Scent Organ Development in *Creatonotos* Moths: Regulation by Pyrrolizidine Alkaloids

Abstract. *7-Hydroxy-6,7-dihydro-5*H*-pyrrolizine-1-carboxaldehyde is the major volatile component of the scent organs in males of two species of* Creatonotos *(Lepidoptera, Arctiidae). The biosynthesis of this presumed pheromone depends on the presence of pyrrolizidine alkaloids in plants that are ingested by the larvae. In addition, these secondary plant substances control the morphogenesis of the scent organs. This morphogenetic effect of an alkaloid has not been observed previously.*

Males of two species of Asian arctiid moths, *Creatonotos gangis* L. and *C. transiens* (Walker), expand large abdominal hair-covered tubes (coremata), not only in the presence of a luring female, but when resting near artificial lights (*1*) (Fig. 1). This behavior contrasts with that of other male moths and butterflies having analogous scent organs. For other Lepidoptera the aphrodisiac effect of the odor of such organs is unambiguous (*2*), but for *Creatonotos* the biological function of the coremata is still undetermined although it seems likely that the male odor of these species will also prove to have a pheromonal role.

Field-caught males of both *Creatonotos* species (collected in Sumatra) show considerable variation in the sizes of the coremata (Fig. 2, A and B), which have weights varying from 0.2 to 2.0 mg. We observed that dietary alkaloids influence the morphology and chemistry of the coremata.

Analyses of carbon disulfide extracts (*3*) of coremata from males of both *Creatonotos* species revealed that they contained 7-hydroxy-6,7-dihydro-5*H*-pyrrolizine-1-carboxaldehyde (Fig. 3)—up to 400 μg per male; the quantities appeared to be correlated to the size of the coremata. This compound has already been characterized as a pheromone from the coremata of another arctiid moth of the genus *Utetheisa* (*4, 5*) and also from hairpencils of danaine butterflies of the genus *Euploea* (*6*); it has been given the trivial name hydroxydanaidal (*7*) (Fig. 3). Previously studied insects appear to be unable to synthesize hydroxydanaidal or the two related danaine pheromones, danaidone and danaidal, unless pyrrolizidine alkaloids (PA's) of plant origin are present as precursors (*5, 7, 8*). These alkaloids are found most frequently in certain genera of the plant families Asteraceae, Boraginaceae, and Fabaceae (*9*); they are ingested either by larvae [for example, *Utetheisa* (*5*)] or by adults [for example, in Danainae (*10*)], which extract PA's from withered plant material.

We now report that in *Creatonotos*, hydroxydanaidal biosynthesis is also dependent on PA's ingested by the larvae. Since PA's provide a chemical defense in various Lepidoptera (*5, 11–13*) and since *Creatonotos* is aposematically patterned, PA's may protect these moths as well. The coremata of males reared from larvae that had fed on plants rich in PA's, such as *Crotalaria*, *Senecio*, or *Eupatorium* (*9*), contained up to 450 μg of hydroxydanaidal. The few available food-plant records for *Creatonotos* indicate that the larvae feed on both PA-containing and PA-deficient plants. The coremata of males reared from larvae

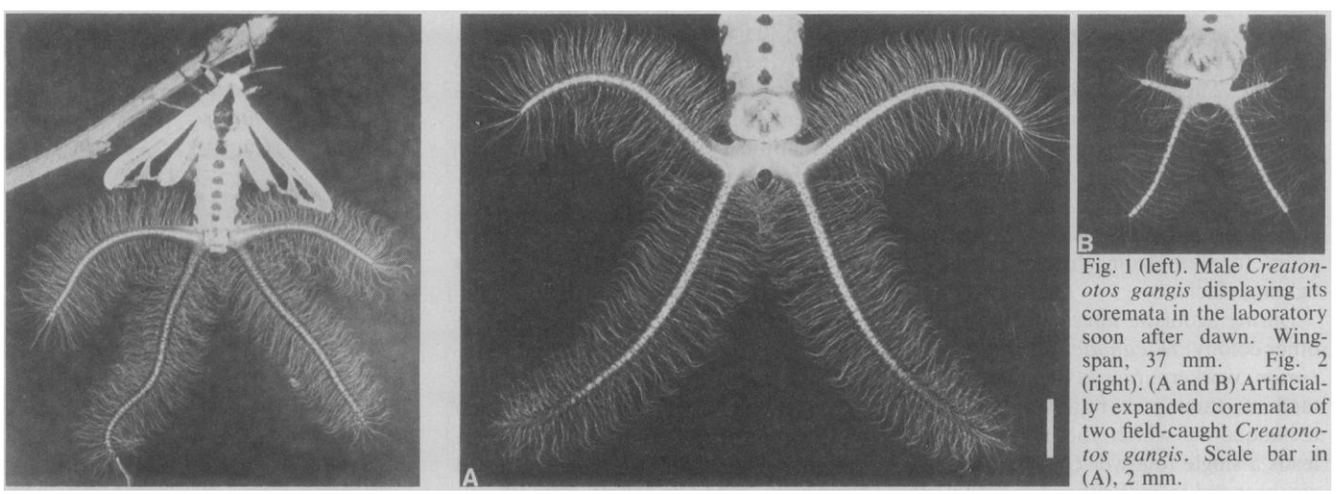

Fig. 1 (left). Male *Creatonotos gangis* displaying its coremata in the laboratory soon after dawn. Wingspan, 37 mm. Fig. 2 (right). (A and B) Artificially expanded coremata of two field-caught *Creatonotos gangis*. Scale bar in (A), 2 mm.

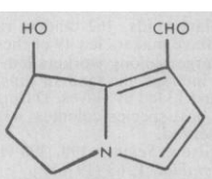

Fig. 3 (left). Structure of hydroxydanaidal.

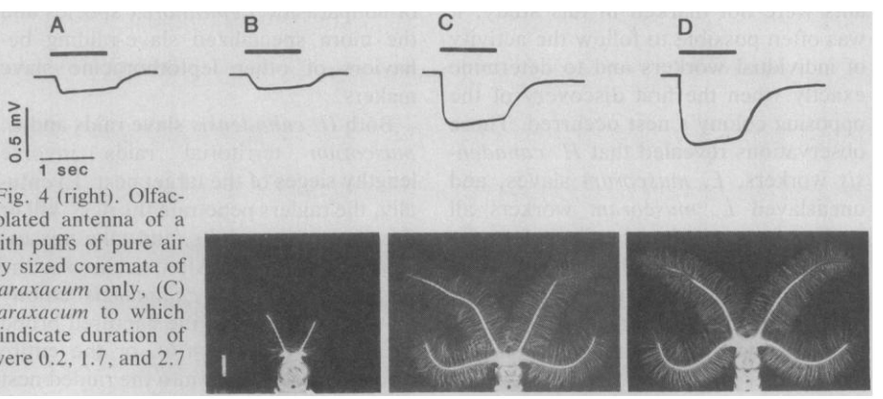

Fig. 4 (right). Olfactory responses (electroantennograms) of an isolated antenna of a female *Creatonotos gangis* to (A) stimulation with puffs of pure air (control) and with puffs of air led over differently sized coremata of males that as larvae had been raised on (B) *Taraxacum* only, (C) *Taraxacum* plus *Senecio jacobeae*, and (D) *Taraxacum* to which about 1 mg of monocrotaline was added; bars indicate duration of stimuli. The respective weights of the coremata were 0.2, 1.7, and 2.7 mg; scale bar, 2 mm.

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raised on PA-deficient *Taraxacum* contained only traces (or none) of this aldehyde (less than 5 ng per male) (*14*). The size of the coremata, which ranged from very small to large, also depended on the access of the larvae to PA-containing plants. We added the monocrotaline to the *Taraxacum* diet of final-instar larvae and we found hydroxydanaidal in the coremata, and the scent organs were much larger than those of control males fed on *Taraxacum* only.

We used standard electrophysiological methods (electroantennograms) (*15*) to test the olfactory effectiveness of the coremata of males that as larvae had been fed diets supplemented with monocrotaline. While very small coremata elicited electrical responses indistinguishable from those of control stimuli (air only), properly developed coremata elicited good responses in the antennae of both sexes and both species; the electroantennogram amplitudes indicate a correlation between organ size and stimulatory power (Fig. 4).

The information available so far demonstrates an insect-plant relationship without parallel. Both the biosynthesis of an odorophore and the maximal development of the organ that disseminates and secretes that odorophore require access to a specific secondary plant metabolite.

DIETRICH SCHNEIDER
Max-Planck-Institut für Verhaltensphysiologie, 8131 Seewiesen, Federal Republic of Germany
MICHAEL BOPPRÉ
Zoologisches Institut der Universitat, 8400 Regensburg, Federal Republic of Germany
JONATHAN ZWEIG
STEPHEN B. HORSLEY
THOMAS W. BELL
JERROLD MEINWALD
Department of Chemistry, Cornell University, Ithaca, New York 14853
KURT HANSEN
Max-Planck-Institut für Verhaltensphysiologie
EDUARD W. DIEHL
Goodyear Hospital, Dolok Merangir, North Sumatra, Indonesia

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3. Excised coremata were preserved in ampules containing CS_2. Extracts were studied by gas chromatography–mass spectroscopy (GC-MS) and by high-performance liquid chromatography (HPLC). The GC-MS identification of hydroxydanaidal in coremata extracts was accomplished by direct comparison with an authentic sample (Finnigan 3000 mass spectrometer). Samples were injected onto a silane-treated glass column (0.2 by 91 cm) packed with 3 percent OV-1 on 80/100 mesh Supelcoport operated isothermally at 117°C. Prominent ions in the electron impact spectra of authentic and natural samples were observed at mass-to-charge ratios of 151 (M+), 134, 123, 122, 95, 94, 79, 61, and 51. Gas chromatographic analyses were conducted with a silane-treated glass column (0.2 by 244 cm) packed with 3 percent OV-17 on 100/120 mesh Gas Chrom Q operated isothermally at 150°C with flame ionization detection. As little as 10 ng per animal could be detected. The quantity of aldehyde in each injection was determined either by electronic integration or by measurement of peak heights. Some samples were also analyzed by HPLC on a μBondepak C_{18} column (0.4 by 30 cm) eluted with a mixture of water and acetonitrile (50:50) with detection by ultraviolet absorbance at 280 nm (0.01 absorbance units full scale). As little as 0.5 ng per injection (2.5 ng per animal) was detected by this method.
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14. We have not yet established whether these small amounts are due to contamination (in the course of excising the organs or from some other source) or if small amounts of PA's are transferred to the larvae by the eggs and subsequently utilized for hydroxydanaidal synthesis; larvae of *Nyctemera* ingest PA's from *Senecio*, which appear in the adult moths and even in their eggs (approximately 1 μg per egg) [M. Benn, J. DeGrave, C. Gnanasundersam, R. Hutchins, *Experientia* **35**, 731 (1979)].
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16. We thank C. Adrian and H. Mayr-Söchting for technical assistance. Supported in part by NIH grant AI-12020 (to J.M.) and by the Schering Corporation.

12 May 1981; revised 13 October 1981

Testosterone Uptake in the Brainstem of a Sound-Producing Fish

Abstract. *Three nuclear areas in the medulla were implicated in the control of sound production in the oyster toadfish* Opsanus tau. *The sonic motor nucleus was labeled by retrograde transport of horseradish peroxidase injected into swimbladder sonic muscles, and an adjacent ventrolateral and a more anterior periventricular nucleus of the medulla were revealed by autoradiography with* ^3H*-labeled testosterone. These androgen uptake sites occur in brainstem areas corresponding to areas predicted to contain the neural centers controlling the duration and fundamental frequency of the toadfish mating call.*

Neurons that concentrate steroids have been found in the forebrain, brainstem, and spinal cord of a number of vertebrates (*1, 2*). The functions of these neurons are largely unknown, although in frogs, birds, and mammals the androgen concentrating motor nuclei of the final common pathway to the sonic muscles are involved in sound production (*3*).

The oyster toadfish (*Opsanus tau*) produces an agonistic grunt and a courtship boatwhistle. Although most fish sounds, including the grunt of the toadfish, are of short duration and pulselike, the boatwhistle is unusual in that it is of relatively long duration and tonal (*4*). Behaviorally relevant aspects of the boatwhistle are its fundamental frequency, duration, and repetition rate (*5*). In view of the close association of sound production and reproductive behavior in toadfish, we examined the question of whether the neurons that innervate the sonic muscles concentrate labeled testosterone.

Steroid target neurons in homologous brain regions of fish, amphibians, reptiles, birds, and mammals show certain similarities (*1–3*). However, in four teleosts, no brainstem steroid-binding neurons were described; these teleosts were the paradise fish (*Macropodus opercularis*) (*6*), goldfish (*Carassius auratus*) (*7*), platyfish (*Xiphophorus maculatus*) (*8*), and green sunfish (*Lepomis cyanellus*) (*2, 9*). The absence of steroid target neurons in these species has not been explained. Of these species only the sunfish is known to produce sound (*10*), although it is typically thought of as visually oriented (*11*). Brain stimulation in the sunfish has evoked nest building, color change, aggression, courtship, and sperm release (*9*), but not sound production (*12*). However, the correlation of steroid uptake with song in birds and mating calls in frogs suggests that brainstem target cells might well be present in a vocal teleost such as the toadfish.

Pattern generators controlling the duration of the boatwhistle call have been localized grossly within the anterior medulla, whereas those controlling fundamental frequency have been found in the

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