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BEE DEPARTMENT

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In August L. Bailey attended the 8th International Congress for Microbiology in Montreal and served as chairman of a Symposium on Insect Microbiology. H. Yvette Spencer-Booth left at the end of September to take up a post in Cambridge.

Behaviour and Physiology

Swarming. Honeybee colonies readily swarm when kept in small hives. When a colony becomes too big for its hive, some bees may eventually cluster outside, but this does not happen until bees have crowded into the hive and reached a density of three to five times the normal on the combs. This congestion of adult bees might seem the most likely cause of swarming, but it has been suggested that lack of room for brood is the principal factor. Consequently, it has been thought important that the part of the hive to which breeding can be confined by a queen-excluder should be big enough to accommodate all the eggs the queen can lay.

The effect of restricting total hive space and space for breeding were compared by restricting either or both in groups of five colonies for 3 weeks. Restricting total hive space greatly promoted swarming, whereas restricting space for breeding had little, if any, effect—either by itself or by augmenting the effect of restricting total hive space.

Small brood chambers are evidently not an important cause of swarming, provided their use does not decrease the total hive space.

The colonies that swarmed in this experiment all did so within 3 days of its beginning. Only colonies that had begun queen rearing before the experiment began contained queen cells occupied at the time of swarming, so crowding seems to induce swarming more quickly than queen rearing. (Simpson)

Queen pheromones. Earlier work (Butler, *J. Insect Physiol.* (1961), **7**, 258–264) suggested that the queen scent which acts synergistically with 9-oxodecenoic acid to inhibit queen rearing by workers is produced in glands widely distributed over the queen's body and not in her mandibular glands, where the 9-oxodecenoic acid is produced. Further work has shown, however, that most, if not all, of this "inhibitory scent" does come from the queen's mandibular glands and is distributed over her body along with the 9-oxodecenoic acid, by which it is readily absorbed and in which it can persist at brood-nest temperature for several days. It seems increasingly unlikely, however, that this is the only, or perhaps even the chief, way in which these substances become available to worker bees, and other possible methods of distribution are being examined.

It is now clear that it is incorrect to equate the term "queen substance",

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defined as material inhibiting the rearing of queens, with 9-oxodecenoic acid alone. (Butler)

Observations by Gary (*Science* (1962), **136**, 773–774) on the mating of queens have been confirmed. Queens mate while flying at heights of 15 ft or more. Drones are attracted upwind to the vicinity of a queen flying at this height by the odour of her 9-oxodecenoic acid, despite its low vapour pressure. Having been attracted by scent in this way, the drones, often a large number of them, form a comet-like group following and darting at objects, particularly dark-coloured ones about the size of honeybees, moving at this height. (Butler and Fairey)

The quantities of 9-oxodecenoic acid in the mandibular glands of virgin and mated queens of different ages were determined by gas-chromatography on ethanol extracts of their heads. Newly emerged queens had little, but the quantity increased rapidly, reaching about 130 μg by the time a queen was 5–10 days old and became nubile. It usually remains large until queens are old. Measurements are being made to see whether it is less than usual, both in superseded queens and those from uncrowded colonies that swarm, as their failure to inhibit queen rearing may reflect their lessened ability to produce this acid. (Butler and Dr. P. N. Paton, National Institute for Medical Research)

Injecting synthetic 9-oxodecenoic acid into individual worker bees kept in queenless groups did not inhibit them from queen rearing, but significantly inhibited the development of their ovaries. (Butler and Fairey)

The odour of 9-oxodecenoic acid, although highly attractive to drones flying 15 ft or higher in the air, did not attract workers. However, experiments with different parts of queens' bodies showed that the scent of the queen that attracts workers is also secreted by her mandibular glands. When mixed with the other constituents of a queen's mandibular gland secretion, it is very persistent even at brood nest temperature, but soon disappears when worker bees can reach it. Although a queen's "attractive scent" is probably distinct from her "inhibitory scent", this has not yet been established. (Butler)

Temperature regulation. A colony of honeybees maintains the temperature of its cluster within fairly narrow limits under wide extremes of outside temperature. Some degree of temperature control results from expansion of the cluster in warm weather and contraction in cold weather, but it has been suggested that bees also control their cluster temperature by adjusting their heat production. This has been shown to be true with groups of 200 or fewer bees (Free & Spencer-Booth, *J. exp. Biol.* (1958), **35**, 930–937), but evidence with whole colonies has been conflicting (Simpson, *Science* (1961), **133**, 1327–1333). The effect of low temperatures on the metabolism of whole colonies is now being investigated at the Low Temperature Research Station, Cambridge, where a room that can be cooled to −40° has been kindly provided. (Free and Simpson)

The effects of exposing individual worker bees for 1–20 hours to high temperatures at 15, 50 or 100% relative humidities were studied. For 1-hour exposures at 45–49° survival increased as relative humidity decreased, presumably because the less the humidity the better the bees

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could cool themselves by evaporating water. Bees younger than 2 weeks survived high temperatures better than older ones taken directly from the hive, but bees of all ages behaved similarly after acclimatisation to the same temperature. For 20-hour exposures at 38–42° survival increased with increasing relative humidity. Desiccation was probably the principal cause of death; loss of body weight increased with decrease in relative humidity. Live bees lost more weight than dead ones after exposure to high temperature for 1 hour, but less after exposure for 20 hours. These results illustrate the need of overheated bees for water to cool themselves and a low relative humidity to evaporate it. In very hot weather colonies can be helped by providing them with water in the hive and giving extra ventilation. (Free and Spencer-Booth)

Worker-bee pheromones. Boch and Shearer (*Nature, Lond.* (1962), **194**, 704–706) showed that geraniol is the main constituent secreted by the abdominal scent (Nassanoff) gland of the worker honeybee. However, although the scent of geraniol attracted food-seeking bees in the field, it did so much less than scent-gland odour, so the odour of the secretion from this gland owes its attractiveness not only to geraniol but also to one or more as yet unidentified volatile substances. (Free)

Feeding mechanism. Studies on the functions of honeybee salivary glands were continued by examining the way in which a bee discharges saliva on to its food. Saliva does not, as has been suggested, travel down the tongue canal while food is moving up the outside of the tongue. Discharge of saliva alternates with food uptake, the same channels being used for both purposes. A bee can raise and lower the bristles on its tongue, which probably enables it to act like a sponge, expanding to absorb fluid and contracting to expel it. The mechanisms of bristle erection, ligula retraction and tongue folding were elucidated. The salivary pump at the outlet of the labial glands is dilated by muscles, but seems to be flattened by its elasticity alone. It has no inlet valve and probably functions by a combination of an outlet valve and friction in the ducts leading from the glands. The pump can work with the ligula almost fully retracted and the tongue folded back, so explaining how labial-gland secretion is supplied to the mandibles during chewing. (Simpson)

Pollination and Field Behaviour

The constancy of individual worker honeybees to nectar and pollen collection, and to flower species visited for pollen on successive trips and days, was studied by catching marked bees at the hive entrance and removing and identifying their pollen loads. On first becoming foragers, bees showed no tendency to prefer to collect pollen rather than nectar, or vice versa. The likelihood of an individual bee continuing to collect pollen was lessened by removing the pollen loads from her corbiculae, but those that continued to collect pollen did so as regularly as before. Ninety-four per cent of the loads contained only one species, confirming that during a foraging trip a bee usually collects one kind of pollen only.

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The percentage of pollen-gatherers that continued to collect the same kind of pollen they had when they were marked decreased on each successive day; the rate of change differed in different experiments. After 7 days only about half the bees were still collecting their original pollen. Bees collecting the most common pollen tended to be the most constant. On days when a particular pollen was temporarily unavailable bees accustomed to collect it usually either did not forage or returned home without pollen; very few collected pollen of another species. No bees consistently collected different pollens at different times of the day, although many returned from some trips with pollen and other trips with nectar. A few bees with loads containing two kinds of pollen collected the same two kinds on later trips, but most with mixed loads, although more inclined than others to collect mixed loads later, collected different mixtures on different trips, so presumably they were dissatisfied with their crops and were sampling others.

When their colonies were moved to a new site with a similar flora to the old site most bees visited the same species at both; changes were usually associated with the relative abundance of the different species at the old and new sites, and the species to which a bee changed after being moved was often one it had occasionally visited before. However, when colonies were put beside a large area of a crop most bees that had not previously foraged on that species began to do so. (Free)

Bee Diseases

European Foul Brood disease. *Streptococcus pluton* grown on culture media was fed to normal larvae and was then recovered from the brood-comb cell walls of surviving pupae, which, therefore, had voided the bacteria in their faeces. Cells of *S. pluton* multiplied when passaged in this way, and they became increasingly virulent. After two such passages bacteria were about as pathogenic as those from naturally diseased larvae. The other bacteria associated with European Foul Brood disappeared when passaged in this way. (Bailey)

American Foul Brood disease. Spores of *Bacillus larvae* germinated best at low redox potential, but vegetative growth was best when aerobic. Glucose greatly stimulated vegetative growth but decreased sporulation. Simple media suiting germination, vegetative growth and sporulation, and giving consistent results, were developed. They have proved more reliable for isolating and identifying *B. larvae*, especially from material containing few spores, than previously recommended media. For example, some brood disease in Australia had been considered possibly a new type because afferent bacterial spores in the diseased larvae failed to grow on media recommended for *B. larvae*, but the organism was readily isolated from samples of larval remains with the aid of the new media and seemed indistinguishable from commonly occurring *B. larvae*.

Spores of *B. larvae* germinated in young honeybee larvae, but apparently not in old ones. Vegetative growth was slow in the growing larvae, and the bacteria migrated to the gut epithelium, to which they became closely

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applied but which they did not penetrate until the larvae pupated. These observations on the behaviour of *B. larvae in vitro* and *in vivo* suggest that, although the environment in the gut of the youngest larva is suitable for spore germination, it soon lacks enough air for vigorous vegetative growth and contains too much glucose for sporulation; only when the motile bacteria penetrate to the aerobic tissues of the prepupa, can they grow vigorously and sporulate. (Bailey)

Chalk Brood disease. Spores of *Ascophaera apis*, like those of *Bacillus larvae*, germinated best at low redox potentials; mycelial growth and sporulation occurred only aerobically. Because of these similarities *in vitro*, tests were made to see whether growth of *A. apis* paralleled that of *B. larvae in vivo*. Food of normal larvae, of ages from 0–4 days, was inoculated with 10^5 spores per larva, but in all of several tests larvae of all ages proved highly resistant to infection; only 1 or 2% of several hundreds were killed and there was no indication that larvae of a particular age were susceptible. (Bailey)

Paralysis. Further work was done with the virus causing acute paralysis and death of adult bees. The pathogenicity (expressed as LD_{50}) of preparations of bee paralysis virus was closely correlated ($r = 0{\cdot}92$) with the number of virus particles they contained, irrespective of their source or the method by which they had been purified. Bees were more readily infected when the virus was injected (LD_{50} $1{\cdot}3 \times 10^2$ particles/bee) than when it was fed (LD_{50} 10^{10} — 10^{11} particles/bee). The virus was transmitted between bees by food exchange, and it accumulated in the faeces of the recipients, but it is unlikely that a dose of virus large enough to cause acute disease is transferred in this way in nature.

Bee paralysis virus was isolated from several colonies from the Harpenden locality and one from Scotland by injecting seemingly healthy bees from each colony with extracts of individuals from the same colony. The injected individuals became paralysed, and extracts from them contained many particles resembling those of bee-paralysis virus. All these extracts reacted with an antiserum prepared against a bee-paralysis virus from the Harpenden bees. In the same way, apparently healthy bumblebees became paralysed when injected with extracts of similar bumblebees, and the paralysed bumblebees contained many virus particles serologically related to those from paralysed honeybees. Bumblebees also became diseased when injected with virus from honeybees, but adult wasps (*Vespula* spp.), larvae of greater and lesser wax moths (*Galleria mellonella* and *Achroia grisella*), cockroaches (*Blatta orientalis*) and flour beetles (*Tenebrio molitor*) did not.

Apparently normal bees also developed symptoms of acute paralysis when injected with some foreign materials (0·1–1·0 μg/bee), such as purified tobacco mosaic or turnip yellow mosaic viruses, whether infective for plants or after being inactivated by exposure to ultra-violet light. The paralysed bees contained many bee-paralysis virus particles, but the injected plant viruses had apparently not multiplied. It seems that bee-paralysis virus is present in small amounts in many apparently healthy bees. The virus can be detected in such individuals either by injecting

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extracts of them into other bees or by stimulating the virus to multiply in them by injecting into them relatively large amounts of foreign material.

When bees were injected with extracts of naturally diseased bees (equivalent of 10^{-4} bees/bee) they developed symptoms of the acute paralysis described above, whereas those injected with more dilute extracts (10^{-7}–10^{-8} bees/bee) developed a chronic form of paralysis, which was more like the naturally occurring disease. This disease has now been serially transmitted for several months, using the dilute extracts to avoid contamination with acute paralysis virus. Bees with chronic paralysis live for several days after first showing symptoms, which have some differences from those of acute paralysis. They contain a characteristic type of particle quite unlike that in bees with acute paralysis. (Bailey and Gibbs, Plant Pathology Department)

Acarine disease. From reports that bacteria increase in the haemolymph of bees infested with *Acarapis woodi* and that the micro-organisms are representative of those from the tracheae but not from elsewhere, it could be supposed that mite-infested bees would be more susceptible than uninfested ones to paralysis virus or other pathogens when these invade *via* the tracheae. Accordingly, samples of bees, some of which were infested with *A. woodi*, were sprayed with preparations of acute paralysis virus or of *Pseudomonas apiseptica* (Burnside). However, both preparations killed equal numbers of infested and uninfested bees. (Bailey and Lee)

Wax moth. Because of reports that the greater wax moth (*Galleria mellonella*) is susceptible to *Bacillus thuringiensis* and that honeybees are not, wax foundation containing spores of this bacillus was prepared with the assistance of Messrs. E. H. Taylor Ltd. The foundation was drawn normally into comb by bees and brood then reared in it. Some died, perhaps more than in control combs, in the first generation of brood in combs with the highest concentrations of spores, but not in later generations. Treated comb that had one generation of brood reared in it was mothproof. Further tests will be made with lesser wax moth and with combs that have been in colonies for long periods. (Bailey and Dr. D. H. Burges, Pest Infestation Laboratory, Slough)

Fumigation of combs. Methyl bromide, which is sometimes used for fumigating combs against wax moth, and propylene oxide, a fumigant used to sterilise foodstuffs, were tested as fumigants against spores of *Nosema apis* and *Bacillus larvae* and cells of *Streptococcus pluton*, all of which depend for their survival on dormant stages carried on comb. Viability of treated spores of *N. apis* was tested by feeding them to caged bees. Bacteria were tested by plating serial dilutions in suitable media. At 15° vapour of methyl bromide (8% v/v) or propylene oxide (1 ml liquid per litre) killed *N. apis* spores between 16 and 24 hours and *S. pluton* between 1 and 16 hours. Very nearly all (99·99%) spores of *B. larvae* were killed between 1 and 16 hours with either fumigant, but a few spores survived 2 days' fumigation.

Adult and larval wax moths are very susceptible to propylene oxide; tests against wax moth eggs are in progress. (Bailey and Lee)

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