Sex Pheromone of the Queen Butterfly: Electroantennogram Responses

Abstract. *Olfactory receptor responses (electroantennograms) were recorded from antennae of danaid butterflies. Antennae of male and female queen butterflies* (Danaus gilippus berenice) *respond equally strongly to the hairpencil of queen males, to its crude extract, and to one of its two identified secretory components (the ketone). Responses to the second component (the diol) are weak. Hairpencils of a related species,* Lycorea ceres, *which also contain the ketone, are equally effective in eliciting electroantennograms from both sexes of the queen. Antennae of another related species, the monarch* (Danaus plexippus), *respond to the same stimuli as does the queen. Monarch hairpencils, which lack the ketone, do not elicit electroantennograms in monarch or queen antennae.*

A pheromonal secretion produced by the male queen butterfly (*Danaus gilippus berenice*) is an aphrodisiac that induces the female to accept its mate. The secretion is produced by the glandular hairs of a pair of tufted stalks (hairpencils) in the male's abdomen (*1*). During courtship, the male everts the hairpencils and deposits a cuticular "dust" (which bears the secretion) onto the antennae of the female (*2*). Two compounds in the secretion have been identified (*3*)—a crystalline pyrrolizidinone (hereafter called the ketone), and a viscous terpenoid alcohol (the diol). The ketone is a releaser-pheromone, and the diol is essential for adhesion of the ketone to the dust, and for adhesion of the dust to antennae of the female (*2*). The female senses the pheromone with olfactory receptor cells belonging to thin-walled sensilla on the antenna (*4*).

We studied the chemosensory responses of the antennae with electrophysiological techniques (*5*). The butterfly antenna (either amputated, or for more prolonged recordings, as an intact part of the mechanically immobilized insect) was mounted between capillary electrodes. Stimuli were applied by blowing air over pieces of filter paper onto which given amounts of the test compound had been spread. The pieces of paper were put into short glass tubes (cartridges) that were fitted to the air outlet. Direct-current recording was conventional. The response observed was a slow electrical potential (electroantennogram or EAG), which is known (*5*) to be the sum of simultaneously elicited receptor potentials of several or many olfactory receptor cells. The relative electrical negativity of the peripheral part of the antenna was used as a measure of the potency of the odor stimuli.

Elicited EAG's (Fig. 1A) are similar in their shape, as well as in their amplitude-response characteristics, to those obtained with sex attractants in moths (*5*). However, whereas in moths only the males have odor receptors for the female pheromone, in butterflies the antennae of both sexes respond equally to the ketone (as they do to all other odorants tested so far). Consequently, in the response curves (Fig. 1B), male and female EAG's have been combined. Electroantennograms comparable to those obtained with the ketone can also be obtained by blowing air over dissected hairpencils of wild queen males, or by the use of crude methylene chloride extracts of such hairpencils. Quantitatively, a milligram of the ketone on filter paper gives approximately the same EAG amplitude as five hairpencils (of wild males) or their crude extract.

Hairpencils of wild queen males lose their power to elicit EAG's very slowly. One cartridge holding five such pencils was used as a stimulus in hundreds of experiments over a period of 14 months without significant decrease of its power (the cartridge was refrigerated between tests). Hairpencils of laboratory-raised males [which lack ketone in chemically detectable amounts, and whose mating competence is impaired (*2*)], elicited EAG's of significance, but lost their stimulative capacity in several weeks. We interpret this to indicate that the ketone deficiency of the laboratory-reared males is merely partial, and that the amounts of ketone present, although chemically undetectable, are nevertheless sufficient to elicit EAG's.

Hairpencils of wild and laboratory-reared queen males have an odor (to humans) that is attributable neither to the ketone nor the diol. This odor persists in the hairpencils of laboratory-reared males even after these have lost their power to elicit EAG's. This suggests that the unknown odorous component or components is not an important stimulus for the queen's olfactory receptors.

Hairpencils of another danaid butterfly (*Lycorea ceres ceres*, from Trinidad) were as effective as queen hair-

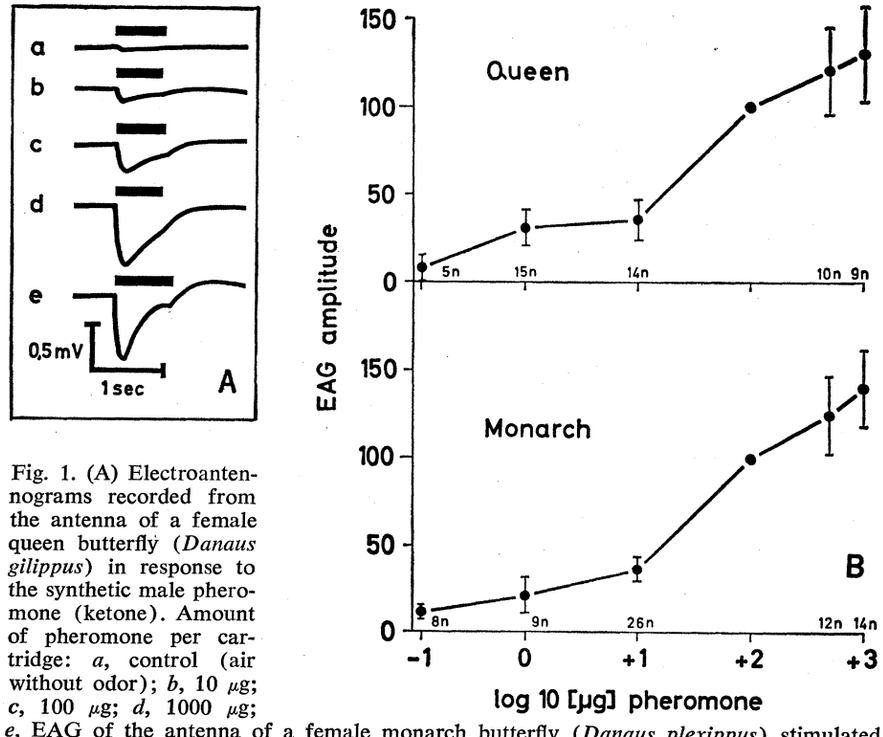

Fig. 1. (A) Electroantennograms recorded from the antenna of a female queen butterfly (*Danaus gilippus*) in response to the synthetic male pheromone (ketone). Amount of pheromone per cartridge: *a*, control (air without odor); *b*, 10 µg; *c*, 100 µg; *d*, 1000 µg; *e*, EAG of the antenna of a female monarch butterfly (*Danaus plexippus*) stimulated with 1000 µg of pyrrolizidinone. Black bars above each trace indicate the duration of the odorous air current. (B) Amplitudes of the EAG responses in queen (upper curve) and monarch (lower curve) butterfly antennae as a function of ketone stimulus-concentration; 100-µg amplitudes set at 100 percent. Mean values of a number of measurements (see the numerals), ± two times standard deviation of the means.

Table 1. Electroantennogram responses to a stimulation of male and female queen antennae with puffs of dust impregnated with different chemicals. Talcum powder, or the extracted cuticular dust from hairpencils, was used as dust. See text for details.

Test stimulus	Response
Dust alone	0
Dust + ketone	+
Dust + diol	0
Dust + mineral oil	0
Dust + ketone + diol	+
Dust + ketone + mineral oil	+

pencils in eliciting EAG's from queen antennae, while hairpencils of the monarch butterfly (*Danaus plexippus*) were totally ineffective. This is in accord with the known chemistry of these butterflies —*Lycorea* hairpencils contain the ketone (*6*), whereas monarch hairpencils lack it (*7*). More surprising was the finding that monarch antennae are sensitive to the ketone, even though this species does not produce the compound; full-size EAG's were elicited in male and female monarch antennae in response to pure ketone, as well as queen and *Lycorea* hairpencils, but never in response to hairpencils of laboratory-reared specimens of the monarch itself. In this context it is noteworthy that the monarch is thought by some to belong to a different genus than the queen (*1*).

The fact that both *Lycorea* and the queen have the same ketonic pheromone, and that the monarch, which lacks the ketone, nevertheless responds to it with EAG's, is not in agreement with the original claim that pheromones (and consequently also the pheromone receptors) are species-specific (*8*). This lack of specificity was also found in experiments with diverse moths (*9*).

Electroantennogram tests with the diol suggested that this compound is at most a weak olfactory stimulant for both the queen and the monarch. Responses reached an EAG amplitude only slightly above that of the control, even when high concentrations of the diol were used. The diol response curve was essentially similar to that obtained with farnesol, another terpene alcohol. The relative insensitivity to these terpenoids is not indicative of a general insensitivity to compounds other than the ketone. Significant EAG's were obtained with all three danaid species tested, in response to fruity and flowery odors and fatty acids.

In nature the male of the queen administers its pheromonal secretion to the female on a powdery dust that acts as a carrier. In an effort to imitate this procedure, we stimulated queen antennae with puffs of dust, blown at close range from a metal tube (1 mm in diameter) that had been charged with dust. The dust consisted either of talcum powder, or of the actual cuticular dust from queen hairpencils [freed of intrinsic secretion by chemical extraction (*2*)].

The dust was variously coated chemically, using the same selection of coatings as was used in parallel behavioral experiments (*2*). Only the ketone-containing samples elicited EAG's (Table 1) (*10*). The agreement with the behavioral tests (*2*) is complete, with one exception. Whereas in our case the dust sample bearing ketone alone was fully effective, in the behavioral tests this sample failed to have an effect. Failure was attributed, not to a lack of an effect on the female, but to an inadequate stickiness of the sample, which made it impossible to endow the courting males with a proper load of dust (*2*). Because we administered the dust to the antennae directly, rather than by way of the male, the sample was bound to take effect.

In conclusion, we agree with Pliske *et al.* (*2*) that the ketone is the only sexual releaser-pheromone of the male queen butterfly. Our evidence is that (i) ketone-EAG's are sufficiently large to be attributable to the activity of many odor receptor cells; (ii) diol-EAG's are too small to be based upon the activity of many such cells; and (iii) the unidentified odorous component of the hairpencils is apparently not an odor for the butterflies. Mineral oil, which has the capacity to serve in place of the diol by acting as a substitute glue (*2*), is not an odor for the butterflies (Table 1).

The fact that sensitivity to the queen pheromone occurs in both sexes, rather than just in the sex opposite from the one producing the substance, is not without precedent, since a similar situation is known to prevail in the queen honeybee (*11*). It remains to be seen, however, whether in the queen butterfly the male makes actual use of its potential ability to monitor its pheromonal output.

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10. Because of the difficulty of controlling quantitatively the amount of dust administered to the antennae, the results are only of qualitative significance.
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12. We thank Mrs. U. Heinecke for technical assistance. T. Eisner (Cornell University) suggested this electrophysiological approach. He and his colleagues, J. Meinwald and T. E. Pliske, supplied live monarch and queen butterflies, hairpencil extracts, hairpencil dust, and synthetic hairpencil compounds. We thank L. P. Brower (Amherst College) for *Lycorea* butterflies to test the EAG effect of their hairpencils on the queen and monarch antennae.
13. *Note added in proof*: We have extended our EAG studies to the African danaid *Danaus chrysippos*. Antennae of males and females of this species responded equally to hairpencils of this species, to hairpencils of queen and *Lycorea* butterflies, and to the ketone. This suggests that this Old World species of family Danaidae also possesses the ketone as the male releaser-pheromone. Final proof, however, can only come from chemical analysis. We thank the Natural History Society (Mrs. E. Carswell, Master in Charge) of the Michaelhouse School at Balgowan, Natal, South Africa, for collecting butterflies for us at their locality.

24 January 1969; revised 3 March 1969

Sex Pheromone of the Queen Butterfly: Chemistry

Abstract. *Two major components in the "hairpencil" secretion of the male of the queen butterfly* (Danaus gilippus berenice) *have been identified. One, a crystalline ketone (2,3-dihydro-7-methyl-1*H*-pyrrolizin-1-one), is known from another danaid butterfly. The other, a viscous terpenoid alcohol* (trans,trans-*3,7-dimethyldeca-2,6-dien-1,10-diol), is new; its structure is confirmed by an unambiguous synthesis.*

The secretion associated with the extrusible brushlike "hairpencils" found in abdomens of males of the queen butterfly (*Danaus gilippus berenice*) plays an important role in the courtship of these insects (*1*).

To isolate the pheromonal components, hairpencils were removed from live males caught in Florida (*1*) and were extracted with methylene chloride. The infrared spectrum of the extract showed prominent carbonyl absorption at 5.90 μ, and resembled that of 2,3-dihydro-7-methyl-1*H*-pyrrolizin-1-one