

## Honey bees dance with their super-sisters

BENJAMIN P. OLDROYD\*, THOMAS E. RINDERER\* & STEVEN M. BUCO†

\*United States Department of Agriculture, Agricultural Research Service, Honey-Bee Breeding, Genetics and Physiology Research Laboratory, 1157 Ben Hur Road, Baton Rouge, Louisiana 70820, U.S.A.

†Statistical Resources, 7332 Highland Road, Baton Rouge, Louisiana 70808, U.S.A.

(Received 28 August 1990; initial acceptance 15 October 1990;  
final acceptance 10 December 1990; MS. number: A5865)

**Abstract.** Four colonies of honey bees, *Apis mellifera*, each composed of two subfamilies were separately placed in a screen cage. Bees of each subfamily were found at different frequencies on a pollen feeder, a sucrose feeder and on the roof of the cage, indicating subfamilial genetic variance for foraging preferences. The colonies were then placed in observation hives, and communication dances were observed. The type (pollen or no pollen) and subfamily of dancers and the subfamily of recruits were recorded. Subfamilial variance for nectar or pollen preference and propensity to dance were observed in every case. There was a strong tendency for recruits to follow dances performed by a member of their own subfamily, indicating subfamily recognition. However, at least some of this positive assortment was due to a complex interaction of genotypic differences among subfamilies in their foraging preferences and tendencies to dance.

A colony of honey bees, *Apis mellifera*, consists of 6-17 subfamilies, each composed of workers sired by a different drone (Laidlaw & Page 1984). As a consequence of haplodiploidy, daughters of each sire are super-sisters, while daughters of different sires are half-sisters (Page & Laidlaw 1988). It is becoming increasingly clear that as a consequence of genotypic variance, workers of particular subfamilies tend to have a genetic predisposition to specialize in various tasks (Page et al. 1989a). Such specialization has now been demonstrated or suggested for the following hive duties: grooming (Frumhoff & Baker 1988; Kolmes 1989), guarding and undertaking (Robinson & Page 1988) and brood care (Kolmes et al. 1989). Propensity for members of particular subfamilies to forage for nectar or pollen (Calderone et al. 1989; Robinson & Page 1989), and scout for a new home (Robinson & Page 1989), has also been demonstrated. This specialization, resulting from polyandry, may allow colonies to be more productive and have higher reproductive success than if they were composed of just one subfamily. Thus, one factor contributing to the evolution of polyandry in honey bees may have been the benefit of within-colony genotypic variance and task specialization (Crozier & Page 1985; Sherman et al. 1988).

When honey bees forage, they encounter a wide range of floral types and distances to floral patches.

Floral patches vary in their productivity over the course of a day, and colonies respond to these varying conditions to maximize colony profit (Visscher & Seeley 1982; Seeley 1985). Specialization in foraging for pollen, or nectar, at long or short distance from the colony may increase overall colony efficiency. Bumble bee, *Bombus* spp., foragers specialize in particular plant species, and this is somewhat dependent upon forager size. Thus, larger individuals and species tend to specialize in larger flowers with longer calyx tubes or larger nectar crops, while smaller bees specialize on smaller flowers (Heinrich 1979, page 152). Using theoretical models, Oster & Heinrich (1976) have shown that specialization in foraging activity can increase overall colony efficiency.

Kerr & Hebling (1964) divided newly emerged worker honey bees into three weight classes (light, medium and heavy) and then observed the age at which the heaviest and lightest bees commenced various tasks. Heavy bees commenced all tasks at a younger age than light bees. They concluded that size variation affects the ontogeny of labour in honey bees. Waddington (1989) suggested that variation in body size could reduce colony efficiency if bees of dissimilar size recruited each other during communication dances, because bees of different size might interpret dance information inaccurately. Using the length of a wing vein to

estimate body size, he was able to show a significant positive correlation between dancer size and recruit size.

Taking these observations together, we constructed the following hypotheses. (1) Honey bee foragers tend to specialize on the basis of flower type or distance at which resources are situated from the colony. (2) Inter-individual variability in patterns of task specialization has a genetic component. This genetic component is observable as differences between subfamilies with respect to patterns of task specialization. (3) If hypotheses 1 and 2 are correct, then workers from given subfamilies should preferentially communicate information as to the nature and location of food resources to other members of their own subfamily, because this would increase overall colony efficiency.

A body of literature now exists (reviewed by Sherman et al. 1988), which suggests that honey bee workers possess the ability to determine the subfamily of their nestmates. Further, it has been suggested that this ability to discriminate super-sisters from half-sisters is employed by bees so that they can act nepotistically towards super-sisters (e.g. Evers & Seeley 1986; Noonan 1986; Page et al. 1989b). If this hypothesis is correct, and if our hypotheses 1 and 2 are also correct, then it would be reasonable to expect that honey bees would use their powers of subfamily discrimination to follow appropriate dancers (that is, members of their own subfamily). By doing so, individuals would be directed to food sources for which they have a genetic predisposition to work. As the size of honey bees has a genetic component (Rinderer et al. 1990), and therefore super-sisters are likely to be more similar in size than half-sisters, recruits might also interpret dance information provided by super-sisters more accurately than that provided by half-sisters (Waddington 1988, 1989).

Our experiments were designed to provide further data on subfamily specialization in foraging by honey bees, and to determine whether scouts preferentially communicate information about food sources to super-sisters. Task specialization may cause a higher number of behavioural interactions between members of specializing subfamilies, than between members of different subfamilies. Therefore, merely to observe a higher proportion of interactions between members of a certain subfamily than is observed between members of different subfamilies does not necessarily demonstrate a functional genetically based sub-

family recognition system. The present report evaluates the two alternative models (task specialization and subfamily recognition) for their ability to explain subfamilial associations during communication dances by honey bees.

## METHODS

We established four colonies headed by sister queens homozygous for the recessive integument colour mutant, cordovan. These queens had been artificially inseminated with two drones, one cordovan (*cd*) and one wild-type (+). The cordovan drones were unrelated to the queens. The wild-type drones were not related to each other or to the cordovan queens or drones. Thus, each colony was composed of two subfamilies, distinguishable by the presence or absence of the cordovan cuticle colour. (For further details of how to establish colonies of two distinguishable subfamilies see Frumhoff & Schneider 1987.) Queens were at least 6 months old before testing, which increases the stability of subfamily relative frequency (Page et al. 1984).

Each colony (containing 5000–10 000 bees) was placed separately against one wall of a screen cage measuring 5 × 5 × 1.5 m. Four to five days prior to data collection, we placed a feeding station (Danka et al. 1990) containing a 50% sucrose solution at the end of the cage opposite to the colony and a container of freshly ground pollen in the middle of the cage. The feeders were replenished ad libitum. The delay ensured that the colonies were well trained to the feeding stations.

Starting at about 1000 hours, the entrance of the colony was reduced to 1 cm in width, and the subfamily membership of approximately 500 returning foragers was recorded using an event counter. These counts provided a good estimate of the ratio of the two subfamilies in each colony's field force. Random collections of about 50 individuals were made at the sugar feeder and roof by passing a collecting bottle through an aggregation of bees either at the sucrose feeder or at the cage corners. Collections were exhaustive at the pollen feeder. Bees were preserved in alcohol before being sorted into subfamilies on the basis of cuticular colour.

We then transferred our colonies into glass-walled observation hives, with free access to the field. Bees were observed executing communication dances. We recorded the phenotype of each dancer and whether or not she carried pollen on her

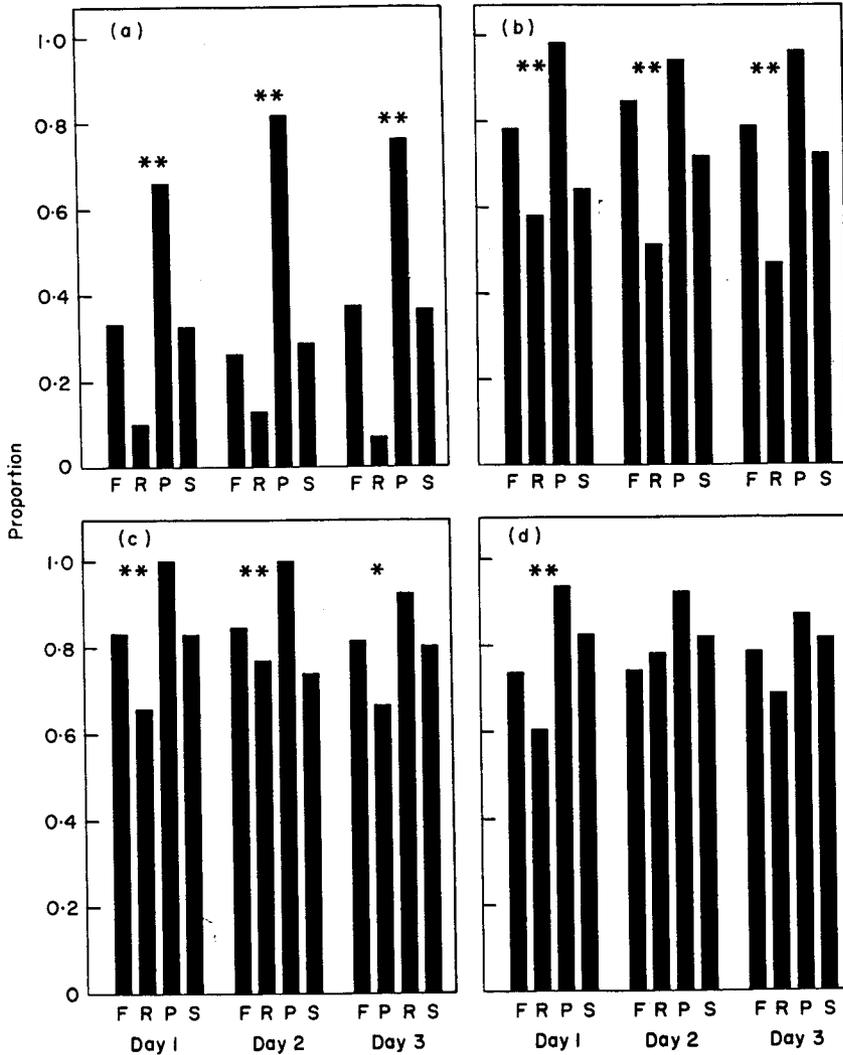


Figure 1. Proportion of bees of the wild-type subfamily found in returning foragers (F), on the roof of the screen cage (R), at the pollen feeder (P) and at the sugar feeder (S). Results of chi-squared tests of independence of sub-family and location are as follows: \*\* $P < 0.001$ ; \* $P < 0.01$ . (a) Colony 41; (b) colony 42; (c) colony 53; and (d) colony 68.

corbiculae. Bees that followed the dancer for more than one revolution of the dance were deemed followers, and their phenotype was also recorded. Dance participants were recorded until the dancer paused. Dancers were recorded only once for any one dancing event, but because bees were not individually identified, we probably recorded the same bees as dancers and followers more than once over the course of a day. Observations were made by a variety of people, including visitors and technicians, who at least initially, were unaware of the hypotheses being tested. From time to time, while

these data were being collected, a second observer recorded the subfamily of 500 arriving foragers, and whether or not they carried pollen on their corbiculae.

Dancing data were first analysed using the following nested model for ANOVA:

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \delta_k + (\alpha\delta)_{ik} + \epsilon_{ijk}$$

where  $Y_{ijk}$  = the number of bees of the  $k$ th subfamily that followed a dancer of the  $i$ th subfamily in the  $j$ th dance;  $\mu$  = the overall mean;  $\alpha_i$  = the effect of the  $i$ th subfamily of the dancer;  $\beta_{j(i)}$  = the effect of

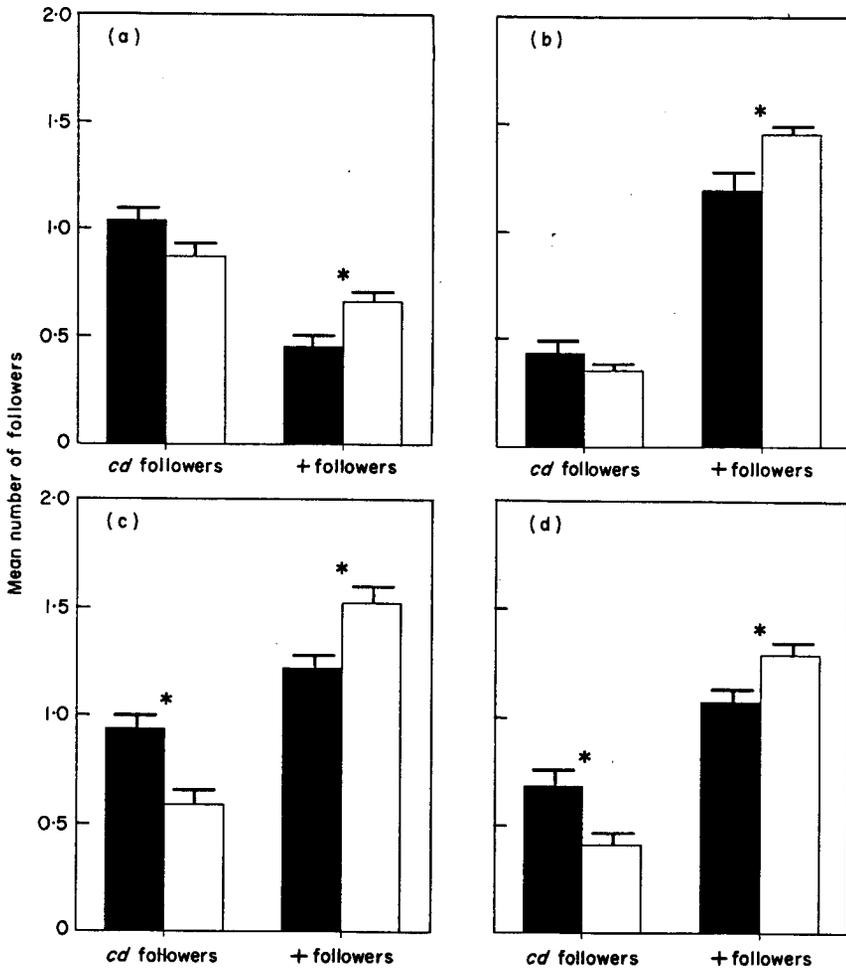


Figure 2. Mean number of followers of each subfamily ( $\pm$ SE) that followed a *cd* dancer (■) and a + dancer (□). \*Significantly more followers attended dances of super-sisters than half-sisters at the 5% level (two-tailed *t*-test). The number of dances observed was (number of *cd* dancers, number of + dancers): (a) colony 41, *cd* = 140, + = 143; (b) colony 42, *cd* = 88, + = 396; (c) colony 53, *cd* = 224, + = 128; and (d) colony 68, *cd* = 175, + = 374.

the *j*th dance within the *i*th dancer subfamily;  $\delta_k$  = the effect of the *k*th subfamily of the followers;  $(\alpha\delta)_{ik}$  = the effect of the interaction of the follower's and dancer's subfamilies;  $\epsilon_{ijk}$  = experimental error.

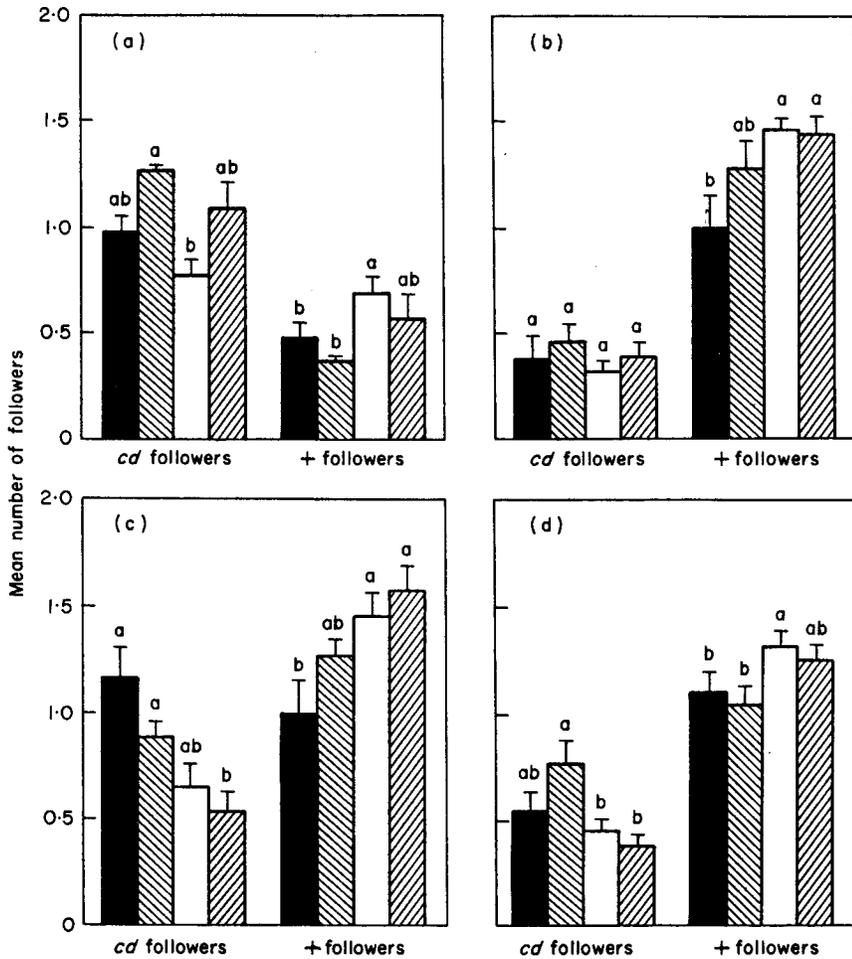
To explore the effects of dancer kind (pollen or no pollen) on levels of recruitment, the data were reanalysed to include the effects of dancer kind and all interactions of dancer kind with dancer and follower subfamily.

## RESULTS

As shown in Fig. 1, there was a remarkable consistency over time of subfamily proportion at each col-

lection point. The following statements can be made with respect to subfamily proportion in the foraging populations. In all colonies, the cordovan subfamily was found at a higher proportion (based on returning forager counts) on the roof of the cage and at a much lower proportion on the pollen feeder. In three of four colonies, the subfamily proportions at the sugar feeder were similar to those of returning foragers. However, in colony 42, the cordovan subfamily was found at higher than the expected proportion at the sugar feeder.

In the first ANOVA, the interaction  $(\alpha\delta)_{ik}$ , between the subfamily of dancers and the subfamily of followers was significant ( $P < 0.05$ ) for all



**Figure 3.** Mean numbers of followers of each subfamily (+SE) that followed dancers of different subfamilies and kinds. (■) *cd* dancer with no pollen; (□) +dancer with no pollen; (▨) *cd* pollen dancer; (▩) +wild-type pollen dancer. Results of chi-squared tests ( $df=1$ ) of independence between dancer subfamily and dancer kind are as follows: (a) colony 41,  $P=0.074$ ; (b) colony 42,  $P<0.001$ ; (c) colony 53,  $P<0.001$ ; and (d) colony 68,  $P=0.003$ . Within a subfamily, columns not sharing the same letter are significantly different ( $P<0.05$ , two-tailed  $t$ -test).

colonies, indicating that recruits did not follow dancers of each subfamily in equal proportion. In every case, a higher proportion of recruits followed dancers in their own subfamily relative to recruits of the other subfamily; the differences were statistically significant in six of eight comparisons (Fig. 2).

These positive subfamilial associations might have been due to some subfamily recognition phenomenon, or alternatively might have been due to a task specialization phenomenon. That is, if one subfamily had a genetic predisposition to collect pollen, then members of that subfamily might be attracted to pollen-bearing dancers, which would

be predominantly of the pollen specializing subfamily. As illustrated in Fig. 3 there was a tendency for followers of particular subfamilies to attend particular kinds of dances. For example, in colony 41, the cordovan subfamily followed more pollen-bearing dancers than nectar dancers, whatever the subfamily of the dancer, while the wild-type subfamily followed more dancers without pollen, whatever the subfamily of the dancer. Nevertheless, where a subfamily preferentially followed pollen bearers, they tended to attend more pollen dances performed by members of their own subfamily than of the other subfamily. Cordovans following

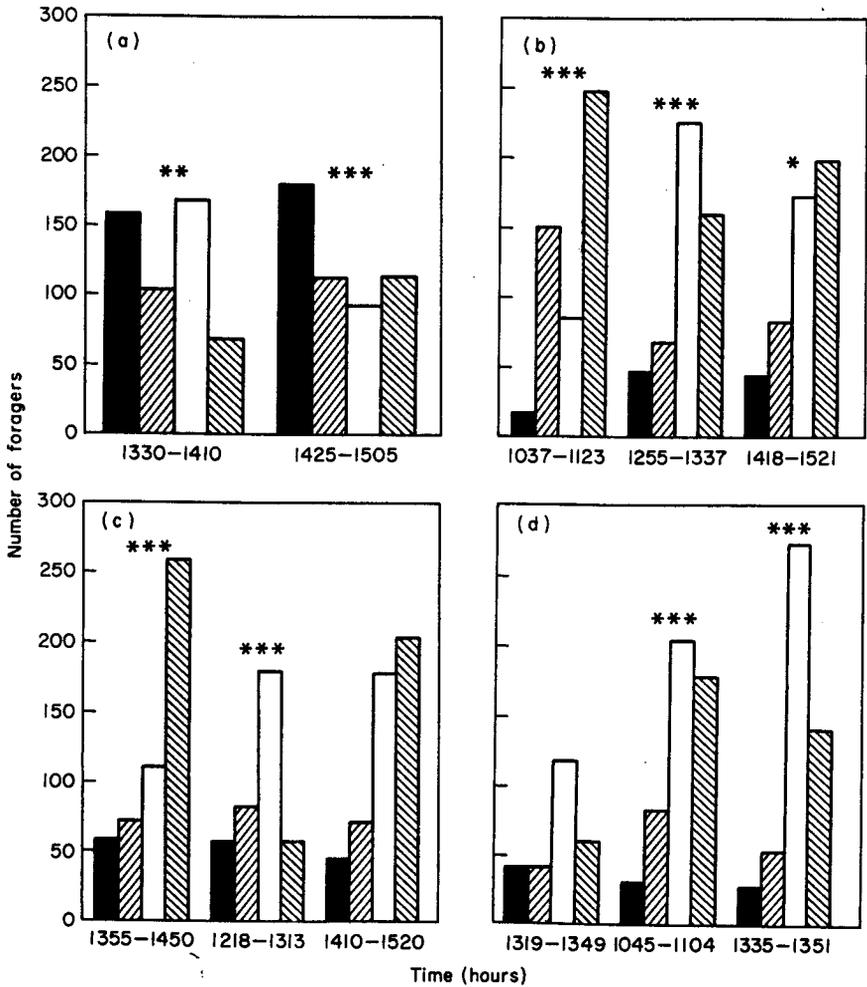


Figure 4. Number of *cd* foragers with (▨), and without (■), and the number of *+* foragers with (▩) and without (□) pollen that returned to observation hives at various times indicated on the horizontal axis. Observations were made in April 1990. Lack of independence between forager subfamily and what it was carrying (chi-squared test with  $df=1$ ) is indicated as follows: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ . (a) Colony 41; (b) colony 42; (c) colony 53; and (d) colony 68.

nectar dances show this phenomenon particularly strongly in colony 53.

To explore the possibility that the apparent subfamily recognition was in fact an artefact of task specialization, we reanalysed the data with a second ANOVA using a more complete model, to investigate how the subfamily and kind (pollen bearing or not pollen bearing) of dancers influenced the number of recruits of each subfamily.

A significant mean square for the follower subfamily by dancer subfamily interaction term from this analysis would indicate that recruits of each subfamily preferentially followed dancers of a particular subfamily, independent of whether dancers

carried pollen. These interactions were significant ( $P < 0.02$ ) for all colonies. A significant mean square for the interaction between follower subfamily and kind of forager (pollen or no pollen), would indicate that dancers carrying pollen varied in their attractiveness to recruits of the different subfamilies, independent of the subfamily of the dancer. These interactions were significant in colonies 41 and 53 ( $P < 0.05$ ), but not in colonies 42 and 68 ( $P > 0.3$ ).

Figure 3 illustrates the results of chi-squared tests of independence of dancer subfamily and whether or not it carried pollen. These were significant in all cases, indicating that both the subfamily and

what the dancer carried affected the tendency to dance.

Counts of foragers returning to observation hives were analysed with chi-squared tests of independence (Fig. 4). The striking feature of these data is the high level of heterogeneity (estimated from the three-way interaction between subfamily, time and day from a log-linear model) between counts at different times of day and on different days for colonies 41 and 53 ( $P < 0.001$ ). However, during any short period of time (counts normally took about 1 h), it is quite clear that subfamilies differed in whether they were collecting pollen or nectar, or perhaps just making orientation flights.

## DISCUSSION

These data suggest a new interpretation for the findings of Waddington (1988, 1989). He showed that dancing honey bees attract recruits of a similar size and postulated that this would reduce communication errors. Our data suggest that his reported size variation may have been due to sub-familial size variation, with dancers attracting a higher proportion of super-sisters than half-sisters. Honey bees show large inter-racial variance in the dialects of dance language (von Frisch 1967). It is thus possible that intra-colonial variance in dance language also exists, and that errors of communication can be reduced if genotypically similar bees (super-sisters) are involved in communication dances.

These data also support the results of Robinson & Page (1989) and Calderone et al. (1989) in demonstrating intra-colonial subfamilial variation in foraging behaviour. Counts of returning foragers showed strong tendencies for members of one subfamily to bear pollen at a higher frequency than members of the other subfamily. These tendencies can be extraordinarily strong, as shown by the extremely low counts of cordovans found at the pollen feeder in the cage. However, it is simplistic to assert that specialization occurs only at the level of pollen or nectar preference. Subfamilial proportions of pollen-bearing foragers varied from day to day, even within a day, presumably reflecting the availability of forage. Our data suggest that one area of specialization between subfamilies might be the tendency to forage at sites at varying distances from the colony. For all colonies, the cordovan subfamilies were found at higher frequencies than expected on the roof of the cage, possibly suggesting they might have preferred to

forage further from the colony. (Another plausible explanation is differential mortality of the two subfamilies. Older bees may behave differently in a cage from younger bees.) Cordovans were also found at very low frequency on the pollen feeder. However, this was not because they were averse to pollen collection in general. All forager counts from observation hives (except those for colony 41) showed that the majority of cordovan returning foragers carried pollen. The proportion of cordovan pollen bearers often exceeded the proportion of wild-type pollen bearers in the observation hive forager counts.

Subfamilial variance in dancing behaviour is also demonstrated by these data. The tendency to dance was strongly affected by both subfamily and forager type (pollen or no pollen; Fig. 3). Similarly, the tendency of a recruit to follow a dancer was affected by the subfamily of the recruit, the subfamily of the dancer, and by what the dancer was carrying (Fig. 2). These data might therefore be seen as supporting the hypothesis that members of subfamilies recognize each other.

The apparent subfamily recognition (Fig. 2) can be at least in part explained by task specialization. Consider colony 41, and accept for the moment that there was no subfamily recognition. Can we reasonably interpret the data on a task-specialization model?

The cordovan subfamily preferentially followed pollen dancers of either subfamily, while wild-types preferentially followed dancers with no pollen (Fig. 3). Let us assume that members of the cordovan subfamily were 'pollen specialists', while the wild-type subfamily was composed of 'nectar specialists'. Preference may be overshadowed by availability, and many returning cordovan foragers may have returned to the hive carrying nothing. Note also, that the cordovan subfamily was found at a higher frequency than the wild-type subfamily in the foraging bee population (Figs 1 and 4), and that most dancers carried no pollen, perhaps because it was not readily available (Fig. 3). Task specialization coupled with unequal subfamily frequency could act in this case to minimize interactions between unlike subfamilies, while maximizing interactions among like subfamilies, in the following way: cordovan nectar dancers attracted a small number wild-type recruits, since the nectar specialist wild-type bees were at lower frequency in the colony. Wild-type nectar dancers attracted a small number of recruits of both subfamilies, but a higher

proportion of wild-types than would be predicted from their colony frequency, as a result of nectar specialism. Cordovan pollen dancers attracted a large following of pollen seeking cordovan recruits. Wild-type pollen dancers attracted a large following of cordovan recruits, but the number of wild-type pollen dancers was low owing to foraging specialization, colony frequency and pollen availability. These effects combine, when the data are pooled in Fig. 2, to suggest the appearance of (or the enhancement of real) subfamily recognition. Similar reasoning can be used on the data from other colonies, but with decreased certainty.

The presence of significant subfamily interaction mean squares (dancer by follower) in all colonies (where dancers were classified by both subfamily and what they carried) indicates that we cannot completely explain apparent subfamily recognition on the basis of some subfamilies preferring to collect, dance for, and follow recruitment dances for pollen or nectar. This result is not surprising. The classification of 'pollen' or 'no pollen' is extremely crude. If we had classified dancers better according to such factors as distance directed by the dance, plant species foraged and availability of the food resource, a clearer pattern of task specialization by subfamilies might have emerged. This, in turn, might have led to a better untangling of the alternative hypotheses that explain apparent subfamily recognition. Nevertheless, it is quite clear that our data should not be taken as unequivocal evidence for subfamily recognition.

In conclusion, our data support previous studies (Calderone et al. 1989; Robinson & Page 1989) that have shown that honey bee subfamilies tend to specialize in various foraging tasks. The hypothesis that specializing sub-families preferentially share information on food sources with their super-sisters is also strongly supported. The mechanism (subfamily recognition or an inevitable consequence of task specialization) by which super-sisters are preferentially attracted to communication dances remains to be fully elucidated. However, this is irrelevant to the welfare of the colony. The fact that it does occur is the important issue. This is expected to reduce errors of communication, and enhance a colony's overall fitness.

#### ACKNOWLEDGMENTS

We thank G. Perrone, R. Herbert, D. Pursifull, L. Beaman and R. Riggio for data collection. L.

Beaman prepared the figures. The manuscript has benefited from detailed criticism by J. Harbo, R. Hellmich and N. Calderone, and the referees. This work is in cooperation with the Louisiana Agricultural Experimental Station.

#### REFERENCES

- Calderone, N. W., Robinson, G. E. & Page, R. E. 1989. Genetic structure and division of labour in honey bee societies. *Experientia*, **45**, 765–767.
- Crozier, R. H. & Page, R. E. 1985. On being the right size: male contributions and multiple mating in the social Hymenoptera. *Behav. Ecol. Sociobiol.*, **18**, 105–115.
- Danka, R. G., Williams, J. L. & Rinderer, T. E. 1990. A bait station for survey and detection of honey bees. *Apidologie*, **21**, 287–292.
- Evers, C. A. & Seeley, T. D. 1986. Kin discrimination and aggression in honey bee colonies with laying workers. *Anim. Behav.*, **34**, 924–945.
- von Frisch, K. 1967. *The Dance Language and Orientation of Bees*. Cambridge, Massachusetts: Harvard University Press.
- Frumhoff, P. C. & Baker, J. 1988. A genetic component to division of labour within honey bee colonies. *Nature, Lond.*, **333**, 358–361.
- Frumhoff, P. C. & Schneider, S. 1987. The social consequences of honey bee polyandry: the effect of kinship on worker interactions within colonies. *Anim. Behav.*, **35**, 255–262.
- Heinrich, B. 1979. *Bumble Bee Economics*. Cambridge, Massachusetts: Harvard University Press.
- Kerr, W. E. & Hebling, N. J. 1964. Influence of the weight of worker bees on the division of labor. *Evolution*, **18**, 267–270.
- Kolmes, S. A. 1989. Grooming specialists among worker honey bees *Apis mellifera*. *Anim. Behav.*, **37**, 1048–1049.
- Kolmes, S. A., Winston, M. L. & Fergusson, L. A. 1989. The division of labor among worker honey bees (Hymenoptera: Apidae): the effects of multiple patriline. *J. Kans. Entomol. Soc.*, **62**, 80–95.
- Laidlaw, H. H. & Page, R. E. 1984. Polyandry in honeybees (*Apis mellifera* L.): sperm utilization and intracolony genetic relationships. *Genetics*, **108**, 985–997.
- Noonan, K. C. 1986. Recognition of queen larvae by worker honey bees (*Apis mellifera*). *Ethology*, **73**, 295–203.
- Oster, G. & Heinrich, B. 1976. Why do bumblebees major? A mathematical model. *Ecol. Monogr.*, **46**, 129–133.
- Page, R. E., Kimsey, R. B. & Laidlaw, H. H. 1984. Migration and dispersal of spermatozoa in spermathecae of queen honey bees (*Apis mellifera* L.). *Experientia*, **40**, 182–184.
- Page, R. E. & Laidlaw, H. H. 1988. Full sisters and super sisters: a terminological paradigm. *Anim. Behav.*, **36**, 944–945.
- Page, R. E., Robinson, G. E., Calderone, N. E. & Rothenbuhler, W. C. 1989a. Genetic structure, division of labor, and the evolution of insect societies. In: *The Genetics of Social Evolution* (Ed. by M. D. Breed &

- R. E. Page), pp. 15-30. Boulder, Colorado: Westview Press.
- Page, R. E., Robinson, G. E. & Fondrk, M. K. 1989b. Genetic specialists, kin recognition and nepotism in honey-bee colonies. *Nature, Lond.*, **338**, 576-579.
- Rinderer, T. E., Daly, H. V., Sylvester, H. A., Collins, A. M., Buce, S. M., Hellmich, R. L. & Danka, R. G. 1990. Morphometric differences among Africanized and European honey bees and their F1 hybrids (Hymenoptera: Apidae). *Ann. entomol. Soc. Am.*, **59**, 346-351.
- Robinson, G. E. & Page, R. E. 1988. Genetic determination of guarding and undertaking in honey-bee colonies. *Nature, Lond.*, **333**, 356-358.
- Robinson, G. E. & Page, R. E. 1989. Genetic determination of nectar foraging, pollen foraging and nest-site scouting in honey bee colonies. *Behav. Ecol. Sociobiol.*, **24**, 317-323.
- Seeley, T. D. 1985. The information-center strategy of honeybee foraging. In: *Experimental Behavioral Ecology and Sociobiology* (Ed. by B. Hölldobler & M. Lindauer), pp. 75-90. Stuttgart: G. Fischer Verlag.
- Sherman, P. W., Seeley, T. D. & Hudson, H. K. 1988. Parasites, pathogens and polyandry in social hymenoptera. *Am. Nat.*, **131**, 602-610.
- Visscher, P. K. & Seeley, T. D. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology*, **63**, 1790-1801.
- Waddington, K. D. 1988. Body size, individual behavior and social behavior in honey bees. In: *Interindividual Behavioral Variability in Social Insects* (Ed. By R. L. Jeanne), pp. 385-417. Boulder, Colorado: Westview Press.
- Waddington, K. D. 1989. Implications of variation in worker body size for the honey bee recruitment system. *J. Insect Behav.*, **2**, 91-103.