired (Fig. 3 *G* and *H*). The very first strand is coiled in such fashion as to provide cover for the front.

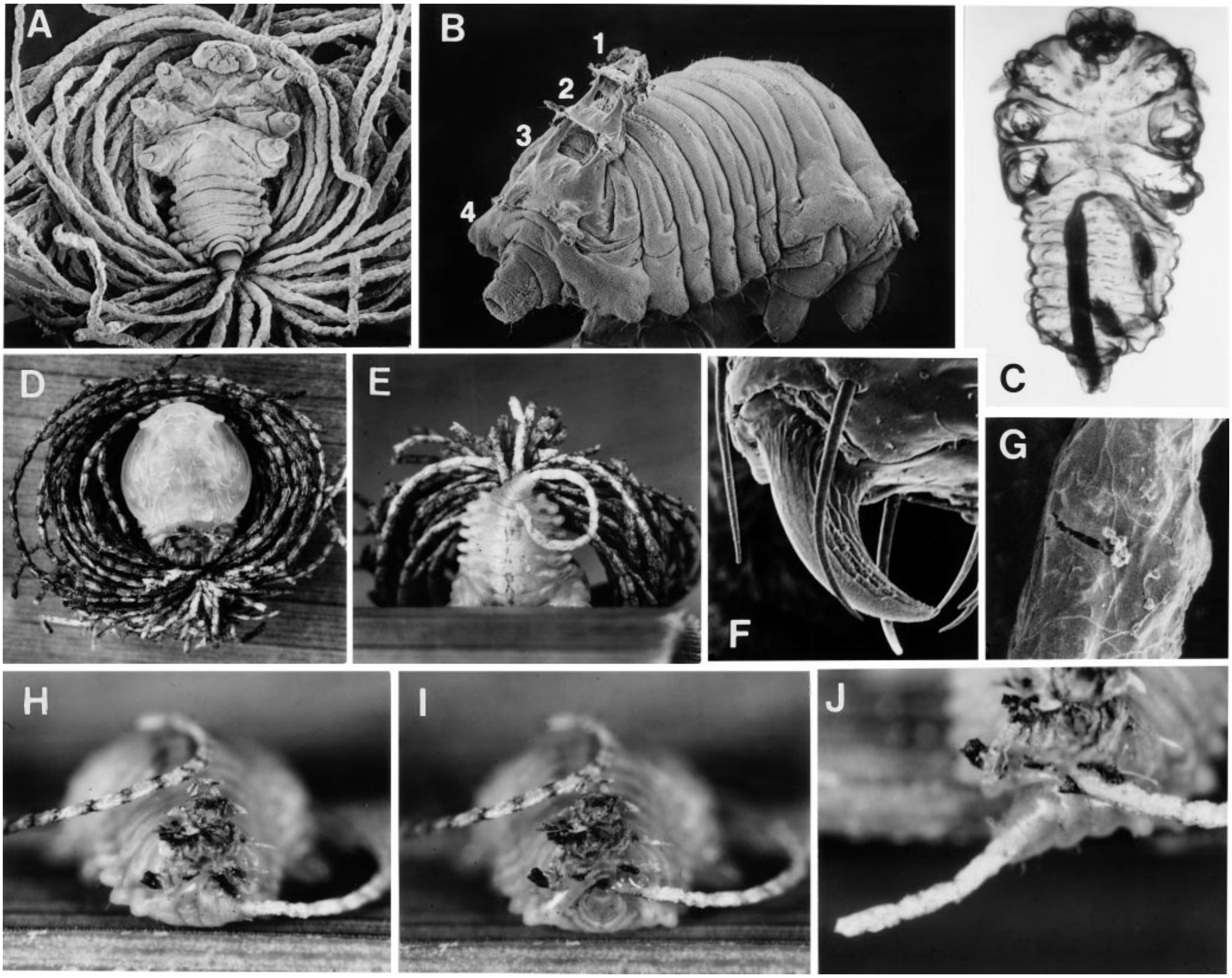

Fig. 2. *Hemisphaerota cyanea*. (*A*) Larva (third instar) in ventral view showing thatch, and single fecal strand emerging from anal turret. (*B*) Larva (fourth instar) showing caudal fork above anal turret; the fork is a composite of the individual forks from instars 1, 2, 3, and 4, stacked one on top of the other (the thatch has been detached from the larva). (*C*) Larva, showing the lengthy recurved hindgut in which the fecal strands are formed (preparation treated with aqueous KOH; the hindgut is cuticle lined and has survived the treatment). (*D*) Ventral view of pupa that has been detached from a palmetto frond. (*E*) Posterior view of a larva that was caused to raise its rear (and therefore to push down the front of its thatch) in response to poking of the anterior margin of the thatch. (*F*) Tarsal claw of larva. (*G*) Close-up view of a fecal strand, showing small tear in the enveloping membrane. (*H–J*) Three consecutive stages in strand production (larva has been deprived of its thatch and is producing new strands). In *H*, it has nearly completed the first strand (the strand coils around the larva). Note that the anal turret is deflected to the right. In *I*, it has pinched off the first strand and has fastened it to the base of the fork with a glistening droplet of glue. In *J*, it has turned the turret in the opposite direction and has begun to produce the next strand. (*A*, ×12; *B*, ×20; *C*, ×15; *D* and *E,* ×5; *F*, ×620; *G*, ×150; *H* and *I,* ×10; *J,* ×16.)

Defense. Once the thatch is constructed, the larva is concealed and physically shielded. Moreover, the larva is anchored to the plant at virtually all times. The anchorage is provided by the sharp tarsal claws (Fig. 2*F*), which the larva keeps inserted in the substrate. It takes considerable leverage to pry a larva loose (Fig. 1*I*). The larva is vulnerable when on the move, but it is hardly ever ambulatory. It feeds at most times, and when so doing moves sideways at an extremely slow pace, without disengaging all claws at a time.

By resorting to postural adjustments of the body, the larva is able to put its thatch to active defensive use. Thus, by appropriate flexion and rotation of the abdominal tip, it is able to tilt the thatch toward the right or left when one side of the thatch is poked, or upward when the rear is poked, or downward anteriorly if the front is poked (Fig. 2*E*). Such postural adjustments are executed already by first instar larvae, even before they have completed construction of the thatch.

Predation Tests: *Cycloneda* and *Stiretrus*. The tests with *Cycloneda* involved placing one individual of this coccinellid larva (last instar) in a Petri dish (9 cm diameter), together with two *Hemisphaerota* larvae (last instar), one with thatch intact, the other denuded (thatch removed with forceps). Events were monitored for 1 h. The test was replicated five times, with five freshly collected last-instar *Cycloneda* larvae. The results were consistent. The denuded larvae were all eaten (Fig. 3*I*) and the thatched larvae all survived. The *Cycloneda* made repeated contact with the thatched larvae, but they consistently ignored these. They did not attempt to bite the thatch or to force themselves into it. Nor were they prompted, on touching the thatch, to undertake the sort of cleansing activities that many insects execute when coming in contact with a chemical irritant.

The tests with the predaceous pentatomid *Stiretrus* were comparable, in that they involved presenting prey to single individuals of the predator in Petri dishes (9 cm diameter), but

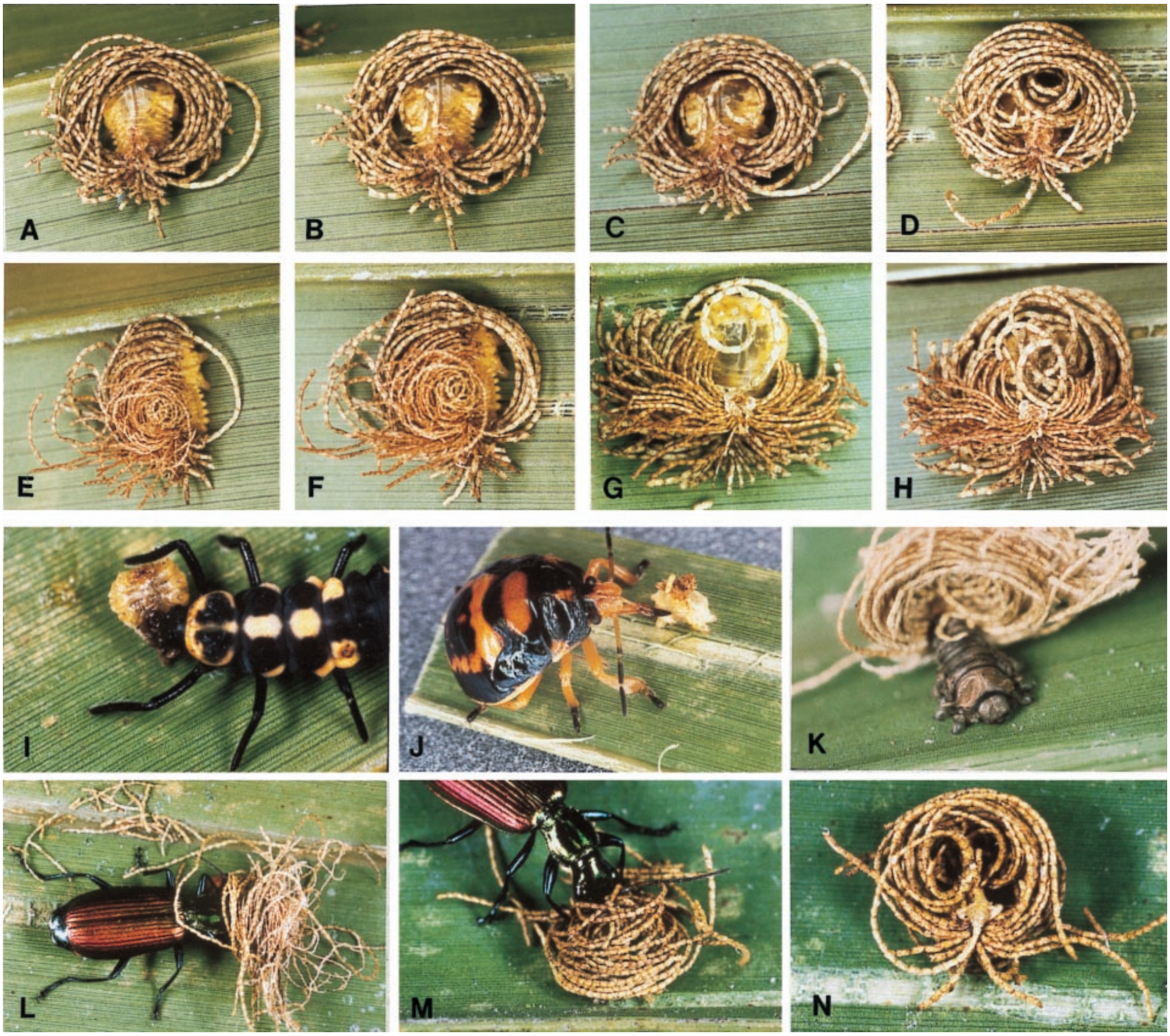

Fig. 3. (*A–H*) *Hemisphaerota cyanea* larvae: thatch repair. (*A–D*) Repair of a window cut into the top of the thatch (*D* is 23 h after mutilation). The first strand, seen to the right of the thatch in *A,* has been laid into place in *B*. A second strand has been positioned in *C* (while a third is in the making), and in *D* the window has been all but repaired. (*E* and *F*) Repair of the right side of the thatch (*F* is 23 h after mutilation). (*G* and *H*) Repair of the front of the thatch (*H* is 22 h after mutilation). (*I*) *Cycloneda sanguinea* larva eating a denuded *Hemisphaerota* larva. (*J*) *Stiretrus anchorago* nymph sucking out a denuded *Hemisphaerota* larva. (*K*) *Hemisphaerota* larva found dead in the field. Discoloration may indicate that death was from microbial infection. (*L–N*) *Calleida viridipennis,* attack behavior. In *L,* the beetle is shown feeding on the *Hemisphaerota* larva by pushing itself beneath the margin of the thatch, whereas in *M* it has reached the larva by biting its way through the top of the thatch. After the meal (*N*), all that is left is the thatch and the anal turret. (*A–H,* ×3.2; *I,* ×7; *J,* ×3.5; *K, M,* and *N,* ×4; *L,* ×3.)

the offerings here consisted of two thatched and two denuded *Hemisphaerota* larvae (last or penultimate instar). Four freshly collected *Stiretrus* nymphs (last instar) were used. Tests were of 2 h duration. The results were again consistent, in that all eight denuded larvae were eaten, and the thatched larvae were spared. Typically, the *Stiretrus* located first one denuded larva, then the other, and ate each by impaling it on the proboscis and sucking it dry (Fig. 3*J*). Although the *Stiretrus* came into repeated contact with the thatched larvae, it consistently ignored these. It did not attempt to probe the thatch, nor was it caused, on contacting the thatch, to extend its proboscis, as predaceous pentatomids typically do when anticipating a meal.

The Counter-Strategy of *Calleida*. We became interested in this beautiful purplish-green, iridescent carabid beetle when we discovered one individual in the field beside a *Hemisphaerota* larva, its head buried in the thatch. We brushed beetle and larva into a vial, only to note that this did not cause the beetle to withdraw from the thatch. Eventually it did, upon which we examined the thatch and saw that the larva had been eaten. We maintained the beetle in a Petri dish (5 cm diameter) and presented it with a total of 15 *Hemisphaerota* larvae (various instars) over a period of 20 days. The larvae were all eaten. The beetle initiated the attack the moment it came in contact with the thatch. It either forced its way beneath the margin of the thatch to reach the larva by that route (Fig. 3*L*), or it bit its way through the top of the thatch and reached the larva from above (Fig. 3*M*). Events then proceeded invisibly, because the beetle "worked" on the larva with its front end hidden beneath the thatch. Suffice it to say that when the beetle finally withdrew, examination of the thatch revealed that the larva had been entirely eaten, except for the anal turret, which was left behind (Fig. 3*N*). The beetle's behavior was rigidly consistent. We eventually captured two more *Calleida*, also at the Archbold Station, and offered each a *Hemisphaerota* larva. They dispatched these quickly by chewing their way into the thatch from above.

Discussion

The *Hemisphaerota* larva is evidently well adapted for survival. The anchorage provided by the tarsal claws, the maneuverability of the thatch, and the possession itself of the thatch all combine to bestow defensive capability on this insect. The list of enemies potentially deterred by the thatch must extend beyond the coccinellid larva and predaceous pentatomid tested by us. Ants must certainly constitute a hazard and might well be thwarted by the thatch, as they are by the fecal shield of other cassidines (1, 5). It is noteworthy that, whereas the *Hemisphaerota* thatch appears to be pentatomid-proof, the shield of other cassidines offers only limited protection against heteropterans (2).

Such evidence as we have suggests the thatch to be chemically inert and to act by virtue of physical deterrency alone. This would be contrary to what has been proven for another cassidine larva, in which chemicals from the foodplant, specifically mono- and sesquiterpenes, are present in the fecal shield and contribute to the defensive effectiveness of the device (1). Dorsal fecal loads carried by other chrysomelid beetle larvae appear also to derive their deterrency from compounds (e.g., fatty acids, tannins, saponins, and alkaloids) derived either unchanged or with modification from the diet (13, 14). We cannot rule out the possibility that the thatch of *Hemisphaerota* is also in some measure chemically offensive.

Calleida is notable in that it circumvents the defense of *Hemisphaerota.* Its thatch-breaching behavior is so stereotyped that one wonders whether the beetle is specialized to feed on *Hemisphaerota. Hemisphaerota* larvae are a common and conspicuous staple on their palmetto hosts, and *Calleida*, with its large eyes, agility, flight capacity, and apparent diurnal feeding habits, may have little difficulty locating the larvae in numbers. We do not know whether other predators feed on *Hemisphaerota* larvae as well. In our many field trips to the Florida scrub, beginning in 1958, we failed to find evidence for the existence of such enemies. However, *Hemisphaerota* larvae are often found dead, still anchored to the plant, their bodies intact but darkly discolored, in a condition suggestive of death from microbial infection (Fig. 3*K*).

Strategies for circumvention of prey defense have evolved in a number of arthropods, including both herbivores and carnivores. Thus, for example, a number of insects that feed on the leaves of latex-producing plants prepare the leaves for ingestion by severing latex canals and causing the latex to drain from the leaves (15). Similarly, grasshopper mice that feed on certain beetles with dischargeable defensive glands overpower these beetles by holding them in such fashion that their glandular discharges are misdirected into the soil (16). Ant lions, when feeding on formicine ants, kill these without risking exposure to the ants' defensive acidic secretion (17), and orb weaving spiders, when feeding on bombardier beetles, encase these in silk, thereby shielding themselves against the beetles' hot quinonoid discharges (18). A refined strategy is practiced by phengodid beetle larvae, which feed on millipedes. They overrun these and paralyze them with a quick injection of enteric fluid into the neck, thereby preventing the millipedes from discharging their defensive secretion (19).

The ability to repair the thatch could be of use to the larva under real circumstances. One could readily envision the thatch being damaged by wind, for instance, or by torrential rain. As a rule, however, one finds larval thatches to be minimally damaged in nature.

Given the inclemencies of the scrub habitat, where episodes of downpour can alternate with periods of extreme heat, one can imagine the thatch serving also in other capacities. It could, for instance, shield the larva from excessive sunlight or serve for retention of moisture following rain or after dawn. There is evidently more to be learned about the "overhead sewer system" of the *Hemisphaerota* larva.

We are indebted to the personnel of the Archbold Biological Station for numerous kindnesses extended to us over the years. Mark Deyrup and Jerrold Meinwald provided helpful comments on the manuscript. This study was supported in part by National Institutes of Health Grant AI02908. This paper is no. 169 from the series, *Defense Mechanisms of Arthropods*; paper no. 168 is ref. 20.

1. Gómez, N. E., Witte, L. & Hartmann, T. (1999) *J. Chem. Ecol.* **25,** 1007–1027.
2. Olmstead, K. L. & Denno, R. F. (1993) *Ecology* **74,** 1394–1405.
3. Olmstead, K. L. (1994) in *Novel Aspects of the Biology of Chrysomelidae*, eds. Jolivet, P. H., Cox, M. L. & Petitpierre, E. (Kluwer, Dordrecht, Netherlands), pp. 311–318.
4. Olmstead, K. L. (1996) *Chrysomelidae Biology,* Ecological Studies, eds. Jolivet, P. H. & Cox, M. L. (SPB Academic Publishing, Amsterdam), Vol. 2, pp. 3–21.
5. Eisner, T., van Tassell, E. & Carrel, J. E. (1967) *Science* **158,** 1471–1473.
6. Blum, M. S. (1994) in *Novel Aspects of the Biology of Chrysomelidae*, eds. Jolivet, P. H., Cox, M. L. & Petitpierre, E. (Kluwer, Dordrecht, Netherlands), pp. 277–288.
7. Beshear, R. J. (1969) *J. Georgia Entomol. Soc.* **4,** 168–170.
8. Deyrup, M. & Eisner, T. (1993) *Nat. Hist.* **102** (12), 42–47.
9. Hilker, M. (1994) in *Novel Aspects of the Biology of Chrysomelidae*, eds. Jolivet, P. H., Cox, M. L. & Petitpierre, E. (Kluwer, Dordrecht, Netherlands), pp. 263–276.
10. Selman, B. J. (1994) in *Novel Aspects of the Biology of Chrysomelidae*, eds. Jolivet, P. H., Cox, M. L. & Petitpierre, E. (Kluwer, Dordrecht, Netherlands), pp. 69–74.
11. Engel, H. (1935) *Z. Morph. Oekol. Tiere* **30,** 42–96.
12. Wigglesworth, V. B. (1972) *The Principles of Insect Physiology* (Chapman and Hall, London), 7th ed.
13. Morton, T. C. & Vencl, F. V. (1998) *J. Chem. Ecol.* **24,** 765–785.
14. Vencl, F. V. & Morton, T. C. (1998) *Chemoecol.* **8,** 25–32.
15. Dussourd, D. E. & Eisner, T. (1987) *Science* **237,** 898–901.
16. Eisner, T. (1966) *Nat. Hist.* **75** (2), 42–47.
17. Eisner, T., Baldwin, I. T. & Conner, J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6716–6720.
18. Eisner, T. & Dean, J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1365–1367.
19. Eisner, T., Eisner, M., Attygalle, A. B., Deyrup, M. & Meinwald, J. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 1108–1113.
20. Eisner, T., Eisner, M., Rossini, C., Iyengar, V. K., Roach, B. L., Benedikt, E. & Meinwald, J. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 1634–1639.