

Chemical attraction of kleptoparasitic flies to heteropteran insects caught by orb-weaving spiders*

(milichiid flies/Hemiptera/defensive secretion/trans-2-hexenal/kairomone)

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Contributed by Thomas Eisner, June 24, 1991

ABSTRACT Insects of the heteropteran families Pentatomidae (stink bugs) and Coreidae (squash bugs), when being eaten by the orb-weaving spider *Nephila clavipes*, attract flies of the family Milichiidae. The flies aggregate on the bugs and, as kleptoparasites, share in the spider's meal. Stink bugs and squash bugs typically eject defensive sprays when attacked; they do so when caught by *Nephila*, but the spray only minimally affects the spider. Evidence is presented indicating that it is the spray of the bugs that attracts milichiids to the spider's catch.

Spiders are slow eaters. Unable to swallow solids, they triturate their prey extraorally, drench it in disgorged digestive fluid, and suck up its liquified contents. The process may take hours, even with prey substantially smaller than the spider. In nature, prey lengthily exposed is often secondarily exploited by kleptoparasites. *Vis á vis* spider prey, such usurpers include both other spiders and insects. Chief among the latter are certain tiny flies of the families Cecidomyiidae, Phoridae, Chloropidae, and Milichiidae, which have long been known as regular visitors to spider prey (1, 2). There is good evidence, in the case of cecidomyiids, that the flies are attracted chemically to the prey, possibly by factors contributed by the spider or liberated in the course of the prey's digestion (1). Olfactory stimuli were also suspected to be involved in milichiid and chloropid attraction (1–3), but the most important evidence to date was an unpublished presentation by P. L. Mitchell, F. L. Mitchell, and J. R. Aldrich, reporting attraction of milichiids to insects of the heteropteran families Pentatomidae (stink bugs) and Coreidae (squash bugs).[¶] These bugs spray defensive chemicals when disturbed, and it had been noted that when they were induced to spray, they attracted milichiids. We here present data that attempts to place this peculiar phenomenon in an adaptive context. Specifically, we demonstrate that milichiids are drawn to heteropterans when the latter are being consumed by orb-weaving spiders and that the spray of the bugs, emitted in futile efforts to repel the spiders, attracts the flies.

Our experiments were done in Florida with a single species of orb-weaving spider, the large, widely distributed *Nephila clavipes*, and an assortment of stink bugs and squash bugs. The milichiids attracted in our tests were of several species. We show that (i) *Nephila* can cope with heteropterans despite the spray these eject when attacked, (ii) heteropterans being eaten by *Nephila* attract milichiids, (iii) *Nephila* prey ordinarily unattractive to milichiids can be rendered attractive by baiting it with components of heteropteran spray, and (iv) a chemical component of heteropteran spray can itself attract milichiids.

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MATERIALS AND METHODS

The study was done in January 1989. Laboratory observations were made at the Archbold Biological Station, Lake Placid, Florida. Tests with the spiders were at Highlands Hammock State Park, Sebring, Florida, a preserved site densely shaded by live oak and cabbage palm, where *Nephila* webs were abundant.

Feeding of *Nephila*. Presentation of prey to the spiders involved flipping individual insects from vials directly into the web and monitoring the course of events. Over the years this technique has been used by one of us (T.E.) to test for the acceptability of dozens of insect species to *Nephila*.

The feeding behavior of *Nephila* is known (4). Typically, the spider darts toward the entrapped insect, bites it with the chelicers to inject venom, envelops it lightly in silk, carries it to the hub of the web, envelops it in more silk, suspends it from the hub, and eats it. Consumption is by suctorial uptake of fluid from the ground-up predigested prey and may take several hours, even with prey only a third the mass of *Nephila*.

In the tests that follow, special care was taken to insure that the heteropterans did not eject spray before being offered to the *Nephila*. To this end, when collected in the field, they were coaxed gently into vials without being directly grasped.

Nephila were given single prey presentations and not reused in any tests.

Acceptability of a Heteropteran to *Nephila*. Thirteen individuals (nine females and four males) of a single species of Pentatomidae, *Nezara viridula*, were offered to *Nephila*, to observe in some detail, with a representative heteropteran, how the spider subdues such chemically defended prey.

Attraction of Milichiids to Heteroptera Caught by *Nephila*. An assortment of 38 bugs (three species of Pentatomidae and three of Coreidae) was fed to *Nephila*; whether or not milichiids alighted on the individual prey items when these were being consumed by the spiders was recorded.

For nine *N. viridula* (all females), the density of milichiid assemblage was recorded as a function of time since prey presentation to the spider. The *Nezara* were offered one after another to their respective spiders, and the webs were inspected thereafter to count the numbers of flies alighted on the prey. Times of inspection fell within the following intervals: 0–½ hr, ½–1 hr, 1–2 hr, 2–4½ hr. At 4½ hr, all spiders had finished their meal. Time 0 was the time of the spider's first contact with the prey.

To obtain milichiids for identification, five *N. viridula* and two Coreidae (one *Acanthocephala confraterna* and one unidentified *Acanthocephala*) that had been fed to *Nephila*

*This is paper no. 106 in the series *Defense Mechanisms of Arthropods*. Paper no. 105 is ref. 20.

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[†]Mitchell, P. L., Mitchell, F. L. & Aldrich, J. R., Annual Meeting of the Entomological Society of America, December 9, 1985, Hollywood, FL.

were plucked away from the spiders once milichiids had assembled on the bugs and were abruptly transferred to vials with ethanol, capturing a substantial fraction of the aggregated flies.

Attraction of Milichiids to Chemically Baited Prey. Previous work had revealed that a moth in culture in our laboratories, the arctiid *Utetheisa ornatrix*, is highly acceptable to *Nephila*, when it has been raised on an appropriate larval diet. Raised on its natural food plant of the genus *Crotalaria*, which contains toxic pyrrolizidine alkaloids, the moth is rejected by the spiders, but it is eaten when its diet is an alkaloid-free semisynthetic formulation based on pinto beans (5).

We used *Utetheisa* moths raised on our alkaloid-free pinto bean diet (5) as *Nephila* prey to test whether addition of chemical components from stink bug spray would render such items attractive to milichiids.

The two chemicals used as additives, *trans*-2-hexenal and hexanal, had been characterized as major components of the spray of a substantial number of Pentatomidae and Coreidae (6, 7).

We offered three categories of *Utetheisa* moths to *Nephila*: (i) baited with 4 μ l of *trans*-2-hexenal ($n = 8$), (ii) baited with 4 μ l of hexanal ($n = 8$), and (iii) unbaited controls ($n = 6$).

The moths had one costal vein cut when offered to the spiders to prevent escape after being flipped from their vials into the webs. Addition of chemical to the treated moths was effected with calibrated micropipettes; the fluid was trickled onto the body of the moths as soon as these had been transported to the hub of the web and the spiders had settled to commence their meal. The procedure did not deter the spiders.

Density of milichiid assemblage over time was monitored as with the *Nezara* feedings in the preceding test by inspecting the webs periodically and counting the flies gathered on the moths. Times of inspection fell within the same intervals as with the *Nezara* tests, except that the overall duration of the trials was shorter because *Utetheisa* were consumed faster than *Nezara*. Time 0 for the tests was the time of application of chemical to the moth (or, for controls, arrival of the spider at the hub of the web with the moth).

Attraction of Milichiids to a Component of Heteropteran Spray. One of two chemicals used in the baiting experiments was also tested for intrinsic attractancy to milichiids. Eight pieces of cardboard (4 \times 7 cm), covered on one side with an adhesive paste (Tanglefoot), were suspended by threads (long dimension vertical) at \approx 1.5 m above ground (common *Nephila* web height) and 15–20 m from one another at our spider test site. Four of the cards (experimentals) were outfitted with a microcapillary tube (both ends open, attached horizontally to the sticky side of the card) laden with 4 μ l of *trans*-2-hexenal. Comparable but empty tubes were affixed to the controls. The cards were taken down after 4 hr and checked for insects trapped in the adhesive.

RESULTS

Acceptability of a Heteropteran to *Nephila*. All except one of the *Nezara* were eaten by the spiders. The capture behavior was typical (see *Materials and Methods*), except that the spiders were sometimes momentarily deterred when the *Nezara* sprayed. Ejections occurred early in the encounters, when the spiders first manipulated the bugs or bore down to inflict their bites, and they were always detectable by their characteristic odor. When sprayed, the spiders briefly backed away from the bugs to clean themselves, usually by drawing appendages through the mouthparts. On a few occasions a spider even moved to the web edge to wipe its mouthparts against a branch (or palm frond) serving as a point of web attachment. The single *Nezara* that escaped (a

Table 1. Species of Heteroptera fed to *Nephila*, number of individuals of each tested, and portion of this number that attracted milichiid flies

Heteropteran prey	Offered, no.	Attractive, no.
(P) <i>N. viridula</i>	22	19
(P) <i>Piezodorus guildiini</i>	4	2
(P) <i>Euschistus</i> sp.	6	2
(C) <i>Acanthocephala confraternata</i>	2	2
(C) <i>Chelinidea vittiger</i>	3	1
(C) <i>Acanthocephala</i> sp.	1	1

Families are Pentatomidae (P) or Coreidae (C).

female) did so before being bitten, by working itself out of the web in 4 min, while the spider was preening after being sprayed. *Nezara* that were eaten were reduced to small pellets of compacted remains, as is typical for spider prey. Consumption times for the 12 eaten *Nezara* ranged from 1½ to >4 hr.

Attraction of Milichiids to Heteropteran Prey. Table 1 lists the heteropterans fed to *Nephila* and the incidence of milichiid visitation to these offerings. All species evidently showed some attractiveness. The bugs all noticeably sprayed (odor apparent) when attacked by the spiders, and none escaped. No milichiids were detected on the spiders before prey presentation, and none appeared on the prey until after spraying. These results contrasted sharply with those obtained over past years by one of us (T.E., unpublished work) in comparable tests, in which *Nephila* were offered dozens of butterflies, moths, beetles, flies, cockroaches, winged termites, earwigs, and mayflies. Although visitation of an occasional milichiid could have been missed in these tests, convergence of flies in numbers such as occurred with stink bugs was never witnessed.

Data from the nine *Nezara* watched over time are given in Fig. 1 (left-hand plot). Milichiids evidently arrived within minutes of the spider's attack, soon achieved peak densities, and then declined in numbers toward the end of the spider's meal. No determination could be made of the turnover rate of the flies on the prey, although it seemed from ongoing observation of a few individuals that these remained alighted for at least several minutes at a time. Observation of approaching flies (Fig. 2A) revealed that they invariably arrived

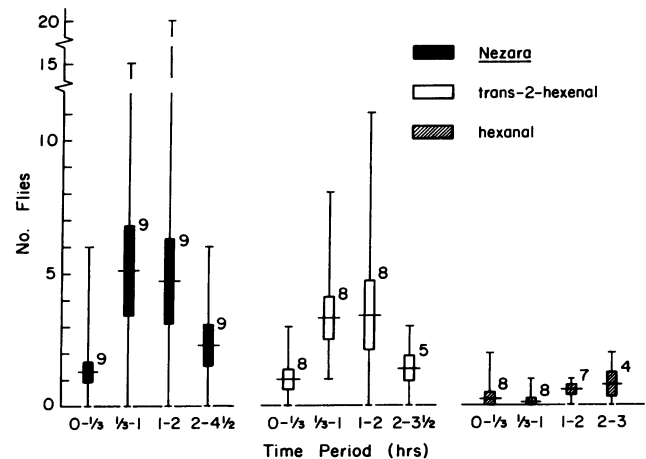


FIG. 1. Attractiveness of three categories of *Nephila* prey to milichiid flies: the bug *Nezara* ($n = 9$), and *Utetheisa* moths baited with *trans*-2-hexenal ($n = 8$) or hexanal ($n = 8$). Data give number of flies (mean \pm SEM in black or white bar; range is given by vertical line length) per prey item, as function of time since presentation of item to spider (see *Materials and Methods* for definition of presentation time). Decrease in sample size n with time indicates early completion of consumption of some prey items.

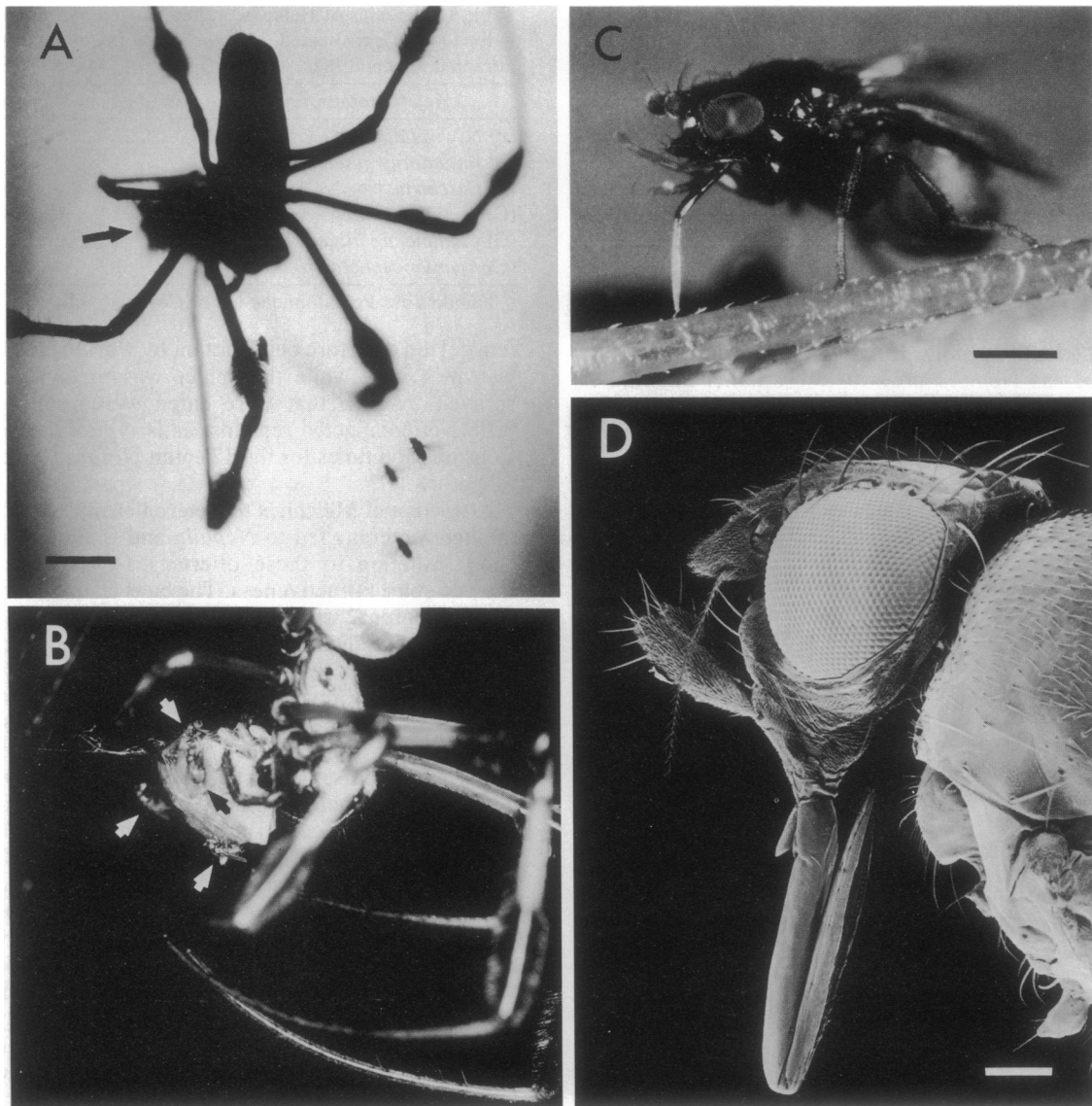


FIG. 2. (A) Milichiid flies converging on a *Nephila* spider that had caught a bug (*N. viridula*, arrow). Three flies are approaching; one has landed on the spider's leg. (B) Later stage of a comparable event; flies have alighted (four are denoted by arrows) on the captured *Nezara*. (C) Milichiid (*Paramyia nitens*) feeding on surface of *Nezara*; proboscis is fully extended (*Nezara* had been pulled from chelicers of the spider and taken to the laboratory for photography; the fly, temporarily removed from the *Nezara* during the transfer operation, resumed feeding the moment it was replaced on the bug). (D) Scanning electronmicrograph of *P. nitens* head, showing the flexed condition in which proboscis is ordinarily held. (Bars: A = 5 mm; C = 0.5 mm; and D = 0.1 mm.)

from downwind. Detectable as tiny specks from a distance, these flies slowly zigzagged their way toward the spider's prey, correcting their trajectory at times as gusts of breeze deflected them from their path. Their flight was reminiscent of that of male moths homing in on females along an aerial pheromonal plume.

Arriving flies ran little risk of entanglement in the web because by the time of their approach, the spiders had already taken their prey to the web center and positioned themselves at the hub. The hub is spun of nonviscid thread that did not encumber the flies.

Close-range observation of flies on *Nezara* (Fig. 2B) revealed that they fed upon landing. Using their proboscis to extract fluids that seeped from the prey, they gorged themselves to the point of utmost abdominal distention. The proboscis is ordinarily kept flexed beneath the head (Fig. 2D). When unflexed for feeding, it extends to substantial length (Fig. 2C).

The *Nephila* reacted only minimally to the flies. They generally appeared oblivious to their presence and reacted to

them by attempting to brush them away with the legs only when the flies clustered around their mouth; flies dispersed by such maneuvers quickly realighted.

The 41 milichiids collected for identification were of three genera, and almost all were female (Table 2).

Attraction of Milichiids to Chemically Baited Prey. Results were clear: the two sets of *Utetheisa* baited, respectively, with *trans*-2-hexenal and hexanal attracted milichiids (Fig. 1, center and right-hand plot), whereas the six unbaited *Utetheisa* that served as controls attracted none (data not shown in Fig. 1). Time course of attractiveness of the *Utetheisa* baited with *trans*-2-hexenal closely matched that of *Nezara* prey. The hexanal-baited *Utetheisa* drew fewer flies despite the fact that this compound is more volatile than *trans*-2-hexenal (8).

Attraction of Milichiids to a Component of Heteropteran Spray. The traps baited with *trans*-2-hexenal all attracted milichiids (total for the four traps: 11 ♀♀ *P. nitens*, 1 ♀ *Milichiella* sp.). Control traps attracted none. Treated traps also attracted three phorid flies (*Dohrniphora* sp.) and one chloropid (*Olcella* sp.).

Table 2. Milichiid flies taken from three species of heteropteran prey being eaten by *Nephila*

Heteropteran prey	n	Milichiids attracted			
		<i>P. nitens</i>	<i>Neophylomyza</i> sp.	<i>Milichella</i> sp.	<i>Desmometopa</i> sp.
(P) <i>N. viridula</i>	5	6♀	10♀		
(C) <i>Acanthocephala confraterna</i>	1	15♀, 1♂	4♀		
(C) <i>Acanthocephala</i> sp.	1	1♂	1♀	2♀	1♀

Only a fraction of flies assembled on prey were caught for these counts. Families are Pentatomidae (P) and Coreidae (C). n, number per species.

DISCUSSION

The chemical basis of an unusual association between a spider, kleptoparasitic flies, and a common prey seems established. Stink bugs and squash bugs are a vast resource of flying insects. For both a predator able to deal with their defenses and kleptoparasites able to exploit the predator's catch, they are an ideal food source.

The finding that milichiids were attracted to all species of bugs tested was not unexpected. Stink- and squash bug secretions are of fundamentally similar composition. They contain primarily low-molecular-weight carbonyl compounds, of which a number, including *trans*-2-hexenal and hexanal, are broadly shared (6, 7). Milichiids, although to a degree differentially sensitive to the individual components (witness the difference in attractiveness of *trans*-2-hexenal and hexanal in the baiting tests), could be broadly attuned to the full spectrum of the compounds and essentially equally responsive to the various combinations in which these occur in the sprays. Heteropteran "stink" to the flies could, therefore, be a more or less invariant chemical beacon, a common cue to a broad diversity of trophic options.

It should be noted that the two secretory components proven attractive to milichiids, *trans*-2-hexenal and hexanal, occur in the spray of the very species (or close relatives of these) used as prey in our tests. *N. viridula*, as well as species of *Piezodorus* and *Acanthocephala*, produce *trans*-2-hexenal (9–12); *Chiliniidea vittiger* secretes hexanal (13). [*Trans*-2-hexenal is a component of the sex pheromone of at least some Pentatomidae (14, 15), and pheromone traps for these species catch a diversity of milichiids and chloropids (J. R. Aldrich, personal communication). We believe that this phenomenon may be a nonadaptive by-product of the adaptive attraction to dead or injured pentatomids and that at natural concentrations the pheromones may not be sufficiently attractive to draw the flies.]

One wonders, given that stink bugs eject their spray at the outset of the spider's attack, why milichiids should continue to be drawn to the bugs for lengthy periods thereafter. The primary reason may be that the glands are not depleted by the discharges and that they continue to emit secretion as the bugs are slowly ground to a pulp by the spider. Immediately after discharge there may also be evaporation from secretion trapped around the gland openings, a highly sculpted region fashioned as a physical sponge for retention of discharged fluid (16, 17).

No estimate was made of the nutritional cost of milichiid kleptoparasitism to *Nephila*. To young *Nephila*, and especially when many flies are present, the trophic cost could be substantial. The flies appear only minimally at risk through their behavior. They are unendangered by the spiders and

seemingly able, under most circumstances, to avoid web entanglement.

In the absence of further information on the reproductive behavior of the flies and on any discrepant nutritional demands of the sexes, no explanation can be ventured for the sharply female-biased nature of milichiid kleptoparasitism. We do not even know whether a proteinaceous meal is optional or obligatory in adult milichiids, much less the range of foods they consume. And little can be said about the few flies of other families (Chloropidae, Phoridae) also lured to our *trans*-2-hexenal traps. Flies of these families do visit spider prey (1) [and even mate on such prey (2)], and it is conceivable that these flies, too, cue in on the glandular emission of captured Heteroptera.

The kleptoparasitic exploits of milichiids are doubtless more varied than indicated by our study. The flies have been reported to feed on heteropteran prey of spiders other than *Nephila* (including other orb-weavers, a thomisid, and an oxydopid) (1, 18), and they have been repeatedly noted on hymenopteran prey (bees, including the honeybee) taken not only by spiders (1, 18, 19) but also entirely different predators (for example, Reduviidae) (1, 2, 19). Their feeding convergence on Hymenoptera, a group of insects richly endowed with integumental glands, raises the question whether they detect these prey also from chemical cues. Chemically mediated kleptoparasitic exploitation of insect prey could be much more prevalent than suspected. Arthropods possess the most varied exocrine glands, and many could be locatable by the chemicals they eject or that leak from their bodies when they are captured. One could envision any number of kleptoparasitic insects homing in on food resources by such cues.

The personnel of the Highlands Hammock State Park kindly allowed us to work on their grounds. J. R. Aldrich and P. L. Mitchell provided helpful comments during the study. W. Mitchell Masters and Athula Attygalle critically reviewed the manuscript. This study was supported, in part, by Grant AI-02908 from the National Institutes of Health.

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