Reports

Chemical Mating Attractants in the Queen Honey Bee

Abstract. Drone attraction to ether extracts of virgin queens (*Apis mellifera* L.) demonstrated that chemical communication enables the drones to orient themselves to queens during mating flights. The primary source of queen mating attractants is the mandibular glands. Fractionation of mandibular gland lipids yielded several attractive fractions that may act jointly. One fraction was queen substance (9-oxodec-2-enoic acid).

Studies of mating activities in the honey bee (*Apis mellifera* L.) have been hindered severely because mating occurs in flight at heights not easily observed. Furthermore, opportunities to observe mating are rare, since the mating activities of the queen normally occur during one to four brief mating flights, taken usually during the second week of adult life (*1*). A queen ordinarily mates with several drones on each of the mating flights, which average 13 minutes in duration (*2*), although queens are known to mate with drones from sources as far as 10 miles from the queen's hive (*3*). The flight distances of queens are not known. However, if a queen were to fly only ½ mile from the hive at heights of 15 to 75 feet, then drones would have to locate the queen within approximately 50 million cubic yards of flight space. Efficient means of communication between drones and queens have evolved, thus compensating for the barrier that flight space poses to mating.

This report presents the first experimental evidence demonstrating the existence of volatile chemicals that function as mating attractants [pheromones (*4*)] in the queen bee.

In early experiments virgin queens 1 to 3 weeks old were tethered to fine monofilament nylon lines attached to balloons filled with helium. Each tether line, approximately 3 feet long, was affixed to the disc (scutum) of the thorax of the queen with a droplet of acetate cement; such queens were able to fly in a small circle. Each afternoon during periods of drone flight tethered queens were elevated to heights of approximately 30 to 80 feet within 100 yards of an apiary. Drones were attracted quickly to the tethered queens, around which they swarmed in large numbers. The attraction of drones under these circumstances agreed with results obtained in previous experiments with tethered queens (*5*) and showed that the proper experimental requirements had been satisfied for subsequent bioassays of queen extracts for mating attractants. The necessity of conducting such bioassays high in the air was shown convincingly. Drones were not attracted to queens below approximately 15 feet from the ground, and drone swarms attracted at greater heights dispersed within seconds if the queens were lowered too near the ground.

In a subsequent experiment virgin queens squashed on small pieces of filter paper were tested for drone attraction under the same conditions; queen squashes were very attractive to drones. Control papers alone attracted only the occasional transient drone, thus indicating that drones were attracted primarily by a chemical stimulus.

In more definitive studies of attractants, experimental conditions were refined by erecting two poles 30 yards apart, between which attractant materials were suspended from a horizontal monofilament nylon line that could be elevated to various heights. All queen extracts tested for drone attraction were impregnated on 25- by 60-mm filter papers inserted into 15- by 50-mm wire-cloth tubes. Four tubes were placed at 10-foot intervals and elevated to 38 feet. The bioassay for drone attraction was a visual assessment of the number and duration of drone visits to queen extracts during a period of 2 to 10 minutes. The erratic flight behavior of drones precluded the use of photography to record drone visits. Most of the drones observed in the experiments reported below presumably came from an apiary approximately one-quarter mile from the experimental area.

An experiment was conducted to elucidate the source of the drone attractant. Mandibular glands (*6*), heads minus mandibular glands, thoraces, and abdomens from 24 virgin queens 3 weeks old were pooled and extracted with ether. These lipid extracts were biosassayed for drone attraction and were ranked in order of their attractiveness to drones. Five replicate rankings were made independently by two observers; tube positions were randomized for each replicate. The mandibular gland lipids ranked first in attraction in every replicate, indicating that the primary source of attractant is the pair of mandibular glands in the head of the queen (Table 1). During dissection some leakage of mandibular gland contents into the head capsule was unavoidable and could account for most of the drone attraction associated with the head fraction.

Attempts were made to characterize the attractants chemically. Mandibular glands were removed from 125 virgin queens 6 to 33 days old. The ether-soluble lipids were extracted from the glands and fractionated by silicic acid column chromatography (*7*). Drones were attracted to two fractions, one material contained in the fatty acid fraction and the other found in the phospholipid fraction. A third fraction demonstrated marginal activity on one occasion. The fatty acid was tentatively

Table 1. Relative drone attraction to extracts of body parts of virgin queens. Numbers indicate replicates in which the rank was observed.

Distribution of ranks in order of attractiveness			
First	Second	Third	Fourth
Mandibular glands			
10	0	0	0
Heads minus mandibular glands			
0	10	0	0
Abdomens			
0	0	7	3
Thoraces			
0	0	3	7

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Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

identified as 9-oxodec-2-enoic acid, known as queen substance (*8*), by its identical retention time value compared to synthetic queen substance (*9*) when submitted to analysis by gas chromatography. In later experiments the foregoing identification was confirmed when synthetic queen substance was found to be attractive to drones in quantities of 0.1 mg per assay tube. A reconstituted mandibular gland lipid complex was considerably more attractive to drones than individual fractions were.

In addition to demonstrating communication by chemicals between drones and queens, these experiments provided evidence indicating multiple mating attractants that may have synergistic action. The attractant chemicals are apparently specific for the species; no other insects were observed to be attracted to the queens or queen extracts. Mating attractants from the queen probably will be useful for surveying drone populations in extensive areas to determine the genetic origin, flight distribution, and flight range.

The remarkably large size of the mandibular glands of the queen bee, relative to other social Hymenoptera, may be explained in part by the important function they serve in mating activities. Chemical communciation between queens and drones on mating flights provides an efficient mechanism permitting rapid, multiple mating of the queen with a minimum exposure to predators (*10*).

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References and Notes

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9. Synthetic queen substance was kindly supplied by R. K. Callow, National Institute for Medical Research, England.
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Effect of Monochromatic Rearing on the Control of Responding by Wavelength

Abstract. The wavelength of a discriminative stimulus exerted no control over the behavior of ducklings raised in a monochromatic environment. Stimulus control was established, however, when responding was reinforced in the presence of one wavelength and not in the presence of another.

When a response is reinforced in the presence of a stimulus, the response is more likely to occur when that stimulus is presented again. Changes in the physical parameters of the controlling stimulus yield orderly changes in the probability that the response will be emitted; to the extent that behavior is invariant under these changes, stimulus generalization is said to occur.

The conditioning procedures traditionally employed in the study of stimulus generalization have in common that a response is reinforced in the presence of a discriminative stimulus (S^D) but differ as to whether responding is also explicitly extinguished in the presence of one or more additional stimuli (S^Δ) prior to generalization testing. Because the home-cage environment of the experimental subjects is rarely controlled, it is unclear whether all conditioning procedures do not perforce involve some extinction of responding to the relevant stimulus property. Perhaps the traditional gradient of stimulus generalization would not be obtained if the stimulus environment were controlled to preclude such differential reinforcement of responding.

Six Peking ducklings, deprived of water for 22 hours, served individually in daily sessions lasting from 1 to 2 hours. Four of these ducklings (birds 1 to 4) were raised in monochromatic light: their wire-mesh home cages were located in an icebox (3 by 2 by 2 feet) illuminated by a sodium lamp (589 mμ). The walls of the box were white (reflectance approximately 0.8) and had a luminance of approximately 1 ft-lam. The remaining two ducklings (birds 5 and 6) were maintained in similar cages located in the experimental room. Four 200-watt tungsten filament lamps, located 3 feet from the cages, provided approximately 1 ft-ca of illumination.

The experimental space was a 12 inch-cubicle painted flat black and illuminated solely by light transmitted through a translucent Plexiglas disk, 1.5 inches in diameter, mounted 8 inches above the floor. A force of 12 or more grams applied to this disk (key) constituted a response and served to control the presentation of water reinforcement through an opening in the wall directly below.

During all of the conditioning sessions, the key was transilluminated only by monochromatic light (589 mμ). During both conditioning and testing the luminance of the response key was changed during a 3-second blackout every 30 seconds so that it varied randomly over a range of 10^{-4} to 10^{-1} lam.

During the first experimental session for each duckling the response of pecking the monochromatic key was conditioned by making the presentation of water contingent upon responses that

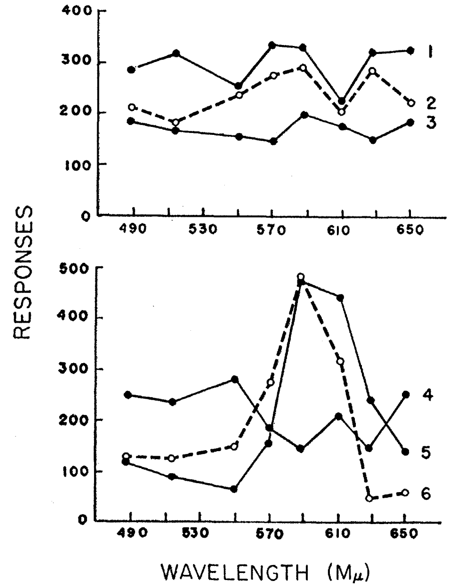

Fig. 1. Stimulus generalization gradients for individual ducklings. Birds 1 to 4 were raised in a monochromatic environment; birds 5 and 6 were not.

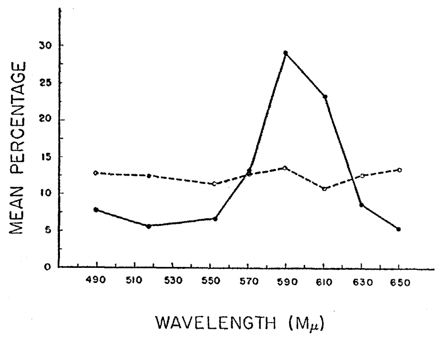

Fig. 2. Mean normalized stimulus generalization gradients. The broken line represents the mean gradient for the four birds raised in a monochromatic environment; the solid line represents the mean gradient for the two birds raised in an environment whose chromaticity was not controlled.