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0014-4754/92/010094-04$1.50 + 0.20/0

Chemical basis of pupal cannibalism in a caterpillar (*Utetheisa ornatrix*)[1]

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Received 2 April 1991; accepted 25 June 1991

Abstract. The moth *Utetheisa ornatrix* derives protection against predation from systemic pyrrolizidine alkaloids (PAs) that it sequesters as a larva from its foodplants (Leguminosae, *Crotalaria* spp.). We here show, in laboratory tests, that *Utetheisa* deficient in body PA can make up for the chemical shortfall by cannibalizing pupae. We present evidence indicating that cannibalism in larvae is elicited not by hunger, but possibly by PA deficiency itself, and that in making cannibalistic choices larvae prefer PA-containing over PA-free pupae. PAs themselves, either in crystalline form or as additives to food items, proved phagostimulatory to larvae. In nature *Utetheisa* tend to pupate away from their foodplant, essentially out of reach of larval attack. The threat of cannibalism may have contributed to the evolution of this pupation behavior.
Key words. Lepidoptera; Arctiidae; pyrrolizidine alkaloids; cannibalism; acquired defense; phagostimulation; specific hunger.

The moth *Utetheisa ornatrix* (family Arctiidae), as a larva, feeds on poisonous plants of the genus *Crotalaria* (family Leguminosae). The toxic principles of *Crotalaria* are pyrrolizidine alkaloids (PAs), present in highest concentrations in the seeds of the plant[3]. *Utetheisa* can tolerate PAs. Larvae feed preferentially on the seeds of *Crotalaria*, storing the PAs in their body and retaining the chemicals systemically through the pupal and adult stages[4,5]. Adult *Utetheisa* also bestow PAs upon the eggs[6]. The acquired PAs protect *Utetheisa* against predation. Adult moths are cut loose from the webs by orb-weaving spiders, and eggs are rejected by coccinellid beetles. Both types of predator are deterred by PA added topically to edible food items, and they respectively accept *Utetheisa* adults and eggs, if these are offered to them free of PA[4,6].

Utetheisa in nature have variable systemic PA loads, and may consequently be presumed to be variably vulnerable to predation. Net PA content of adults varies from near zero to several hundred µg per moth (B. Roach, unpublished data). We interpret this to indicate that *Utetheisa*, as larvae, compete for access to the alkaloid-rich seeds of their foodplant, and that they are variously successful in this competition. We know that larvae have the sensory capacity to detect PAs and that they preferentially choose food containing PAs (addition of as little as 10^{-4}% monocrotaline, a PA from *Crotalaria*, will induce *Utetheisa* larvae to feed on agar (Blankespoor, Firlik, Pressman, Conner, Eisner, unpublished). We also know, from field observations, that *Crotalaria* seeds may at times be in short supply. For instance, on *Crotalaria mucronata* in central Florida, we noted that when *Utetheisa* populations are dense, and particularly when the larvae are in intense competition with other seed-eaters (such as the larvae of *Etiella zinckenella*, a pyralid moth), virtually the entire seed set of the plants may be eaten away. Under such circumstances *Utetheisa* may have no option but to feed primarily on the foliage of the plants. While consumption of leaves does not preclude *Utetheisa* larvae from achieving functional adulthood, it does result in their acquiring substantially lesser quantities of PA[5].

We present evidence here indicating that larval *Utetheisa* that lack systemic PA can make up for the chemical deficiency, even relatively late in larval life, by turning to cannibalism. Specifically, we show that PA can be acquired by larvae by feeding on pupae. Moreover, we show that larvae turn to cannibalism not as a consequence of hunger, but when their diet is *Crotalaria*-free (and therefore PA-free), and that in exercising cannibalistic choices they prefer PA-laden over PA-deficient dietary items. While our experiments were carried out almost exclusively in the laboratory, the findings, we feel, have a bearing on understanding the natural history of *Utetheisa*. Specifically they may help explain why larvae pupate at sites away from their foodplant.

Materials and methods

Experimental animals. The *Utetheisa* stemmed from our laboratory culture, established with stock taken near Lake Placid, Highlands County, Florida. Voucher specimens are on deposit (lot 1154) in the Cornell insect collection.

The moths were raised on two diets: a) PB diet, a semisynthetic diet[7] based on pinto beans and devoid of *Crotalaria* content (and therefore PAs), and b) CS diet, identical to PB diet, but with 10% (by weight) of its pinto bean content replaced by ripe *Crotalaria spectabilis* seeds. *C. spectabilis*, one of the principal foodplants of *Utetheisa* in the southeastern USA, contains the PA monocrotaline, mostly in its seeds[3].

We had previously determined that, as expected, adult *Utetheisa* raised on PB diet are PA-free, while those raised on CS diet contain monocrotaline [628 ± 48 (SEM) μg monocrotaline/moth (N = 31); B. Roach, unpublished data]. We also knew that the CS diet is an appropriate substitute for the natural foodplant. Adult *Utetheisa* reared outdoors on seed-bearing *C. spectabilis* contain 701 ± 59 (SEM) μg monocrotaline/moth (N = 22)[5]. Individuals reared outdoors on immature (seedless) *C. spectabilis* contain only 98 ± 10 (SEM) μg monocrotaline/moth (N = 46)[5].

In what follows we denote *Utetheisa* larvae and pupae by (−) and (+) signs, to indicate respectively that they are PA-free (PB-diet raised) or PA-containing (CS-diet raised).

The pupae of cabbage butterflies (*Pieris rapae*) used as choice items in some of the experiments, were obtained from a laboratory culture, established with stock from Ithaca, NY, and maintained on an artificial diet[8].

Chemical samples. Monocrotaline free base and heliotrine free base were obtained commercially, and by courtesy of J. Meinwald, respectively. Monocrotaline N-oxide was prepared in J. Meinwald's laboratory by treating the free base with hydrogen peroxide[9].

Basic experimental protocol. All feeding tests were done with medium-sized *Utetheisa* larvae [15–18-day-old; mean body mass of a representative sample of 15 larvae = 115 ± 15 (SEM) mg] The larvae were individually transferred directly from their cultures (no starvation time) to cylindrical plastic chambers (85 mm diameter, 35 mm height), and offered a choice of two food items. The items were presented on opposite quadrants of circular pieces of filter paper that fitted the bottom of the chambers. The chambers were otherwise empty.

Results were checked at the end of 24 h, and scored as consumption of one item or the other, of both items, or of neither. All tests were repeated 25 times, using previously untested larvae for all trials.

Test series 1: Presentation of whole pupae. Larvae were given a choice between two pupae (fully hardened, less than 24 h old). The pupae were fastened to the filter papers with small squares of double-sided sticky tape. Fate of pupae was scored as eaten (if they were partially to nearly totally hollowed out by the larvae following perforation of the pupal shell) or uneaten (if they were uninjured). Each test was undertaken in parallel with (−) and (+) larvae. In tests where *Utetheisa* pupae were sexed, differentiation was done on the basis of external genitalic features.

The series comprised five tests, in which the choice situations were as follows:

Test 1A: between a (+) and a (−) pupa (pupae unsexed)
Test 1B: between two (+) pupae, one male and one female
Test 1C: between two (−) pupae, one male and one female
Test 1D: between a (+) pupa and a *Pieris* pupa (pupae unsexed)
Test 1E: between a (−) pupa and a *Pieris* pupa (pupae unsexed)

Test series 2: Presentation of pupal squashings. The tests were comparable to the preceding, except that pupae were offered as squashings rather than whole. The intent was to see whether the larvae might be more prone to feed on pupae if these were free of their enclosing shell. The pupae were forcibly squashed on the filter papers and the cuticular parts removed. In consuming the squashings, the larvae always ingested a portion of the impregnated filter paper as well, providing a visual testimony of their feeding activity. A squashing was scored as eaten, if part of the paper was thus chewed away, and uneaten, if the impregnated zone remained imperforate. The tests were carried out in parallel with (+) and (−) larvae.

The choices were as follows:

Test 2A: between a (+) and a (−) pupal squashing, both male
Test 2B: between a (+) and a (−) pupal squashing, both female
Test 2C: between a (+) and a *Pieris* pupal squashing (both unsexed)
Test 2D: between a (−) and a *Pieris* pupal squashing (both unsexed)

Test series 3: Presentation of crystalline PAs, and of PA-supplemented Utetheisa samples. These tests were intended to clarify further the phagostimulatory capacity of PAs. The alkaloids were tested in crystalline form, and as topical or systemic additives to pupal samples.

Two PAs were selected for testing: monocrotaline and heliotrine. Monocrotaline was tested in the two chemical forms (free base, N-oxide) in which it occurs naturally, both in *Crotalaria spectabilis* and in *Utetheisa*[5]. Heliotrine, not found in either of the primary foodplants of *Utetheisa* in the USA (*C. spectabilis*, *C. mucronata*)

(B. Roach, unpublished), but present in other plant sources[10], was tested as free base only.

The tests, carried out with (−) larvae only, were of three types, repeated in parallel with each of the three PA samples (monocrotaline free base, monocrotaline N-oxide, heliotrine free base):

a) Crystalline PA. Paired offering of PA (500 µg in 25 µl methanol solution) and solvent control (25 µl methanol), applied directly to the filter paper.

b) PA-supplemented pupal squashing (topical addition). Offering of two (−) pupal squashings (prepared as in test series 2), one treated by topical addition of 500 µg PA in 25 µl methanol, the other (solvent control) treated by addition of 25 µl methanol only.

c) PA-supplemented pupal squashing (systemic addition). Offering of two pupal squashings (as in (b)), one derived from a (−) larva that received a systemic dose of 600 µg PA (three 10-µl injections of aqueous suspension, into late 4th and/or early 5th instar), the other (control) derived from an untreated (−) larva.

Field tests. Laboratory-raised *Utetheisa* pupae were affixed (August 26–September 7, 1990) outdoors to *Crotalaria* plants, under conditions where they faced potential cannibalistic attack by free-living *Utetheisa* larvae. The experimental site was a stand of *Crotalaria mucronata* shrubs, grown from seeds on the grounds of the Archbold Biological Station (Lake Placid, Florida), where both *C. mucronata* and *Utetheisa* occur naturally. The stand covered an area of 10 × 10 m, and included nearly 500 plants. The plants were mature and bore seed pods at all developmental stages. They were only moderately infested with *Utetheisa*. Intact seed pods were in ample supply, and *Utetheisa* larvae were visually estimated to number only a few per plant. In heavy infestations, such as we have seen at other times and sites in Florida, *Utetheisa* larvae may be present in substantial numbers on single plants.

Pupae were individually affixed to main and lateral branches of the plants, in roughly even spacing throughout the stand (0.5–1.5 m apart). They were individually attached with a small anchoring square and a narrow girdling strip of two-sided sticky tape. A total of 75 (+) and 75 (−) pupae were thus exposed, for an average exposure time of 97 h per pupa. During the exposures, pupae were individually inspected at irregular intervals of several hours, between 07.00 h and 23.00 h.

Results

Test series 1 (fig. 1). The results with these tests provided a first indication of what was to become clear also in test series 2. It was the (−) larvae that did most of the feeding, and they tended to feed selectively on (+) pupae (fig. 1 A, B, D and fig. 4 A). Moreover, (−) larvae chose male and female (+) pupae with comparable frequency

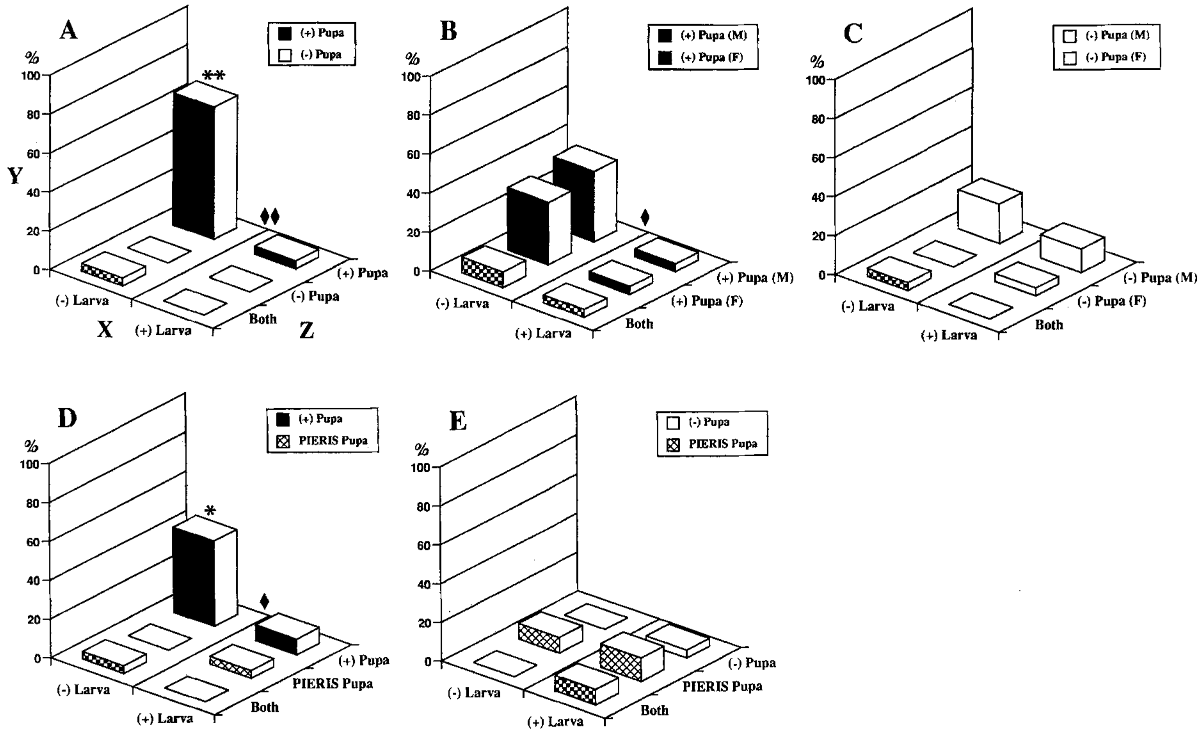

Figure 1. (Tests 1 A–1 E). Choice preference of (+) and (−) larvae offered paired food items in various combinations (choices are given in boxes). Each plot compares parallel tests (X-axis) undertaken with (+) and (−) larvae (N = 25 of each type). Z-axis gives breakdown of choices (non-feeders are omitted); Y-axis gives percent distribution of choices (rectangles flush with base plane are O values). Statistical conventions (also applicable to figs 2,3): *asterisk(s)* indicate significance of difference from alternative choice (and in most cases also from the 'both' choice) in same test (* = $p<0.01$; ** = $p<0.001$); *diamond(s)* indicate significance of difference from same choice in parallel test (♦ = $p<0.01$, ♦♦ = $p<0.001$) (G-test, with p-value conversions[11]).

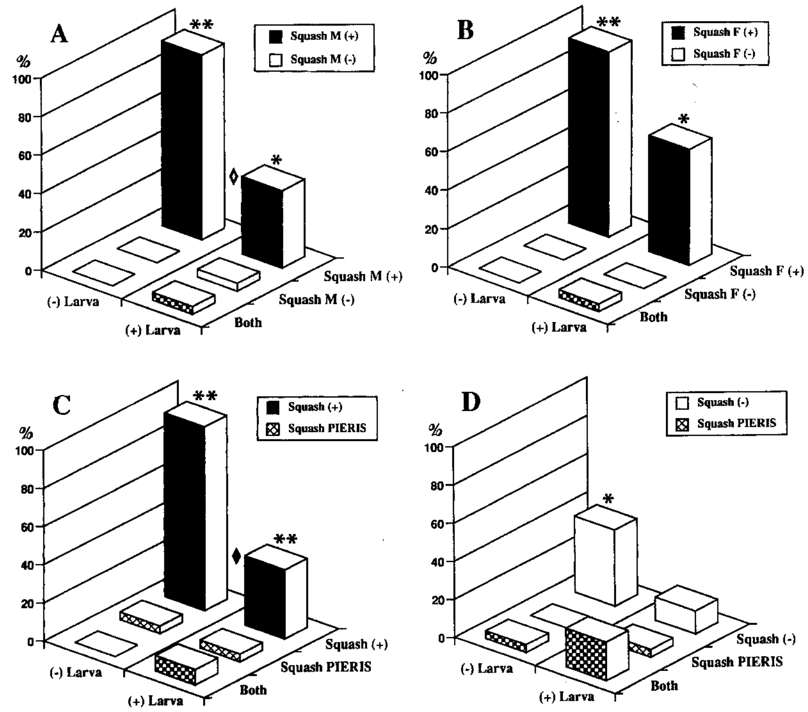

Figure 2. (Tests 2 A–D). Choice preference of (+) and (−) larvae offered paired food items in various combinations (choices are given in boxes). Conventions as in figure 1, except in 2 A, where ◇ is $p < 0.025$.

($p > 0.5$; fig. 1 B). PA-free choice items, whether in the form of (−) pupae or *Pieris* pupae, remained relatively uneaten by either type of larva (fig. 1 C, E), although to the extent that there was any consumption of these items, both larvae seemed to show a slight preference (for unclear reasons) for male over female (−) pupae (fig. 1 C). *Test series 2* (fig. 2). The results were consistent with those from the previous test series. (−) Larvae did most of the feeding, and they fed preferentially on (+) pupal squashing (fig. 2 A,B,C and fig. 4 B). (+) Larvae, although showing overall lesser feeding incidence, shared the bias for (+) squashings. For each type of larva, feedings were about equal on squashings of either sex (fig. 2 A,B). PA-free items were again relatively ignored by larvae of both types [except for (−) squashings by (−) larvae, where no (+) squashings were available (fig. 2 D)]. On the whole, however, there was a higher incidence of feeding in these tests than in test series 1, possibly because in the present series pupae were offered as squeezed out contents, unprotected by the pupal shell. *Test series 3* (fig. 3). The results were remarkably similar for all three PA samples tested. All three elicited strong phagostimulatory responses in crystalline form. The larvae ate large holes into the filter papers where PA solution had been applied, and left imperforate the control zones treated by solvent alone. With the pupal squashings the results were similar, whether the PA was added topically to the squashing itself, or systemically into the larva prior to pupation.

Field test. A substantial fraction of the 150 pupae affixed to the *Crotalaria* plants fell victim to attack by predators, including ants (*Solenopsis invicta, Conomyrma bureni*), a tettigoniid grasshopper (*Odontoxiphidium apterum*), and a sphecid wasp (*Mischocytta cubensis*). Details of these attacks, as well as other data pertaining to the vulnerability of *Utetheisa* to predation, will be presented elsewhere. Suffice it to say, that we noted 4 instances of *Utetheisa* larvae feeding on staked-out pupae. In each case this involved a nearly full-grown larva feeding on a (+) pupa. In 2 of the cases we came upon the encounter when the pupae were barely perforate, indicating that the feeding larvae had themselves initiated the attack. In the other 2 cases, the pupae had already undergone extensive injury, leaving some doubt as to whether the larvae were the primary assailants.

Discussion

It seems established, that under the simple choice conditions of our laboratory tests, *Utetheisa* larvae may opt to consume conspecific pupae. The primary factor driving larvae to such cannibalism appeared to be not food deprivation, but a deficiency in dietary *Crotalaria*. All larvae were equally well fed at the time of testing, having been introduced into the test chambers directly from their cultures. It was (−) larvae that consistently showed the higher propensity for cannibalism. Moreover, in making their cannibalistic choice, (−) larvae selected

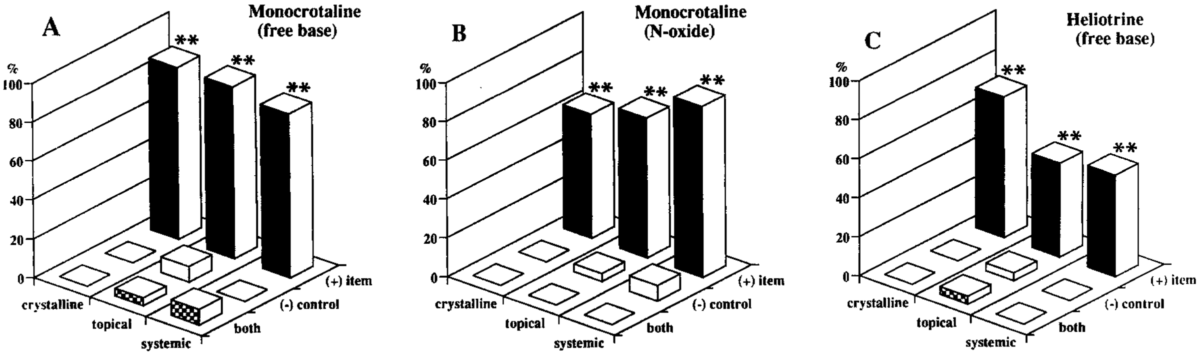

Figure 3. (Test series 3). Choice preferences of (−) larvae offered PA-bearing samples [(+) items] paired with PA-free controls [(−) controls]. The three PAs (monocrotaline free base, monocrotaline N-oxide, heliotrine free base), were each tested in three ways (X-axis: crystalline, topical, systemic; N = 25 larvae per test; details in text). Z-axis and Y-axis give, respectively, the breakdown and the percent distribution of choices. Statistical conventions (asterisks) as in figure 1.

almost solely (+) pupae or pupal squashings, leaving the paired (−) alternatives essentially ignored. (+) Larvae were less disposed to be cannibalistic. They fed minimally on whole pupae and only moderately on pupal squashings, although when they did feed on the latter, they shared with (−) larvae the predilection for (+) squashings. (+) Larvae, although doubtless no less able than (−) larvae to chew through the cuticular shell of whole pupae, seemingly lacked the drive manifested by (−) larvae to engage in this task. It is tempting to conclude from these results that the larvae can 'sense' their own PA content, and that they are driven to cannibalism when they perceive this content to be low. However, we cannot rule out the possibility that they gauge their PA content indirectly, by monitoring some other dietary factor from *Crotalaria* that correlates quantitatively with PA. Either way, the adaptive result is the same – the larvae are driven to remedy PA shortages when they are deficient in PA.

The tests with crystalline PAs and with PA-supplemented items, showed that the PAs themselves were in all likelihood the phagostimulatory agents responsible for the cannibalistic attacks on (+) pupal samples. Moreover, judging from the data with monocrotaline, both the free base and N-oxide forms of PA can be equally stimulating. The fact that even heliotrine showed potency is of interest. Heliotrine does not occur in either *C. spectabilis* or *C. mucronata*, *Utetheisa*'s primary foodplants in Florida. Also, heliotrine has a 7-S configuration while monocrotaline is 7-R[10], indicating that *Utetheisa* larvae have the capacity to 'recognize' PAs in both their natural stereoisomeric forms. The further difference, that heliotrine is a single ester, while monocrotaline is a macrocyclic lactone[10], is evidently also of no phagostimulatory consequence to the larvae.

The fact that *Utetheisa* larvae can seemingly gauge their body PA content, can respond to PAs as feeding cues, and further, can take corrective action to remedy systemic PA deficiencies by feeding differentially on PA-laden items, has adaptive connotations. Without systemic PA, *Utetheisa* are vulnerable to predation. Sequestering the chemicals as larvae provides the insect with protection throughout life as well as with the ability to protect its eggs. It makes sense, therefore, that larvae should have the capacity to detect PAs both exogenously and endogenously (albeit in the latter case possibly indirectly), as well as the drive to 'go after' the compounds

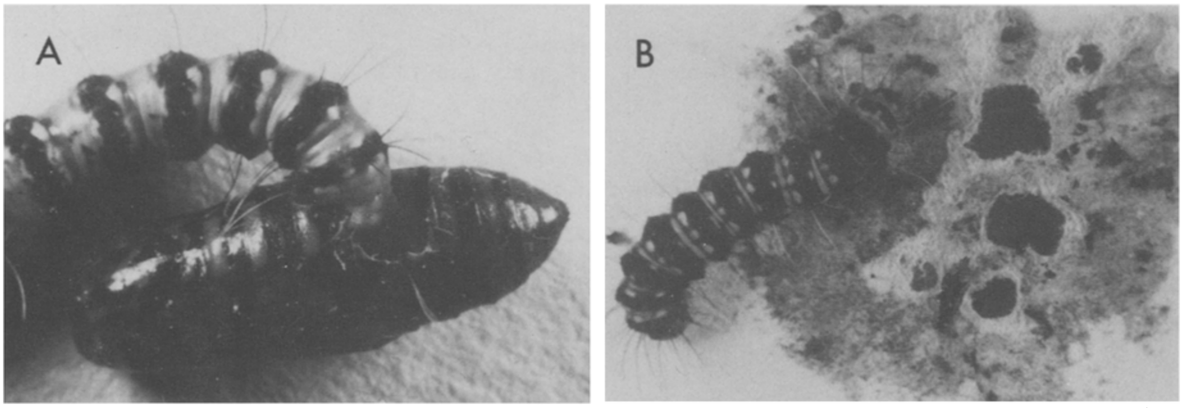

Figure 4. *A* Cannibalistic (−) larva feeding on a (+) pupa. *B* (−) Larva chewing into filter paper impregnated with a (+) pupal squashing. Note that three holes have already been chewed into the paper.

when they 'need them'. For male *Utetheisa*, the PAs have the added function of serving as precursors for the sex pheromone they use in courtship[4].

In nature, of course, larvae might remedy systemic PA deficiencies chiefly by seeking out the seeds of their foodplant rather than pupae. In our field test only very few pupae were actually cannibalized. We attribute this to the special circumstance that seeds were in abundance on our experimental *Crotalaria* at the time, and that the larvae on these plants were therefore not sufficiently PA-deficient to be highly cannibalistically inclined. But pupal cannibalism may be rare as a matter of course in *Utetheisa. Utetheisa* larvae do not, as a rule, pupate on their foodplant. As we ourselves determined, and was earlier noted by others (W. J. Conner, personal communication), mature *Utetheisa* larvae crawl away from their *Crotalaria* host to pupate on shrubs and herbs nearby, or under the bark of trees. Cannibalism in *Utetheisa* is much more likely to befall the eggs. As we will be reporting elsewhere, consumption of eggs by *Utetheisa* larvae is subject to the same 'rules' as reported here for pupal cannibalism. Thus, for instance, larvae tend to feed on eggs only if these contain PA and the larvae themselves are PA-deficient (Bogner and Eisner, unpublished).

While we do not here provide supporting data, we know from chemical analyses that (−) larvae that cannibalize (+) pupae acquire substantial loads of systemic PA. Actual assays of moths which as (−) larvae had each eaten a (+) pupa, gave a mean value of 546 ± 169 (SEM) μg monocrotaline/moth ($N = 10$) (J. Kopecky, unpublished data), a value closely matching that obtained with moths that acquired their PA naturally from seed-bearing *C. spectabilis*, or from our PA-containing laboratory diet (see 'Materials and methods').

Acquired PAs evidently play a dual role in the life of *Utetheisa*. While on the one hand they can convey protection against predation, they can on the other, increase vulnerability to cannibalism. One wonders to what extent, under changing natural conditions, the trade-off between these potentially conflicting functions may undergo shifts. Are there times when it 'pays' for *Utetheisa* to be deficient in systemic PA, times when the threat from cannibalism outweighs that from predation, times perhaps when foodplant seeds are scarce, larvae abundant, and PA to be found most concentrated in the body of conspecifics? In the absence of appropriate field data these questions remain open. What is clear is that the tendency of *Utetheisa* to pupate away from its hostplant provides a mechanism for minimizing the risks of cannibalistic attack from larvae.

Acknowledgments. Study supported by NIH grant AI02908, Hatch funds (191-7402), and the Deutsche Forschungsgemeinschaft (BO 944/1-2). We thank Patricia Bogner, Glen Scoles, and Maria Eisner for much help, B. Roach and J. Kopecky for analytical data, B. Roach for preparing monocrotaline N-oxide, J. Meinwald for the heliotrine sample and useful discussion, and an anonymous reviewer for a helpful suggestion. The field tests were carried out at the Archbold Biological Station, Lake Placid, Florida; Mark Deyrup kindly planted the *Crotalaria* stand.

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0014-4754/92/010097-06$1.50 + 0.20/0

Male production induced by antibiotic treatment in *Encarsia formosa* (Hymenoptera: Aphelinidae), an asexual species

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Received 21 January 1991; accepted 23 May 1991

Abstract. The production of large numbers of males in the thelytokous species *Encarsia formosa* was induced by feeding antibiotics to their mothers. The males induced by antibiotic treatment produce sperm and sometimes mate with females, but insemination does not occur.
Key words. Thelytoky; microorganisms; autoparasitoid; biological control; sex ratio; parthenogenesis.