Evidence for an Antiaphrodisiac in the Sweat Bee *Lasioglossum* (Dialictus) *zephyrum*

Abstract. *Male* Lasioglossum (Dialictus) zephyrum *in the field are attracted to models impregnated with the odor of virgin females. The number of males responding increases with increasing odor concentration. Once a male has contacted a model, its attractiveness to other males is reduced, suggesting that the male may contribute an antiaphrodisiac cue so that other males avoid mated females.*

The term antiaphrodisiac was used by Happ (*1*) to describe a pheromone produced by male mealworm beetles (*Tenebrio molitor* L.) that inhibits the responses of other males to the scent of conspecific females. Later investigation did not confirm this work (*2*), and it was thought that the term would be short lived (*3*). It was revived, however, for a male-contributed odor that may help enforce monogamy in the butterfly *Heliconius erato* L. (*4*). Antiaphrodisiacs have not been reported in the Hymenoptera with the exception of an anecdotal account (*5*). I now describe an antiaphrodisiac odor cue in a halictine bee, *Lasioglossum (Dialictus) zephyrum.*

Lasioglossum zephyrum is a primitively eusocial halictine bee (*6*) that nests in large aggregations in bare, vertical clay banks. Males patrol these aggregations in search of females and respond to them and similar visual stimuli by pouncing (*7*). This apparently indiscriminate behavior is increased by the presence of female odor (*8*), which is considered to be an aphrodisiac (*9*). Since numerous pounces and few matings are seen in the field, it was thought that *L. zephyrum* females will mate only once (*10*). However, evidence from electrophoretic studies suggests that some females may mate twice (*11*).

Preliminary field observations with tethered females (*12*) revealed that the attractiveness of a particular female changes quickly. Up to seven males would rapidly converge on a tethered female and then disperse in 2 minutes or less, leaving behind a mating pair or the female alone. When the female was then moved to another part of the aggregation (30 m upwind), she was temporarily unattractive. Because dead females also attract males (*13*), it seemed unlikely that females were actively turning on and off some pheromone cue. Therefore an alternative possibility—that in *L. zephyrum* contact with a male decreases a female's attractiveness—was investigated.

Female models, consisting of black nylon wrapped around the tip of a 15-cm long wooden applicator stick and impregnated with one of six different odor cues, were tested in the field (*14, 15*). To obtain the Dufour's gland extract from *Colletes* sp., six field-captured adult females were dissected and their Dufour's glands placed in a vial containing 0.5 ml of methylene chloride; 41.6 μl of this extract was placed on a model. The odor of *L. zephyrum* females was obtained by placing individual virgin females ($N = 35$) in 20-ml glass vials, the corks of which contained pollen, honey, and water. Daily, each female was transferred to a clean vial and the used vial was rinsed three times with a total of about 3 ml of methylene chloride yielding one female day equivalent (FDE) of odor. These rinses were frozen and 570 FDE were accumulated. Excess solvent was evaporated under a gentle stream of nitrogen gas, leaving 1 ml total volume. Three different concentrations of this material were applied to three different female models. The fourth model was impregnated with 22.8 FDE of female odor and was placed for 5 minutes in a 20-ml vial containing five *L. zephyrum* males, they walked on, touched their antennae to, and pressed their abdomens against the model. Two other models served as a standard and a control.

Table 1. Individual 95 percent confidence intervals for the means of the number of *Lasioglossum* (*Dialictus*) *zephyrum* males that passed within 20 cm of female models during field trials of the six odor cues. These are based on the pooled standard deviation.

Treatment	Confidence interval
Control	17.52, 30.12
Standard	16.68, 30.28
22.8* FDE	20.88, 32.68
5.7 FDE	23.05, 34.95
11.4 FDE	27.55, 40.45
22.8 FDE	25.72, 37.62

*Indicates the presence of male odor.

Table 2. The results of a Student–Newman-Keuls test of the significance of differences between the mean numbers of *Lasioglossum* (*Dialictus*) *zephyrum* males hovering near female models with six odor cues. Letters indicate means that are not significantly different.

Treatment	Mean
28.8 FDE	9.43 a
14.4 FDE	5.43 b
28.8* FDE	5.0 b
7.2 FDE	2.86 b, c
Control	0 c
Standard	0 c

*Indicates presence of male odor.

A full series of six models, each with a different odor cue, was prepared at 10 a.m. on 7 days: 25, 26, 29, 30, and 31 August and 1 and 2 September 1982 (a back-up series was prepared each day also). To create a randomized block design, the stimuli were presented individually in a random order to free flying males at a nest aggregation. The stimuli had been coded beforehand so that the observations were made without knowledge of the cue involved. Each applicator stick was placed on the ground so that the nylon model projected 1 cm over the edge of the top of the vertical bank containing the aggregation. Small nail markers were placed 20 cm to either side of the model and an imaginary line, creating a half circle, was drawn on the bank's surface connecting these markers. Since male *L. zephyrum* tend to fly near the bank's surface, any male flying over the semicircular area could be easily observed, and this response was recorded as a pass. When a male approached within 1.5 cm of the model and stayed facing it for more than 5 seconds, the response was recorded as a hover. Each male entering the semicircle was recorded. Males rarely landed on a model in the field (*16*).

The data were analyzed by two-way analyses of variance (*17*, pp. 324–327). There was a statistically significant effect due to treatments (odor cues) for pass responses ($F(5, 30) = 3.175$; $0.05 > P > 0.01$). The 95 percent confidence intervals for the means of each treatment (Table 1) reveal a trend toward an increased number of pass responses as the concentration of female odor increases, and a reduction of responses due to male contact with the odor stimulus.

Two-way analysis of variance of the hover response also showed a statistically significant treatment effect ($F(5, 30) = 8.66, P < 0.01$). When the control and the standard were omitted from the analysis (no males hovered near them), there were still significant treatment effects ($F(3, 18) = 5.87$; $P < 0.01$). An *a posteriori* Student-Newman-Keuls test of the differences between treatment means (*17*, pp. 239–242) showed that the highest concentration of female odor (22.8 FDE) elicited more responses than any of the other stimuli (Table 2). The high concentrations of female odor elicited more responses than either of the two lower concentrations of female odor. More importantly, contact with males produced a statistically significant reduction in hover responses but did not eliminate them.

The attractiveness of female models impregnated with the 22.8 FDE odor cue is decreased after contact with males. Since most *L. zephyrum* females mate only once but some (<36 percent) will mate again, the male donor of an antiaphrodisiac odor cue protects his mate from superfluous male responses and reduces the likelihood that she will mate again. A male that detects such a cue on a female would gain more fertilizations by avoiding her, especially in sites like a *L. zephyrus* aggregation where there are large numbers of other females.

The material deposited by males on females may be a specific pheromone or pheromone blend (an antiaphrodisiac) or it could be some multipurpose material present on the surface of males, such as cuticular hydrocarbons. Antiaphrodisiac pheromones are most likely to evolve in aggregated species, with low to moderate levels of polygamy and sperm mixing. *Lasioglossum zephyrum* is aggregated and appears to have low to moderate levels of polygamy (*18*). Whether there is sperm mixing is not known.

PENELOPE KUKUK
Department of Entomology, Cornell University, Ithaca, New York 14853

References and Notes

1. G. M. Happ, *Nature (London)* **222**, 180 (1969).
2. C. J. August, *J. Insect Physiol.* **17**, 739 (1971).
3. M. C. Birch, in *Pheromones*, M. C. Birch, Ed. (North-Holland, London, 1974).
4. L. E. Gilbert, *Science* **193**, 419 (1976).
5. Male *Centris adani* may deposit a citrol-like odor on females during copulation; the response of other *C. adani* males to this odor is not known [G. W. Frankie, S. B. Vinson, R. E. Coville, *J. Kans. Entomol. Soc.* **53**, 837 (1980)].
6. The social behavior of *L. zephyrum* has been well studied. See C. D. Michener, *The Social Behavior of the Bees* (Belknap, Cambridge, Mass., 1975) chap. 24; M. D. Breed, *Insectes Soc.* **22**, 375 (1975); M. D. Breed, J. M. Silverman, W. J. Bell **24**, 351 (1977); L. Greenberg, *Science* **206**, 1095 (1979); P. F. Kukuk, M. D. Breed, A. Sobti, W. J. Bell, *Behav. Ecol. Sociobiol.* **2**, 319 (1977); G. Buckle, *Insectes Soc.* **29**, 125 (1982).
7. E. M. Barrows has described patrolling behavior (*13*) and pouncing behavior [*J. Kansas Entomol. Soc.* **49**, 105 (1976)].
8. E. M. Barrows, thesis, University of Kansas, Lawrence (1975).
9. ———, *Behav. Biol.* **15**, 57 (1975).
10. G. C. Eickwort and H. S. Ginsberg, *Annu. Rev. Entomol.* **25**, 421 (1980).
11. Electrophoretic examination of 31 apparently singly founded *L. zephyrum* colonies indicates that in 36 percent of the colonies either the founding female was doubly inseminated or there was more than one foundress.
12. Females were tethered by taping their wings to a 15-cm long applicator stick with Scotch brand strapping tape. Such a female was held by hand in an area containing an abundance of patrolling males. Five females were used in all. The field site for this and other experiments was the Monkey Run site, a steep, east-facing clay bank rising about 9 m above a stream (Fall Creek) in Tompkins County, Ithaca, N.Y. The *L. zephyrum* aggregation contains hundreds of nests, and thousands of males patrol the site in July and August.
13. E. M. Barrows, *Insectes Soc.* **22**, 307 (1975).
14. The odor cues consisted of: control, 50 μl of methylene chloride; standard, methylene chloride extract half of a *Colletes* sp. Dufour's gland; 5.7 FDE, 10 μl of vial rinse; 11.4 FDE, 20 μl of vial rinse; and 22.8 FDE, 40 μl of vial rinse; and 22.8 FDE, 40 μl of vial rinse plus male contact.
15. A methylene chloride extract of the Dufour's glands of six *Colletes* sp. was used as a standard because in the Collitidae the Dufour's gland secretions are dominated by macrocyclic lactones. Similarly, in the Halictidae, the Dufour's gland secretes macrocyclic lactones [C. D. Andersson, G. Bergstrom, B. Kullenberg, D. Stallberg-Stenhagen, *Ark. Kemi.* **26**, 191 (1966); G. Bergstrom, *Chem. Sci.* **5**, 39 (1974); A. Hefetz, H. M. Fales, S. W. T. Batra, *Science* **204**, 415 (1979); R. M. Duffield, A. Fernandes, S. McKay, J. W. Wheeler, P. R. Snelling, *Comp. Biochem. Physiol.* **67B**, 159 (1980); A. Hefetz, M. S. Blum. G. C. Eickwort, J. W. Wheeler, *Comp. Biochem. Physiol.* **61B**, 129 (1978); R. M. Duffield, A. Fernandes, C. Lamb, J. W. Wheeler, G. C. Eickwort, *J. Chem. Ecol.* **7**, 319 (1981)].
16. There were two instances in which males landed on a model, it was the one with the high female odor concentration. After the landings occurred, the contaminated model was removed and replaced with a fresh model. The time of the observation was extended to compensate for time lost between a male's landing and the replacement of the model.
17. R. A. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, ed. 1, 1969).
18. E. M. Barrows, W. J. Bell, C. D. Michener. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2824 (1975).
19. I thank G. C. Eickwort, W. L. Roelofs, and P. W. Sherman for reading an earlier draft of this manuscript. Supported by NIH-NRSA grant 1-F32-MH08722-01.

20 April 1984; accepted 18 October 1984

Descending Efferents from the Superior Colliculus Relay Integrated Multisensory Information

Abstract. *By means of their efferent projections to motor and premotor structures, the cells in the deep superior colliculus are intimately involved in behaviors that control the orientation of the eyes, pinnae, and head. These same efferent cells receive multiple sensory inputs, thereby apparently enabling an animal to orient its receptor organs in response to a wide variety of cues. This sensory convergence also provides a system in which motor responses need not be immutably linked to individual stimuli but can vary in reaction to the multitude of stimuli present in the environment at any given moment.*

That the superior colliculus (SC) plays a role in visual orientation has been known since the late 19th century (*1*), but only recently has its multisensory nature been demonstrated. Behaviorally, it is evident as a tendency to neglect contralateral sensory stimuli after the removal of one SC (*2*)—an observation that prompted investigators to explore the organization and properties of its constituent neurons. Consequently, the organization of the sensory (visual, auditory, and somatosensory) and motor representations in the SC has been described in detail, and these representations have been shown to be in register with one another (*3*). For example, the upper portions of visual, auditory, and body space are represented in the same region in the SC, and electrical stimulation here produces upward movement of the eyes, pinnae, and head—an elegant yet simple organizational plan.

For the SC to transform sensory input into motor output, sensory input ultimately must reach the deep efferent cells that produce orientation by way of projections (Fig. 1) to the brainstem and spinal cord (*4*). Yet with few exceptions (*5*) little attention has been directed to-

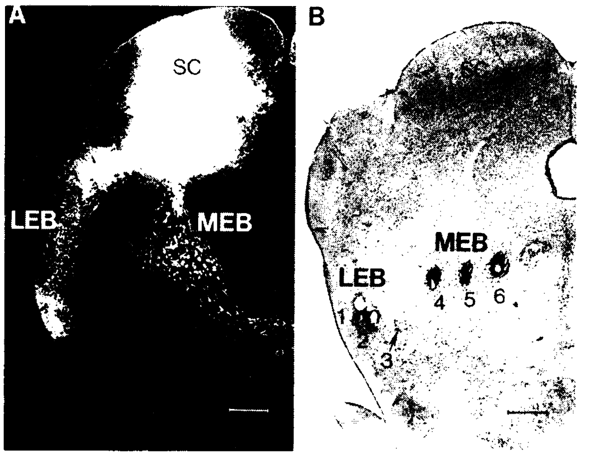

Fig. 1. (A) Dark-field micrograph showing the two descending efferent pathways labeled with tritiated leucine [the medial efferent bundle (*MEB*) and the lateral efferent bundle (*LEB*)], as they exit from the superior colliculus (*SC*) and course toward their brainstem and spinal cord targets. Calibration bar, 1 mm. (B) SC neurons with descending efferent axons were activated antidromically by electrical stimulation delivered through arrays of implanted electrodes. The tips of the electrodes in each array were spaced approximately 1 mm apart in the anterior-posterior and medial-lateral planes to permit the delivery of discrete electrical stimuli at a variety of points within each of these efferent SC pathways. This micrograph illustrates the most caudal point at which the electrical stimuli were presented (electrode 3). The tips of the remaining electrodes (1 and 2 and 4 to 6) were located in sections as far as 1.0 mm rostral to the one shown here [corresponding to levels depicted in (A)]. Calibration bar, 1 mm.