Millipede defense: Use of detachable bristles to entangle ants*
(prevention/mechanical defense/Diplopoda/Polyxenida/Formicidae)

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ABSTRACT The millipede Polyxenus fasciculatus (Diplopoda; Polyxenida) defends itself against ants by use of a pair of bristle tufts at its rear. When attacked, it wipes the tufts against the ants, thereby causing these to become enmeshed by bristles that detach from the tufts. Ants contaminated with bristles desist from their assault. The bristles have grappling hooks at the tip by which they lock onto setae of the ants and barbs along their length by which they interlink. In attempting to rid themselves of bristles, ants may succeed only in further entangling themselves by causing the bristles to become enmeshed. Ants heavily contaminated may remain entangled and die. Most millipedes have chemical defenses; polyxenids, instead, have a mechanical weapon.

Millipedes (Arthropoda; Diplopoda) are an ancient group. They are also a hardy group, for they have survived since Silurian times (1) despite the evolutionary onslaught of insects. Insects are among their chief enemies, and millipedes are well-protected against insect attack. Most possess defensive glands, in the form of integumental sacs, from which they discharge fluids when disturbed. The fluids are of variable composition. Some millipedes discharge p-benzoquinones (orders Julida, Spirobolida, and Spirostreptida), others eject phenols (order Callipodida), and still others emit cyanogenic compounds (order Polydesmida), quinazolinones (order Glomerida), or alkaloids (order Polyzonida) (2). One group not known to be chemically protected are the polyxenids (order Polyxenida). Comprising some 60 species in 4 families (3, 4), polyxenids are only millimeters in length, furtive in habits, and for the most part little known. We report here on the defense of one species, Polyxenus fasciculatus (family Polyxenidae), that protects itself against ants by entangling these with detachable bristles that it sheds from two brush-like tufts at its rear (Fig. 1).

MATERIALS AND METHODS

The Millipede. We collected P. fasciculatus on the grounds of the Archbold Biological Station, Lake Placid, Highlands County, FL, under loose bark of slash pine (Pinus elliottii). Such habitat may be favored by polyxenids generally. The European Polyxenus lagurus has been reported from under bark of conifers, sycamore, beech, and maple (5). Ants also frequent such habitat. While probing for P. fasciculatus, we routinely encountered foraging workers of two myrmecine ants (Crematogaster ashmeadi and a closely related undescribed congener).

P. fasciculatus has the typical appearance of a polyxenid (Fig. 2A and C). In common with other members of its order but unlike millipedes generally, the body of P. fasciculatus is densely beset with setae. These are arranged in transverse rows along the back and into sets of flower-like clusters along the flanks. The two tufts that constitute the defensive apparatus form what appears to be a single caudal projection (Fig. 2A). The bristles within these tufts are extremely fine in comparison to the body setae, and barely resolved at lower magnifications. Because they glint in light, the tufts appear brighter than the body itself.

We maintained P. fasciculatus individually in small containers with pieces of slash pine bark and a source of moisture (wetted cotton). The animals were used in predation trials within at most a few days of being collected.

The Ants. Two species of myrmecine ants, C. ashmeadi and Xenomyrmex floridanus, both collected on the grounds of the Archbold Biological Station, were used in the predation trials. C. ashmeadi were brought into the laboratory with leaves of turkey oak (Quercus laevis) on which they had been tending aphids. X. floridanus were taken as a colony within the twig in which they were nesting. The ants were tested in trials on the day they were collected.

Predation Trials. These were staged in small Petri dishes (5 cm diameter). Each trial consisted of introducing first a P. fasciculatus into the dish and then, after some minutes, two to four ants. Events were monitored with a stereomicroscope until at least one millipede–ant encounter took place.

Fourteen millipedes were tested: 10 in individual trials with C. ashmeadi and 4 in individual trials with X. floridanus. New sets of ants were used in all trials.

Scanning Electron Microscopy. In preparation for scanning electron microscopy, individual P. fasciculatus and C. ashmeadi were killed (immersion in 70% ethanol), dehydrated (100% ethanol), and critical-point dried (6). The ants were dropped in ethanol without being directly touched immediately after they were incapacitated by a millipede in a predation trial.

RESULTS

The Caudal Tufts. Ordinarily, in the undisturbed animal, the caudal tufts are closely appressed, so that they form what is essentially a single bundle of bristles. The bristles are aligned largely along the surface of the bundle and are absent from the

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FIG. 2.  *P. fasciculatus* (C–K are scanning electron micrographs).  (A) Live millipede; the caudal tufts form the light-colored projection on right. (B) Ant (*C. ashmeadi*) incapacitated by contact with a millipede's tufts. (C) Whole millipede. (D) Close-up of bristles in tufts; note barbs on shaft of bristles. (E) Oblique view of tufts. (F) Distal portion of a bristle, showing barbs on shaft and terminal "grappling hook." (G) Entangled ant (*C. ashmeadi*), showing the mesh of bristles that has immobilized the legs. (H) "Grappling hook" fastened to an ant's seta. (I) Same, fastened to a number of setae. (K) Detail of a tangle of bristles. (*A* and *B*, bar = 0.5 mm; *C*, *E*, and *G*, bar = 0.1 mm; *D*, *F*, and *H–K*, bar = 10 μm.)
center (Fig. 2E). Individually, the bristles consist of a filamentous shaft and a modified tip. The shaft is densely beset with barbs, all pointing hook-like at an angle toward the bristle tip (Fig. 2D). The tip itself is fashioned as a multipronged grappling hook (Fig. 2F).

When we prodded live *P. fasciculatus* with a fine probe, we noted that as the animals moved to evade the disturbance, they often retracted their rear toward the probe, while at the same time abruptly splaying their caudal tufts. In doing so, they seemed always to succeed in touching the probe with the tufts. The splayings were of the briefest duration, and occurred sometimes repeatedly in quick succession. We did not succeed in photographing the tufts in their splayed condition.

While experimenting with live *P. fasciculatus*, we also noted that the bristles of the tufts detach with relative ease. We found, for instance, that we could readily pluck away bunches of bristles simply by tugging at the tufts with fine (watchmaker's) forceps.

**Predation Trials.** A total of 32 ant—millipede encounters were witnessed in the course of the 14 predation trials with ants. The results were fundamentally similar with the ants of both species. The encounters ranged in severity from mere antennal probings by individual ants to more intense "inspections" in which ants brought their mouthparts to bear on the millipede in evident anticipation of biting. The millipedes responded quickly and consistently. They flexed their rear toward the ant, momentarily splayed their tufts, and immediately moved away.

All ants discontinued their assaults when touched by the tufts, and all were affected by the bristles that came to cling to them as a consequence of being "stroked." Those that received only a few bristles, as for instance on an antenna or leg, sometimes succeeded in cleaning themselves within minutes. But others, whose oral palps, antennae, and forelegs had all received bristles, were sometimes literally immobilized. They attempted to preen themselves, but the efforts seemed only to aggravate their plight. As they wiped antennae with forelegs, drew appendages through the mouthparts, or stroked forelegs against one another, they succeeded only in further entangling themselves. It appeared as if through preening they were merely spreading a "glue." Such ants would sometimes eventually lose their footing and come to lie motionless on a side (Fig. 2B). We preserved four such severely incapacitated individuals (two of each species) for scanning electron microscopy. Another four that we kept live (all *C. ashmeadi*) were found to be still partly entangled and no longer living when checked after 37 h. Of the remaining 24 ants, which had all received lesser "dosages" of bristles, 4 (all *C. ashmeadi*) were preserved. The other 20, when checked after 37 h, were found to be disentangled, essentially bristle-free, and ambulatory. Residual pieces of bristles were noted on only some of them.

Most millipedes in the trials had encounters with more than one ant. Two had encounters with three ants, and one with four ants. In none of these millipedes that endured multiple encounters were the tufts noted to have undergone more than a partial loss of bristles.

The scanning electron micrographs (Fig. 2 G–K) provided evidence of how the bristle entangling mechanism works. The bristle tips are the functional units that insure that the bristles become anchored to the ant. They appear designed to grasp setae as grappling hooks. Indeed, scrutiny of the surface of entangled ants with the scanning electron microscope revealed numerous sites where bristles had become fastened by their tips to individual setae or groups of setae (Fig. 2 H and I).

Another important function is fulfilled by the barbs on the bristles. These act as hooks by which the bristles can become cross-linked to form loose aggregates, in a virtually haphazard fashion. The bristle tip can themselves hook onto the bristles, thereby providing yet additional possibilities for bristle link up (Fig. 2K). The formation of bristle aggregates appears to play a basic role in the incapacitation of the ants. Entangled appendages in encumbered ants were always noted to be "strung" together by loose masses of interconnected bristles (Fig. 2G).

**DISCUSSION.**

The defensive function of the caudal tufts of *P. fasciculatus* seems established, certainly *vis à vis* ants. Given that the tufts are of general occurrence in Polyxenida, one can imagine them serving for defense throughout the order, providing the benefit that in other millipedes is offered by chemical weaponry. Ants are doubtless among the principal enemies of millipedes; ants are ubiquitous in the habitats frequented by millipedes and they often compound their threat by foraging in groups. To the minute individual polyxenid, even a single ant poses a hazard. It is notable, therefore, that *P. fasciculatus* proved capable of thwarting the attacks of several ants in succession.

The possibility that the polyxenid tufts serve for defense had been raised by others. Reinecke (7) and Latzel (8) noted that polyxenids, when prodded, splay the tufts and revolve them toward the offending instrument. Bode (9) found these responses still to occur after decapitation of the millipede, indicating that they are under reflex control. Drawings that Reinecke (7) provides of the caudal bristles of *P. lagurus* show these structures to be essentially identical to those of *P. fasciculatus*.

We envision the mechanics of ant entanglement by polyxenid bristles to involve two actions. The first action occurs when the millipede brushes its tufts against the ant. This causes numbers of bristles to become fastened to the ant, and then to be pulled from the tufts as the millipede moves away. The second action is triggered when the ant attempts to clean itself. These attempts, instead of enabling the ant to rid itself of the bristles, cause only a further snarling of the bristles, with the result that the ant becomes increasingly encumbered. Ants that in our trials became heavily contaminated with bristles did not survive.

Polyxenids are bound to have enemies other than ants. Since setae and other hair-like elaborations are a standard feature of the arthropod integument, virtually any small arthropod could prove vulnerable to the polyxenid defense. Potential enemies of polyxenids could include spiders, centipedes, pseudoscorpions (which we found to be relatively abundant under bark with *P. fasciculatus*), and predacious beetles. Remarkably, tropical ants of the aberrant ponerine genus *Thaumatomyrmex* hunt polyxenids as a matter of routine. When encountering a polyxenid, *Thaumatomyrmex* grasps it with its specialized mandibles and kills it with a sting, then strips off its bodily setae (including sometimes the tufts) and eats it. The tufts in these encounters seem to provide no defense (10).

An important feature of polyxenid tufts is that their bristles are renewable. Millipedes characteristically continue to molt as adults, and polyxenids are no exception. Studies done with *P. lagurus* have shown that when this millipede emerges from a molt it invariably has fully constituted tufts, even if it lost some of its bristles before the molt. Interestingly, it is specifically the loss of bristles in *P. lagurus* that hastens the advent of the next molt (11). Other polyxenids, including *P. fasciculatus*, could well have the same capacity to "rearm when in need of armament."

Detachable integumental outgrowths serve for defense in a number of arthropods. Tarantulas, for instance, have loose hairs on their back that they flick with their legs toward predators when assaulted (12). Some caterpillars have detachable urticating hairs, which when not already expended in defense are detached by the larva itself and placed on the surface of the cocoon at pupation (13). Because they easily detach when coming into contact with sticky spider silk, the scales of moths prevent moths from becoming readily en-
trapped in spider webs (14). Especially noteworthy is the 
defense of larvae of beetles of the family Dermestidae (e.g., 
species of Trogoderma and Anthrenus). These larvae, which are 
similar in size and appearance to polyxenids (15), also protect 
themselves by use of detachable bristles. Their bristles have 
tips specialized for anchorage, and barbs along their length by 
which they can interlock. They serve effectively in defense 
against ants and other arthropods (16–18). The parallel with 
polyxenids is obvious, and illustrative of a striking case of 
evolutionary convergence.

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