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Subfamily recognition and task specialisation in honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae)

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Abstract. A honey bee (*Apis mellifera*) queen mates with about ten haploid drones, thus producing colonies composed of about ten subfamilies of super-sisters. An increasing but controversial body of literature supports the views that: (1) Members of each subfamily within a colony can recognise each other, and distinguish super-sisters from half-sisters. (2) Members of each subfamily use this recognition information and increase the reproductive fitness of their own subfamily at the expense of half-sisters through behaviour termed nepotism. A mathematical model is developed that shows that task specialisation by subfamilies, and bees that repeatedly undertake the behaviour within subfamilies, can influence the numbers of interactions among super-sisters, relative to the numbers of interactions between half-sisters. The model is then evaluated using a data set pertaining to trophallaxis behaviour in a two-subfamily colony. It is concluded that with this data set, task specialisation and subfamily recognition were indeed confounded, suggesting that the apparent subfamily recognition could easily have been an artefact of task specialisation.

Key words: Honey bee – Kin recognition – Nepotism – Trophallaxis – Supersisters – *Apis mellifera*

Introduction

Honey bee (*Apis mellifera*) queens mate with about 10–17 drones to produce 10–17 subfamilies (Adams et al. 1977; Koeniger 1987). Members of each subfamily are super-sisters with an average relatedness G , of 0.75, while members of different subfamilies are half-sisters ($G = 0.25$) (Laidlaw and Page 1984). Workers are sub-fertile, and under normal circumstances only reproduce via their queen mother. Thus, the possibility exists for reproduc-

tive conflict among subfamilies over which virgin queen should inherit the nest (Ratnieks 1989). A subfamily can increase its reproductive success through queen production by a factor of 3 when one of their number becomes the hive's new mother.

Getz (1981) was the first to point out that if members of each subfamily within a colony could recognise each other, they could increase their reproductive success by ensuring that one of their own super-sisters became the new queen. This nepotism hypothesis has led to widespread inquiry to try to discover whether (as is necessary for the hypothesis) honey-bee workers can discriminate between super- and half-sisters (reviewed by Moritz and Southwick 1992). Such discrimination has been termed "kin recognition" by most authors, although "subfamily recognition" is more appropriate, as all colony members are kin to greater or lesser degrees.

In addition to reproductive conflicts, haplodiploidy and polyandry can lead to "task specialisation" (Robinson and Page 1988) among subfamilies. On average, super-sisters share 3 times as many genes in common by descent as half-sisters. As a consequence, workers of particular subfamilies tend to have a genetic predisposition to specialise in various tasks for behaviour that is heritable. Such specialisation has now been demonstrated or suggested for the following hive duties: grooming (Kolmes 1989, Frumhoff and Baker 1988, Kolmes et al. 1989), guarding, undertaking, (Robinson and Page 1988), fanning, comb building (Kolmes et al. 1989), queen rearing (Noonan 1986; Page et al. 1989) and scouting for a new nest site (Robinson and Page 1989). Propensity for members of particular subfamilies to forage for nectar or pollen (Calderone and Page 1988; Calderone et al. 1989; Robinson and Page 1989; Oldroyd et al. 1991a), for different plant species (Oldroyd et al. 1992), for different sucrose concentrations (Oldroyd et al. 1991b), or for different distances from their colony (Oldroyd et al. 1993) has also been demonstrated.

Oldroyd et al. (1991a, 1991b, 1992, 1993) suggested that under some experimental circumstances, task specialisation and subfamily recognition could be confound-

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ed. Genetic specialisation might increase the numbers of interactions among bees of like subfamilies, while reducing the numbers among unlike subfamilies. For example, a subfamily that specialises in pollen collection would tend to perform communication dances for pollen sources at higher frequency than bees of other subfamilies. Bees of this same subfamily might also be attracted to pollen dances (Oldroyd et al. 1991a). Thus a human observer might confuse task specialisation with subfamily recognition. Here we explore this hypothesis in depth by developing a mathematical model of behavioural interactions among bees in a two-subfamily colony, in which subfamily proportions are unequal. The model formalises the intuitive possibility of confounding, demonstrating not only how the appearance of subfamily recognition can arise through task specialisation, but also the circumstances under which the artifact subfamily recognition can be statistically significant. Observations from a two-subfamily colony in which every bee was individually identifiable, and subfamily proportions were known, are then used in conjunction with the model to show that task specialisation and subfamily recognition can be completely confounded, and that either or both phenomena could have caused the results obtained.

Materials and methods

The model. Consider the way in which some subfamily "recognition" data are collected and analysed (Evers and Seeley 1986; Frumhoff and Schneider 1987; Hoogendoorn and Velthuis 1988; Noonan 1986; Noonan and Kolmes 1989; Oldroyd et al. 1991a, b, 1993). Details differ widely, but in essence an observer scans an observation hive containing a colony comprised of two identifiable subfamilies, seeking two bees involved in the behaviour of interest. The observer records occurrences of the behaviour, noting the subfamilies of the participants. The observer assumes the presence of subfamily recognition where the probability of a bee interaction between like subfamilies does not equal their colony proportion. The null hypothesis, that neither subfamily demonstrates subfamily recognition, may be written as $H_0: P_{11} \times P_{22} = P_{12} \times P_{21}$ versus $H_1: P_{11} \times P_{22} \neq P_{12} \times P_{21}$, where P_{11} is the probability of interaction between subfamily-1 bees, P_{12} is the probability of interaction between subfamily-1 initiating bees and subfamily-2 recipient bees, P_{22} is the probability of an interaction between subfamily-2 bees, and P_{21} is the probability of an interaction between subfamily-2 initiating bees and subfamily-1 recipient bees.

The χ^2 test statistic is used to test for subfamily recognition based on a 2×2 contingency table, similar to the one shown below, where frequency of behaviour, n_{ij} , are recorded with respect to the initiating and receiving bees.

		Receiver's subfamily	
		1	2
Initiator's subfamily	1	n_{11}	n_{12}
	2	n_{21}	n_{22}

In this context, the traditional χ^2 test statistic is used to test the hypothesis of subfamily recognition. However, the null hypothesis can be rejected as a consequence of task specialisation rather than subfamily recognition and nepotism.

Let the proportion of subfamily-1 bees in a colony be X and the proportion of subfamily-2 bees be $1-X$. The probability that a ran-

dom worker is a member of subfamily-1 is X and the probability that it is a member of subfamily-2 is $1-X$. Let the probability of a random worker performing the task during the observation period be a if it is a member of subfamily-1 and ra if it is a member of subfamily-2. The factor r is the rate at which bees of subfamily-2 perform the task relative to bees of subfamily-1 (that is, task specialisation). Combining these probabilities, the probability of observing a member of subfamily-2 that performs the task during the observation period is $ra(1-X)$, and the probability of observing a member of subfamily-1 that performs the task is aX .

If there is no genetic variance between the two subfamilies for receiving behaviour, the probability that a receiving bee is a member of subfamily 1 or 2 is equal to their respective colony proportions. Thus the probability that a subfamily-1 bee will interact with a subfamily-1 bee, P_{11} , is

$$aX(X) = aX^2 \quad (1)$$

The probability that a subfamily-1 worker will interact with a subfamily-2 worker, P_{12} , is

$$aX(1-X) = aX - aX^2 \quad (2)$$

The probability that a subfamily-2 worker will interact with a subfamily-2 worker, P_{22} , is

$$ra(1-X)(1-X) = ra(1-X)^2 \quad (3)$$

and the probability that a subfamily-2 worker will interact with a subfamily-1 worker, P_{21} is

$$ra(1-X)(X) = ra(X-X^2). \quad (4)$$

The behaviour of individual bees due to genetic variance among subfamilies can therefore cause any of the following to be true:

$$P_{11} \neq P_{12} \quad (5.1)$$

$$P_{21} \neq P_{22} \quad (5.2)$$

$$P_{11} \neq P_{21} \quad (5.3)$$

$$P_{12} \neq P_{22} \quad (5.4)$$

$$P_{11} + P_{22} \neq P_{12} + P_{21} \quad (5.5)$$

any of which could be interpreted as evidence for subfamily recognition. In terms of the probable behaviour of individual bees Eqn. 5.5 may be written as

$$aX^2 + ra(1-X)^2 \neq aX(1-X) + ra(X-X^2) \quad (6)$$

which reduces to

$$2X^2 + 2rX^2 - 3rX + r - X \neq 0. \quad (7)$$

This inequality will exist whenever $X \neq 0.5$ or $r/(r+1)$, subject to the condition that $r \geq 1$. Equation 7 shows that: (1) when the most common subfamily has a genetic tendency to perform a task where bees interact together, a high frequency of interaction between members of that subfamily will be observed; (2) if the two subfamilies are in roughly equal proportion, no bias in frequency of interactions between members of same and different subfamilies will be observed; (3) when the specialising subfamily is at low relative frequency, it will appear to interact with half-sisters more than super-sisters. Thus if the behaviour is used as a measure of subfamily recognition, then the relative proportion of each subfamily in a colony will influence the frequency of intra- and inter subfamily interactions. Thus it is possible to ascribe a high frequency of intra-subfamily interactions to subfamily recognition when it is really a result of task specialisation and subfamily relative frequency. This effect cannot in itself result in a significant value of χ^2 , since $[aX^2][ra(1-X)^2] = [aX(1-X)][ra(1-X)X]$ (that is, $P_{11} \times P_{22} = P_{21} \times P_{12}$ for all values of X and r). However, there are two ways that task specialisation can bias the overall frequencies and result in a significant χ^2 . First, if the behaviour of receiving bees is genetically variable, then some subfamilies will receive the behaviour of interest at higher frequency than others, leading to biases in P_{ij} that are unrelated to subfamily recognition. Second, since individual bees are not identified in experiments, the behaviour of just a few bees

Table 1. Numbers of behavioural interactions by eight bees in a hypothetical colony composed of two subfamilies

Bee	Subfamily	Number of donating incidents		Number of receiving incidents	
		To subfamily 1 bees	To subfamily 2 bees	From subfamily 1 bees	From subfamily 2 bees
A	1	15	15	1	1
B	1	2	2	20	20
C	1	12	12	2	2
D	1	4	4	16	16
E	1	3	3	4	4
F	1	0	0	1	1
G	2	4	4	2	2
H	2	3	3	1	1

Note that each individual bee interacts with bees of both sub-families at equal frequency displaying no nepotism. When numbers are pooled, however, subfamily-1 appears to behave nepotistically towards its own subfamily (see text)

could cause significant values of χ^2 , and be erroneously interpreted as evidence for subfamily recognition.

As a simplified hypothetical example of the problems that can be caused by repeated observations, consider the data in Table 1, in which the behaviour of eight bees is repeatedly observed, either knowingly or unknowingly. In this colony, we will assume that all eight bees in Table 1 never interact with each other, and that the two subfamilies are at equal frequency. Subfamily-1 bees have a higher genetic tendency to perform the task than subfamily-2 bees. Bees A and C are very active bees of subfamily-1, which repeatedly initiate the behaviour of interest. They interact at colony frequency with other bees. Bees B and D are very active bees of subfamily-1 that receive the behaviour of interest. The following contingency table combines the frequency information for the eight bees. The χ^2 test statistic and associated probability are given, with the expected cell frequencies reported parenthetically.

		Receiver's subfamily		
		1	2	
Donor's subfamily	1	80 (86.6)	39 (32.4)	$\chi^2 = 5.5$
	2	51 (44.4)	10 (16.6)	

Clearly, in this example, no subfamily recognition is evident. However, the combined χ^2 analysis might lead one to incorrectly infer subfamily recognition for subfamily-1. The only way to avoid violating the assumptions of the χ^2 test, is to individually identify bees, and to record an individual's behaviour only once.

Application of the model. A queen homozygous for the recessive body color mutant *cordovan* (*cd*) was inseminated with mixed semen of two drones, one wild-type (+) and one *cd*, to produce a colony with two identifiable subfamilies (Frumhoff and Schneider 1987). Two brood combs covered with bees were removed from this colony, and gently shaken so that older (foraging) bees flew back to their colony. The two combs were then placed in an incubator at 35°C and 75% RH.

Groups of about 100 bees were cooled on ice until they were immobile. Using a combination of numbered disks glued to the thorax and four different paint colours (plus no abdominal mark), we marked bees so that they were individually identifiable, and placed them in an observation hive. One thousand *cd* workers and 1500 + workers were placed in the observation hive. This process took 4 days. The colony's queen was then placed in the observation hive, along with one comb of the colony's brood, and the hive given free access to the field.

Three days after establishment, the experimental colony was functioning normally. We then scanned the colony for up to 8 h for each of 6 days. A voice recording was made of the identity of each bee involved in trophallaxis, and the time that the interaction occurred. Receiving bees were identified as those individuals with extended tongues.

Results and discussion

Although the behaviour observed was associated with subfertile workers, it is not selectively neutral. Korst and Velthuis (1982) showed that bees which received more food via trophallactic interactions were more likely to become laying workers.

Trophallaxis was observed 366 times. The number of bees of each subfamily involved in trophallaxis is given in Table 2. After correcting for subfamily proportion, there were 3.09 times more bees of the + subfamily involved in trophallaxis as donors than of the *cd* subfamily, indicating subfamilial genetic variance or "task specialisation" for trophallaxis in our colony.

Equation 7 predicts the appearance of subfamily recognition for the + subfamily in this experimental colony: task specialisation was present, and the two subfamilies were at unequal frequency. As predicted, the highest number of interactions observed was between members of the + subfamily.

Table 2 provides the results of χ^2 tests of independence from 2×2 contingency tables of donor/recipient counts. Where all observations were analysed, the χ^2 is significant, and could be taken as evidence of subfamily recognition and nepotism. However, where repeated measures on the same bees are excluded from the analysis, significance is much reduced or eliminated. When data on second and subsequent donations by particular bees are excluded from the analysis, there is no significant association between the subfamily of donor and receiver bees (Table 2). When second and subsequent observations of receiving bees are excluded from the analysis the association is marginally significant ($P = 0.051$, Table 2), and when second and subsequent donation and receiving events are excluded, significance is completely lost (Table 2).

Table 2. Subfamilies of bees involved in trophallaxis in a two-subfamily colony

	All observations		Excluding repeated observations of						
	Subfamily of Recipients		Donors		Recipients		Both		
	cd	+	cd	+	cd	+	cd	+	
Subfamily of donors									
cd	37	28	26	26	33	26	25	24	
+	124	177	75	113	113	156	72	109	
χ^2									2.0
Probability of observed χ^2			5.4		1.7		3.7		0.16
			0.02		0.19		0.05		

In this data set, subfamily recognition was confounded with task specialisation, and either or both effects could have caused the highly significant χ^2 reported for all data in Table 2. Of course some of the loss of significance when data from repeated observations were excluded was due to reduced power of the test due to reduced sample size. The high number of wild-type/wild-type interactions that remained after exclusion of repeated observations is may well be due to differences in subfamily proportion and task specialisation as predicted by Eq. 7. However, this kind of experimental design, which has so often been used, cannot disentangle the effects of task specialisation from those of subfamily recognition. We do not claim therefore, that there was no bias towards super-sister interactions due to subfamily recognition in our colony; only that such a bias cannot be proven on this evidence.

Subfamily discrimination by honey bees will be selected for if it facilitates the rearing of super-sisters as queens. However, some experimental methodologies like that used here cannot conclusively demonstrate the operation of a useable super-sister recognition system in natural honey bee colonies, as the actions observed have confounded origins in either task specialisation or subfamily recognition. As such, these experiments provide only equivocal evidence in support of the theory that subfamilial nepotism or the underlying subfamily recognition system were forces in the development of, or now help to maintain, sterility and eusociality in the honey bee. For this theory to endure, unambiguous experimental evidence is required. Page et al. (1989) probably used the best of all possible experimental designs to uncover subfamily recognition and demonstrate its linkage to enhanced reproductive success of one subfamily (Seeley 1985, p. 56). They measured differences in the proportion of subfamilies among workers and offspring queens, and concluded that the relative proportions changed. On this basis they claimed that subfamily discrimination and nepotism had occurred at a small but ubiquitous and detectable rate. However, Oldroyd et al. (1990) have shown that these results are expected on the basis of sampling error alone, and that nepotism probably did not occur in that experiment.

Future experiments in this field should guard against confounding genetic specialisation and subfamily recog-

inition, and avoid the possibility of repeated observations on the same bee. Experiments should focus on reproductive individuals since workers are subfertile, and worker-worker interactions may be of little evolutionary significance. Experiments like that of Page et al. (1989) are ideal, and the opportunity to use RAPDS (Fondrk et al. 1993), microsatellites (Estoup et al. 1993) or DNA fingerprinting (Moritz et al. 1991) in order to determine genetic relatedness of workers and virgin queens should be taken up.

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