Some Pheromones Controlling Honeybee Behaviour.

C. G. Butler (Rothamsted Experimental Station, Harpenden, Hertfordshire, England)

During the last twenty years, the control of honeybee behaviour by pheromones has received much attention. This is, perhaps, a good time to review and try to co-ordinate some of the discoveries and to consider whether any general conclusions can be drawn about the ways in which pheromones operate.

Nassanoff pheromone

One of the first honeybee pheromones to be discovered was the scent produced in the Nassanoff gland of the worker.[1] When a worker finds a source of sucrose syrup, or even drinking water,[2] or (very occasionally by *Apis mellifera*) a source of floral nectar,[3,4] she distributes scent from this gland merely by exposing its surface, so attracting other bees to exploit her discovery. Again, in what appears to be a completely different context, an individual worker bee who has been foraging or has for some reason been separated from other workers or her queen often exposes her Nassanoff gland when she rediscovers them.[5] However, distribution of the scent is then often, but not always, encouraged by the bee using her wings to fan a current of air over the exposed gland. It is interesting that both *Apis cerana* ssp. *indica* and *A. florea* workers, unlike those of *A. mellifera*, frequently expose their scent glands when visiting flowers.[6]

---

[1] Sladen, F.W.L. 1902, Ent. mon. Mag. 38, 208-211.
[2] Free, J.B. 1969, Personal communication.
[3] Frisch, K. von and Rösch, G.A. 1926, Z. vergl. Physiol. 4, 1-21.
[4] Free, J.B. and Racey, P.A. 1966, J. apicult. Res. 5, 177-182.
[5] Renner, M. 1960, Z. vergl. Physiol. 43, 411-468.
[6] Butler, C.G. 1954, *The World of the Honeybee*, London. Collins.

The Nassanoff pheromone, as many from the honeybee, seems to operate over a short range only, probably not more than 100 mm.[7] It is not colony specific,[5] and it will be interesting to find out whether it is even species-specific or is common to some, or all, of the four species of *Apis*.

The composition of this olfactory pheromone has been the subject of some controversy[8,9,10,11] but the biologically active principle has now been shown to be a mixture of citral and geraniol, of which citral is by far the more important component although much less abundant than geraniol.[12,13] Recent electrophysiological work shows that one type of olfactory receptor cell on the honeybee antenna responds, not only to the Nassanoff scent, but also to citral, geraniol, geranic acid, and citronellol.[14]

'Footprint' pheromones

When homecoming bees first succeed in finding the entrance to their hive when it has been displaced during their absence (for example, by turning the hive through 90°), they distribute Nassanoff scent both with and without wing fanning. Another pheromone, which we have called the 'footprint' pheromone, also assists such bees to find the entrance. It is distributed on the hive, around the entrance, by the bees that crawl there, and it attracts other

---

[7] Butler, C.G. In preparation.

[8] Boch, R. and Shearer, D.A. 1962, Nature, Lond. 194, 704-706.

[9] Boch, R. and Shearer, D.A. 1964, Nature, Lond. 202, 320-321.

[10] Free, J.B. 1962, J. apicult. Res. 1, 52-54.

[11] Shearer, D.A. and Boch, R. 1966, J. Insect. Physiol. 12, 1513-1521.

[12] Weaver, N., Weaver, E.C. and Law, J.H. 1964, Prog. Rep. 2324, Texas A & M University.

[13] Butler, C.G. and Calam, D.H. 1969, J. Insect Physiol. 15, 237-244.

[14] Kaissling, K.-E. and Renner, M. 1968, Z. vergl. Physiol. 59, 357-361.

workers and stimulates them to enter the hive.[15] The homecoming bees are also attracted by an odour in the hive atmosphere, which may be that of the 'footprint' substance and is certainly perceived olfactorily and possibly also chemotactically. This pheromone is persistent but probably not colony, perhaps not even species, specific. Although deposited by the workers' feet, and possibly to some extent by the tip of the abdomen, as we have recently discovered by making bees leaving and entering their hives crawl over smoked glass plates,[16] it is probably produced in glands on most parts of a bee's body.[15] It is soluble in, and recoverable from, ethanol and methanol but not ether.[15] It has not yet been identified.

A similar, but chemically distinct, 'footprint' pheromone, which also assists homecomers to find their way, is deposited, probably involuntarily, around the nest entrance by the wasp, *Vespula vulgaris*, and probably by many other social insects and some solitary ones also.[15]

Worker honeybee 'footprint' pheromone persists for at least 4 hours at 23°C and over twenty times as long at 5°C,[15] many times longer than Nassanoff pheromone,[17] with which dishes of food are seldom, if ever, contaminated because the feeding bees disseminate the pheromone in the air rather than on the food or substrate. For example, bees that were feeding at a glass Petri dish and disseminating Nassanoff pheromone were made to stand on, and feed through, large mesh wire-gauze that completely covered the dish. After removing the wire-gauze, through which some of the Nassanoff scent in the atmosphere could be expected to have passed and reached

---

[15] Butler, C.G., Fletcher, D.J.C. and Watler, D. 1969, Anim. Behav. 17, 142-147.

[16] Butler, C.G. and Watler, D. Unpublished.

[17] Butler, C.G. 1970. In preparation.

the dish and its contents, these were found to be no more attractive to bees than a clean dish. However, the wire-gauze on which the feeding bees had stood attracted other bees and in this respect was comparable to a similar piece of wire-gauze on which about the same number of bees had stood for a similar length of time without exposing their Nassanoff glands.[17]

Worker honeybees seem not to use their mouthparts to deposit a pheromone at a feeding-place, because when bees were allowed to lick a dish of sugar syrup clean by thrusting their tongues through wire-gauze on which they were standing, no attractive substances were subsequently demonstrable on the dish. The salivary glands are not, therefore, the source of an attractive pheromone.[18]

Nassanoff pheromone that has just been released by bees flying around, or feeding at, a dish of syrup, apparently attracts other bees from a short distance away, but it is the 'footprint' pheromone deposited on a dish by bees that makes such a dish more attractive than a clean one. This is a point requiring consideration both when planning experiments and interpreting the results.

Both drone and queen honeybees deposit attractive 'footprint' pheromones on surfaces on which they crawl, and their pheromones are indistinguishable from those of worker honeybees when tested on food-seeking bees in the field. Also, those of the queen and drone are, like that of the worker, found on the surface of all parts of the body, but especially on the thorax.[17] However, when tested in a different context, namely in the hive, as an attractant for workers with or without a queen, it became clear that a queen's 'footprint' pheromone differs from, and in this situation was much more attractive than, those of worker or drone.[17] Presumably, the worker, drone, and queen honeybee share a common 'footprint'

---

[18] Simpson, J. Personal communication.

pheromone, but the queen has an additional substance that strongly attracts bees in the hive. This special, additional, 'footprint' pheromone of the queen occurs on all parts of her body and is produced mainly, if not exclusively, in her mandibular glands.[17,19] It is at least as persistent as worker honeybee 'footprint' pheromone, probably very much more so when mixed with such 'fixatives' as the fatty acids in her mandibular gland secretion. However, it is readily removed and destroyed by worker bees who would otherwise remain unaware of the loss of their queen for a considerable time after she had gone, instead of realising her absence within 30 minutes or sooner after her removal from the hive.[6] It operates over a short range of perhaps as much as 20 mm and is soluble in ethanol and some other organic solvents.[17] It plays a very important part in the hive in attracting workers to the queen, the source of the "queen substance" which, when available to the workers of a colony in sufficient quantity, inhibits queen rearing and development of workers' ovaries.[20,21,22,23] We now know that this honeybee "queen substance" is a mixture of at least two biologically active substances, 9-oxodec-trans-2-enoic acid and another that we originally called "inhibitory scent"[24,25] and now think to be the

---

[19] Gary, N.E. 1961, Science 133, 1479-1480.

[20] Butler, C.G. 1954, Trans. R. ent. Soc. Lond. 105, 11-29.

[21] Butler, C.G. 1959, Proc. R. ent. Soc. Lond. (A), 34, 137-138.

[22] Pain, J. 1961, Ann. Abeille 4, 73-158.

[23] Voogd, S. 1955, Experientia 11, 181-182.

[24] Butler, C.G., Callow, R.K. and Johnston, N.C. 1961, Proc. R. Soc. B, 155, 417-432.

[25] Butler, C.G. 1961, J. Insect Physiol. 7, 258-264.

odour of 9-hydroxydec-trans-2-enoic acid,[26] which is probably the "pheromone II" of Pain.[22]

Swarm attraction and stabilization

The odour of 9-oxodecenoic acid seems not to attract worker honeybees in the hive,[22,27] nor does that of 9-hydroxydecenoic acid,[26] although that of a mixture of the two sometimes does outside the hive.[28] Indeed, in 1964 Velthuis and van Es reported that swarming worker honeybees that had lost their queen could be attracted by the odour of her 9-oxodecenoic acid.[29] We were able to confirm this with experiments in which the queens of swarms in large, open fields were removed and the queenless bees flew 100 m upwind to a source of synthetic 9-oxodecenoic acid hidden in a wire-gauze cage attached to a cane and about 1·3 m above the ground.[28] Worker honeybees paid no obvious attention to the odour of this acid when it was offered at the same site but 4 m above the ground, although many drones did so as the odour of this acid is the queen's olfactory sex attractant.[30,31] Although the workers seeking their lost queens were attracted by the odour of 9-oxodecenoic acid and some alighted on the cages containing it, they did not form quiet, persistent clusters. When, however, we added 9-hydroxydecenoic

---

[26] Butler, C.G. and Callow, R.K. 1968, Proc. R. ent. Soc. Lond. (B), 43, 62-65.

[27] Butler, C.G. 1960, Experientia 16, 424-426.

[28] Butler, C.G. and Simpson, J. 1967, Proc. R. ent. Soc. Lond. (B), 42, 149-154.

[29] Velthuis, H.H.W. and Es, J. van. 1964, J. apicult. Res. 3, 11-16.

[30] Gary, N.E. 1962, Science, N.Y. 136, 773-774.

[31] Butler, C.G. and Fairey, E.M. 1964, J. apicult. Res. 3, 65-76.

acid (another pheromone from the queen's mandibular glands that has been shown to be responsible for stabilizing the swarm cluster[32]) to the 9-oxodecenoic acid in the cages 1·3 m above the ground, the combined odours of these acids attracted the bees and caused them to form compact, stable, queenless clusters on the wire-gauze cages containing them.[28]

Sex attraction

Virgin queen and drone honeybees (*A. mellifera*) pay no attention to one another either in the hive, or at or near its entrance when leaving on their nuptial flights, in spite of the fact that at least from the time she becomes nubile onwards throughout her life a virgin (or mated) queen has on the surface of her body, and is therefore continuously releasing into the atmosphere, olfactory sex attractant, 9-oxodec-*trans*-2-enoic acid, produced in her mandibular glands.[30,31] This must impinge on and, presumably, stimulate sense cells on the drones' antennae that are tuned to respond even to a very few molecules of this acid.[33] Not until a queen reaches a minimum height above the ground do the drones pay attention to her. This height depends on the speed of the wind at any given time and so tends to vary considerably.[31] It ranges from about 4 m to 25 m or more.[31,34] The stronger the wind the nearer the ground drones and, presumably, queens fly.[31] The heights at which drones and queens are making their nuptial flights at given times are usually, perhaps always, greater than those at which the worker bees are flying at these times and, indeed, when passing through the workers' flight zones, queens are liable to be attacked by them.[34]

---

[32] Butler, C.G., Callow, R.K. and Chapman, J.R. 1964, Nature, Lond. 201, 733 only.

[33] Kaissling, K.-E. and Renner, M. 1968, Z. vergl. Physiol. 59, 357-361.

[34] Ruttner, F. 1966, Bee World 47, 93-100.

On reaching the altitude appropriate to the wind speed, a drone probably flies about at random until he perceives the odour of 9-oxodecenoic acid, the queen's sex attractant, whereupon he turns and flies directly upwind for about 9 m looking for her. Because of his small visual acuity, he must fly within about 1 m of a queen to see her. If he fails to see her, or to obtain a further scent stimulus, he stops flying directly upwind and casts around for her scent. On finding it he flies directly upwind once more, looking for her. This sequence of events continues until the drone gets near enough to a queen to see her and chase her, or until he loses her scent altogether, or becomes fatigued or short of food, and returns home.[31] The biological significance of this system is apparent when one realises that, in whatever direction a queen flies, a drone will be more likely to find her if he flies upwind whenever he smells her than if he flies about at random. This is because a queen always starts and finishes her flight at the same point. Therefore, whatever she does during her flight, the sum of the distances she moves in various directions is zero relative to the ground. This means that her upwind air distances are longer than her downwind ones, so that she leaves longer scent trails when travelling upwind than when travelling downwind. Therefore, when a drone smells a queen she is more likely to be upwind than downwind of him and he is more likely to get nearer to her by turning upwind than by flying about at random.[35]

In some mountainous parts of Austria and Germany,[34,36] and in similar terrain in some other countries,[37] flying drones from

---

[35]Butler, C.G. 1967, Biol. Rev. 42, 42-87.

[36]Ruttner, F. and Ruttner, H. 1968, Z. Bienenforsch. 9, 259-265.

[37]Lecomte, J. Personal communication.

apiaries as far as 8 km away[34] aggregate in the same restricted areas year after year. Attempts to find similar drone aggregation places in various parts of England, including fenland, moorland, woodland, arable and grass areas, have failed.[38] At the places regularly surveyed in England during the summer of 1967, the number of drones visiting on consecutive days when drones were flying very strongly ranged from about ten to many thousands.[38] The presence of one or more queens at an observation place on a few occasions could, it is thought, have explained the variation in the number of drones observed at different times. Further, the regular occurrence of aggregation places in some areas and their apparent absence in others, may not be as contradictory as it appears if one assumes that in those areas where drones seem to be widely distributed there are many, ecologically suitable aggregation places; so many, in fact, that aggregation is not apparent. By contrast in those areas where aggregations regularly occur, suitable sites may be few so that aggregation becomes obvious.

9-Oxodecenoic acid is very persistent, and only a few molecules are necessary to stimulate a flying drone to turn upwind. For example, in the summer of 1962 I dipped a glass rod into a dilute solution of 9-oxodecenoic acid in ethanol. Since then it has been kept unprotected on an open shelf in my room. In June 1969 it still attracted drones when suspended 5 m above the ground on a sunny afternoon.

In 1966 we found that ethanol extracts of queens of _Apis cerana_ ssp. _indica_ and of _A. florea_ attracted drones of _A. mellifera_, and were able to show that each queen contained about 10 µg 9-oxodec-_trans_-2-enoic acid.[39] Unfortunately, we had no _A. dorsata_ queens.

---

[38] Butler, C.G. 1968, Rep. Rothamsted exp. Stn. for _1967_, 212-213.

[39] Butler, C.G., Calam, D.H. and Callow, R.K. 1966, Nature, Lond. _213_, 423-424.

We suggested that all four species of *Apis* may share the same, or similar, olfactory sex attractant and pointed out that, although inter-specific attraction may perhaps occur in regions where two or more of these species co-exist, inter-specific matings are unlikely because of disparity in size and anatomical differences between the drones. It has since been demonstrated that *Apis cerana* drones can be stimulated by the odour of *A. mellifera* queens.[40]

A drone of *A. mellifera* can be attracted from a distance as great as 60 m downwind by the odour of the sex attractant of a queen.[31] On seeing the queen he darts towards her and, keeping to leeward of her, examines her very closely, often touching her with his antennae and sometimes also with his front-legs.[40] Experiments show that he will rarely mount her unless her sting-chamber is open[40,41] and, further, that the odour of the 9-oxodecenoic acid on her body is also an important stimulus to mounting.[40] It seems, therefore, that, on her mating flight, a virgin queen's 9-oxodecenoic acid serves both as an olfactory sex attractant stimulating a distant drone to turn upwind and fly towards her and, when he sees her and approaches her closely, as an aphrodisiac stimulating him to mount her. The results of these experiments led me to conclude that the odour of 9-oxodecenoic acid was probably the only aphrodisiac produced by a queen.[40] But it seems possible that an olfactory, or perhaps gustatory, substance that is produced in gland pockets of tergites 2, 3 and 4 of the queen, and which are most active when she is ready to mate,[42] also acts as an aphrodisiac that is, perhaps, perceived by a drone when he touches the queen's body with his antennae or front-legs.[40]

---

[40] Butler, C.G. 1967, Proc. R. ent. Soc. Lond. (B), 42, 71-76.
[41] Gary, N.E. 1963, J. apicult. Res. 2, 3-13.
[42] Renner, M. and Baumann, M. 1964, Naturwissenschaften 51, 68-69.

Alerting pheromones

As worker honeybees reach foraging age, a substance with a strong odour accumulates in their mandibular glands and eventually becomes abundant in those of older foragers.[43,44] It has been identified as 2-heptanone,[45] shown to alert guard bees when presented at the hive entrance[45,46,47,48] and to act as a repellent when presented to foraging honeybees at a feeding place,[43,49,50] although its function then is not, as might be supposed, to divert bees away from exhausted food sources, as these proved more attractive than unexhausted ones.[49]

The mandibular gland pheromone of worker honeybees is several times less potent as an alerting substance than the odour of their crushed sting apparatus[48] which contains isoamyl acetate,[51] a powerful alerting substance for honeybees.[46,51] However, although the odour of isoamyl acetate is probably the principal alerting component of the secretion of the sting complex of the worker honeybee, it is not the only one.[46]

The biological function of the mandibular gland pheromone, 2-heptanone, of the older worker honeybee remains a matter for conjecture, as it seems doubtful whether it serves as an alerting pheromone at, or near, the hive in the way that isoamyl acetate from the sting complex does. Perhaps it acts as a warning substance, enabling would-be robber bees to avoid strongly guarded colonies,[49]

---

[43] Simpson, J. 1961, Rep. Rothamsted exp. Stn. for 1960, 172-173.

[44] Boch, R. and Shearer, D.A. 1967, Z. vergl. Physiol. 54, 1-11.

[45] Shearer, D.A. and Boch, R. 1965, Nature, Lond. 206, 530 only.

[46] Free, J.B. and Simpson, J. 1968, Z. vergl. Physiol. 61, 361-365.

[47] Maschwitz, U.W. 1964, Nature, Lond. 204, 324-327.

[48] Maschwitz, U.W. 1964, Z. vergl. Physiol. 47, 596-655.

[49] Simpson, J. 1966, Nature, Lond. 209, 531-532.

[50] Butler, C.G. 1966, Nature, Lond. 212, 530 only.

[51] Boch, R., Shearer, D.A. and Stone, B.C. 1962, Nature, Lond. 195, 1018-1020.

or is used by bees that have entered the nest of another colony and are stealing its carbohydrate stores to deter the colony's defenders from attacking them. Another possibility is that it plays a part in regulating brood feeding, its presence near a larva informing nurse bees that approach it that this larva has just been fed. Against this last hypothesis must be set the fact that 2-heptanone is more abundant in the mandibular glands of field bees than in those of nurse bees, but perhaps this is just fortuitous.

Control of development of sexual maturity and queen rearing

Experimental work leading to isolation and identification of the pheromones inhibiting ovary development in adult worker honeybees and the rearing of superfluous queens by them has been thoroughly reviewed by several workers,[22,35,52] and need only be summarized.

Both oogenesis in worker honeybees (A. mellifera) and queen rearing by them are, except under the special circumstances mentioned later, inhibited by pheromones[21,23,53] produced in the queen's mandibular glands.[54,55] Butler[20] called the mixture of these pheromones together with other substances that are soluble in ethanol and some other organic solvents, 'queen substance'. The pheromones concerned have since been identified as 9-oxodec-trans-2-enoic acid[24] and 9-hydroxydec-trans-2-enoic acid.[26] They, together with the other substances in the secretion of the queen's mandibular glands, are spread by the queen over her body when grooming,[56] so becoming available to those of her workers who are

---

[52] Verheijen-Voogd, C. 1959, Z. vergl. Physiol. 41, 527-582.
[53] Butler, C.G. 1957, Experientia 13, 256-257.
[54] Butler, C.G. and Simpson, J. 1958, Proc. R. ent. Soc. Lond. (A) 33, 120-122.
[55] Butler, C.G. 1959, Proc. R. ent. Soc. Lond. (A) 34, 137-138.
[56] Butler, C.G. 1956, Proc. R. ent. Soc. Lond. (A) 31, 12-16.

attracted to her by an unidentified pheromone also present in her mandibular gland secretion.[17,19] Workers attracted by a queen's odour lick the inhibitory pheromones from her body and share them widely in regurgitated food with other members of their colony.[20,56] In this way all the workers remain inhibited until the supply of inhibitory material either fails (e.g. the queen becomes lost or dies) or diminishes (e.g. before supersedure of a failing queen[57]), or there is serious interference with its collection and distribution (e.g. when overcrowding of the hive leads to swarming[58]).

Some general conclusions

Several important conclusions, which probably apply to pheromones in general, can be drawn from studying those of honeybees.

First, it is essential to present any given pheromone in the right context, and often at about the right concentration, for a normal biological result to be obtained (e.g. although 9-oxodecenoic acid is very important both in inhibiting workers from rearing superfluous queens and in preventing unnecessary development of their ovaries, it does not attract workers in the hive.[22,23,24] However, in the field its odour assists swarming workers to find their queen, provided it is presented in the height zone in which they are flying[28]). Second, it must be realised that a given pheromone may have several distinct functions and produce different results in different situations (e.g. 9-oxodecenoic acid not only inhibits queen rearing and helps attract swarming bees, but also serves as a sex attractant for drones helping them to find a nubile, flying queen,[31] and as an aphrodisiac when a drone finds such a queen.[40] It must, however, be presented in the correct height zone[31]). Third, some pheromones consist of mixtures of at least two substances of which some, but not all, act synergistically (e.g. the olfactory pheromone that assists swarming workers to find

---

[57]Butler, C.G. 1957, Insectes soc. 4, 211-223.

[58]Butler, C.G. 1960, Proc. R. ent. Soc. Lond. (A) 35, 129-132.

their queen consists of a mixture of the odours of 9-oxodecenoic acid and 9-hydroxydecenoic acid acting additatively, not synergistically.[28] However, when inhibiting queen rearing by workers these substances act synergistically[26]). Fourth, a mixture of two, perhaps more, pheromones, each controlling different, distinct phenomena, is sometimes released (e.g. a queen's scent, attracting workers in the hive to her, is released together with her inhibitory 9-oxodecenoic and 9-hydroxydecenoic acids and with her 'footprint' pheromone[17,19]). Fifth, pheromones are not necessarily species-specific (e.g. queens of *Apis mellifera*, *A. cerana* and *A. florea* share a common olfactory sex attractant, 9-oxodecenoic acid[39]).
