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Evolution

Pheromonal advertisement of a nuptial gift by a male moth (*Utetheisa ornatrix*)*

(mate choice/male pheromone/pyrrolizidine alkaloid/hydroxydanaidal)

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ABSTRACT **Male *Utetheisa ornatrix* produce a courtship pheromone (hydroxydanaidal) that they derive from systemic pyrrolizidine alkaloid of plant origin. Pheromone titers in males correlate with systemic levels of alkaloid and with the quantity of alkaloid transmitted to the female at mating. The male's emission of pheromone during courtship may therefore communicate his possession of protective alkaloids and his capacity to provision the female. By mating preferentially with males endowed with hydroxydanaidal, females may ensure their acquisition of an alkaloidal gift for use in egg defense.**

In many insect species, males contribute resources to the female at mating (2). Benefits to the female from these nuptial gifts include increased fecundity (3–5) and enhanced offspring survivorship (6). When male donations are of variable quality, females may assess the gifts and mate preferentially with males able to bestow the most worthy offerings. Precopulatory gift assessment by the female can occur visually when offerings are presented overtly, as in the form of prey items (3, 7). But if gifts are bestowed covertly, by transfer with the spermatophore, their prenuptial evaluation becomes problematic.

Covert gift presentation occurs in the arctiid moth *Utetheisa ornatrix*, in which the males transmit protective pyrrolizidine alkaloids (for example, monocrotaline; structure I) to the females by seminal infusion (6). Mated females transfer alkaloid thus received, together with systemic alkaloid of their own, to the eggs, which are protected against predation as a result (6). Both males and females obtain their alkaloid from their larval food plants (*Crotalaria* spp.), thereby acquiring protection both for themselves (8, 9) and their offspring. Males, in addition, use the alkaloid metabolically to produce a pheromone, hydroxydanaidal (structure II), that they emit from a pair of brushes (coremata) everted during close-range precopulatory interaction with the female (10). Males endowed with hydroxydanaidal have a higher mating success than those devoid of the pheromone (10). The females possess antennal chemoreceptors by which they could gauge hydroxydanaidal (11).

The finding that hydroxydanaidal mediates male acceptance in courtship led to the suggestion that female *Utetheisa* might assess their suitor's defensive vigor (that is, their alkaloid content), as well as the male's alkaloid donating capacity, by quantitative appraisal of the pheromone (6, 8, 10). If so, males should contain pheromone titers proportional both to their intrinsic alkaloid load and to the quantity of alkaloid they bestow upon females. We here present laboratory data demonstrating that these quantitative relations hold and that males do indeed advertise their alkaloidal "worth" in the context of courtship.

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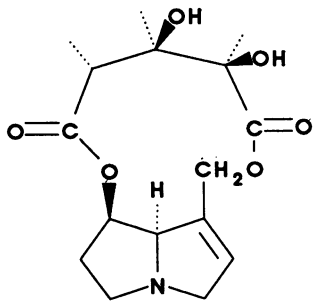

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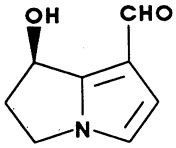

II

MATERIALS AND METHODS

Our approach involved rearing males on diets containing various levels of monocrotaline and then assaying chemically for (*i*) the hydroxydanaidal content of the coremata, (*ii*) the quantity of monocrotaline transferred to females at mating, and (*iii*) the quantity of monocrotaline remaining in males after mating.

In nature, *Utetheisa* obtain monocrotaline from *Crotalaria spectabilis*, one of the insect's principal food plants in the southern United States (12). *Utetheisa* feed preferentially on the seeds of the plant, where monocrotaline is concentrated (13, 14). In the laboratory we are able to raise *Utetheisa* on a semisynthetic diet based on pinto beans, devoid of pyrrolizidine alkaloid (10). To obtain males of graded monocrotaline content, larvae were raised on seven diets containing increasing percentages (dry weight) of *Crotalaria* seeds relative to pinto beans (0, 0.1, 1, 3, 5, 10, and 100%; the diets will henceforth be referred to by these percentages). The seeds and beans were ground by a blender prior to inclusion in the diets [*Crotalaria* seeds were first scarified in concentrated sulfuric acid (3 hr) and then soaked in water (18 hr) (15); the pinto beans were only soaked in water]. For the 100% diet, the *Crotalaria* seeds were included whole (after preparatory scarification and soaking), since larvae survived poorly when ground seeds were used. Larvae were transferred to the various diets near the end of the second instar; up to that age they were maintained on the alkaloid-free 0% diet (the pinto bean stock diet). Only females raised on the 0% diet were used in the matings. To ensure that the reared individuals did not contain alkaloids acquired by inheritance, their parents were selected from a laboratory line raised for several generations on the 0% diet.

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Four days after emergence, males weighing between 70 and 85 mg were paired individually with females and allowed to mate overnight. The next day, between 11 a.m. and 2:30 p.m., their two coremata (long spatulate scales plus underlying integumental membrane) were excised (12), and the males (n = 3 per diet), their female partners, and the paired coremata were stored frozen for subsequent chemical analysis. Our experiment assumes that hydroxydanaidal titers are not appreciably altered by courtship and mating, since pheromone levels were measured after mating, whereas corematal assessment on the part of the female occurs before coupling. To determine if mating affects hydroxydanaidal titers, coremata were also removed from 5-day-old virgin males (1, 10, and 100% diets; n = 3 males per diet).

The monocrotaline content of adults was determined by gas chromatographic analysis, using methods previously described (6), except that a different instrument and temperature program [Shimadzu (Columbia, MD) GC-mini-2 capillary column, 180–220°C at 2°C/min; eicosane as internal standard] were used for some of the determinations.

Coremata were extracted in carbon disulfide for 9 hr. The extracts were filtered, dried, redissolved in pyridine, and assayed by gas chromatography (Varian 2100; 6% Carbowax, 120–200°C at 8°C/min). Hydroxydanaidal levels were quantified by comparing peak areas of extracts with standard curves generated by using synthetic hydroxydanaidal.

By use of Spearman rank correlation coefficients (16), we tested for significance of correlation between the following parameters: prenuptial monocrotaline content of male, quantity of monocrotaline remaining in males after mating, quantity of monocrotaline transferred to female at mating, and hydroxydanaidal titer of coremata. The prenuptial monocrotaline content of males was calculated by summing the postmating monocrotaline contents of the mating partners (on the assumption that the amount detected in females represented the full amount relinquished by the males at mating).

RESULTS

The monocrotaline content of the males differed broadly (0–888 μg) and varied in accord to the *Crotalaria* seed content of the larval diet (Fig. 1). Maximal alkaloid levels were already achieved with the 10% diet. Males reared on the 0% diet lacked monocrotaline entirely, as did females that mated with these males. We conclude that dietary *Crotalaria* was the sole source of monocrotaline in our samples and that monocrotaline detected in females stemmed entirely from their male partners.

Corematal hydroxydanaidal levels also differed widely (0–21.8 μg) and correlated with the prenuptial monocrotaline content of the males (r = 0.95; $P < 0.01$) (Fig. 2*A*). The quantity of monocrotaline transferred to the female also correlated with the male's prenuptial monocrotaline load (r = 0.91, $P < 0.01$) (Fig. 2*B*), as well as with levels of monocrotaline remaining in males after mating (r = 0.89, $P < 0.01$). In addition, the quantity of monocrotaline transferred at mating correlated with the corematal hydroxydanaidal levels (r = 0.88; $P < 0.01$) (Fig. 2*C*), although variation was substantial.

The comparison of corematal hydroxydanaidal levels of the mated males with those of virgin controls revealed no significant differences ($P > 0.4$ for each diet; t test) (Fig. 3).

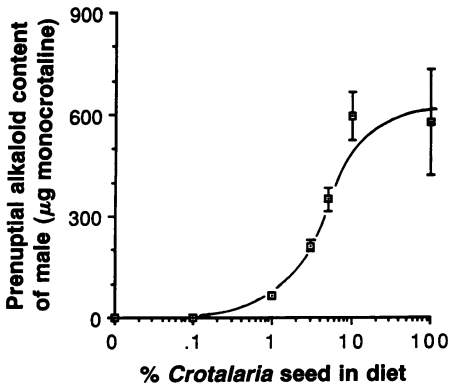

FIG. 1. Relationship between adult male alkaloid (monocrotaline) content (mean ± SEM) and *Crotalaria* seed content of larval diet. Percentages denote the fraction of *Crotalaria* seeds relative to pinto beans in the dietary formulations.

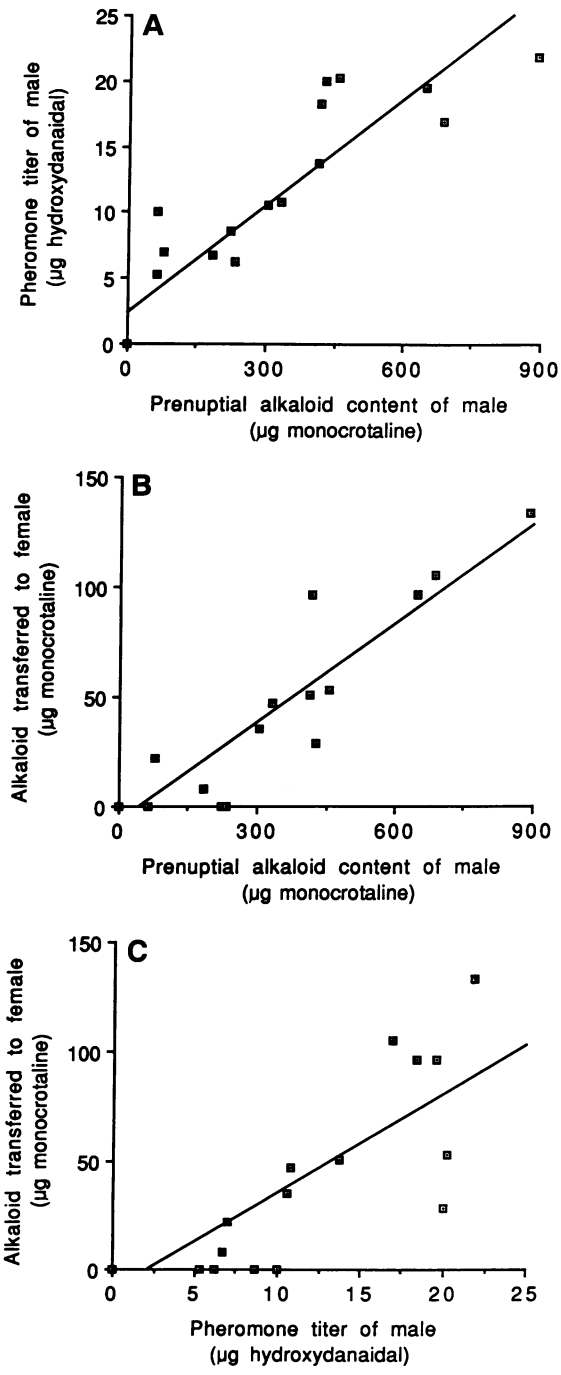

FIG. 2. Relationships between adult male alkaloid (monocrotaline) content, adult male pheromone (hydroxydanaidal) titer, and quantity of alkaloid (monocrotaline) transferred to the female at mating. The single points at the origin of *A*, *B*, and *C* are each a composite of six data points; the single point in *B* immediately to the right of the origin on the x axis is a composite of two data points.

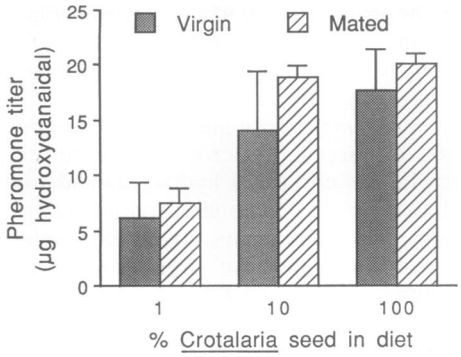

FIG. 3. Pheromone (hydroxydanaidal) titer (mean + SEM) of virgin and once-mated males, at 5 days after emergence, plotted as a function of larval diet. Conventions for diet percentages are as in Fig. 1.

Evidently, hydroxydanaidal is either not lost in significant measure during courtship or is maintained at relatively constant levels in the coremata by secretory replenishment.

DISCUSSION

While female choice has been demonstrated for a number of insect mating systems, it is often unclear how preferences promote female reproductive success (2). In *U. ornatrix*, given the quantitative relationships demonstrated here, the male pheromone has the potential to communicate the male's dietary history (alkaloid content of larval food), his state of chemical defendedness (systemic alkaloid content), and his alkaloid donating capacity. By favoring males of high hydroxydanaidal titer, females could benefit by accruing large alkaloidal gifts for investment in egg protection. But they could reap other gains as well. The alkaloid received from the male could in part bolster the female's own defenses, and the very act of coupling with an alkaloid-rich mate could confer protection during the lengthy period (often over 8 hr) that the female remains immovably, and therefore vulnerably, paired in copulation (10). By choosing males of high alkaloid content, females could also be selecting for a genetic capacity. *Utetheisa* larvae could differ in their ability to compete for the alkaloid-rich seeds of *Crotalaria*, as well as in their capacity to sequester ingested alkaloid (10, 12).

Field data indicate that *Utetheisa* males differ broadly in their systemic alkaloid content. Detected adult levels in individuals raised on *C. spectabilis* plants ranged from 0 to 1010 μg per individual (12), a span closely matching that of our laboratory males. Differences in alkaloid content in field-raised individuals have been shown to be a function of differences in dietary intake. Adults that as larvae fed on seedless, immature *Crotalaria*, and therefore had access to leaves only, attained substantially lower systemic alkaloid loads than individuals raised on seed-bearing mature plants (12). Hydroxydanaidal levels in wild males also differed widely, over a range (0–35 μg) closely in line with that of our laboratory males (12). The data presented here are therefore reflective of the variation normally present in *Utetheisa* populations.

The strategy of *Utetheisa* is complex, and a number of questions remain open. Both males and females mate multiply (17, 18). Do females remain equally receptive and selective, irrespective of how often they mated and how much total alkaloid they received from males? Does the proportionality of hydroxydanaidal to alkaloid remain constant as males become progressively depleted of alkaloid through repeated matings? [In the laboratory, males mate up to six times in 8 days, transferring alkaloid with each mating (17)]. Is the amount of nutrient received with the spermatophore another parameter potentially ascertainable by the female through hydroxydanaidal assessment? *Utetheisa* spermatophores are substantial, averaging 5.5 mg (for the male's first mating; $n = 30$) or ≈7.5% of male precopulatory mass.** Spermatophore mass correlates with male mass ($r = 0.52$, $P < 0.01$; $n = 30$; Pearson product moment correlation) (D.E.D., unpublished results), and mass (in males raised on *Crotalaria* plants) correlates with hydroxydanaidal levels (12).

Existing evidence indicates that pheromonal advertisement of male worth occurs in other insects as well. Indeed, pyrrolizidine alkaloids, together with pheromonal derivatives thereof, play a role in the courtship and defense of a number of butterflies and moths (19–27). Although critical quantitative data are not at hand for most of these species, we have suggested that female appraisal of potential paternal contribution may be a common feature of their mating strategies (17, 19–21). Particularly relevant are the findings with another arctiid moth, *Creatonotus transiens*, in which the male also provides pyrrolizidine alkaloid to the eggs and the coremata produce hydroxydanaidal and in which both hydroxydanaidal titer and coremata*l size* have been shown to bear a quantitative relation to systemic alkaloid content (26, 28).

**Spermatophore mass was calculated by taking the male copulatory mass loss and female copulatory mass gain, correcting each for spontaneous mass loss (measured in the same individuals on the previous night, over a time span comparable to the mating period), and averaging the two figures (17).

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