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THE CENTRAL ASSOCIATION
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*Chemistry of Communication
in the Honeybee*

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Chemistry of communication in the honeybee

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The study of insect behaviour has developed with tremendous rapidity in the last ten years, very largely as a result of better understanding of the way in which signals from the outside world reach insects through their senses of sight, hearing, touch, taste and smell, and release behaviour patterns. In this lecture attention is given to one section alone of the range of external stimuli that influence behaviour, namely the chemical one of smell.

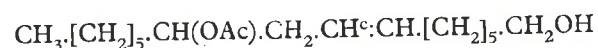
A useful term was introduced ten years ago, namely "pheromone" that defines the concept of a substance secreted by an animal into the outside environment. It then induces a behavioural or physiological response in other individuals of the same species. The simplest and best known pheromones are more familiar under the name of sex attractants. "Assembling" by male moths to a newly emerged female has been known for many years: scientific investigation may probably be said to begin with Fabre (1923), who gives a vivid and picturesque account of assembling of the emperor moth *Saturnia pavonia* and of the oak eggar *Lasiocampa quercus*.

Specific and exact knowledge dates from the isolation by Butenandt, (Butenandt, Hacker, Hopp and Koch, 1962), after some twenty years of work and extraction of some half million insects, of a compound from the female of the silkworm moth *Bombyx mori* that attracted and excited the male. This "bombykol" was found to be hexadeca-10-*trans*-12-*cis*-dien-1-ol :



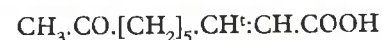
In the U.S.A. (Jacobson, Beroza and Jones, 1961) the sex pheromone of the female gypsy moth *Parthetria dispar* was isolated. "Gyptol"

had the constitution (+) 10-acetoxyhexadec-7-*cis*-en-1-ol :



At this time some half-dozen insect sex attractants have been identified. It soon became clear that very many activities of animals—not only mating in Lepidoptera—were wholly or partially controlled by pheromones that could be isolated as simple, specific chemical compounds. Thus there are trail substances, alarm substances, aggregating scents and others. Very special importance attaches to pheromones in social insects in which not only mating but the very complex organization of the community has to be regulated.

C. G. Butler in 1954 (Butler 1954a, b) put forward the hypothesis of a "queen substance" that was distributed in the hive by the queen and had two effects. It inhibited the development of ovaries in the workers and it prevented the workers from constructing queen cells and rearing new queens. This hypothesis was amply supported by work in France and Holland and, with the development of a reliable method of assaying activity, the way was made smooth for a successful attack on the problem of isolation by measuring the activity of fractions prepared from crude extracts of queens. Guided by the discovery that activity was concentrated in the queen's mandibular glands, and using paper-chromatographic methods that deal with quantities of less than a milligramme, a pilot experiment showed the way to deal with extracts from large numbers of queens' bodies or heads. The active material was found to be an unsaturated ketonic acid, 9-oxodec-*trans*-2-enoic acid :



This compound was synthesised and the synthetic material found to be highly active (Butler, Callow and Johnston, 1961). French and Swiss workers reached the same conclusion. This was, however, not the complete story of "queen substance". Synthetic or natural 9-oxodecenoic acid had the same kind of activity as a live queen, but did not inhibit queen rearing to quite the same extent. It was not until seven years later that the missing factor was identified. 9-Oxodecenoic acid works by contact, but there was also a scent factor derived from the queen, and this was identified (Butler and Callow, 1968) as the scent of the reduction product of 9-oxodecenoic acid, 9-hydroxydec-*trans*-2-enoic acid :



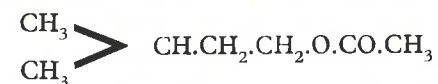
The full behavioural response is produced by a mixture of the two substances, one of which is contacted and the other smelt.

Gary (1962), who had been studying the mating of drones and queens, confirmed earlier speculations as to the existence of a sex attractant in the queen honeybee and traced its source to the mandibular gland. Trials with a synthetic sample of the recently isolated pheromone in the mandibular gland showed that this had a powerful sex-attractant action. Gary doubted whether 9-oxodecenoic acid was the sole attractant, but subsequent work by Butler and Fairey (1964) supplemented Gary's observations and removed this doubt. It must be emphasised, however, that sex attraction leading to mating is not simply a matter of chemical control. 9-Oxodecenoic acid inside the hive does not excite the drone to mating behaviour. The source of the pheromone must be between 5 and 15 m above the ground, according to the speed of the wind; the drone will then approach upwind to an object of the size of a queen, and finally, will copulate with her when the object is a live queen with her sting chamber open (Butler, 1969). Chemical and physical factors are effective only when combined.

This combined action is evident in further observations with 9-hydroxydecenoic acid. Artificial swarm clusters are stabilized (Simpson, 1963) by the presence of a queen, by a cage in which a queen has been kept, but not by 9-oxodecenoic acid, although this has a transitory attraction. 9-Hydroxydecenoic acid, however, does stabilise the cluster completely (Butler, Callow and Chapman, 1964). By contrast, queenless swarming bees will be attracted by the odour of 9-oxodecenoic acid but will not settle, whereas 9-hydroxydecenoic acid alone has little attractive power at a distance. When both acids are present together the swarming queenless bees act as though a queen were present. They will attract others by dispersing Nasonov gland scent and form a quiet, stable cluster. The attractive effect is dependent on the height of the artificial queen above the ground: 1.3 m is optimal; at 4 m the odour is ignored (Butler and Simpson, 1967). Again, the chemical stimulus is active only when presented in a suitable context.

The Nasonov gland scent of the worker honeybee has been elucidated very largely by the work of Shearer and Boch (1966), who devised a method of collecting it by exposing the gland of the anaesthetized bee and wiping its surface with a small piece of filter paper. Combining many of these wipes and extracting them with ether yields a material that can be examined by gas chromatography. Geraniol, citral and the related nerolic and geranic acids were detected and subsequent work (Butler and Calam, 1969) indicates that citral is the main attractive component.

The sting gland of the worker produces an alarm pheromone, the function of which is to alert all adjacent worker bees to the attack that has been made on the first bee to give the alarm. Boch, Shearer and Stone, (1962) and Gunnison and Morse (1968) have investigated this material. It is complex, but the main active constituent is isopentyl acetate :



The worker bee has a mandibular gland that contains 10-hydroxy-decenoic acid, an important constituent of larval food, and also a volatile, strongly smelling constituent, which was identified by Shearer and Boch (1965) as heptan-2-one, $\text{CH}_3.[\text{CH}_2]_4.\text{CO}.\text{CH}_3$.

This compound is already known to be an alarm pheromone and attractant for a species of ant, *Iridomyrmex pruinosus*, and it has been suggested that in the honeybee it is an alarm pheromone that supplements the sting gland pheromone. Simpson (1966) obtained results that emphasize the repellent action of heptan-2-one on honeybees. It might conceivably have a function as a mild deterrent, stopping nurse bees from giving more food to larvae that have just been fed : when the volatile heptanone has evaporated another meal can be given. At the hive entrance, its deterrent action may be used against robbers.

Yet other honeybee pheromones have been postulated by the biologists studying bee behaviour. There is a nest-entrance marking substance that attracts the worker bees to the entrance already used by others (Butler, Fletcher and Watler, 1969). This is probably closely connected with the "hive atmosphere" and the "colony odour", all materials as yet intangible to the chemist. There are still problems to be solved in connection with the queen. There is the Koshevnikov gland, the purpose of which is not known and the secretion from which has not yet been properly investigated. There is also the pleasant smell said by Renner and Baumann (1964) to be associated particularly with nubile, or newly mated queens. There is also an attractive odour of a queen to which workers of queenless colonies respond immediately.

The chemical work at Mill Hill and Rothamsted has had as its object the more thorough analysis of materials that can be obtained from honeybees. Callow, Chapman and Paton (1964) extended the sensitivity of the analysis by using gas-liquid chromatography, by which as little as a two-hundredth of a microgramme of methyl 9-oxodecenoate can be recognized and quantitatively assessed.

Detection of compounds is not, however, matched by ability to recognise them and, in the paper mentioned, thirty-one compounds appeared to be present in significant amount in extracts from queens' heads, but only thirteen of them were identified. Work in progress is using the technique of analysis by a gas chromatograph linked with a mass spectrometer. In many cases the very sensitivity of the method is a disadvantage, for contaminants, ranging from plasticisers to constituents of propolis or even, possibly, fingerprints, can be picked up. Examination of washings of the bodies of honeybees indicates a basic cuticular waxy layer of long-chain paraffins, from $\text{C}_{13}\text{H}_{28}$ to $\text{C}_{31}\text{H}_{64}$, which seems to act as a fixative for more volatile compounds. The queen clearly has the most complicated mixture on her body. This may differ from one individual to another, and our endeavour is to try to correlate our analytical findings with "performance", age, and physiological state of the queen.

So far, we have been dealing with the emission of signals. Their reception is equally important, but does not fall in the sphere of chemistry. Nevertheless, mention must be made of the physiological work of very great delicacy being carried out in Germany, at the Max-Planck Institute at Seewiesen, by Schneider and Kaissling on sensory perception of air-borne chemicals by sensilla in the antennae of insects, (Boeckh, Kaissling and Schneider, 1965 ; Schneider, 1969). A single one of these cells seems to be sensitive to a single molecule of a pheromone. This is paralleled by the sensitivity of receptors in the eye to single photons. In either organ an accumulation of these minimal stimuli is necessary before a sensation is produced. Nevertheless the threshold of reaction of an insect may be of the order of 10,000 molecules—actually one molecular hit on rather less than every one of the 10,000 hairs on the antenna of the male silkworm moth. We have to think in terms of a hundred million million effective doses in a gramme. Kaissling and Renner (1968) find that there are receptors in queens, workers and drones for 9-oxodecenoic acid and for Nasonov gland scent. In the Tropical Products Institute and the Anti-Locust Research Centre this electro-antennographic technique has been used to monitor the effluent from a gas chromatograph (Moorhouse, Yeadon, Beevor and Nesbitt, 1969) and there are exciting possibilities in the future of direct detection of active substances and their location in a complex mixture.

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