

The use of CSMA fly rearing medium failed to elicit any eye color modification. The custard-like texture of the milk-yeast gel (which was similar to that used by Ward and Hammen) provided a matrix permitting larval necrophagy. In other control experiments, the feeding of dead 3rd instar gn/gn-larvae to developing gn/gn-larvae produced no photopigment modification. And similarly, feeding experiments utilizing +/gn as a supplement produced results identical to those reported in the +/+ feeding tests.

Additions of 0.5 to 2.5 mg of d-l-kynurenine (Sigma) to the surface of food vials in which gn/gn-larvae were reared produced adults with increasing degrees of photopigment

modification. Adults from each treatment were homogeneous but the eye color produced was an increasing intensity of brown and not the yellow-green to light red pattern resulting from larval necrophagy. From visual observation, it appears that Ziegler's suggestion that the substance secreted into the medium by the +/+ -larvae in Ward and Hammen's study was kynurenine was not confirmed. The reddish color suggests that the pterin and not ommochrome synthesis was affected by larval necrophagy.

We may conclude that in the photopigment development of gn/gn-house flies, the genotype, age and quantity of the sib it eats determines the extent of phenocopied modification.

- 1 Supported by C.U.N.Y., Faculty Research Award Program Grant 1103 and N.I.H. Biomedical Research Support Program 5-SO5-RR07064.
- 2 We thank Alice Chabora and B. Wallace for their discussions and Sandra Smolin for her technical assistance. I.C. McDonald of the USDA Metabolism and Radiation Research Laboratory, Fargo, N.D., kindly supplied marker strains of house fly eye color mutants.
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- 6 The eye color of the normal gn/gn-fly is a pale yellow-green and tests have shown that this gene was identical to that of the gn/gn-strain in culture at the ARS, USDA Laboratory, Fargo, N.D. D. Wagoner, Genetics 57, 729 (1967) places the gn-locus on the 3rd autosome. Wild type flies have a dark red-brown eye color.
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A sex pheromone from the mandibular glands in bumblebee queens

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Summary. In this paper it is shown that the mandibular glands of young bumblebee queens produce a species-specific sex pheromone. From our results it becomes obvious that the pheromone is a releaser for the mating attempts made by the conspecific males.

During the past 3 decades, several authors investigated behavioural and physiological aspects of mating in bumblebees. After Frank's² description of flight paths characteristic for male bumblebees, much attention was paid to this phenomenon³. Krüger⁴ presumed that the function of the scents deposited on marking spots is to attract females. Stein⁵ isolated the marking secretion from the heads of *Bombus terrestris* males and identified the active component as farnesol. Many other substances from male bumblebee glands have since been identified⁶.

The role of virgin queens in bumblebee mating biology was studied by Krüger⁴ and Free⁶, who described the queen as rather passive. Free suggested that males might recognize queens by their size and colour pattern.

Preliminary experiments with virgin queens, either flying free or tied to a line, gave us the impression that the queen is able to communicate to the male her receptiveness. If tethered no copulation occurred. For such a signal, pheromones from the mandibular glands could be involved. In this paper we report on such a sex pheromone from the mandibular glands of bumblebee queens.

The experiments were carried out in flight rooms either at the Zoological Institute II, Würzburg (Federal Republic of Germany) or at the Laboratory of Comparative Physiology, Utrecht (Netherlands). In Würzburg we used *Bombus terrestris* males and females emerging from nests initiated in

the Institute. In Utrecht we used males and queens produced in nests of *B. hypnorum*, *B. pratorum* and *B. terrestris* which were collected in the field previously and kept in confinement.

A few dozen of males were set free in a flight room. After a few days they had set out a flight path generally having only a few marking spots. These marking spots were readily detectable as they were visited frequently by the males. In the Utrecht experiments, males of more than one species were kept together. They shared most of the marking spots. Once the flight path was established, normal young queens were introduced into the room. The behaviour of the males towards such queens was studied. To the same males we offered also:

1. Living virgin queens whose mandibular glands were extirpated.
2. Similar queens without mandibular glands but impregnated with an acetonic extract of mandibular glands of a queen of their own species.
3. Queens without mandibular glands which had been dead for at least 2 weeks. These were impregnated with acetonic extracts of mandibular glands of queens of their own species or of another species.

The extirpation was done under CO₂-narcosis; a window was made in the malar area which was closed again after removal of the mandibular gland. Operated queens were

Results of mating experiments

Species	Number of queens tested	Reaction of conspecific males Approach	Inspection	Attempt to copulate	Copulation
<i>Bombus hypnorum</i>					
I. Living, intact queens	8	8	8	6	2
II. Living, without mandibular glands	18	18	18	3	0
III. Living, without mandibular glands but with secretion of m.g. returned on body	4	4	4	4	3
IV. Dead, but with secretion of m.g. returned on body	8	8	7	5	0
<i>Bombus pratorum</i>					
I. Living, intact queens	13	13	13	13	6
II. Living, without mandibular glands	8	8	8	1	1
III. Living, without mandibular glands but with secretion of m.g. returned on body	1	1	1	1	0
<i>Bombus terrestris</i>					
I. Living, intact queens	15	15	15	14	10
II. Living, without mandibular glands	6	6	6	1	1
III. Living, without mandibular glands but with secretion of m.g. returned on body	2	2	2	2	0
Reaction of <i>Bombus hypnorum</i> males					
<i>Bombus pratorum</i> Without m.g. and killed. Before the experiment m.g. extract of <i>B. hypnorum</i> was given on the body	9	9	6	4	2

The reactions of males towards the indicated number of queens are represented. Each queen was scored only once.

given at least 24 h to recuperate. Control queens had a CO₂-treatment of comparable duration.

During our experiments we observed that the males could behave in 2 very different ways towards offered queens. Many males that had been confined in large numbers, or during a period exceeding a week, were very eager to seize any particular queen, even during her flight. Furthermore males of *B. hypnorum* were seen chasing *B. terrestris* queens which they attempted to seize. They were also chasing each other. These reactions were considered to be abnormal and were therefore not included in our results.

With fewer males in the cage, attention was paid to queens only after they had landed on a marking spot. The subsequent behaviour of these males was then rather uniform. After approaching a queen, the male hovers in the air at a distance of approximately 1–2 cm, and is inspecting her with his outstretched antennae. Only the conspecific male persists in his inspection and will land next to her, whereas males of a different species resume their flight along their pathway. After landing the male continues his antennal display for about 10 sec, paying special attention to the head and abdomen. Then he touches her abdomen with his front legs and mounts the queen, mostly from the rear. After mounting the male bends his abdomen ventrally which position is kept for a period ranging from a few sec to several min. Eventually copulation follows. A copulation takes up to 1 h in *B. pratorum*, but in *B. hypnorum* it is somewhat shorter (20–40 min). In all 3 species examined, males were seen tapping with their legs on the abdomen of the queen for 2–3 sec in 10-sec intervals. Tapping frequency was about 5 times per sec.

For an analysis of the role of the mandibular glands, the mating behaviour was divided into the following steps:

1. Approach: the distant orientation; flight of the male towards the female once she has landed (or was placed) on an odour mark of the flight path.
2. Inspection: the hovering flight of the male with antennal inspection of the queen.

3. Attempt to copulate: a male mounted on a queen and with his abdomen bent ventrally for a period exceeding 20 sec.

4. Copulation: only when we saw the male inserting his genital apparatus into the genital opening did we designate the behaviour to fall into this category.

The results of our experiments with *B. hypnorum*, *B. pratorum* and *B. terrestris* are represented in the table. From the table it becomes evident that the mating process consists of 2 distinct parts, namely an approach and inspecting phase, followed by an attempt to copulate. It must be emphasized that copulation itself is not an adequate parameter for the attractiveness of the queen. Even unmutated queens who were fully attractive were not always mated, although males in almost all cases attempted to copulate. The releaser for the mounting and copulation attempt is apparently a chemical one derived from the mandibular gland secretion. Under our conditions, even the dead queen of the dark variety of *B. pratorum*, once provided with the mandibular secretion of *B. hypnorum* queens, does stimulate *B. hypnorum* males to mount and to attempt to copulate with these objects. Such interspecific mating attempts have not been seen with unmutated queens for any of the combinations that could have occurred. This supports the opinion that the chemical releaser is species-specific.

- 1 Supported by The Netherlands Organisation for the advancement of Pure Research ZWO.
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