FA-16
M-1659

BIOLOGICAL STUDIES OF THE QUEEN PHEROMONE OF THE RED IMPORTED FIRE ANT

B. MICHAEL GLANCEY

Insects Affecting Man and Animals Research Laboratory, Agricultural Research, SEA, USDA, Gainesville, Florida 32604

Ant behavior is, to a large extent, controlled by odors or pheromones, an aspect of ant biology that was reviewed by Wilson (1963) and Blum (1969, 1974). To date, most studies of ant pheromones have emphasized the trail, alarm, defense, territorial, aggregation and flight initiation pheromones. However, the presence of a queen "recognition" pheromone(s) in the red imported fire ant, *Solenopsis invicta* Buren was recently demonstrated (Glancey, unpublished data; Jouvenaz et al. 1974.) This pheromone appears to have potential for control of fire ants since it can be used to induce the worker ants to carry foreign objects into the nest. As a result, a high priority has been placed on its isolation and chemical characterization. In my paper today, I will (1) review the literature pertaining to queen pheromones; (2) describe the observations that led to the discovery of the queen pheromone of the imported fire ant; and (3) discuss the progress on the development of bioassay techniques.

Literature Review

Wheeler (1910) stated that a queen of *Formica consocians* was accepted by *F. incerta* (=*pallidefulva nitridiventris* Emery) because of her resemblance to them, because of her passive demeanor, and because of her neutral or soothing odor. In an elegant piece of research, Stumper (1956) reported that queen ants elicit 3 responses from workers: (1) grouping, (2) licking of the body by the workers, and (3) transport of the queen after the nest is disturbed. Since these 3 responses are continued after the death of the queen, he inferred that they could be interpreted as a form of chemotropism, a series of positive complex reactions released by chemical stimuli. Moreover, he reported that these responses are facultative since all workers did not automatically react in the same manner. Only a variable number, on meeting their queen, bestowed their care, and after a certain amount of time, they left for other occupations. Thus the individual worker's response was dependent on their physiological and psychological states.

In addition, in his work with *Lasius alienus* and *Pheidole pallidula*, Stumper made various ether and alcohol extracts of queens to obtain the "ectohormone" (= pheromone). These extracts were applied to filter paper, sponge, or elder pith and these surrogate queens were placed in the colony. The ants treated the control (similar material without ectohormone) with marked indifference whereas the surrogate queens caused assembly, licking, biting and moving though the responses were less pronounced than the responses toward a living or dead queen. In fact, dead *Lasius* queens treated with extracts of *Pheidole* queens were adopted as queens by *Pheidole* workers; but after a day, the cadavers were recognized as strangers and pulled to pieces. In contrast, the surrogate queens placed with *Pheidole* were still effective after 8 days.

Stumper noted that the response of the *Pheidole* workers depended on contact with the surrogate queen, particularly antennal palpitation. However, he believed that distant chemoreception (odor) on the part of the workers was operating since there were always concentrated chains of assembly by the workers. He thus distinguished an attraction reaction, exclusive of the royal "ectohormone" reaction, but he noted that some of the assembly could be caused by interattraction among the workers. Since he had obtained a response to extracts of alate virgins, though a much weaker one than that to the fertile queen, he inferred that the royal ectohormone was passed around by grooming of the queen and the brood and therefore had a role in caste or colony regulation.

Schneirla (1957) reported that the attraction of the workers to the odor of the queen helped hold colonies of the army ant colony together. He observed that the queens were usually surrounded by a nucleus of workers with the workers constantly grooming the queen by licking, mouth contact, and antennal stroking. Watkins and Cole (1966), in their work with 6 species of the army ant, *Neivamyrmex*, showed that workers were attracted to secretions of their own queen and of alien queens when the queens were confined on filter paper. In addition, workers housed without a queen were more responsive to queen secretions than workers from the same colonies housed with queens. Workers were still responsive to the secretions after the material had aged for 72 hours.

Brian (1973) reported that in *Myrmica rubra* the broodrearing workers would respond to the queen recognition pheromone. However, he concluded that volatile vapors are not used in this recognition and that contact with the queen was necessary. He stated that the pheromone was probably produced in the thorax or abdomen, not in the mandibular or pharyngeal

gland.

Evidence for a Queen Pheromone in *S. invicta*

In late 1971 and early 1972, while collecting field queens of the red imported fire ant, *Solenopsis invicta* near Gulfport, Mississippi, my co-workers and I observed that inseminated queens held in an open jar attracted workers from other field colonies. If the jar was capped, there was no attraction. These observations led to the hypothesis that the queens were producing a queen "recognition" pheromone, and a series of studies was conducted to test this theory (Glancey, unpublished data). Worker ants responded to air drawn over a mated queen, and pentane extracts of dealated queens applied to pieces of filter paper were attractive. Dealated (inseminated) queens, squashed or alive, were more attractive than alate virgins. Also squashes of the abdomen were always more attractive than squashes of the head or thorax, and thorax squashes were more attractive than squashed heads. Subsequently, in laboratory tests, dead alates (male or female) were carried (by worker ants) into nests when they had been treated with queen extract while untreated dead alates were immediately carried to the refuse pile. Also, small sections of round applicator sticks treated with the queen extracts were usually carried into the nest.

In other tests, live worker ants of 4 species (*Solenopsis richteri*, *S. geminata*, *Camponotus caryae discolor*, and *C. pennsylvanicus*) were treated with benzene extracts of mated queens and placed in colonies of the red imported fire ant. The *S. invicta* workers immediately killed untreated ants but led or pulled treated ants into the nest. There they groomed the pseudoqueen and cared for her about 2 hours. During this time, the pseudoqueen appeared to be quite agitated, as if she knew she was in a strange nest. If she tried to get away, the workers dragged her back to the nest, but there was no apparent hostile action. After about 2 hours of grooming, the queen recognition substance apparently had dissipated, and the workers then killed her. One of the effects of the grooming seemed to be a "quieting" of the workers since they stopped moving about and remained still until the whole colony was quieted down.

At about the same time Jouvenaz et al. (1974) observed that workers of *S. invicta* and *S. geminata* were attracted to portions of blotter paper on which their queens had been confined. Workers from a given colony were more strongly attracted to areas where their own queen was confined than to areas occupied by an alien queen. They also reported that *S. invicta* was

attracted to areas occupied by *S. geminata* queens, but that *S. geminata* was only poorly attracted to areas previously occupied by *S. invicta* queens.

Bioassay Techniques

In view of the observations in Mississippi and those of Jouvenaz et al. (1974), effort was begun during 1975 and 1976 to develop a bioassay test that could be used to measure the activity of the queen pheromone. First, however, it was necessary to define the colony behavior toward the queen. In a normal laboratory colony, i.e., a colony with a queen, brood and over 2,000 workers that are maintained in a Wilson cell in a plastic tray, the queen is visited by 3 or 4 workers at a time. These workers groom and antennate her and probably feed her. Over time there is a gradual interchange of these workers with other workers who continue the grooming sequence. However, the queen is never completely covered by workers, as Schneirla (1971) described for army ants, even if the colony is disturbed. If the queen was removed from her colony and placed about 10 inches away from the cell, she wandered randomly until she contacted some of her workers. Within 2 or 3 minutes, the movements of the foragers became oriented, and a trail of workers appeared that connected the queen with the cell. The queen moved along this trail until she contacted the brood cell. At this point she was surrounded by a large cluster of workers and attempted to gain entrance to the cell by climbing up its smooth sides. If she failed, the ants formed a chain and helped pull her back into the cell.

In the field, when a colony is opened and soil and ants are scattered about, the worker ants seem to cluster around the queen immediately. Then by pulling her, they guide her to safety in the soil.

In developing a bioassay, use was made of these laboratory and field observations. The first assay consisted of treating a small piece of applicator stick, about the same length as a queen, with a benzene extract of mated queens. This stick, or surrogate queen, was then placed at a standard distance from the cell or nest in a laboratory colony. Activity was determined by (1) the number of ants clustering at 0.5, 1, 2, 3, and 4 minutes; (2) the distance the stick was moved during the same periods; (3) the time required to move the stick to the nest opening; and (4) the time required to get the stick into the nest.

With this technique and extracts of physogastric queens, it was determined that concentrations of 0.1 queen equivalent were very attractive.

Then the bioassays were used to determine whether the pheromone was specific to queens or whether other castes were capable to eliciting the response. In this case, 2 types of benzene extracts: whole body and crushed ants on filter paper, were prepared from 100 each of all castes of *S. invicta*, minors, majors, or replete workers and 10 each of female alates, male alates, and mated queens. The concentrations tested were 1.0, 0.1, 0.01 and 0.001 ant equivalents in 20 µl benzene. Only extracts of mated queens elicited the defined responses from workers. Also, the responses to some queen extracts were effective with as little as 0.01 queen equivalent. However, a clustering response was occasionally observed with some caste extracts. Therefore queen and caste extracts were compared by exposing worker ants to sticks treated with a 0.1 ant equivalent of the caste extracts and sticks treated with a 0.1 queen equivalent. The mated queen extracts were always the most active in eliciting worker response. The extracts of majors, repletes and female alates elicited only a very low response.

The data thus supported the postulate of a queen recognition pheromone, but the results at times were occasionally erratic. Sometimes the worker ants would carry the untreated stick into the nest. At other times they would carry the treated stick to the refuse pile and drop it there, and shortly thereafter, other workers would retrieve the stick and carry it to the next or workers would cluster about a stick and groom it, but would not move it. The bioassay therefore did not provide satisfactory separation of the behavioral responses of attraction and movement and the system needed revision so it would be possible to separate responses to volatiles and to contact chemicals.

Present Methodology

During the past 6 months, a variety of techniques have been evaluated for determining the activity of the queen pheromone. These evaluations are still in progress, but it appears that several different bioassays will be needed to adequately support efforts to identify the substance chemically. Since this work is still in progress, I will not discuss it in detail at this time, but the techniques will certainly fall into the 3 following categories.

(1) Attraction to volatiles — A system has been devised to detect and measure the response of individual worker ants or groups of worker ants to volatiles in air blown over queens or queen extracts. The ants respond by clustering around the source of emission of the air and volatiles.

(2) Feeding responses — Because of the observed "quieting" effect when worker ants have access to stocks treated with queen extracts, a simple feeding test has been devised to detect ingestion of components of the queen extract.

(3) Movement of objects treated with queen extracts — Tests similar to those described previously have been modified for use with groups of worker ants or with colonies. Fractions of the queen extracts that are active will also be tested against ants in the field.

Summary

In summary, red imported fire ant queens definitely secrete a substance that is attractive to their workers. The material can be extracted with solvents. When the extract is applied to inanimate objects, these objects induce attraction and clustering of the workers. In most instances, the treated objects are moved into the nest where the worker ants lick and antennate them for many hours or days. The extracts contain a volatile component that can be carried in an air stream and attracts workers. The extracts induce responses consistently at concentrations of 0.1 queen equivalent and erratically at 0.01 queen equivalent.

Literature Cited

Blum, M. S. 1969. Alarm pheromones. Ann. Rev. Entomol. 14:57-80.

Blum, M. S. 1974. Pheromonal sociality in Hymenoptera In Pheromones. Am. Elsevier Publ. Co., Inc., N. Y. 495 pp.

Brian, M. V. 1973. Queen recognition by brood-rearing workers of the ant, *Myrmica rubra* L. Anim. Behav. 21: 691-698.

Jouvenaz, D. P., W. A. Banks, and C. S. Lofgren. 1974. Fire ants: Attraction of workers to queen secretions. Ann. Entomol. Soc. Amer. 67: 442-444.

Schneirla, T. C. 1957. Theoretical consideration of cyclic processes in doryline ants. Proc. Amer. Philos. Soc. 101: 106-133.

Schneirla, T. C. 1971. Army ants. W. N. Freeman and Co., San Francisco. 349 pp.

Stumper, R. 1956. Etudes myrmecologiques. LXXVII. Les secretion attractives des reines de fourmis. Mitt. Schweiz. Entomol. Ges. 29: 373-380.

Watkins, J. F., II and T. W. Cole. 1966. The attraction of army ant workers to secretions of their queen. Texas Sci. 18(3): 254-265.

Wheeler, W. M. 1910. Ants: Their structure. Development and behavior Columbia Univ. Press, New York. 663 pp.

Wilson, E. O. 1963. The social biology of ants. Ann. Rev. Entomol. 8: 345-368.