Postmating Female Odor in *Heliconius* Butterflies: A Male-Contributed Antiaphrodisiac?

Abstract. *Genetic crosses between races of* Heliconius erato *that differ in the odor of female abdominal glands show that the odor originates in the male and is transferred to the female at mating. Behavioral observations strongly suggest that the odor is an antiaphrodisiac that helps to enforce monogamy among females.*

Male insects have evolved a variety of devices which function to prevent or inhibit further mating by females that have mated once. These include the production of a proteinaceous plug or sphragus by some male butterflies (*1*) and the injection of male accessory gland substances, which presumably bind to sites in the female nervous system and thereby induce monogamous behavior in females of certain flies, mosquitoes (*2*), and moths (*3*). Male *Tenebrio* beetles also use chemical methods to enforce monogamy, but by means of an antiaphrodisiac pheromone, transferred to the female at mating, which repels other males (*4*).

I report observations and experiments which indicate that males of the neotropical butterfly *Heliconius erato*, like *Tenebrio molitor* males, transfer antiaphrodisiac pheromones to females at mating. To my knowledge, no other lepidopterans are known to do so. Unlike *Tenebrio*, *H. erato* apparently possesses structures specifically evolved for the transfer (both sexes) and dissemination (females only) of the pheromone.

It has long been known that female *H. erato*, when handled, produce a strong odor that is said to resemble phenylcarbylamine, or witch hazel. The odor is present in mated but not virgin females and is seldom detected in males, possibly because it is retained internally and not displayed. It emanates from two structures known as "stink clubs," which are adjacent to the extrusile abdominal glands of the female (*5*). The odor has been variously ascribed to defensive as well as courtship functions, with little evidence for either (*5*).

Evidence that the odor of female *H. erato* after mating is of male origin was obtained as follows. Four races of the butterfly (*H. erato adanus*, Trinidad; *H. e. petiveranus*, Mexico; *H. e. phyllis*, Brazil; and *H. e. chestertonii*, Colombia) were maintained in tropical insectaries under seminatural conditions. The odor of the Colombian race, *H. e. chestertonii*, after mating is highly distinctive, being much more fragrant than that of any other race tested. During March 1972, males of *H. e. chestertonii* were introduced into an insectary containing *H. e. adanus* and, likewise, *adanus* males were introduced into the *chestertonii* house. Interracial matings (Fig. 1A) were readily obtained (*6*) and the odor of mated females was recorded. All *chestertonii* females ($N = 3$) mated by *adanus* males produced the odor characteristic of most races of *erato* including *adanus* (*5*); all *adanus* females ($N = 4$) mated by *chestertonii* males produced the distinctive fragrance of *chestertonii*. Thus, in all seven matings, the odor of mated females was determined by the race of the males which had mated with them. Several hundred first-generation hybrids were subsequently obtained, all of intermediate color pattern phenotype. Three females (*adanus*, $N = 1$; F_1 hybrid, $N = 2$) mated to F_1 hybrid males each possessed an intermediate odor (*7*). Reciprocally, a hybrid female backcrossed to an *adanus* male had the typical *adanus* odor.

The evidence for the antiaphrodisiac function of the odor is more anecdotal but is highly suggestive. Male *H. erato* waiting on female pupae (Fig. 1B) are normally oblivious to both visual and tactile stimuli. I have, on numerous occasions in the insectary, battered about clusters of such males quite violently without any taking flight. Yet, when I bring the abdomen of a mated female *H. erato* to within

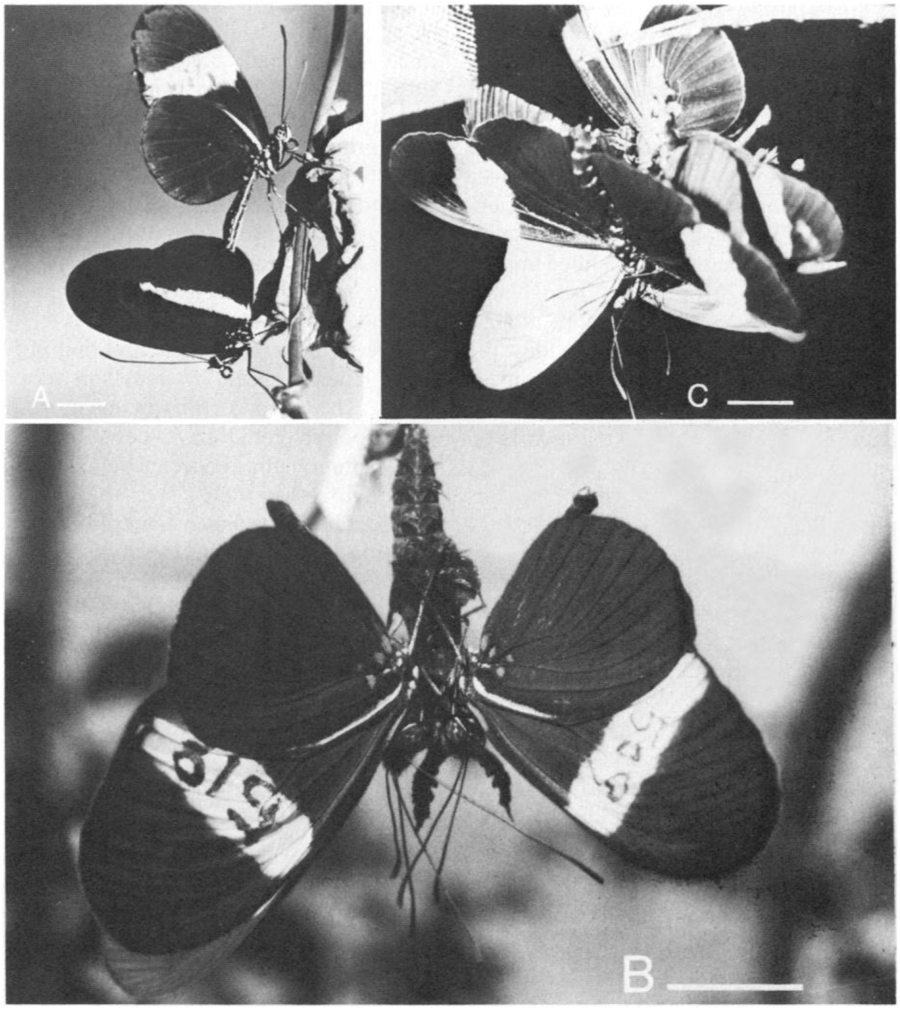

Fig. 1. (A) Interracial mating in *Heliconius erato*. A mating behavior between a male of the Trinidad race *adanus* (above) and a female of the Colombian *chestertonii* (below). (B) Three *H. erato adanus* males await the emergence of a female in tropical insectary. Note the antler-like projections at the anterior (lower) end of the chrysalis. Such structures are present in pupal mating species (*H. erato, H. charitonia*) but are reduced or absent in heliconiines not mating at the pupa. They probably function to give the semihelpless female a foothold while males attempt to copulate. (C) Eight *H. erato adanus* males mob a pupal male *H. erato chestertonii* which is about to eclose. Such behavior has not been observed within races, thereby suggesting differences in chemical communication. Scale bar, 1 cm.

a few centimeters of male clusters, the males always become highly agitated, rapidly moving the antennae and palps, and generally disperse in a matter of seconds. While I have at times observed that actual contact between female abdominal glands and male antennae is required to dislodge the most persistent male from the pupa, the repulsive quality of the mated female seems certain. I have observed males of another pupal mating species, *H. charitonia*, to behave in the same way. These responses of "pupa-sitting" males are not consistent with Crane's conclusions on the basis of her observations of males courting mature mated females—that is, that the "odor has no apparent effect on the courting male" (*5, 8*).

Given that the "stink club" odor is transferred from the male to the female at mating and that the odor of mated females lasts for weeks (*5*) and even months (*9*), it is likely that (i) a large amount of the substance or its precursor is transferred at mating and (ii) special structures are involved in transfer and storage.

While no storage organ is known, the yellow abdominal gland of the female is a possible candidate. In fact, this gland enlarges and its color intensifies after mating, indicating that it may be filled up during copulation. Moreover, Eltringham's early study (*10*) of the structure of *Heliconius* abdominal glands provides the probable answer as to how the transfer occurs. Inside the male clasper Eltringham found and described a pouch lined with glands. It appears that the female's "stink club" would fit neatly into this pouch during mating.

The understanding of why these complex behavioral and morphological traits have coevolved between the sexes of *H. erato* is relevant to elucidating the nature of selection pressures that act on courtship behaviors (*11*). With respect to the *H. erato* system described here, two evolutionary questions emerge. (i) Why should males pay attention to antiaphrodisiac pheromones, especially if, as in *Euphydryas* butterflies and other insects (*1*), males that mate after the first mating gain sperm precedence? (ii) Why should the female sex "cooperate" by evolving structures for receiving and disseminating the pheromone?

The answers to these questions present no problem if the prevention of further matings is in the interest of both male and female parents. The female may be accepting assistance from the male in reducing time-wasting future courtship and avoiding the possible injury (*12*) attendant on subsequent matings. Males that respond to the pheromone and restrain from extended courtship of mated females or prolonged perching on male pupae may also avoid wasting considerable time and energy.

However, the fact that additional spermatophores probably increase female fecundity (*13*) suggests that monogamy may not be in the interests of the female, and may be enforced by her mate. If so, the "stink clubs" must have evolved in the context of other unknown functions, and it is more difficult to understand why subsequently courting males respond to the pheromone. One possible scenario involves the evolutionary derivation of the pheromone. Male *H. erato* assemble on young conspecific pupae of both sexes, and also sometimes (in insectaries) on pupae of *H. melpomene*, the males of which are not attracted to pupae. As the pupae develop, those of *H. melpomene* and *erato* females receive further attention, which continues during and after eclosion. This results in the mating of female *erato* (usually before the wings are fully expanded) but in general *melpomene* of both sexes may be dislodged from their grip on the pupal cases and killed by falling. Conversely, male *erato* pupae become either repellent or unattractive some time before emergence, and the adults are not harassed while expanding their wings. This suggests that there is a male identification pheromone that is effective in late *erato* pupae and that protects teneral males by repelling other males. The possession by males of such a compound could have constituted a preadaptation for rendering females repellent to other males, and the pheromone could thus have acquired a dual function. Males would therefore be subjected to complex selection pressures in their responses to and synthesis of repellent pheromones, with the possible result that they would be deterred from mating with nonvirgin females with which it would be in their interest to mate. One further piece of circumstantial evidence supports this hypothesis. I have already referred to the detectable difference between the odors of mated female *H. e. adanus* and *H. e. chestertonii*. The fact that the only observed case of intraspecific harassment of an eclosing male *erato* occurred between these two races (Fig. 1C) suggests interracial variation in the hypothesized male identification pheromone.

LAWRENCE E. GILBERT
Department of Zoology,
University of Texas, Austin 78712

References and Notes

1. P. A. Labine, *Evolution* **22**, 799 (1968); G. A. Parker, *Biol. Rev. Cambridge Philos. Soc.* **45**, 525 (1970).
2. R. H. Barth and L. J. Lester, *Annu. Rev. Entomol.* **18**, 445 (1973). See references 32, 51, and 81 for examples.
3. G. E. Swailes, *Ann. Entomol. Soc. Am.* **64**, 176 (1971).
4. G. M. Happ, *Nature (London)* **222**, 180 (1969).
5. J. Crane, *Zoologica (N.Y.)* **40**, 181 (1955).
6. Female pupae of *H. erato* attract males which then sit on the pupae until eclosion (Fig. 1B). Mating usually occurs on the pupal case as the female emerges. Thus, even though the adult color pattern of *H. e. chestertonii* (blue-black and yellow) differs greatly from that of *H. e. adanus* (black and red), interracial matings were easily obtained when these races were mixed.
7. In spite of the distinctive odor of the parent types, it was nevertheless difficult to distinguish hybrid odor from either parent after a few minutes of sniffing.
8. It should be pointed out that Crane was apparently unaware of the pupal mating behavior in *H. erato* and conducted all of her experiments on fully eclosed individuals.
9. Female *H. erato* mated only once still have perceptible odor after 3 months in the laboratory.
10. H. Eltringham, *Trans. R. Entomol. Soc. London* (1925), p. 263.
11. E. O. Wilson, *Sociobiology* (Belknap, Cambridge, Mass., 1975), chap. 15.
12. L. M. Roth, *Science* **138**, 1267 (1962); H. Ikeda, *Mem. Ehime Univ. Nat. Sci. Ser. B* **7**, 18 (1974).
13. Evidence for the nutritional role of spermatophores is as follows. Females mated to males fed solutions of ^{14}C amino acid produced ^{14}C-labeled eggs. (There was no significant difference in the number of counts per minute between fertilized and unfertilized eggs, indicating that eggs are labeled independently of the sperm by other components of the spermatophore.) Moreover, after several days most of the label exists in the female hemolymph (L. E. Gilbert, in preparation).
14. I thank K. Brown, who obtained the *chestertonii* stocks, and the National Science Foundation for support of my more general work on *Heliconius* that made such "side projects" possible. Support was also provided by the University of Texas, Austin (B.S.S.G. RR-07091-06). I thank B. Barth, B. Cade, C. Norris, M. C. Singer, J. Strassman, and, in particular, D. Otte, all of whom read the manuscript and made numerous improvements.

1 March 1976; revised 13 May 1976

Trajectory of the Mt. St. Augustine 1976 Eruption Ash Cloud

Abstract. *The ash clouds from the 23 January 1976 eruption of Mt. St. Augustine in Alaska drifted over Arizona on 25 January at an altitude of 6 kilometers as deduced from air trajectories and the observation of an unusual cloud.*

A major eruption of Mt. St. Augustine (59.36°N, 153.43°W) occurred on 23 January 1976. The eruption produced two major ash clouds, the first emitted at 0700 A.S.T. (1700 U.T.) and the second at 1630 A.S.T. (0230 24 January U.T.). The event was observed locally and also by the NOAA weather satellite, the weather being unusually clear at the time of the eruptions. The second eruption was photographed by Kienle (*1*) from an altitude of 3.0 km and showed an anvil structure