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Bees

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BEE DEPARTMENT

C. G. BUTLER

A. J. Durrant, Elaine M. Fairey and Inge B. M. Riedel left, and P. A. Racey and N. Wilding were appointed. Dr. G. Knerer (University of Toronto) is working in the department on wild bees.

The immediate practical aim of most of our work is to make beekeeping methods more efficient, to find the best ways of using honeybees and other insects as pollinators of crops, and to improve the control of honeybee pests and diseases. However, some results have implications extending beyond beekeeping, implications that become more important as increasing attention is paid to seeking alternative methods to insecticides for controlling crop pests. For example, research on the social organisation of the honeybee colony, both in and outside the hive, show how the behaviour of insects depends on chemicals (pheromones) they produce and over what distances such chemicals as sex attractants can be expected to act. Similarly, the work on bee disease is relevant to the use of pathogens to control insect pests.

Behaviour and Physiology

Swarming. The most common cause of swarming in beekeeping is that the adult bees become overcrowded because the hive is too small to accommodate the colony as it increases; this increased density has been measured. A few colonies swarm when the hive space per adult is not limited, perhaps because of local overcrowding. This was caused in hives by taking away some of the brood, when the adult bees concentrated themselves around the remaining brood. In untreated colonies it may be a result of rearing unusually many queens, which means that much less worker brood is produced. Colonies with plenty of total hive space were made to swarm by two different treatments. In one experiment the queens were removed from 7 colonies to make them raise emergency queens. When the first young queens were ready to emerge most of the worker brood was removed from the colonies. Many queens were heard piping in 6 colonies, at least 4 of which swarmed. In the second experiment 3 large colonies not raising queens were united with one only of the queens. Queens were reared in at least 12 primary queen cells (i.e., cells specially built for queen rearing and not modified from worker cells) and the colony swarmed with a virgin queen 36 days after it was united. This behaviour agrees with experience that prime swarms from uncrowded colonies are usually headed by a virgin queen from hives in which the old queen has died or been killed at about the time the new queens emerged.

The density at which honeybees cluster depends on the temperature. This was shown both by weighing bees in different parts of hives kept at different temperatures and by measuring the changes in length of a colony

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in a cylindrical cage when the temperature was changed. The mean density of a colony with combs can range from 4 bees/ml at $-20°$ C to 1 bee/ml at 25° C. As a colony may need twice as much space at 25° C as at 15° C to prevent overcrowding, temperature rises are obviously significant to swarming. (Simpson)

The characteristics of winter bees. Winter bees characteristically have large hypopharyngeal glands rich in invertase, but the reason for this is unknown. It now seems that it may partly be explained by the fact that winter bees have little or no brood to feed. Evidence for this came from experiments in which the amount of brood allowed to colonies in May was varied. Bees 14 days old in colonies that were deprived of brood contained as much invertase as autumn bees of the same age; bees in colonies given extra brood had less invertase than in colonies with the usual amount of brood. There is still no explanation for breeding slowing so much in late summer when forage is still plentiful. (Simpson and Riedel)

Pheromones of worker honeybees. Pheromones were studied from the sting-chamber glands, mandibular glands, Nassanoff glands and body surfaces of worker honeybees. It was confirmed that one or more of the glands opening into a worker's sting-chamber produces a scent (either isoamyl acetate or containing isoamyl acetate) that is released by guard bees, alerts other workers and recruits them to defend the hive. The olfactory pheromone from the mandibular glands of older workers, particularly foragers, was identified as 2-heptanone, as first reported by Shearer & Boch (*Nature*, *Lond.* (1965) **206**, 530). It has been reported to act in a similar, but less pronounced, way as isoamyl acetate—i.e., as a weak alarm substance—and also as an attractant for worker honeybees (Maschwitz, *Nature*, *Lond.* (1964) **204**, 324; Shearer & Boch, *Nature*, *Lond.* (1965) **206**, 530). We could not confirm either of these conclusions, but instead obtained clear evidence supporting Simpson's conclusion (*Rothamsted Report* for 1961, p. 172) that it repels worker honeybees. Its biological function remains a matter for conjecture.

An unidentified olfactory pheromone left on dishes or other objects crawled over by foraging bees was shown to be present on most parts of a worker's body. It is very attractive to foraging honeybees, possibly even more so than the odour from the Nassanoff gland of the worker, and persists. When exposed in a dish covered with perforated zinc in the open air at 67–75° F, the odour of a plaster of Paris plate (15 $\times$ 15 mm $\times$ approx. 4 mm thick) over which 20 foragers had just run continued to attract foragers, visiting a training-table in search of food, for at least 10 minutes, and often for twice as long. Failure to realise the existence of this pheromone could explain the apparently contradictory results of some behaviour experiments described in the literature.

The biologically active part of the secretion of the Nassanoff gland of the worker honeybee, first reported to consist of geraniol alone and later of geraniol plus nerolic and geranic acids (Boch & Shearer, *Nature*, *Lond.* (1962) **194**, 704; *Nature*, *Lond.* (1964) **202**, 320), was shown to consist of a mixture of geraniol and citral (geranial), with citral being much the more

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important constituent. Weaver, Weaver and Law (*Prog. Rep. Tex. agric. Exp. Stn* (1964) No. 2324, 1) independently reached the same conclusion. (Butler, with Drs. R. K. Callow and J. R. Chapman, National Institute for Medical Research)

Pheromones of queen honeybees. Some progress was made in attempts to isolate and identify the scent in the secretion of the queen's mandibular glands that attracts worker bees to their queen and prevents them from becoming restless. This pheromone probably plays an essential part in the process by which the workers of a colony obtain from their queen the important pheromones, such as queen substance, controlling their behaviour. (Butler, with Dr. J. R. Chapman)

The attraction of free-flying drones by the olfactory sex attractant of the queen, 9-oxodecenoic acid, was further studied. Drones cannot readily be attracted to such places as small clearings in woods or in tall scrub, but once they have been attracted to a place by the odour of a queen's sex attractant they will continue to visit it for several days, probably, indeed, for the rest of their lives. Also, drones that have been attracted to a particular small area in this way quickly learn to associate with the attractive odour various inanimate and animate objects, such as a leaf suspended by a nylon thread from the top of a 20-foot pole or even a white-coated experimenter. (Butler)

Stings. In the hope of finding breeding stock from which to select a strain of honeybees with useless stings, the stings of 11,446 workers from 484 colonies were examined, but the only defect found was dislocation of the interlocking sliding joint between the lancets and the stylet in 16 bees, each from a different colony. Such dislocation is much commoner in queens than in workers, judging from the number that have been found during instrumental insemination. Possibly it is caused by failure of the tongues of the stylet to enter the grooves of the lancet during growth, or by damage during the last moult when the sting is still soft. Evidently other sting defects are so rare that there is little hope of finding them, but as dislocated stings are almost certainly useless to the bees it may be worthwhile to find out whether they occur more frequently in some colonies than others. (Simpson)

Use of water by adult honeybees. Honeybees add saliva from their labial glands to nectar or syrup, thus making it less viscous and facilitating its uptake through the proboscis. In some conditions bees also take water. To find how much syrup and how much water bees take at different temperatures and to what extent they dilute or evaporate their honey-stomach contents, groups of bees were kept in cages without combs but provided with separate feeders containing sucrose syrup and water respectively. The amounts of syrup and water taken at various incubator temperatures were measured. The extent to which the syrup in the bees' honey-stomachs was later either diluted or concentrated (by evaporation) was also determined. Irrespective of its concentration, more syrup was taken at low than at high temperatures, indicating increasing metabolism with decreasing

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temperature. The more dilute the syrup offered, the greater the total amount taken by the bees, but the smaller the quantity present in any individual's honey-stomach at any given time. Dilute syrup is probably less attractive to bees than concentrated syrup, although more of it is needed to maintain life.

Little water was taken below 30° C, but above this temperature the amount taken increased with increasing concentration of the syrup. Below 30° C the bees diluted the syrup with saliva; the greater the concentration, the more water they added.

Although the bees added water to the syrup in their honey-stomachs at temperatures above 30° C, they later evaporated off enough, by exposure to the hive atmosphere, to increase the syrup concentration until it again equalled or exceeded that of the original syrup. Probably, therefore, in the hive, where temperatures usually exceed 30° C, nectar is always concentrated by the bees to some extent. Despite the fact that bees add more water to concentrated than to dilute syrup and then must get rid of the surplus water, this entails less work than concentrating very dilute syrup and leads to the practical conclusion that it is at all times better to feed concentrated than dilute syrup. (Free)

Pollination and Field Behaviour

Apples. The individual foraging areas, varietal preferences and constancy of honeybees in an orchard containing 5 varieties of apples (Cox, Sunset, Laxton's Fortune, Merton Worcester, Tydeman's Late Orange) were studied. The number of bees visiting each variety was proportional to the number of its flowers, but also depended on its stage of flowering. When some varieties were at a more advanced stage of flowering than others and the early flowering varieties began to lose their petals the proportion of the total population of bees visiting them decreased. Thus bees originally visiting trees of early flowering varieties tended later to move to other varieties and, consequently, the total foraging area of each bee became quite large. However, with those varieties that retained or increased their attractiveness the same bees continued to visit them consistently; many bees were seen throughout the experiment only on or near the same trees they were first seen to visit. Many of these bees kept to the same trees, even when nearby trees of another variety became slightly more attractive to bees in general.

Because bees that remain constant to one variety can only pollinate its flowers if it is self-fertile, these observations re-emphasise the importance of choosing compatible polliniser and main variety trees whose flowering periods closely coincide, and interplanting them in such a way as to encourage the bees to include trees of both varieties in their foraging areas.

Blackcurrants. Further work confirmed previous observation that blackcurrants get little, if any, benefit from cross-pollination.

Field beans. The pollination requirements of field beans (*Vicia faba*) were studied by comparing the seed yields of plants in the open and when caged

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with and without bees. Plants caged with bees produced significantly more pods per plant and more beans per plant than plants caged without bees, but many fewer than uncaged plants. The environment in the cages seemingly limited the total production capacity of plants.

Red clover. Experiments were made to determine the ability of bumblebees and honeybees to pollinate red clover flowers. Developing heads were enclosed in bags and, after the flowers had opened, the bags were removed from a few heads at a time and each watched until it was visited by a bee, when it was bagged again. The behaviour of each bee and the number of florets it visited were recorded.

When newly opened florets were exposed, 70–80% set seed, but delaying the exposure decreased the percentage set. Provided a bee entered the florets, the same amount of seed was set by a honeybee or a bumblebee. Different bumblebee species were equally effective. Bees carrying pollen tended to visit more florets per head and to be more efficient pollinators than those that were collecting nectar only, and bumblebees visited more florets per head than honeybees. The number of florets visited per head also varied with the availability of nectar and pollen.

The number of visited florets that set seed did not decrease when the number of florets per head increased, indicating that the compatible pollen a bee has collected from other red clover plants is not quickly rendered ineffective by extensive dilution with pollen of the flower the bee is visiting. (Free)

Bee Diseases and Pests

Paralysis. The queens of several colonies were replaced in autumn, 1964, with queens obtained from colonies with chronic paralysis in various parts of Britain. In May 1965 some bees with chronic paralysis were collected each day in traps at the entrance of the hives of all but one of these colonies. Three colonies each produced about 50 paralysed bees daily, but the others produced many fewer. Pupae found in the traps of hives housing paralytic bees contained as much chronic bee paralysis virus (CBPV) as paralysed adults. Only one of the colonies continued to produce undiminished numbers of paralysed bees throughout the summer. During late August the number of chronically paralysed bees increased briefly in some others, but at this time bees with chronic paralysis were trapped from several colonies with normal queens, including colonies beyond the flight range of those in which paralysis had been introduced. These observations do not contradict previous results indicating that worker bees inherit susceptibility to paralysis from certain queens, and confirm earlier results showing that infection is widespread. Moreover, they show that some loss is caused by this disease in colonies that would ordinarily be considered healthy.

Environmental factors, such as high temperature or toxins, may be more important than genetic susceptibility in making CBPV active. For example, almost all of many bees from one colony, previously considered healthy, developed chronic paralysis on one occasion when they were incubated at

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35° C. However, on later occasions bees from this and other colonies that had produced paralytic bees did not develop detectable amounts of CBPV when they were incubated, even at 37° C, or when they were fed slow-acting toxins (heated honey), although they died sooner than control bees and showed symptoms not unlike those of paralysis.

Tests on samples of adult bees sent to the National Agricultural Advisory Service Bee Advisory Unit at Rothamsted showed that all those diagnosed, according to external signs, as having, or probably having, paralysis contained much CBPV. At least 70% of all samples with no other obvious cause of sickness were suffering from chronic paralysis. Of 15 samples of adult bees found to be severely infested with *Acarapis woodi*, three also had chronic paralysis. Almost all the many bees of one of these samples developed chronic paralysis after they had been kept at 35° C for a few days.

Preparations of bees, with a disease resembling chronic paralysis, sent from Ottawa (Canadian Department of Agriculture) were tested for CBPV. Local bees responded to injections with these preparations in exactly the same ways as with CBPV, and antiserum to CBPV neutralised the infectivity of the Canadian virus. It is clear, therefore, that the virus causing bee paralysis, which has already been found in bees from Europe, Scandinavia and the Far East, also occurs in North America.

Sacbrood. Nearly 200 local colonies were surveyed during spring for sacbrood. Extracts of dead prepupae were tested in gel-diffusion plates against antiserum prepared against sacbrood virus. About 15% of colonies had sacbrood, although only very few dead prepupae were usually found at one time in any colony. Forty per cent of the colonies headed by daughters of one breeder queen had sacbrood. This was significantly larger than the incidence in the other colonies, and indicates a measurable difference between strains of bees either in their susceptibility to this disease or in their ability, usually considerable, to eject from the hive the remains of dead larvae.

European Foulbrood. Tests to control EFB were made with nisin, an antibiotic approved for use as an additive in certain foods, and with oxytetracycline (OTC). Although the organism concerned, *Streptococcus pluton*, was more sensitive to nisin than to OTC *in vitro*, nisin, unlike OTC, did not control EFB very well, probably because it is less stable than OTC in honey and may also be easily digested in the larval gut.

Antibiotics used in medicine are not permitted in foods. From this public-health aspect, OTC is probably the least objectionable of those antibiotics known to be effective against EFB and convenient for use in honey-bee colonies, because it loses activity soonest. Tests were made, in collaboration with Pfizer Ltd., to determine how long honey must be left in colonies that have been treated with OTC for all the active antibiotic to disappear. In June, 4 weeks after feeding colonies with OTC in syrup, the antibiotic was hardly detectable in their honey stores (less than 1 ppm). In colonies treated in early May about 1 ppm was still present in samples of their honey 6–8 weeks later. Because of the poor weather during this time,

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the treated colonies had gathered almost no nectar, which otherwise might have diluted the antibiotic in stored honey.

In another experiment, 4 g of OTC (at least four times the usual effective dose) was fed to some colonies in syrup. This syrup was extracted from the combs, immediately after it had been stored there by the bees, and replaced in the same combs in sealed glass containers so that the bees could not dilute it with incoming nectar. The antibiotic activity in these completely isolated stores diminished from about 300 to about 3 ppm after 9 to 10 weeks.

It is concluded that it is inadvisable to remove combs of honey for extraction from colonies until at least 2 months after they have been treated with OTC.

Tests were made with erythromycin against nine strains of *Streptococcus pluton* isolated from diseased larvae obtained from different parts of the world. Each strain was equally sensitive, and growth was prevented by concentrations of 10^{-7}–10^{-8} of the antibiotic. Other organisms resembling *S. pluton* morphologically have been reported to resist much larger concentrations of erythromycin *in vitro*, although EFB responds very well to treatment with this antibiotic. It seems likely that these resistant organisms are secondary invaders and not related to *S. pluton*.

Streptococcus pluton was isolated from a sample of honeybee larvae from Zambia suspected of having EFB. About 90% of bacterial cells present that grew *in vitro* were of *S. pluton*, and the remainder were almost entirely of organisms resembling *Streptococcus faecalis*. This is not an uncommon condition of larvae with EFB in other parts of the world.

Chalkbrood. Honeybee larvae infected with spores of the fungus *Ascosphaera apis* have been shown to be more likely to develop chalkbrood when chilled for a short time immediately after they are sealed in their cells than when chilled for the same period later on in their 2-day prepupal phase. This may be because, at the start of this phase, the fungal hyphae are most numerous in the gut, and so are then most likely to invade the tissues successfully after the chilling process. Although still alive, at least for a while, in the faeces voided during the prepupal phase, the hyphae then probably cannot invade the larval body. In some tests chalkbrood was caused by inoculating larvae at a normal temperature (35° C) but in an atmosphere of oxygen. This supports previous evidence (*Rothamsted Report* for 1963, p. 166) suggesting that the mycelium of *A. apis* does not develop readily in the anaerobic condition of the gut of a normal larva. Chilling, by temporarily inactivating the larva, may perhaps allow enough oxygen to diffuse into its gut to reactivate the mycelium. (Bailey)

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