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HYDROXYDANAIDAL AND THE COURTSHIP OF *HAPLOA* (ARCTIIDAE)

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ABSTRACT. Male *Haploa clymene* display large tubular coremata in the moments before mating. The coremata are deployed in the immediate vicinity of the female's antennae and their eversion is a prerequisite for mating success. The major volatile component associated with the coremata of *H. clymene* and *H. confusa* is the dihydropyrrolizine hydroxydanaidal. In *H. clymene* production of hydroxydanaidal is contingent on larval access to their natural hostplants containing pyrrolizidine alkaloid precursors. The widespread distribution of hydroxydanaidal in arctiid moths suggests a single early evolutionary origin for the ability to produce it. This origin appears to precede the divergence of three arctiid subfamilies: the Arctiinae, Pericopinae, and the Ctenuchinae.

Additional key words: coremata, mating behavior, pyrrolizidine alkaloids.

Male moths of the family Arctiidae possess some of the most morphologically elaborate scent-disseminating structures known in the Lepidoptera (Birch et al. 1990). These structures are most often displayed in the moments before mating and are thought to play a role in sexual selection (Eisner & Meinwald 1995). The pheromonal signals they release have been characterized in several cases, and the compound hydroxydanaidal (or the related compound danaidal) has been identified repeatedly. Hydroxydanaidal is a volatile dihydropyrrolizine (Fig. 1) derived from defensive pyrrolizidine alkaloids (PAs) sequestered from the larval or adult food of each species (Conner et al. 1981, Boppré & Schneider 1985, Krasnoff & Roelofs 1989). We here add *Haploa clymene* (Brown) and *H. confusa* (Lyman) to the growing list of hydroxydanaidal bearers, investigate the dependence of hydroxydanaidal production on larval diet in *H. clymene*, and describe the courtship of this species. We also suggest a single evolutionary origin for hydroxydanaidal production within the Arctiidae.

MATERIALS AND METHODS

Haploa clymene were collected as larvae on *Eupatorium purpureum* L. in Forsyth County, North Carolina. Adults were collected at blacklights and allowed to mate and lay eggs. Larvae were fed fresh leaves of *E. purpureum*, a member of a genus known to contain pyrrolizidine alkaloids (Mattocks 1986), or *Plantago rugelii* Dcne., a plant known to be devoid of pyrrolizidine alkaloids (Cronquist 1981). Additional larvae

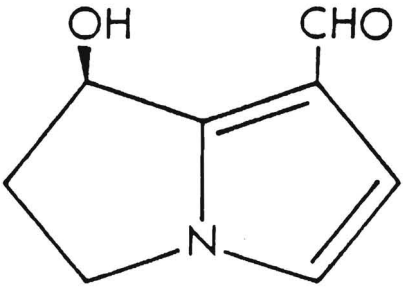

FIG. 1. *R*-(–)- hydroxydanaidal.

were started on *E. purpureum* for their initial instar and then switched to an alkaloid-free *Manduca* artificial diet (Bell & Joachim 1976) until pupation. All larvae and adults were held at room temperature on a 16L:8D photoperiod regime. Final-instar larvae of *H. confusa* were collected on *Eupatorium maculatum* L. and *Lythrum salicaria* L. by Scott Smedley in Ithaca, New York. These were fed *E. purpureum* until pupation.

Coremata are tubular and inflatable scent-disseminating structures found in males of many species of arctiid moths. They were everted from between the 7th and 8th abdominal segments of 2–4 day old male *Haploa* by gently squeezing their abdomens. The coremata were excised with iridectomy scissors, and dropped into 100 µl of methylene chloride. Benzophenone was used as an internal standard. Quantitative chromatography was carried out on a Hewlett Packard (HP) 55790A gas liquid chromatograph with an on-column injection port and flame ionization detector. Ultra I (methyl silicone) and Ultra II (phenyl methyl silicone) columns (Hewlett Packard; 25 m, 0.32 mm ID, 0.52 mm film thickness) were used. The carrier flow was 5.0ml/min with an initial temperature of 50°C held for 2 min; the temperature was then increased to 280°C at 5°C/min and held for 2 min. Authentic samples of *R*-(–)-hydroxydanaidal (99% pure) were provided by Jerrold Meinwald of the Department of Chemistry, Cornell University.

Mass spectrometry and infrared spectroscopy were carried out on an HP 5890 GLC coupled with an HP 5965A infrared detector and an HP 5970 mass selective detector. Compounds were separated on a 60 m, 0.25 mm ID, 0.25 mm methyl silicone column. The initial temperature was held at 100°C for 2 min and increased to 240°C at 2.5°C/min.

Courtship behavior of *H. clymene* was observed and recorded in a laboratory wind tunnel (60 × 60 × 150 cm; windspeed = 25 cm/sec) under deep red illumination (<5 lux). Mating sequences were videotaped using a BGC CCD-500E video camera and a JVC BR9000 video recorder.

Ultrasound was monitored using a QMC S200 bat detector and recorded on the audiotrack of the videotape. Some males were rendered incapable of everting their coremata by applying a cyanoacrylate ester-based glue to their partially everted coremata and allowing the coremata to retract.

RESULTS

Haploa clymene mate between 3 h and 7 h after the onset of scotophase. The courtship is initiated by the female through the release of a typical arctiid sex attractant blend. Female pheromone-releasing behavior is readily apparent as the rhythmic exposure of the openings of tubular sex pheromone glands. The sex attractant, composed of Z,Z,Z-3,6,9-heneicosatriene and related compounds (Davidson 1995), stimulates males to fly upwind and seek females. When the male reaches the female he exposes a pair of inflatable cuticular tubes called coremata. They extend from their origin in the intersegmental membrane between the male's seventh and eighth abdominal segments, often encircle the female's abdomen, and curve together just above the head of the female, presumably stimulating her antennae with male courtship pheromone (Fig. 2). Genital contact is made and copulation ensues. Although *Haploa* have well-developed tymbal organs (Fullard & Fenton 1977, Davidson 1995) like those that have been shown to be involved in the courtship of several arctiid species (Simmons & Conner 1996) their courtship is silent.

The major volatile component associated with the coremata of both *H. clymene* (field collected as adults or fed *E. purpureum* through all their larval stages) and *H. confusa* (collected as final instar larvae on *E. maculatum* or *L. salicaria* and fed *E. purpureum* for the remainder of their larval life) matched an authentic sample of hydroxydanaidal in retention time on all three columns, IR spectrum, and mass spectrum (*m/z* (relative intensity): 151(3.5), 133(59), 104(100), 77(26), 51(35)).

The coremata of male *H. clymene* raised through all larval instars on *E. purpureum* produced an average of 0.88 ± 0.68 µg hydroxydanaidal/individual (n = 13) with a range from 0.08 to 2.18 µg/individual. Males raised on *Plantago rugelii* (n = 1) or the alkaloid-free diet (n = 11) contained no detectable hydroxydanaidal in their coremata (detection limit 0.01 µg/individual). There were no apparent differences in the morphology of the coremata of *H. clymene* raised on the three diets. A composite sample of the coremata of twenty *H. confusa* had a mean corematal titer roughly ten times lower (0.0685 µg/individual).

The courtships of six unoperated male *H. clymene* (reared on *E. purpureum*), each paired with a female, were videotaped in the laboratory wind tunnel. In 5 of 6 cases the coremata of the males were clearly visi-

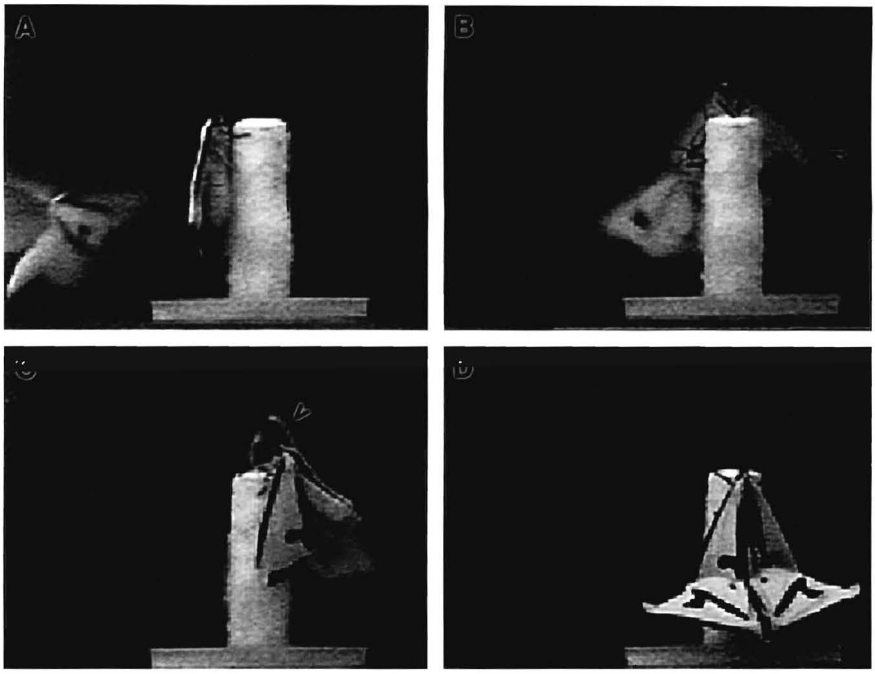

FIG. 2. Freeze-frame video sequence of the courtship of *H. clymene*. A, male approaches female (0.00 sec); B, male everts coremata (1.52 sec); C, coremata reach the antennae of the female (2.24 sec); D, pair *in copulo* (10.89 sec). Arrows mark everted coremata.

ble and the courtship resulted in copulation. In the one case where the male did not evert his coremata, the courtship was unsuccessful because the female evaded the male by flying away. Three courtship sequences involving males rendered incapable of everting their coremata by gluing were all unsuccessful. In each case the female evaded the male and effectively terminated the encounter. The difference between the success rates of unoperated males and the glued males was significant (Fisher exact probability, P = 0.047). Due to the small number of males available sham-operated controls could not be performed for this experiment. However, previous studies indicate that the application of glue one segment forward on the abdominal venter had no effect on courtship success in an arctiid (Conner 1987).

DISCUSSION

It is clear that *Haploa clymene* use hydroxydanaidal-laden coremata during courtship and are similar to *Utetheisa ornatrix* L. (Conner et al. 1981), *Pyrrarctia isabella* (J. E. Smith), *Phragmatobia fuliginosa* (L.) (Krasnoff & Roelofs 1990), and *Cisseps fulvicollis* (Hubn.) (Meyer 1984)

in exposing the coremata briefly just prior to contact between the male and female. Cornematal exposure appears to be critical to courtship success; in its absence females evade males. The antennae of female *H. clymene* have been shown through electroantennogram bioassay to be sensitive to hydroxydanaidal (Davidson 1995), and thus it is likely that the courtship behavior of *H. clymene* is mediated by the hydroxydanaidal associated with its coremata. The coremata of *H. confusa* also contain hydroxydanaidal and, although courtship studies were not carried out on this species, they likely mate in a similar manner. The order of magnitude difference in the hydroxydanaidal titer between the *Haploa* species is probably related to their dietary differences but further experiments will be necessary to verify this.

Like *Utetheisa* (Conner et al. 1981), *Creatonotus* (Wunderer et al. 1986), *Estigmene*, *Pyrrharctia*, and *Phragmatobia* (Krasnoff & Roelofs 1989) production of hydroxydanaidal in *Haploa* is contingent upon dietary intake of PAs. Yet males reared on diets devoid of PAs have coremata that are visually indistiguishable from those of normal males, in striking contrast to the morphogenetic effects of PAs on cornematal development in *Creatonotus* (Boppré & Schneider 1985). The significance of the difference in cornematal development between these genera is not yet clear.

Hydroxydanaidal usage is proving to be widespread within the Arctiidae. Hydroxydanaidal (or the related dihydropyrrolizine danaidal) has been identified from the abdominal coremata of *Estigmene acrea* (Drury), *Pyrrharctia isabella*, and *Phragmatobia fuliginosa* (Krasnoff & Roelofs 1989); the genitalic coremata of *Utetheisa ornatrix* (Conner et al. 1981); the abdominal coremata of *Creatonotus gangis* (L.) and *C. transiens* (Wlk.) (Bell & Meinwald 1986); and the abdominal scent brushes of *Paraeuchaetes pseudoinsulata* Rego Barros (Schneider et al. 1992) within the subfamily Arctiinae, and in the abdominal coremata of *Cisseps fulvicollis* (Krasnoff & Dussourd 1989) and the ventral valve of *Cosmosoma myrodora* Dyar (Ruth Boada, pers. comm.) within the subfamily Ctenuchinae. This broad phyletic distribution for hydroxydanaidal usage in courtship suggests a single evolutionary origin within the Arctiidae. The common ancestor of the Arctiinae, the Pericopinae, and the Ctenuchinae appears to have been a PA-feeder (Jacobsen 1994; Susan Weller, pers. comm.) with, we propose, the ability to produce hydroxydanaidal. We suggest that this single origin set the stage for the repeated evolution of non-homologous scent-disseminating structures throughout the Arctiinae and the Ctenuchinae in a pattern consistent with sexual selection. Although the pericopine *Gnophaela latipennis* (Bdv.) has been shown to contain PAs (L'Empereur et al. 1989) no members of the subfamily Pericopinae have been studied with respect to hydroxydanaidal usage in courtship.

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