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The origin of the odours by which honeybees distinguish their companions

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Groups of 20 to 50 foragers from two different honeybee colonies were trained, in turn, to syrup in two dishes 1 to 3 ft. apart. Both groups were then allowed to visit their dish at the same time, and newcomers were then found to be preferentially attracted to the dish visited by members of their own colony.

This preferential attraction was shown to be a consequence of distinguishable odours emitted by the workers. These odours were not genetically inherited but were derived from metabolic differences between their colonies. These differences were produced by changes in food supply, and probably also through differences in breeding rhythms. They would develop between queenless halves of colonies.

Uniform and distinguishable colony odours are a consequence of widespread food transmission among the foragers of each colony. The role of olfactory recognition in the social life of the bee is discussed.

INTRODUCTION

The existence of different colony odours, distinguishable by honeybees but not by man, used to be assumed by many beekeepers and naturalists (Bethe 1898; v. Buttel-Reepen 1900) and many methods of queen introduction are still based on this belief (Snelgrove 1940). However, conclusive evidence for the existence of such odours has been lacking and honeybee behaviour towards robbers and towards strange queens, formerly expressed in terms of colony odour, appeared capable of other explanations, so the belief in distinguishable colony odour has dwindled. Current opinion on this subject was adequately reflected by Brother Adam (1951) at the 14th International Beekeeping Congress, thus: 'By the term "colony odour" it is assumed that each bee emits a scent which imparts to every member of a colony a uniform and distinctive odour, and that this odour varies from colony to colony. However, no positive evidence has ever been brought forward to warrant this assumption....There may be such a thing as "colony odour", but all the evidence seems to disprove its existence.'

Little attention has been paid to the work of v. Frisch & Rösch (1926), which showed that foraging honeybees preferentially attracted bees from their own colonies, possibly because the authors themselves did not discuss the relevance of this finding to behaviour at and within the hive.

As their experiments were all done on one pair of colonies, which were unrelated although not conspicuously different, no conclusion could be drawn concerning the general validity of their results. They might have been the consequence of a simple genetical difference between the two stocks, and then one might expect that only a small number of different colony odours would exist among honeybees and that no preference would be observed among bees of the same genotype. Alternatively, their findings could have been one example of a general situation, in which the workers of any colony could by their odour attract members of their own colony in preference to all other bees.

In this paper the findings of v. Frisch & Rösch (1926) are confirmed and amplified. Some light is shed on the origin of the differences in colony odour, and the role which olfactory recognition plays in the social life of honeybees is discussed.

Methods

The following basic technique was used.

Two stocks were placed at various distances from each other on a site free from other colonies, and they were left open for a day to enable the foragers to orientate.

Two Petri dishes, *a* and *b*, were placed 1 to 3 ft. apart at a site equidistant from both colonies. In the later experiments a wooden screen (*D*) $1\frac{1}{2}$ yd. long and 1 yd. high, was erected between the dishes in an attempt to reduce the intermingling of the scents around them, but this was not essential (figure 1).

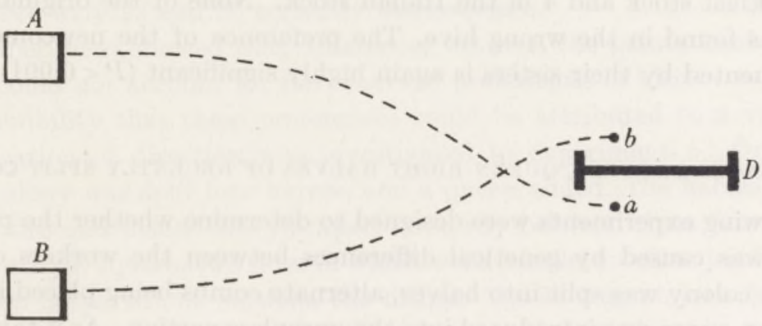

Figure 1. Arrangement of experiments. *A*, *B* = colonies to be tested; *a*, *b* = sites of feeders; *D* = wooden screen.

During an evening the entrance to the hive of one colony (*A*) was blocked. Next morning dish *b* was filled with sugar syrup, and bees from the other colony (*B*) were trained to it. Thirty to fifty of them were marked on the thorax with paint of one colour, and fed for an hour, while all other visitors were killed. That evening colony *B* was blocked, and it remained so during the following day until 30 to 50 workers from *A* had been trained to syrup in dish *a*, and their thoraces painted with a different colour.

Two observers were then employed. Both hives were unblocked, and both dishes supplied with dilute syrup (1 part sugar/5 parts water by weight). Only correctly marked bees were allowed to drink; strays from the wrong dish were transferred, and all unmarked visitors were killed. As soon as at least 6 marked visitors were present at one time on each dish the dilute syrup was replaced by concentrated syrup (2/1) so that dancing and recruiting would be encouraged. Subsequent newcomers to each dish (between 50 and 110) were marked on the abdomen with the same colours as those on the thorax of the trained bees. In the evening both hives were opened, and all marked bees in them were killed and recorded.

Demonstration of preferential attraction to companions

In experiment 1 a yellow Italian and a black Swiss stock were compared. The conspicuous colour difference enabled us to dispense with the marking of newcomers, and simply to kill and record them at each dish. The distance between the hives was 6 yd. and the dishes were 100 yd. from the hives. During 4 h on 3 August 1951, a windy day, 31 yellow and 14 black newcomers were killed at the dish to which marked trained yellow bees were coming, while 13 yellow and 29 black recruits were killed at the dish visited by the marked black bees. This is significantly different from a random distribution of visits ($P = 0{\cdot}001$).

In experiment 2 an Italian and a Buckfast stock were compared. In this and in subsequent experiments the newcomers were marked with different colours and later retrieved from the hives. The two stocks were 300 yd. apart, and the dishes midway between them. At the dish to which Italian bees were trained, 109 recruits were marked; 76 of these were recovered from the Italian stock and 12 from the Buckfast stock. Of the 41 recruits marked at the other dish 32 were found in the Buckfast stock and 4 in the Italian stock. None of the originally trained foragers was found in the wrong hive. The preference of the newcomers for the dishes frequented by their sisters is again highly significant ($P < 0{\cdot}001$).

Differences between queen-right halves of recently split colonies

The following experiments were designed to determine whether the preferential attraction was caused by genetical differences between the workers of the two colonies. A colony was split into halves, alternate combs being placed in opposite halves, and a queen was introduced into the queenless portion. As it takes 21 days for a worker to develop, the adults of both halves covered the same range of genetical diversity for this time, while the presence of a queen in each half ensured normal colony development.

In experiment 3 a colony of Buckfast bees was split on 21 August and the two halves were moved to a new site and placed 300 yd. apart. On 22 August 30 workers from one half were trained to a dish midway between the halves, and marked. This—the queenless half—was then requeened. Next morning 30 bees from the other half were trained to a second dish, and marked. Dilute syrup was then supplied to both dishes and the first half was unblocked. Twenty minutes later both dishes were being regularly visited by correctly marked workers; until this time all newcomers were killed. Concentrated syrup was then supplied to both dishes and all newcomers were appropriately marked. The colonies were opened in the evening with the following result.

In the requeened half 36 correctly marked and 27 incorrectly marked newcomers were found; in the other half there were 26 correctly marked and 9 incorrectly marked newcomers. The deviation from random visits is again highly significant ($P = 0{\cdot}006$).

At this point it seemed advisable to investigate two conceivable sources of error in the technique. The first was that the newcomers might have recognized the different paints used for marking by their colour or smell (although only the pigments of the paints were different). A second possibility was that the communication of direction of a source of food was much more accurate than v. Frisch (1946) supposed.

In experiment 4 the first possibility was tested in the following manner: the same split halves as in the third experiment were used in the same sites. Guides from one half were trained, and their thoraces marked blue, on 26 August; 2 days later guides from the other half were marked pink. Dilute syrup was then supplied at both dishes and all the guides were marked on their abdomens with a pink and blue mixture paint. Concentrated syrup was then supplied, and the abdomens of 66 recruits coming to the dish of the pink guides were marked blue, while 84 newcomers to the other dish were marked pink. In this way the colours were intermingled at both dishes. That evening 55 bees with pink abdomens and 14 with blue ones were found in the hive of the blue guides; the other hive contained 30 bees with blue abdomens and 21 with pink ones. This again shows a significant preference in visits ($P < 0{\cdot}001$). In each hive one wrongly coloured guide was found; there were 23 and 20 correctly marked ones.

This experiment showed that differences between the paints used for marking the bees could not account for the observed preferences in visits.

The possibility that these preferences could be attributed to a very accurate communication of direction was investigated in experiment 5. On 21 August another colony was split into halves, and a queen added. The halves were moved to a new site and placed 300 yd. apart; midway between them four dishes were placed in a 2 yd. square, two sides of which were parallel to a line joining the hives. There were no partitions between the dishes. On 26 August bees from one half were trained to opposite corners of the square, and their thoraces marked either blue or white, while the other two dishes were empty. On the following day bees from the other half were trained to the other two corners, and marked either yellow or pink. Later, when the guides were coming steadily to all four dishes, the syrup concentration was raised and the abdomens of all newcomers were marked in the colours of their guides.

In the hive with pink and yellow guides 60 correct newcomers (45 pink and 15 yellow) and 42 incorrect ones (26 white and 16 blue) were recovered.

In the other hive there were 65 correct (45 white and 20 blue) bees and 32 incorrect (23 pink and 9 yellow) ones.

Chance deviation from random visiting at least as great as the observed one is highly improbable ($P < 0{\cdot}001$); as pairs of dishes were exactly in the same direction from each hive this positive result cannot be explained in terms of accuracy of communication of direction, but only by preferential attraction.

Experiments 3 to 5 excluded the possibility that differences in colony odour, which were inferred from the observed preferences in visiting, might be caused by differences in the genetical constitution of the workers.

Preferential attraction by the workers of queenless colonies

Experiment 6 was designed to determine whether the presence of queens was necessary for the maintenance of the preferential attraction. Two colonies were dequeened on 31 August and placed 300 yd. apart. They were successively trained to a pair of dishes sited midway between them, and they were compared on 9 September. One contained 24 correct and 2 incorrect recruits, the other contained 60 correct and 27 incorrect ones. There were no incorrect guides. This observed preference of visits is highly significant ($P<0\cdot001$), and it can be concluded that a striking difference between the two colonies was maintained for 9 days after both had been dequeened.

Absence of preferential attraction in recently split queenless colonies

The next two experiments were designed to determine whether preferential attraction developed quickly in the split halves of dequeened colonies. Two colonies (A and B) were dequeened on 31 August, and each was split into halves ($A\,1$ and $A\,2$, $B\,1$ and $B\,2$), alternate combs being placed in each half. $A\,1$ and $A\,2$ were housed in a specially constructed double hive, so that the halves were side by side and separated by a vertical partition consisting of two sheets of 3 mm wire gauze, 2 cm apart. The combs were parallel to the partition, and the entrances were close to the partition but on opposite sides of the hive. $B\,1$ and $B\,2$ were similarly housed but separated by only one screen of wire gauze. These hives were designed because it was possible that differences would develop more readily between $A\,1$ and $A\,2$, which could not exchange food, than between $B\,1$ and $B\,2$, which were able to do so.

In experiment 7 guides from $A\,1$ and $A\,2$ were successively trained to two dishes 150 yd. away, and they were compared on 4 September. Twenty-two correctly and 22 incorrectly marked recruits were recovered from $A\,1$, together with 1 incorrectly and 17 correctly marked guides; 17 correct and 8 incorrect newcomers were recovered from $A\,2$, and 18 correct guides. The slight preponderance of correct recruits is not statistically significant ($P=0\cdot2$).

In experiment 8 guides from $B\,1$ and $B\,2$ were successively trained to a pair of dishes. These halves were compared on 7 September. In $B\,1$ there were 22 correct and 40 incorrect newcomers, together with 18 correct and 3 incorrect guides. In $B\,2$ there were 25 correct and 12 incorrect recruits, together with 21 correct guides and 1 incorrect one. Thus, altogether, there were a few more incorrect visitors (52) than correct ones (47).

Production of differences between colonies by feeding

The next three experiments were designed to test the possibility that the preferential attraction was a consequence of differences in colony metabolism.

On 20 September a colony was dequeened and divided frame by frame into three 5-comb nuclei, *D*, *E* and *F*, which were placed in a line at 10 yd. intervals, on a new site. At this time there was very little natural forage. Next day a mixture of $\frac{1}{2}$ lb. heather honey and 1 tablespoonful black treacle was poured over the tops of the frames of nucleus *F*; *D* and *E* were not fed. Two training dishes were placed 25 yd. from the line of nuclei.

In experiment 9 bees from *D* and *F* were compared on 29 September, after the usual preliminary training. In the fed nucleus, 43 correctly marked and 14 incorrectly marked recruits were found and killed; in *D*, the unfed nucleus, there were 11 correct and 6 incorrect newcomers. Both nuclei contained large numbers of correctly marked guides, but there was 1 incorrectly marked guide in *F*, and 2 in *D*; these 3 were killed.

Thus 1 week after feeding a highly significant difference ($P = 0{\cdot}006$) had developed between the fed third and one unfed third.

In experiment 10 the two unfed nuclei (*D* and *E*) were compared on 5 October, after the usual training. In *D*, 22 correctly and 20 incorrectly marked newcomers were found and killed; in *E* there were 16 correct and 13 incorrect ones. No incorrectly marked guides were found. This difference is not significant ($P = 0{\cdot}7$).

Experiment 11 was done on the following day. Fed nucleus *F* was compared with unfed *E*, using the guides from experiments 9 and 10, which had not been killed. In *F* there were 37 correct and 16 incorrect newcomers, and in *E* there were 35 correct and 5 incorrect ones. Only one incorrectly marked guide was found in *F*. This difference in visiting is highly significant ($P < 0{\cdot}001$).

The results of experiments 9, 10 and 11 show that no significant differences had arisen between the two unfed nuclei, whereas the fed nucleus had developed a distinctive difference in relation to the other two.

The olfactory nature of recruit attraction

In the preceding experiments odour was postulated to be the distinguishing factor between workers of different colonies. This hypothesis was tested in experiment 12, which also yielded additional information.

On a dull, calm day (15 October, maximum temperature 61·3° F, wind velocity 1 to 3 m.p.h., 3·7 h sunshine) 50 mass-marked bees from a colony were trained to a syrup-filled Petri dish *X*, on one side of a screen; no bees were trained to a similar syrup-filled dish *Y* on the opposite side. Throughout this experiment all new recruits at both dishes were killed on arrival.

During the first 70 min, 24 recruits arrived at *X* to join the trained bees; 2 went to *Y*. One drop of strong lavender water was then added to the syrup in both dishes. In the next (second) hour 21 recruits arrived at *X* to join the trained bees, and one went to *Y*. This showed that the presence of this strong extraneous scent did not disturb the preference; the bees were not in this instance searching for the scent contained in the syrup.

The dishes, and small wooden boxes on which they were standing, were then exchanged. The trained bees were jumpy at first, but they soon settled down to the new dish Y.

There were about 20 marked bees at dish Y at any one time. During the first 12 min after the exchange no recruits arrived at Y, to which the trained bees were going, but 6 arrived at the previously visited dish X, which was now without trained bees. Thus the new recruits were not attracted by the sight, sound, or smell of trained bees, but by something pertaining to X, which these bees had previously visited for over 2 h. This could only be an odour.

The persistence of the attractive odour

The foregoing experiment was continued for 1 h, and the number of newcomers killed at each dish in successive 5 min intervals is recorded thus:

Y, with trained bees	0	0	1	4	2	2	1	1	1	6	4	4
X, now without bees	2	1	4	0	2	0	2	0	3	0	2	2

During the first 45 min 12 recruits went to Y, but 14 went to X. During the last 15 min 14 recruits went to Y, but only 4 to X.

At the end of this (third) hour Y was removed, together with its stand, and a similar new dish, Z, with a stand, put in its place. Z had received no previous visits. X, on the opposite side, was not touched.

The trained bees had been returning to their side without mistake, but during the first 10 min after the second change of dishes marked bees had to be driven away from the empty dish X on nine occasions; thereafter they made no mistakes. This indicates that the odour was used by the trained bees for their own guidance, as well as by the newcomers.

During the next (fourth) hour both dishes attracted recruits in about equal numbers. The distribution of the new recruits at 5 min intervals is recorded thus:

Z, with trained bees	1	1	0	1	0	2	0	3	2	1	3	2
X, now without bees	1	0	1	0	3	0	1	3	0	2	2	1

It thus appears that the attracting odour persisted at the Petri dish X for at least 2 h.

Discussion

(a) *Origin of colony odours*

Experiments 1 to 6 and 9 to 11 demonstrated that honeybees going to a feeding place were attracted by bees from their own colony in preference to those of another colony; experiment 12 showed that this was due to odour. The presence of queens in the two colonies was not essential for this result (experiments 6, 9 and 11) and it could be demonstrated between the split halves of a colony (experiments 3 to 5, 9 and 11). The preferential attraction cannot therefore be explained genetically. Experiments 9 to 11 indicate that the differences in odour developed from differences in metabolism of the colonies. Experiment 3 showed that a significant difference might develop within 3 days.

There is a possibility that a characteristic colony odour could be spread within the hive by surface contact between the workers. Steinhoff (1948) has shown that scents readily adhere to the waxy surface layers of the bee cuticle. However, experiment 12 indicates that recruits were not attracted to the guides themselves, but only to the odour which had been emitted, and therefore shows that surface contact was not responsible for the uniformity which produced the present results.

Nixon & Ribbands (1952) have shown by the use of radioactive ^{32}P that there is widespread and rapid food transmission between honeybees of the same colony, and especially between foragers. This implies that the food of the foragers of any colony is fairly uniform, and likely to produce volatile waste products in similar proportion. Differences in the food supply of different colonies would lead to the production of dissimilar odours by the foragers of those colonies.

In our experiments odour differences were in fact produced by feeding additional honey and treacle to one of three equivalent nuclei, but it is likely that various natural changes in diet would produce similar results. Differences in colony metabolism could also arise from differences between queens, which might produce changes in food consumption through variations in breeding rates. This factor may have contributed to the result of experiments 3 to 5.

(*b*) *Mechanics of olfactory recognition*

Differences in olfactory attraction cannot be explained by differences in intensity of a single odour but only by *relative* differences between mixtures of odours, the components of which would often be identical but in varying proportions. The ability of honeybees to discriminate between different mixtures of the same odours (v. Frisch 1919) is in harmony with such an interpretation. If this is accepted one would expect that odours from bees of one colony would attract bees from other colonies but to a lesser degree. Since experiment 12 shows that visual attraction is comparatively slight, the considerable proportion of bees which were attracted to the wrong dish in most of the other experiments demonstrates the potency of the odour of strange bees. Von Frisch & Rösch (1926) thought that the odour of foragers from one colony had no attraction for bees from other colonies. They attempted to eliminate the odour of the guides from one colony by covering their Nasanov gland with shellac; they then found that 11 recruits from this colony were attracted to these guides, while 14 went to a nearby dish visited by bees from the second colony. They concluded that these newcomers were then being attracted to both dishes entirely by vision. In the light of our experiment 12 it seems more likely that covering the Nasanov gland did not completely suppress the odour, and that attraction to both dishes was olfactory.

Sladen (1902) described Nasanov's gland as an odour-producing organ used for communication, and v. Frisch & Rösch (1926) presumed that this gland was the source of the odours relevant to their experiments. In most of our experiments some of the bees were observed exposing this gland, and we think that the scent from this gland was the main attractive factor, but not necessarily the only one.

(c) *Role of colony odour in community life*

Experiments with syrup dishes do not necessarily give an accurate picture of bee behaviour on natural crops. This artificial source of food is more abundant, more constant and more strictly localized than any natural crop, and consequently excites foragers to a higher degree, which results in fanning and scenting. The existence of scenting on natural crops has been doubted (Jacobs 1925), and has only been described by v. Frisch & Rösch (1926). However, it seems reasonable to assume that scenting plays some role in the attraction of newcomers to natural crops, and has not been developed merely to facilitate robbing; it is doubtful how far scenting would help robbers to bring recruits, because in the earlier stages of robbing exposure of scent glands by the robbers would aid the defenders. In addition, experiment 12 shows that the scent is so persistent that it could be used by foragers for their own guidance on return to a crop. The scent marking of localities for their own orientation and that of their fellows reminds one of similar habits among dogs and other social canines.

Fanning by bees with exposure of the Nasanov gland is frequently observed at the hive entrance, especially in conditions of reorientation (Ribbands & Speirs 1952); it seems certain that in these circumstances the scent aids other members of the colony in finding their way home.

The conspicuously jumpy behaviour of robber bees might by itself be sufficient to enable entrance guards to recognize and repel them (Cale 1949), but the distinguishable colony odours certainly help the inmates of a hive to recognize intruders. Observations not yet completed have shown that during the robbing season foragers mistakenly returning to a wrong colony and approaching normally are attacked like robbers, sometimes even when hovering in mid-air, thus illustrating the importance of smell (Ribbands, unpublished). Outside the robbing season foragers blundering into the wrong hive with full honeysacs are seldom attacked (v. Buttel-Reepen 1900).

A situation intermediate between foraging and robbing was described by Kalmus (1941). Bees of two recognizably different strains were trained to a Petri dish containing concentrated sugar syrup overlaying sand. As long as the syrup was plentiful conditions were amicable; when the syrup was becoming exhausted, fighting commenced, but only between bees of different strains. It now seems reasonable to assume that recognition of the enemy was by smell.

Colony odour may be of practical importance for beekeeping operations, e.g. in queen introduction and when uniting colonies, though other factors often predominate and obscure this.

Mutual recognition and the recognition of strangers in certain circumstances is a necessary feature of any complex social organization. We have now demonstrated the origin of distinguishable colony odours, and discussed their role in this respect within honeybee communities. They may have a similar origin and similar functions among other social insects. McCook (1877) described experiments which indicated that the recognition of their fellows by ants was based on smell. The experiments of Miss Fielde (1904, 1905) then led her to the conclusion that mutual recognition by ants was based on the odour inherited from their mother, together with some progressive change in that odour according to their age; our hypothesis could provide a more simple explanation for some of her observations. Emerson (1929) considered that odours were also the basis of mutual recognition in termite communities.

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