of 1 mole of oxygen per mole of cobalt; recycling through at least five oxygenation-deoxygenation cycles is possible. Equilibrium constants were not calculated for these reactions; however, a trend toward increasing formation of the oxygen adduct was noted with an increase in the base strength of the substituted pyridine.

Molecular weight measurements of the species formed on the oxygenation of [1] in pyridine were made by oxygenating the parent compound directly in the cryoscope cell. Complex [1] was dissolved in pyridine under a nitrogen atmosphere and the solution was cooled to below $-10°C$. The cell was then evacuated and filled with oxygen. After the solution was sufficiently agitated, the molecular weight (454) of the oxygenated species was calculated (molecular weight calculated for the pyridine adduct [3] was 392). Within the limits of experimental error, this indicates that a monomeric species is formed on oxygenation of [1] in pyridine solution.

On the basis of these data, we believe that the following equilibrium exists in solution

$$Co(acac)_2en + B + O_2 \rightleftharpoons Co(acac)_2en(B)O_2$$

(where $B = DMF$, py, CH_3-py, CN-py, NH_2-py) and that the oxygenated species formed in solution and the isolated crystalline solid are monomeric 1 : 1 oxygen adducts.

The similarity between the synthetic oxygen-carrying system described here and the naturally occurring iron-oxygen carrier, hemoglobin, is quite striking. The porphyrin-like heme group functions as a planar tetradentate ligand coordinated to the ferrous ion. This serves the same function as the $(acac)_2en$ ligand in the synthetic oxygen carrier. In oxyhemoglobin the iron is thought to achieve an octahedral environment through coordination of a histidine residue in a *trans* position to the coordinated oxygen. This is similar to the function of the Lewis base in the synthetic oxygen adducts described here.

ALVIN L. CRUMBLISS
FRED BASOLO

Department of Chemistry, Northwestern University, Evanston, Illinois 60201

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Sex Pheromone of the Queen Butterfly: Biology

Abstract. *Males of the queen butterfly* Danaus gilippus berenice, *deprived of the two extrusible brushlike "hairpencils" at the rear of their abdomen, are capable of courting females but incapable of seducing them. In normal courtship, an aphrodisiac secretion associated with the hairpencils is transferred by way of tiny cuticular "dust" particles to the antennae of the females. Of the two substances identified from the secretion, one (the ketone) acts as the chemical messenger that induces the females to mate. The only known function of the other compound (the diol) is to serve as a glue that sticks the dust to the female. Males were reared under conditions in which they produced subnormal amounts of ketone and showed reduced seductive capacity. Under certain experimental circumstances, the competence of these males was restored by addition of synthetic ketone.*

Many butterflies and moths possess extrusible brushlike structures which, because they are restricted to one sex and impregnated with secretion, are thought to serve for pheromone dissemination during courtship (*1*). In the queen butterfly *Danaus gilippus berenice* (Fig. 1A), whose mating behavior has been described (*2*), the brushes consist of a pair of tufted stalks ("hairpencils") present in the posterior abdomen of the male, where they are ordinarily tucked away within infoldings of the integument. When, during courtship, the male overtakes the female in flight, he everts the hairpencils (Fig. 1B) and brushes them against the female's antennae and head. She responds by alighting on herbage, whereupon he hovers closely above her and subjects her to further "hairpencilling." Eventually he too alights, and copulation takes place (Fig. 1C).

Hairpencils are glandular structures of considerable complexity. The surface of the individual hairs is irregularly covered with tiny cuticular spherules (Fig. 1D). This "dust" detaches readily and tends to stick to surfaces (including insect integument) against which hairpencils have been brushed. Stickiness of the dust is attributable to a coating of liquid secretion, stemming from specialized gland cells associated with the bases of the hairs. Extraction of hairpencils has led to identification of two components of the secretion (*3*)—a crystalline pyrrolizidinone (hereafter called the ketone), and a viscous terpenoid alcohol (the diol).

The importance of hairpencils for mating became apparent in preliminary tests carried out in the field. Individual males (112 specimens), captured on the wing at a site where queens of both sexes were persistently abundant, were experimentally deprived of their hairpencils (*4*), provided with identifying wing markings, and immediately released at the same site. Control males, captured and released together with the experimentals at the site, consisted of 112 individuals that were merely marked, and another 112 that were marked and subjected to the incidental preoperative manipulations undergone by the experimentals. The site was revisited at 2-day intervals for 16 days, and systematically sampled each time for marked males (captured individuals were noted and released again after each sampling). A high proportion of individuals of all samples (34 percent of experimentals; 40 and 45 percent of the two sets of controls) were recaptured at least once. Moreover, there appeared to be no difference in the viability of the samples (their frequency of recapture remained comparable on successive visits). However, there was a sharp difference in their frequency of recapture *in copula*. Whereas the two sets of controls were mating with similar success (40 and 49 percent of individuals recovered of the two samples were taken *in copula* at least once; several were found paired more than once), the experimentals were decidedly unsuccessful (only one of the males was once taken *in copula*) (*5*).

Comparable experiments were also carried out in a flight cage (Fig. 1E), with essentially similar results. Males without hairpencils are not hesitant to court. Observations made of over 100 of them in the cage and in the field showed that they actively pursue females in flight, engage in the same aerial maneuvers that ordinarily accompany hairpencilling, but then, after having brought their mates to alight, fail to induce them to remain alighted and to copulate. Removal of hairpencils does not impair the fertility of males. They can be hand-paired to females (*6*), and the resulting offspring are viable.

The supposition that hairpencil dust is transferred to the female during normal courtship was verified. Females retrieved from the cage immediately after their first mating had dust particles on the surface (Fig. 1G) and sensory pegs (Fig. 1F) of the antennae. Uncourted virgins lacked such particles.

An unexpected finding was that males raised indoors on a normal food plant *Asclepias tuberosa rolfsii* (fresh and refrigerated cuttings) were relatively unsuccessful sexually. Less than 20 percent of such males, introduced in groups into the cage with an excess of virgin females and kept under frequent daily observation, were ever found in copulation. Comparable tests with males that were raised outdoors on the same plant (living), and others that were caught in the wild, consistently yielded mating incidences of over 50 percent. The indoor-raised males were behaving in much the same way as males without hairpencils. The initial phases of courtship were normal (even the hairpencils were everted on schedule), but during the final phase they too seemed unable to induce the females to remain alighted. A possible explanation for their reduced mating ability is that the secretion associated with their hairpencils was chemically deficient in ketone (*7*). This led to a series of experiments in which attempts were made to restore, by chemical means, the mating competence of these males.

In essence, the experiments consisted of supplying to the hairpencils of the ketone-deficient males additional amounts of hairpencil dust, coated with various chemical samples to be tested, and then releasing the males into the cage together with virgin females, for determination of their mating ability. Seven groups of males were tested, each group bearing dust of a different coating (*8*). The groups numbered from 19 to 50 individuals, and they were tested separately, all members of a group being caged simultaneously at the outset of the 7 days during which they were kept under observation (*9*). The results are given in Fig. 2 (groups I-VII).

As expected, addition of uncoated dust had no effect (group VII), while addition of crude secretion restored mating competence (group I). Unexpectedly, ketone alone (group IV) was ineffectual. However, dust coated with the dry crystalline ketone lacked the adhesiveness of natural dust (*8*), and it appeared not to stick properly to the hairs and might also have failed eventually to stick to the females. Ke-

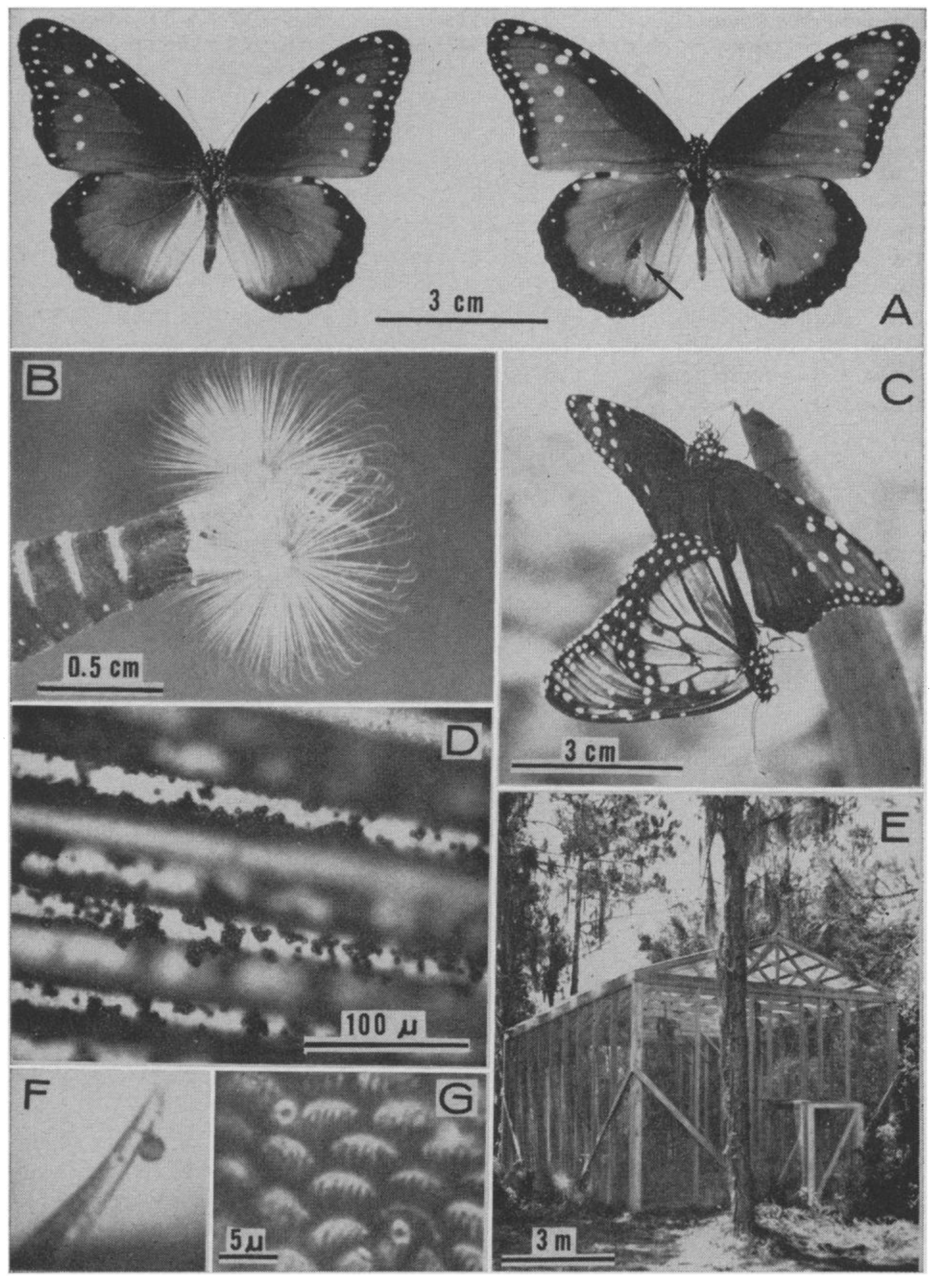

Fig. 1. (A) The queen butterfly (*Danaus gilippus berenice*); arrow points to wing pouch of male. (B) Fully extruded hairpencils on abdominal tip of male. (C) Queens mating; the female hangs from abdomen of male. (D) Hairpencil dust on surface of hairpencil hairs. (E) Experimental cage on grounds of Archbold Biological Station, Lake Placid, Florida, in which many of the courtship tests were done. (F) Sensory peg in antenna of freshly courted virgin female, bearing a dust particle from the male. (G) Two dust particles on surface of antenna of freshly courted virgin female. (F and G are the same magnification.)

Type of male	Group No.	Sample Size	Substance(s) Added with Dust	% Retrieved in Copula	
Reared Indoors (Ketone Deficient)	I	20	Crude Secretion	85	Competence Restored
	II	36	Ketone + Diol	58	
	III	30	Ketone + Mineral Oil	60	
	IV	25	Ketone	12	Competence Unrestored
	V	21	Diol	14	
	VI	25	Mineral Oil	24	
	VII	50	None	14	
Reared Outdoors	VIII	19	None	58	Intrinsically Competent
Wild	IX	30	None	73	

Fig. 2. Mating frequencies of males treated by addition of uncoated or variously coated hairpencil dust. Details in text. The incompetent groups (IV–VII) constitute a homogeneous collection of samples ($P > .60$) and individually differ significantly from each of the competent (both restored and intrinsic) groups ($P < .02$).

tone with diol does impart stickiness to dust, and the males bearing this sample (group II) were competent. Restoration of their competence could not have been due solely to the added diol, since an inert viscous mineral oil, capable of imparting stickiness, could effectively substitute for the added diol (group III). Neither diol alone (group V), nor mineral oil alone (group VI), had significant effect. Control tests (groups VIII and IX) done with intrinsically competent males [wild males, and males reared outdoors], all treated by addition of uncoated dust, yielded high competence values, comparable to those obtained previously with similar males, totally untreated.

Mating competence can evidently be restored, by correction of the ketone deficiency. All samples containing ketone were effective, provided they were borne by sticky dust. All samples lacking ketone were ineffective. We conclude that the ketone is the essential chemical messenger that "seduces" the female. As a pheromone, it can be classed as an aphrodisiac (*10*). The only proven function of the diol is that it imparts the necessary stickiness to the carrier dust (*11*).

It seemed possible that the diol might serve in yet another capacity. Chemically, it has some similarity to farnesol, a compound which, together with related susbtances, has been known to mimic the action of juvenile hormone (*12*). One of the functions of juvenile hormone in adult female insects is to stimulate yolk deposition during oogenesis (*12*). The diol, topically administered by the courting male, might conceivably act as an oogenetic "booster," properly timed to coincide with mating. However, virgin females that were hand-paired to males lacking hairpencils produced viable offspring in normal numbers, suggesting that the effect, if it occurs at all, is at most a relative one (*13*).

Male queen butterflies possess a pair of pouchlike organs on the wings (Fig. 1A, arrow) whose function has eluded us. The resting male everts the hairpencils and thrusts the unsplayed tufts of hair into the pouches several times per day (*14*). The pouches have also been called wing glands, because their walls possess cells resembling insect exocrine cells (*2*). Whatever their function, the pouches do not appear to be essential for mating. Males (outdoor-reared) whose pouches had been excised promptly after emergence from the pupa before the hairpencils could have been thrust into them, mated with normal frequency.

The chemical basis of courtship in danaid butterflies is more complicated than these data imply. Two other species investigated produce secretions that are both similar to and different from that of the queen butterfly. A neotropical form *Lycorea ceres ceres* produces the ketone, but lacks the diol, and instead produces two aliphatic acetate esters (*15*). The monarch butterfly *Danaus plexippus* lacks the ketone, but produces two substances similar to the diol (*16*). Both species have hairpencils homologous to those of the queen, but neither produces hairpencil dust. Moreover, only the monarch has wing pouches.

T. E. PLISKE
T. EISNER

Section of Neurobiology and Behavior, Division of Biological Sciences, Cornell University, Ithaca, New York 14850

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4. The stalks of the hairpencils were severed proximal to their swollen glandular tips (Fig. 1B).
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8. Dust was obtained from wild males (by rinsing their hairpencils with methylene chloride), and freed of intrinsic secretion by sequential washing with methylene chloride, benzene, and ethanol. Recoating with a test sample involved adding dust to a solution of sample in methylene chloride, and letting the solvent evaporate under agitation. Ratio of sample to dust was always about 1 : 1 by weight. In samples consisting of artificially constituted mixtures, the ratio of constituents was always 7 : 1 by weight in favor of the ketone. The ketone and diol used in all samples (except crude secretion) were synthetic. The mineral oil was one of intermediate viscosity (170 cp). Application of dust to the test males involved hand-holding them with their abdomens gently squeezed so as to cause eversion of the hairpencils, and dipping the tufts into the mass of coated dust. Visible amounts of dust were always picked up by the hairs, but there was no quantitative consistency in the dosage applied. Uncoated dust, and dust coated with pure crystalline ketone, lacked the adhesiveness of the other dust samples, and appeared to be taken up in lesser amounts.
9. The males were 3 to 6 days old when given dust and introduced into the cage (3 days is the minimum age at which any males mate). The caged females were all virgins. The cage was checked at intervals of 3 to 4 hours, and mating pairs were removed as found; new virgins were added to replace the females. Males that mated usually did so within the first days. Unmated males remaining in the cage had their hairpencils dipped in their respective dust samples at 2-day intervals.
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