

## Corematal function in *Utetheisa ornatrix*: interpretation in the light of data from field-collected males

Alexander L. Bezzerides<sup>1</sup>, Vikram K. Iyengar<sup>2</sup> and Thomas Eisner<sup>1</sup>

<sup>1</sup>Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

<sup>2</sup>Department of Biology, Villanova University, Villanova, PA 19085, USA

**Summary.** Males of the moth *Utetheisa ornatrix* produce a pheromone, hydroxydanaidal, contained in two brush-like organs, the coremata, which they evert during courtship. Hydroxydanaidal is derived chemically by the males from pyrrolizidine alkaloids that they sequester from their larval food plants. It had been shown previously that the amount of hydroxydanaidal in the coremata correlates positively with a number of male parameters that are a measure of the male's "worth." Hydroxydanaidal could thus be used by the female as a basis for discrimination in courtship. Experiments carried out with virgin *Utetheisa* had shown that females do indeed mate selectively with males of high corematal hydroxydanaidal content, thereby accruing the multiple benefits that are the consequence of such selectivity. Unresolved was the question whether corematal hydroxydanaidal could still provide a basis for female appraisal of suitors when these had previously mated. Given the proven promiscuity of the female, male *Utetheisa* could be expected to be promiscuous as well. Evidence we present here shows that field-collected males, which could be expected to be mostly non-virginal, are of reduced acceptability to females. They are also, on average, in possession of lower quantities of hydroxydanaidal, explaining possibly why they should be held in lesser regard. At low levels, however, hydroxydanaidal does not lend itself for precise assessment of male worth, because as we show herein, at such levels, the compound does not correlate with any of the criteria of male quality (male size, spermatophore mass, male pyrrolizidine alkaloid content, amount of pyrrolizidine alkaloid transmitted to the female). Males that have mated beforehand are therefore likely not to be classed into subcategories by the females, but simply as belonging to one category of "less desirables". The significance of these findings to our understanding of the mating strategy of *Utetheisa* is discussed.

**Key words.** Lepidoptera – Arctiidae – pyrrolizidine alkaloid – sexual selection

### Introduction

Pyrrolizidine alkaloids (PAs; singular, PA) play a fundamental role in the life of the arctiid moth *Utetheisa ornatrix*

Correspondence to: Thomas Eisner, e-mail: te14@cornell.edu

(henceforth referred to as *Utetheisa*) (Eisner & Meinwald 1995; Eisner & Meinwald 2003). The insect sequesters these toxins from its larval foodplants (*Crotalaria* spp., Family Fabaceae), and retains them through metamorphosis into the adult stage. The female transmits PA to the eggs, using not only some of the PA she herself sequestered, but also PA that she receives from the male with the spermatophore at mating (Dussourd *et al.* 1988; Bezzerides & Eisner 2002). As a result of PA possession, all stages of the moth are protected against enemies, the adults and larvae against spiders (Eisner & Eisner 1991; Eisner & Meinwald 2003), and the eggs against ants (Hare & Eisner 1996), chrysopid larvae (Eisner *et al.* 2000), coccinellid beetles (Dussourd *et al.* 1988), and parasitoid wasps (Bezzerides *et al.* 2004). The male produces a pheromone, hydroxydanaidal (HD), that he derives chemically from the acquired PA. The chemical is more volatile than PA, and is aired by the male from two brush-like structures, the coremata, that he everts during close range precopulatory interaction with the female (Conner *et al.* 1981).

We had shown earlier that the male's corematal HD titer correlates positively, both with the male's PA content and the amount of PA that the male transmits to the female as a gift, leading us to postulate that the female might use the male's HD titer as a parameter for gauging male "worth" (Conner *et al.* 1981; Dussourd *et al.* 1991). Further, since the male's PA content correlated also with male mass (as well as with mass of spermatophore transferred), it seemed that what the females could gauge, ultimately, through assessment of the male's HD, was male size (Iyengar & Eisner 1999a; Conner *et al.* 1990; LaMunyon & Eisner 1994). This seemed important, since body size is a heritable trait in *Utetheisa* (Iyengar & Eisner 1999a). The corematal scent, therefore, could provide the female with a means for assessing, on the one hand, the phenotypic benefits that she might accrue from a male (that is, the amount of PA and nutrient contained in his spermatophore) and on the other, the genetic benefits (by favoring large males the female could assure that she produced larger sons, more successful in courtship, and larger daughters, able to produce more eggs) (Iyengar & Eisner 1999b; Eisner & Meinwald 2003). HD, in other words, could be the female *Utetheisa's* ticket to reproductive success.

Such evidence as we had did indeed indicate that female *Utetheisa* mate selectively. If given a choice between males containing PA, and PA-free control males (raised on PA-free

diet) that lacked HD, they showed a significant preference for the former. Such selectivity was exercised by the female not only in laboratory tests, in which the two types of males were offered simultaneously to her for comparative assessment (Iyengar & Eisner 1999b), but in outdoor experiments, in which the two males were presented to females in separate tests and were therefore subjected to independent assessment (Conner *et al.* 1981). PA-free males were not all rejected in these experiments, but they proved distinctly less acceptable than their PA-containing counterparts.

Further evidence indicated that HD was the sole criterion of choice upon which the female based her preference. Females failed to differentiate between males of different size or PA content, if these males lacked HD, but they expressed a preference for males bearing HD, even when these were size-matched and PA-free (Iyengar *et al.* 2001). There was direct evidence, also, that HD had signal value. Contacting a female with HD-bearing coremata elicited abdominal movements on her part typical of those leading to male acceptance (Conner *et al.* 1981). Moreover, the female antennae in *Utetheisa* were found to be beset with chemoreceptors highly sensitive to the (+)isomer of HD, the form in which the chemical ordinarily occurs in the coremata (Grant *et al.* 1989). The evidence for a fundamental pheromonal function for HD seemed compelling. Yet there were questions that remained open.

*Utetheisa* females are promiscuous. Female mating incidence is readily established by counting the number of colla (that is, the tubular remnants of spermatophores) in the bursa (the spermatophore receptacle) of the female. Based on such counts, previous investigators had reported mating frequencies of, on average, 4 to 5 per female *Utetheisa* in nature (Pease 1968). Data that we ourselves obtained, based on females from a single well-established population of the moth (on the grounds of the Archbold Biological Station, Lake Placid, Florida), showed colla counts to average 11 per female, and to range upward to 23 per female (Bezzerrides & Eisner 2002). So far as we know, these are record highs for Lepidoptera.

Data that we had so far, pertaining to male quality and female choice in *Utetheisa*, were obtained for the most part with virginal individuals. The correlation we had found between male body mass, spermatophore mass, body PA content and HD titer, were descriptive, therefore, of the relationship of these parameters in the sexually "unspent" male, that is, the male still in full possession of his alkaloidal and nutritive reserves. But what about in the experienced male? Is there still a correlation between these parameters after the male has mated and lost some of his body mass and PA? And in particular, is HD titer then still a correlate of the other parameters, and therefore a predictor of male worth?

To answer these questions we undertook a simple experiment. We collected *Utetheisa* males from our field population at the Archbold Station, and paired these males individually with virgin PA-free females from our laboratory colony. For pairs that opted to mate, we then made a series of quantitative determinations and looked for underlying correlations. Specifically, we ascertained the following: male wing area (an indicator of "original" male mass, i.e. mass on emergence from pupa); male mass prior to mating (determined directly); spermatophore size (calculated from

mass transferred by male to female at mating); PA received by female (determined by analysis of female); male PA content prior to mating (determined by adding the amount of PA contained in male and female after mating); and coremata HD content (determined by analysis). We found these values for the most part to be lower with these males than they were with virgins, which was to be expected. The parameters also correlated as they did in virgins, but there was an exception. Coremata HD content varied independently.

We here present these results, which added some complication to our interpretation of the sexual strategy of *Utetheisa*.

## Materials and Methods

### Laboratory rearing of females

The virgin female *Utetheisa* used in the matings were derived from our laboratory colony, established years ago from individuals collected at or near the Archbold Biological Station, Lake Placid, Florida. These females were reared on an artificial diet based on pinto beans (Miller *et al.* 1976), rather than *Crotalaria* beans, under which conditions they develop to adulthood entirely PA-free.

### Field collection of males

*Utetheisa* males used in the matings were taken by net at a site at the Archbold Station where the ground cover included dense patches of *Crotalaria mucronata* (whose principal contained PA is usaramine), and an occasional *Crotalaria spectabilis* plant (whose principal contained PA is monocrotaline) (Conner *et al.* 1981). As expected, these males contained PA. Males were paired with PA-free females on the evening of capture, and were weighed prior to being paired.

### Matings

Pairings were effected, in accord to a previously-described protocol (LaMunyon & Eisner 1994), by confining individual field-collected males with individual virginal laboratory-raised PA-free females, in humidified cylindrical containers (0.35 L). Pairs were checked at 6 h intervals beginning at dusk, to verify that mating took place (mating lasts 10–12 h in *Utetheisa*).

A total of 50 pairs were thus caged, and given the opportunity to mate.

### Calculation of spermatophore mass

Paired males and females were weighed before and after mating. For each pair that mated, the mass of spermatophore transferred was calculated by taking the average between the male's weight loss during the period of confinement with the female, and the female's weight gain during that period (both weights being corrected for spontaneous mass loss of the individuals) (LaMunyon and Eisner 1994).

For three of the pairs that mated the calculation of spermatophore mass yielded a negative value. For statistical calculations these values were set to zero.

### Coremata HD content

Following mating, the two coremata of each male were excised, and stored frozen (dry ice) in 200  $\mu$ l dichloromethane. Samples were later analyzed, after transport to our Cornell laboratories, by the technique described elsewhere (Bezzerrides 2004).

### Male PA content and magnitude of alkaloidal nuptial gift

Following excision of the coremata from the males, both males and females were stored frozen (dry ice) for their transport to Cornell, where each individual was extracted in 500  $\mu$ l of buffer solution (González *et al.* 1999), centrifuged at 14,000 rpm for 10 min, (Eppendorf 5415C centrifuge), filtered (Gelman 0.45  $\mu$ m CR PTFE Acrodisc), and analyzed for PA content by high pressure liquid chromatography as described previously (González *et al.* 1999). The quantity of PA detected in the female was taken to represent the magnitude of the male's alkaloidal nuptial gift. The magnitude of the male's prenuptial alkaloidal load was calculated by adding the values obtained for PA content of the mating partners.

Most moths contained usaramine as their sole PA, indicating that they had fed as larvae on *Crotalaria mucronata*, the dominant *Crotalaria* species at the collection site. But monocrotaline was present in very small quantity as an accompanying PA in a portion of the samples, suggesting that some individuals had also fed on *C. spectabilis*. For individuals containing both alkaloids, PA content was calculated by summing the quantities contributed by each. Both PAs were found to occur in their free-base and N-oxide forms.

### Calculation of male wing area

We have established recently that wing area (defined as the sum of the wing area of the two forewings) is a reliable correlate of original adult mass in *Utetheisa*, that is, of adult mass at the time of emergence from the pupa ( $r^2 = 0.38$ , d.f. = 49,  $P < 0.0001$ ; unpublished data based on wing area and body mass determination of 3-day old virgin adults). The values we obtained for body mass of the experimental males used herein could be expected to be low relative to the original body mass, given that these males at the time of capture had in all probability already lost some body mass to previous mating partners. Wing area measurement therefore provided an indirect means for obtaining some indication of original body mass. To determine wing area of our experimental males, the forewings were clipped from the moths and affixed with sticky tape to paper. The paper with wings in place was then photocopied, and the individual wing images (both forewings for each moth) were cut from the paper and weighed. Wing area was then calculated by reference to the known proportionality of paper weight to area.

### Statistics

All variables were measured on a continuous scale, and showed normal distribution of values (Shapiro-Wilk's test,  $P > 0.05$ ), except for the size of the spermatophore and the amount of PA transferred at mating. The distribution of values for these two variables was normal after square root transformation. Pearson's product-moment correlation was used to determine the strength of the relationship between the measured characteristics. All reported  $P$  values are two-tailed. Values cited in text are expressed as mean  $\pm$  S.E.

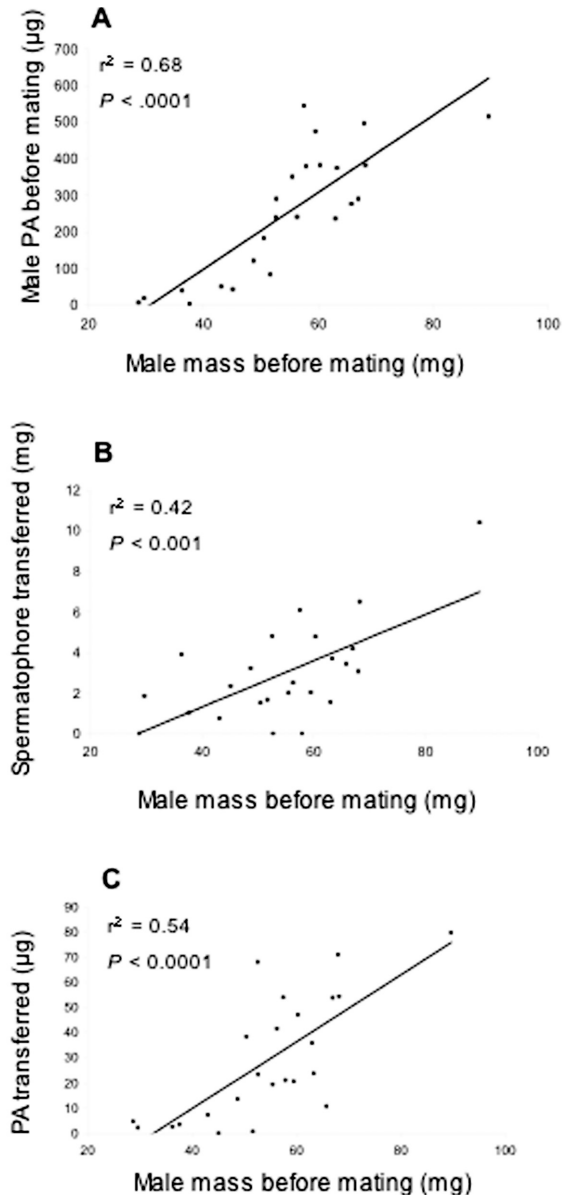
## Results

### Mating incidence

Of the 50 pairs that were set up in the nuptial containers, 24 proceeded to mate. These 24 pairs constituted the sample size  $N$  upon which all determinations were based.

### Relationship of male mass to other male traits

As is evident from Fig. 1 A-C, the amount of PA present in the male prior to mating, the mass of the spermatophore transferred, and the mass of PA bestowed upon the female,

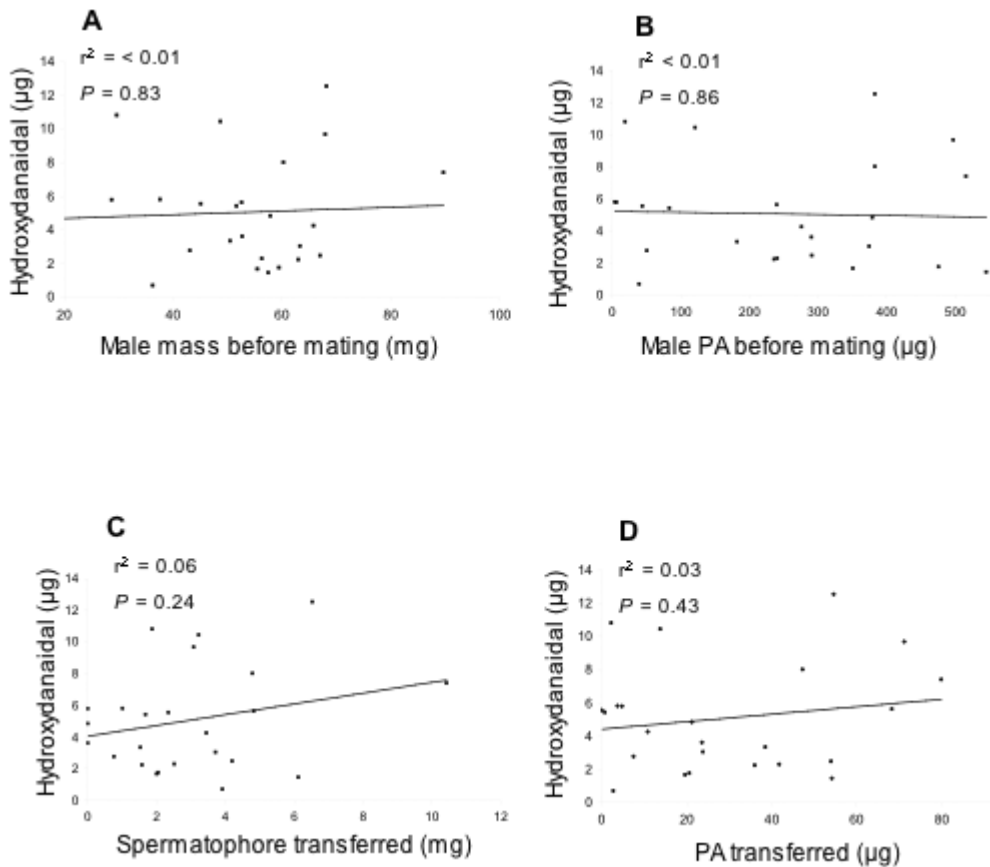


**Fig. 1** (A) Male PA content, (B) spermatophore mass, and (C) PA transferred at mating, plotted as a function of male mass before mating. Each parameter correlated positively with male mass before mating

all correlated positively with the mass of the male prior to mating.

### Relationships of corematal HD content to other male traits

Corematal HD content did not significantly correlate with male mass prior to mating, male PA content before mating, mass of spermatophore transferred, or amount of PA bestowed upon the female (Fig. 2 A-D). Corematal HD level also failed to correlate with male wing area, in other words, with a parameter that is reflective of the original mass of the male (Fig. 3 A). Spermatophore mass, in contrast, did correlate with wing area (Fig. 3 B).



**Fig. 2** Corematernal hydroxydanaidal (HD) content, plotted as a function of (A) male mass before mating, (B) male PA content before mating, and (C) spermatophore transferred at mating, and (D) PA transferred at mating. HD content did not correlate significantly with any of the parameters

## Discussion

The data presented herein raise more questions than they answer, and are not conducive to easy interpretation. They are worth examining, however, in relation to specific queries:

*What do the data tell us about male acceptability?*

Only 24 of the 50 pairs that we set up in the mating chambers proceeded to copulate. This is a low mating incidence, indicative of a male acceptability rating of just under 50%. This figure matched the acceptability rating of PA-free laboratory-reared males offered to laboratory-reared females in tests outdoors (Conner *et al.* 1981). PA-containing males, in contrast, tested under identical outdoor circumstances, proved 100% acceptable (Conner *et al.* 1981). Our present set of males can thus be said to have been judged as decidedly undesirable.

*To what can one attribute this negative ranking?*

We are inclined to believe that the cause lies in the fact that our males were all in considerable measure “spent” at the time of capture, in the sense that they were largely PA-depleted as a consequence of previous matings. We knew the females at the site to be highly promiscuous and assumed the males to be equally so. We also knew laboratory-raised *Utetheisa* males to mate with up to 6 females over the course

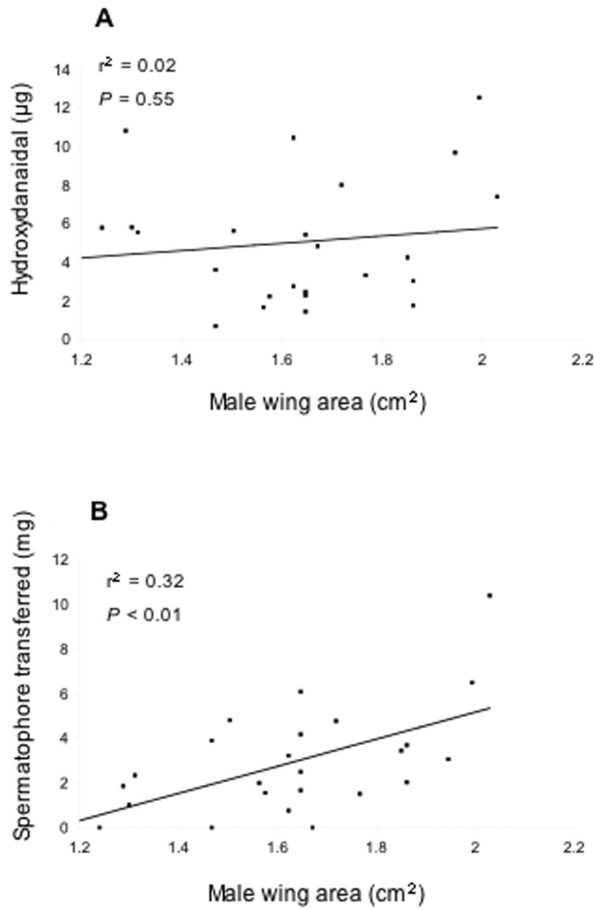
of 8 days if given the chance (Dussourd *et al.* 1991). It was our prediction, therefore, that our males should have low PA contents, produce lesser-sized spermatophores, and contain relatively low HD titers. All these predictions turned out true. The males contained less than half as much PA ( $251 \pm 36 \mu\text{g}$ ) as is average ( $540 \pm 30 \mu\text{g}$ ) for newly emerged virginal *Utetheisa* raised on mature pod-bearing *Crotalaria* plants outdoors (Conner *et al.* 1990); they produced smaller spermatophores ( $2.98 \pm 0.49 \text{ mg}$ ) than are produced by virgin males on their first mating ( $6.82 \text{ mg}$  on average) (LaMunyon 1992), and contained less corematernal HD ( $5.86 \pm 0.67 \mu\text{g}$ ) than is average for field-collected males ( $15.8 \pm 8.2 \mu\text{g}$ ) (Conner *et al.* 1990).

It should be noted further that the females that we paired with our males, on account of being PA-free, might have been expected to be fully accepting of their lowly-endowed male partners, given that these could provide at least some PA. The fact that these females were choosy, despite their total lack of PA suggests that they may be programmed to be fundamentally uncompromising in the matter of mate selection.

*What can we conclude from the fact that corematernal HD-content did not correlate tightly with any of the other assessed parameters in our males?*

Our conclusion that the males in our population were on the whole PA-impoverished and for that reason relatively undesirable, begs the question of how *Utetheisa* females come





**Fig. 3** Corematal hydroxydanaidal (HD) content (A) and spermatophore mass (B), plotted as a function of male wing area. HD content did not correlate with wing area, whereas spermatophore mass did show such a correlation

to ascertain that prospective partners are chemically insufficient. Given, as we here show, that corematal HD level does not correlate with systemic PA level in PA-impooverished males, it is clear that the female cannot gauge the PA content of such males with precision, on the basis of HD titer. But could being able to make a rough assessment not in itself be of use to the female? Could females not simply assign all males of lower HD levels – such as the males in our sample — to the ranks of the “less desirable” and those of high levels to the ranks of the “preferable”? Discrimination could thus be effected coarsely by the female, across the entire quantitative range of PA possessed by the male, albeit with no more than an approximate assurance of success. What our data show, in our judgement, is not that HD is useless as a predictor of male PA content when such content is low, but merely that it is inadequate for fine quantitative resolution of the parameter. Our sample of males, we believe, was truncate, in the sense that it lacked males of high PA content. Had we found such males, our sample would doubtless have included individuals of high HD titer, such as we know to exist in natural *Utetheisa* populations (Conner *et al.* 1990), individuals which we would predict would have met with a high degree of acceptance, and

would have transmitted, on average, large nuptial gifts. Why were such males rare in our population? Probably because males in a setting of high promiscuity do not long endure as virgins, that is, in the condition in which they contain undiminished amounts of PA (and nutrient). Evidence that virginity was indeed of low prevalence in our population was obtained with females: dissection of 64 females taken at the site revealed not a single individual totally lacking in colla (Bezzerides & Eisner 2002).

It is of some interest that spermatophore mass in our males correlated with wing area, and indirectly therefore with male mass at time of emergence from the pupa. This means that a female, even if courted by a resource-impooverished male unable accurately to advertise his quality by way of his HD titer, may have the option, after insemination, to gauge male quality by way of spermatophore size. Female *Utetheisa* are known to exercise sperm selection, and to favor sperm from larger spermatophores. They might therefore, if improperly “informed” of the male’s quality by way of HD, take corrective action and judge the male after mating by the size of its spermatophore.

*What are some of the remaining questions?*

One question of importance that remains totally open is whether a female’s ability to accommodate spermatophores is affected by her mating history. It would obviously have been desirable to have data on the size of spermatophore transmitted when the female’s bursa is partly encumbered by remnants of spermatophores from previous matings. Experiments comparable to those described herein, but with virgin males and previously-mated females are obviously in order. It is hard to imagine how full-sized spermatophores might be accommodated by females if they had repeatedly mated. Bursae that we examined of field-collected females were sometimes noted to be crammed to capacity with colla. And what attention, one might ask, does the female pay to the corematal signal of the male, if she is unable to receive but a fraction of what he has to offer? And why do females mate as often as they do? Do they derive benefits in sufficient measure from a mating no matter how often they have already mated? Do they perhaps heed the message implied in the corematal signal of the male only preparatory to their first set of matings, when they are still able to incorporate the full measure of a male’s gift? There are obviously secrets that *Utetheisa* has yet to share with us.

### Acknowledgements

This research was supported by the National Institutes of Health (AIO2908) and the National Institutes of Mental Health (5T32MIH51793). We are grateful to Janice Beal for maintenance of our moth culture, and to the staff of the Archbold Biological Station for countless favors. This is paper no. 191 of the series Defense Mechanisms of Arthropods.

### References

- Bezzerides A (2004) Phenotypic and genetic benefits of promiscuity in an arctiid moth (*Utetheisa ornatix*). Ph.D. dissertation (Cornell University, Ithaca, NY)

- Bezzerides A, Eisner T (2002) Apportionment of nuptial alkaloidal gifts by a multiply-mated female moth (*Utetheisa ornatrix*): eggs individually receive alkaloid from more than one male source. *Chemoecol* 12: 213–218
- Bezzerides A, Yong T-H, Bezzerides J, Hussein J, Ladau J, Eisner M, Eisner T (2004) Plant-derived pyrrolizidine alkaloid protects eggs of a moth (*Utetheisa ornatrix*) against a parasitoid wasp (*Trichogramma ostrinae*). *Proc Nat Acad Sci USA* 101: 9029–9032
- Conner WE, Eisner T, Vander Meer RK, Guerrero A, Meinwald J (1981) Precopulatory sexual interaction in an arctiid moth (*Utetheisa ornatrix*): role of a pheromone derived from dietary alkaloids. *Behav Ecol Sociobiol* 9: 227–235
- Conner WE, Roach B, Benedict E, Meinwald J, Eisner T (1990) Courtship pheromone production and body size as correlates of larval diet in males of the arctiid moth, *Utetheisa ornatrix*. *J Chem Ecol* 16: 542–552
- Dussourd DE., Harvis CA, Meinwald J, Eisner T (1991) Pheromonal advertisement of a nuptial gift by a male moth *Utetheisa ornatrix*. *Proc Nat Acad Sci USA* 88: 9224–9227
- Dussourd DE, Ubik K, Harvis C, Resch J, Meinwald J, Eisner T (1988) Biparental defensive endowment of eggs with acquired plant alkaloid in the moth *Utetheisa ornatrix*. *Proc Nat Acad Sci USA* 85: 5992–5996
- Eisner T, Eisner M (1991) Unpalatability of the pyrrolizidine alkaloid containing moth, *Utetheisa ornatrix*, and its larva, to wolf spiders. *Psyche* 98: 111–118
- Eisner T, Meinwald J (1995) The chemistry of sexual selection. *Proc Nat Acad Sci USA* 92: 50–55
- Eisner T, Meinwald J (2003) Alkaloid-derived pheromones and sexual selection in Lepidoptera. Pp. 341–368 in Blomquist GH & Vogt RG (eds) *Insect Pheromone Biochemistry and Molecular Biology*. Florida: Academic Press Inc
- Eisner T, Eisner M, Rossini C, Iyengar VK, Roach BL, Benedikt E, Meinwald J (2000) Chemical defense against predation in an insect egg. *Proc Nat Acad Sci USA* 97: 1634–1639
- Grant AJ, O'Connell RJ, Eisner T (1989) Pheromone-mediated sexual selection in the moth *Utetheisa ornatrix*: olfactory receptor neurons responsive to a male-produced pheromone. *J Ins Behav* 2: 371–385
- González A, Rossini C, Eisner M, Eisner T (1999) Sexually transmitted chemical defense in a moth (*Utetheisa ornatrix*). *Proc Nat Acad Sci USA* 96: 5570–5574.
- Hare JF, Eisner T (1996) Cannibalistic caterpillars (*Utetheisa ornatrix*) fail to differentiate between eggs on the basis of kinship. *Psyche* 102: 27–33
- Iyengar VK, Eisner T (1999a) Heritability of body mass, a sexually selected trait, in an arctiid moth (*Utetheisa ornatrix*). *Proc Nat Acad Sci USA* 96: 9169–9171
- Iyengar VK, Eisner T (1999b) Female choice increases offspring fitness in an arctiid moth (*Utetheisa ornatrix*). *Proc Nat Acad Sci USA* 96: 15013–15016.
- Iyengar VK, Rossini C, Eisner T (2001) Precopulatory assessment of male quality in an arctiid moth (*Utetheisa ornatrix*): hydroxydanoidal is the only criterion of choice. *Behav Ecol Sociobiol* 49: 283–288
- LaMunyon CW (1992) Multiple mating and its implications in an arctiid moth (*Utetheisa ornatrix*). Ph.D. dissertation (Cornell University, Ithaca, NY)
- LaMunyon CW, Eisner T (1994) Spermatophore size as determinant of paternity in an arctiid moth (*Utetheisa ornatrix*). *Proc Nat Acad Sci USA*. 91, 7081–7084
- Miller JR, Baker TC, Cardé RT, Roelofs WL (1976) Reinvestigation of oak leaf roller sex pheromone components and the hypothesis that they vary with diet. *Science* 192: 140–142
- Pease RW Jr (1968) The evolutionary and biological significance of multiple pairing in Lepidoptera. *J Lep Soc* 22: 69–73

Received 22 April 2005; accepted 20 May 2005.  
Published Online First 8 July 2005.



To access this journal online:  
<http://www.birkhauser.ch>