Apidologie (1990) 21, 493-500

Original article

Queen discrimination by honeybee (*Apis mellifera* L) workers

JB Free*, AW Ferguson **, JR Simpkins

AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ, UK

(Received 20 March 1990; accepted 30 July 1990)

Summary — A simple new technique for testing worker honeybees' ability to distinguish between honeybee queens is presented. Two queens were caged separately with groups of young sibling workers which were the daughters of another queen. The cages were exposed to the same environmental odours for 10 days. When placed in the test apparatus and given a choice of both queens, workers segregated towards the queen with which they had been caged. This provides further evidence that the distinctive odour of an individual queen is probably partly inherited and is learned by workers. The nature and functioning of queen odours are discussed.

***Apis mellifera* / queen recognition / odour / genetic component**

INTRODUCTION

The distinctive odours common to the members of a honeybee colony are acquired in part by adsorption of the odours present in the hive atmosphere onto the waxes of the body surface (Renner, 1955, 1960). The ability of worker bees to recognise and prefer their own queen to another (Henrikh 1955; Velthuis and Van Es, 1964; Morse, 1972) could be because she has acquired the same odour as the workers themselves (Boch and Morse, 1974).

However, even after queens have been exposed for several days to the same hive atmosphere, worker bees are able to differentiate between them and recognize their own queen (Boch and Morse, 1979). This indicates that adsorption of different odours onto the body surfaces of queens is not entirely sufficient to explain their individual odour characteristics.

Recent behavioural evidence suggests that the distinctive odours of queens are in part genetically determined. In the laboratory small groups of free-moving workers are able to discriminate between queens on the basis of inherited odours (Breed, 1981) or of closeness of kinship of queens to workers (Page and Erickson, 1986). These studies relied upon agonistic responses as indicators of discrimination, and queens were sometimes stung. In the field

* Present address : University of Wales, School of Pure and Applied Biology, Bee Research Unit, Cardiff, CFI 3TL, UK
** Correspondence and reprints

Boch and Morse (1982) demonstrated that recently dequeened swarms clustered around queens that were sisters to their own queen in preference to unrelated queens.

Evesham (1985) used a simple 2-chamber choice test to investigate queen preferences shown by workers of the polygynous ant *Myrmica rubra L.* Here we have adapted this laboratory technique for the study of queen discrimination by worker honeybees in a manner which does not rely upon aggressive responses. We have tested worker differentiation of queens kept in an identical artificial environment.

MATERIALS AND METHODS

Ten days before a test, 2 1-y-old mated laying queens (A and B) were placed in separate cages with groups of 50 1-d-old workers (also A and B). The queens used all originated from vigorous colonies and were of the same Italian strain, but their exact degree of relatedness was not known. The workers were all daughters of 1 other queen and had emerged from the same comb in an incubator maintained at 32 °C. Coloured paint marks (cellulose dope) had been applied to the thoraces of both queens and workers so that bees from each group could be distinguished during the experiment. This paint dries quickly (solvent acetone) but any persisting odour would be common to all individuals. The cages were rectangular (90 x 60 x 50 mm) and constructed of transparent 3-mm thick Perspex (Plexiglass) sheet with 60 ventilation holes (3 mm diameter) in the walls. The bees were fed with odourless sucrose syrup and water supplied from glass tubes inserted through holes in the cage walls. Both cages of bees were maintained in the same incubator at 32°C until the day of the test.

The apparatus used in the test consisted of 4 clean Perspex cages of similar design to those described above. These were interconnected by glass tubes (15 mm internal diameter and 150 mm long) inserted through holes in the cage walls to form a rectangular array of chambers (Fig 1). Access to the glass tubes from 2 cages at opposite corners of the rectangle was restricted with queen excluders through which only worker bees could pass. At the start of the test queens A and B were each placed into 1 of these 2 cages and worker groups A and B were introduced separately into the other cages (fig 1). Each cage contained a supply of odourless sugar syrup and water.

The test was conducted in a dark room at 30 °C. Observations were made using red light which was invisible to honeybees. After 2 h and 24 h the numbers of group A and B workers in each cage containing a queen were counted. The test was replicated 4 times. A different pair of mated laying queens, new workers and clean apparatus were used each time.

RESULTS AND DISCUSSION

At each observation less than half of the workers were found to be in cages with queens. However, in every test, both after 2 h and 24 h, more workers were found in the cage containing their own adopted queen (with which they had been caged for 10 days) than in that containing the foreign queen (table I).

If a honeybee queen is placed in a foreign colony, she is usually treated aggres-

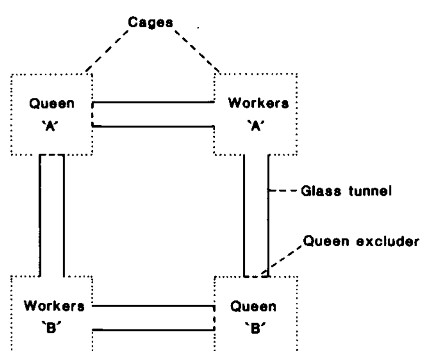

Fig 1. Apparatus used to test workers' ability to discriminate between their own and another queen.

Table I. Distribution of worker bees between cages containing their own and foreign queens.

Exp	*After 2 h* *No worker bees with:*		*After 24 h* *No worker bees with:*	
	Own queen	*Foreign queen*	*Own queen*	*Foreign queen*
1	23	9*	18	12
2	16	22	19	8
3	28	2*	30	18
4	19	8*	23	14
All	86	41*	90	52*

* Significant difference between number of workers with their own queen and the number with the queen foreign to them, $P < 0.05$ (χ^2 test, df = 1; null hypothesis: equal number of workers with own and foreign queens).

sively by workers (Yadava and Smith, 1971; Robinson, 1984). In order to introduce a new queen to a colony, beekeepers often enclose her in a wire mesh cage through which workers can contact but not sting her (Butler and Simpson, 1956). The queen is released after ≈ 24 h, when she is no longer attacked. Probably during the short period of caging the queen loses much of the absorbed odour by which she was recognised as foreign. Worker conditioning to a new queen odour (Breed, 1981) and acquisition by the queen of odours associated with the new colony (Robinson, 1984) are probably also involved in this rapid change of worker response. Although an element of doubt must remain, it seems unlikely that much of the acquired odour associated with the queens used in our experiments remained after 10 d in an incubator. By then, significant differences in the odours of the queens were likely to be genetically determined.

The ability of workers to distinguish queens to which they were related to different degrees was demonstrated by Boch and Morse (1982) and Page and Erickson (1986). Our results lend further support to a partly genetic origin of distinctive queen odours. However, in this study all workers used were siblings and queens were not closely related to the workers; therefore the attraction to queens was not dependent upon the extent of a shared genotype. Breed (1981) and Moritz and Crewe (1988) also demonstrated this discriminatory ability of workers with respect to unrelated queens; they suggested that the queen recognition system was based on learning. In most circumstances workers probably assess the acceptability of a queen according to odours to which they are conditioned, some of which happen to be genetically determined.

In the polygynous ant, *Myrmica rubra L*, loyalty to a queen does not seem to be related to genotype but rather appears to be a learnt response. Very young workers are unable to distinguish their own mother queen either from other queens in the same colony or from foreign queens from another colony (Evesham, 1985). However, the worker ants tend to become conditioned to and form a bond with the first queen they encounter and prefer her to other queens (Evesham, 1984).

The genetic components of honeybee colony odour are probably recognised in a similar manner to queen odour. Worker recognition has been shown to involve inherited odour characters by Breed (1983) and Getz and Smith (1983). Breed *et al* (1985) showed that workers can discriminate closeness of kinship with other workers and probably use their own genotype as a reference point. This leads to the potential for kin selection within a colony and there is evidence that this occurs (Getz and Smith, 1983; Breed *et al,* 1985; Page *et al,* 1989). However, colony odour has a function distinct from this. It is used to distinguish nest-mates from intruders and is probably composed of both acquired environmental cues and bee-derived cues. Disseminated to the whole colony in the wax of the comb and in cuticular hydrocarbons (Breed *et al,* 1988), it seems likely that even the bee-derived, genetically determined components of colony odour are learned by workers in the same way as queen odour (which indeed must form part of it). This would be consistent with a "Gestalt" or group model of colony odour similar to that proposed by Crozier and Dix (1979) but not solely dependent upon inherited odour characters. By contrast, some genotype dependent kin recognition cues appear to remain distinctly associated with individuals (Breed *et al,* 1985). These may be chemicals of lower volatility (Getz *et al,* 1989).

Queens may usually be recognised by only 2 criteria, firstly as queen caste due to pheromones (Velthuis, 1985), and secondly as nest-mates due to a learned odour comprising elements which have been both acquired and inherited by the queen. Our results are in agreement with the suggestion of Boch and Morse (1974), *ie* the odour by which queens from different sources can be distinguished by workers is identical with colony odour. On the relatively infrequent occasions when 2 or more sister queens are present in a colony (*eg* after swarming), the ability of workers to discern their degree of kinship with queens provides a third cue which could be used for kin selection. However, as noted by Page and Erickson (1986), at present there is a lack of evidence for kin selection of adult queens in a natural context.

In the present study the preference of workers for their own adopted queen was not as marked after 24 h as after 2 h. Probably contact with the other worker group and queen had caused some mixing of queen odours and some change in the colony odour conditioning of workers. When a honeybee colony swarms the established queen leaves the nest and is replaced by her daughter. Other components of colony odour (*eg* forage) are also liable to change, and it is therefore necessary for worker conditioning to show a degree of plasticity.

Moritz and Crewe (1988) developed a bioassay in which the metabolic rate of groups of workers was measured and shown to vary in response to genetically determined differences in volatiles from queens. Getz *et al* (1986, 1989) used conditioned reflexes to show that workers can distinguish genetically determined differences in the chemical characteristics of other workers. Conditioned reflexes could also be used to test worker responses to queens. However, the implications of studies using such techniques are difficult to assess in terms of social behaviour. Other bioassays have used agonistic behaviour as a criterion for queen discrimination (Breed, 1981; Page and Erickson, 1986). We suggest that further development of the laboratory technique presented here may be of value in studying the role of genotype and chemical phenotype in social interactions and kin recognition.

The understanding of queen and kin recognition in honeybees is of significance not only for the study of social evolution

(Hamilton, 1964a,b; Breed, 1981) but also for practical beekeeping methods. For instance, further information on the length of time necessary for honeybee workers to become conditioned to the odour of a foreign queen or for the conditioning to be lost after queen removal could be helpful in devising more successful methods of introducing new queens to colonies. The present experimental technique could form the basis of such investigations.

ACKNOWLEDGMENTS

We would like to thank P Tomkins for providing the bees used in this study and anonymous reviewers for helpful comments on the text.

Résumé — Reconnaissance de la reine par les abeilles ouvrières (*Apis mellifera* L). Des travaux récents (Breed, 1981; Boch et Morse, 1982; Page and Erickson, 1986; Moritz et Crewe, 1988) prouvent que les odeurs distinctives des reines d'abeilles sont partiellement d'origine génétique. Nous avons utilisé une nouvelle technique pour étudier si les ouvrières sont capables de différencier des reines maintenues dans un même environnement artificiel. Cette technique utilise des ouvrières qui se déplacent librement, mais ne repose pas sur la réaction agonistique comme critère de reconnaissance.

Deux reines pondeuses accouplées et âgées d'un an ont été encagées séparément avec des groupes de 50 ouvrières marquées à la peinture, qui étaient les filles nouvellement écloses d'une autre reine. Les cages ont été maintenues durant 10 j dans une étuve où elles étaient exposées aux mêmes odeurs ambiantes. On estime qu'au bout de ce temps, seules les différences d'odeur d'origine génétique persistent entre les 2 reines.

Le dispositif de test consiste en 4 cages propres reliées par des tubes de verre pour former un carré (fig 1). Les reines A et B ont été placées dans 2 cages opposées; les ouvrières qui avaient été encagées avec les reines (ouvrières A et B) ont été introduites séparément dans les 2 autres. Les ouvrières pouvaient circuler librement dans l'ensemble du dispositif, les reines étaient maintenues dans leur cage par des grilles à reine (fig 1). Le test a été fait dans l'obscurité à 30°C. Les ouvrières de chaque groupe présentes dans chacune des cages à reine ont été dénombrées au bout de 2 h et de 24 h. Le test a été répété 4 fois.

Pour chaque observation, on a trouvé plus d'ouvrières dans la cage qui contenait leur propre reine (celle avec laquelle elles avaient été encagées durant 10 j) que dans celle qui contenait l'autre reine (tableau I). Ce résultat confirme l'hypothèse selon laquelle les odeurs des reines possèdent une composante héréditaire. Elles sont susceptibles d'être apprises par les ouvrières. D'une façon générale les reines sont vraisemblablement reconnues par 2 critères : 1), appartenance à la caste des reines (par les phéromones), 2), appartenance au même nid (par l'odeur commune de la colonie). L'odeur de la colonie semble être composée à la fois de signaux acquis du milieu et de signaux provenant des abeilles (Breed *et al*, 1988), qui incluent probablement ceux provenant de la reine; elle est susceptible d'être apprise par les ouvrières. Les ouvrières sont également capables de distinguer leur degré de parenté avec les reines (Breed, 1981; Page and Erickson, 1986), ce qui peut constituer un signal pour la sélection de la parenté dans les cas où il y a plusieurs reines dans la colonie (par exemple après essaimage). Cela reste à démontrer en conditions naturelles. De jeunes ouvrières de la fourmi polygyne *Myrmica rubra* L,

sont susceptibles de devenir conditionnées à la première reine qu'elles rencontrent et de la préférer aux autres; elles sont incapables de les reconnaître d'après leur degré de parenté (Evesham, 1984). Dans nos expériences, la préférence des ouvrières d'abeilles pour leur propre reine était moins forte après 24 h qu'après 2 h. Le conditionnement des ouvrières aux reines doit présenter une certaine plasticité chez l'abeille.

Le dispositif expérimental présenté ici doit pouvoir servir à d'autres recherches sur la reconnaissance de la parenté *(kin recognition)* chez l'abeille, importantes tant pour l'étude de l'évolution sociale que pour la pratique apicole (introduction de reines dans les colonies).

***Apis mellifera* / reconnaissance de la reine / odeur / déterminisme génétique**

Zusammenfassung — Die Unterscheidung von Königinnen der Honigbiene durch Arbeiterinnen. Kürzlich vorgelegte Beobachtungen deuten darauf hin, daß der charakteristische Eigengeruch der Bienenkönigin zumindest teilweise erblich bedingt ist (Breed, 1981; Boch und Morse, 1982; Page und Erickson, 1986; Moritz und Crewe, 1988). Wir benutzten eine neue Technik, um zu untersuchen, ob die Arbeitsbienen zwischen Königinnen in einer einheitlichen künstlichen Umgebung unterscheiden können. Bei dieser Technik werden Arbeitsbienen mit freiem Bewegungsspielraum eingesetzt; die Technik beruht aber nicht auf deren feindseligem Verhalten als Anzeiger für die Unterscheidung zwischen den Königinnen.

Zwei einjährige, begattete und legende Königinnen ("A" und "B") wurden getrennt mit je 50 farbmarkierten Arbeitsbienen (frischgeschlüpfte Töchter einer anderen Königin und demnach Geschwister) gekäfigt. Die Käfige wurden 10 Tagelang in einem Brutschrank gehalten, wo sie denselben Umgebungsgerüchen ausgesetzt waren. Es wird festgestellt, daß nach diesen 10 Tagen nur genetisch bestimmte Geruchsunterschiede zwischen diesen beiden Königinnen bestehen blieben. Der Testapparat besteht aus vier sauberen Käfigen, die miteinander durch Glasröhren so verbunden waren, dass sie ein Viereck bilden (Abb 1). Der Zu- und Abgang von zwei Käfigen an den gegenüberliegenden Ecken des Vierecks war mittels Absperrgitter verschlossen, durch das nur Arbeitsbienen passieren konnten. Die Königinnen "A" und "B" wurden in diese beiden Käfige getrennt. Die mit ihnen gekäfigten Arbeitsbienen (Arbeiterinnen "A" und "B") wurden in die beiden verbleibenden Käfige getrennt (Abb 1). Der Versuch wurde im Dunkel bei 30 °C durchgeführt. Zwei und 24 h später wurde die Zahl der Arbeiterinnen jeder Gruppe in jedem der beiden Königinnen-Käfigen gezählt. Der Versuch wurde viermal wiederholt.

Bei jeder Beobachtung wurden mehr Arbeiterinnen der eigenen Gruppe bei "ihrer" Königin (mit der sie 10 Tage lang gekäfigt gewesen waren) gefunden als in dem Käfig der anderen Königin (Tabelle I). Dieser Befund stützt die Annahme, daß individuelle Königinnengerüche eine erbliche Komponente besitzen. Diese Gerüche werden wahrscheinlich von den Arbeiterinnen gelernt. Königinnen werden vermutlich an zwei Merkmalen erkannt, zuerst als Angehörige der Königinnenkaste (durch die bestimmten Pheromone) und zweitens als Nestgefährten durch den gemeinsamen Volksgeruch.

Der Volksgeruch besteht vermutlich aus erworbenen Umweltkomponenten und aus von den Bienen stammenden Anteilen (Breed *et al*, 1988), der wahrscheinlich auch Gerüche der Königin einschließt; der Geruch wird vermutlich von den Arbeitsbie-

nen erlernt. Arbeitsbienen erkennen auch ihren Verwandtschaftsgrad zur Königin (Kinship, Breed, 1981; Page und Erickson, 1986), was ein Signal für die Verwandtschafts-Selektion im Falle der Anwesenheit von mehreren Königinnen im Volk (zB im Nachschwarm) bilden könnte. Das muß jedoch noch unter natürlichen Umgebungen nachgeprüft werden. Junge Arbeiterinnen der Ameise *Myrmica rubra* L werden gewöhnlich zu der ersten Königin, der sie begegnen, konditioniert und sie bevorzugen sie gegenüber anderen Königinnen; sie sind aber unfähig, sie nach dem Grade ihrer Verwandtschaft zu erkennen (Evesham, 1984). Die Bevorzugung der eigenen "angewöhnten" Königin durch die Arbeitsbienen war nach 24 h weniger stark als nach 2 h. Die Konditionierung der Arbeitsbienen an Königinnen zeigt also bei der Honigbiene eine gewisse Plastizität.

Die hier vorgestellte experimentelle Anordnung könnte die Grundlage für weitere Untersuchungen über Verwandtenerkennung *(kin recognition)* bei der Honigbiene bilden, die sowohl für das Studium der sozialen Evolution wie für Methoden der praktischen Bienenzucht, wie Zusetzen von Königinnen, von Bedeutung sind.

***Apis mellifera* / Königinerkennung / Geruch / erbliche Komponente**

REFERENCES

Boch R, Morse RA (1974) Discrimination of familiar and foreign queens by honey bee swarms. *Ann Entomol Soc Am* 67, 709-711

Boch R, Morse RA (1979) Individual recognition of queens by honeybee swarms. *Ann Entomol Soc Am* 72, 51-53

Boch R, Morse RA (1982) Genetic factor in queen recognition odours of honey bees. *Ann Entomol Soc Am* 75, 654-656

Breed MD (1981) Individual recognition and learning of queen odours by worker honeybees. *Proc Natl Acad Sci USA* 18, 2635-2637

Breed MD (1983) Nestmate recognition in honeybees. *Anim Behav* 31, 86-91

Breed MD, Butler L, Stiller TM (1985) Kin recognition by worker honey bees in genetically mixed groups. *Proc Natl Acad Sci USA* 82, 3058-3061

Breed MD, Williams KR, Fewell JH (1988) Comb wax mediates the acquisition of nest-mate recognition cues in honey bees. *Proc Natl, Acad Sci USA* 85, 8766-8769

Butler CG, Simpson J (1956) The introduction of virgin and mated queens, directly and in a simple cage. *Bee World* 37, 105-114, 124

Crozier RH, Dix MW (1979) Analysis of two genetic models for innate components of colony odor in social Hymenoptera. *Behav Ecol Sociobiol* 4, 217-224

Evesham EJM (1984) The attractiveness of workers towards individual queens of the polygynous ant *Myrmica rubra* L. *Biol Behav* 9, 144-156

Evesham EJM (1985) Recognition and aggregation abilities of young workers in the polygynous ant, *Myrmica rubra* L, for their own queens (Hymenoptera: Formicidae). *Entomol Gen* 10, 77-85

Getz WM, Smith KB (1983) Genetic kin recognition: honey bees discriminate between full and half sisters. *Nature* 302, 147-148

Getz WM, Brückner D, Smith KB (1986) Conditioning honeybees to discriminate between heritable odours from full and half sisters. *J Comp Physiol* A 159, 251-256

Getz WM, Brüchner D, Smith KB (1989) Ontogeny of cuticular chemosensory cues in worker honey bees *Apis mellifera. Apidologie* 20, 105-113

Hamilton WD (1964a) The genetical evolution of social behaviour. I. *J Theor Biol* 7, 1-16

Hamilton WD (1964b) The genetical evolution of social behaviour. II. *J Theor Biol* 7, 17-52

Henrikh VG (1955) Can bees recognise their own queen? *Pchelovodstvo* 3, 23-29 (in Russian)

Moritz RFA, Crewe RM (1988) Chemical signals of queens in kin recognition of honeybees, *Apis mellifera* L. *J Comp Physiol* A 164, 83-89

Morse RA (1972) Honey bee alarm pheromone: another function. *Ann Entomol Soc Am* 65, 1430

Page RE Jr, Erickson EH Jr (1986) Kin recognition and virgin queen acceptance by worker bees (*Apis mellifera* L). *Anim Behav* 34, 1061-1069

Page RE Jr, Robinson GE, Fondrk MK (1989) Genetic specialists, kin recognition and nepotism in honey-bee colonies. *Nature* 338, 576-579

Renner M (1955) Neue Untersuchungen über die physiologische Wirkung des Duftorganes der Honigbiene. *Naturwissenschaften* 42, 589

Renner M (1960) Das Duftorgan der Honigbiene und die physiologische Bedeutung ihres Lockstoffes. *Z Vergl Physiol* 43, 411-468

Robinson GE (1984) Worker and queen honey bee behavior during foreign queen introduction. *Insectes Soc* 31, 254-263

Velthuis HHW (1985) The honeybee queen and the social organisation of her colony. *In: Experimental Behavioural Ecology and Sociobiology* (Hölldobler B, Lindauer M, eds) G Fischer Verlag, Stuttgart, 343-357

Velthuis HHW, Van Es J (1964) Some functional aspects of the mandibular glands of the queen honeybee. *J Apic Res* 3, 11-16

Yadava RPS, Smith MV (1971) Aggressive behaviour of *Apis mellifera* L workers towards introduced queens. I. Behavioural mechanisms involved in the release of worker aggression. *Behaviour* 29, 212-236