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Intra-colonial foraging specialism by honey bees (*Apis mellifera*) (Hymenoptera: Apidae)

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Summary. Colonies of honey bees with two identifiable subfamilies were established. Returning foragers were captured and killed at two different sampling times. The mean volume and per cent soluble solids of crop contents were determined for each subfamily, as was the mean weight of the pollen pellets. No significant differences in nectar volume or concentration were detected between subfamilies within colonies. However, in a few colonies, significant subfamily by sampling-time interactions were present, suggesting that in these colonies subfamilies differed in their nectar and pollen collecting behavior at different times of day. The plant genera worked by pollen foragers were also determined. In four of six colonies, bees of different subfamilies were found to be majoring on different plant species (Fig. 1). Implications of this intra-colonial variance in foraging behavior for colony fitness are discussed.

Introduction

Queen honey bees mate with drones from a variety of colonies, which are likely to be genetically diverse. Crozier and Page (1985) proposed eight hypotheses to explain the evolution of social insect polyandry, one of which was that a multiply-inseminated queen may have increased fitness compared to a monandrous queen, since her offspring will have greater genetic variance. Increased intra-colonial genetic variance may increase colony fitness in several ways. First, breadth of genetically-based tolerance to parasites and pathogens may increase the ability of a colony to withstand disease (Sherman et al. 1988). Second, varying physiology among colony members may allow the collective workers to function more effectively under a broader range of environmental conditions than could colonies with a narrower genetic base (Crozier and Page 1985). Third, multiple mating reduces the probability that a queen will have low brood viability due to sex allele homozygosity (Shaskolsky 1976; Page 1980).

An increasing body of evidence demonstrates that behavioral differences do occur among subfamilies within honey bee colonies (reviewed by Page et al. 1989). This is not surprising. Honey bee colonies are composed of 6–17 subfamilies of super-sisters (reviewed by Laidlaw and Page 1984), each sired by a haploid drone. Super-sisters have a coefficient of relatedness of 0.75. Half-sisters, sired by different unrelated drones, have a coefficient of relatedness of only 0.25. The high relatedness of super-sisters will cause them to behave similarly for behavioral traits that are heritable. Half-sisters are much less related and may behave differently in response to the same environmental stimuli.

Foraging behavior is one area in which genetic variance among subfamilies could affect the fitness of the colony (Page et al. 1989) by altering the range of conditions under which a colony can effectively forage. Genetic variance in foraging behavior in known to exist in honey bees. Mackensen and Nye (1969) developed two lines of bees which differed in the rates at which they collected alfalfa pollen, while Mackensen and Tucker (1973) demonstrated that these lines focused on different pollen plants in the absence of alfalfa. Hellmich et al. (1985) selected two lines of bees that differed in the rates at which they collected pollen of all kinds. Bees of these two lines differ in their rates of pollen foraging when released into the same colony (Calderone and Page 1988). Genetic variance in rates of pollen collection among subfamilies also exists in colonies of unselected bees (Calderone et al. 1989; Robinson and Page 1989; Oldroyd et al. 1991). Subfamilial variation has also been demonstrated in the average age at which bees first begin to forage (Calderone and Page 1988).

We speculated that division of labor among subfamilies in foraging tasks might be more subtle than a higher or lower tendency to forage for pollen or nectar. Subfamilial differences may exist in natural colonies in such factors as: average weight of nectar or pollen load, average foraging distance, or plant genera preferred as food

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sources. In these experiments we examined in detail the loads carried by returning foragers to six colonies each composed of two identifiable subfamilies.

Materials and methods

Virgin queens homozygous for the recessive color mutation *cordovan* (*cd*) were instrumentally inseminated with one *cd* drone and one wild type (+) drone. This procedure produced colonies composed of two subfamilies, each identifiable by the integument color of the bees. Virgin queens and drones were obtained from a variety of genetic backgrounds. Colonies 42 and 53 were headed by sister queens, colonies 95 and 100 by unrelated sisters, and colonies 193, 194 and 197 by daughters of a third queen. The + drones used were unrelated to each other or to the queens. The *cd* drones used for each sister group were brothers. All bees used in matings were not inbred.

These experiments were conducted in late summer 1990, on the urban fringe of Baton Rouge, Louisiana. The entrance to each colony (3000–5000 bees) was blocked with a trap $37 \times 6 \times 6$ cm. The side blocking the entrance was 0.25 cm wire mesh, while the other four sides were wooden. Effectively, then, returning foragers entered a dummy entrance. They then aggregated on the wire mesh next to the real entrance. The fact that the bees could smell the entrance through the mesh caused most bees to remain in the open traps, thereby minimizing drifting. All colonies were more than 4 m apart, surrounded by extensive landmarks. These landmarks also minimized drifting between colonies during trapping. After approximately 100 foragers were caught in this way (about 5 min), the trap was closed and placed in a plastic bag together with an open cyanide killing jar (Sylvester et al. 1983). This method of killing bees has no measurable effect on the concentration or volume of honey bee crop contents (Sylvester et al. 1983), and minimal food exchange occurs among aggregating foragers.

1. Weights of nectar and pollen loads. Collections were made on 2 August 1990. Colonies 193, 194 and 195 at site A were sampled at 0815 hours, and again at 1400 hours. Colonies 42, 53, 95 and 100 at site B (4 km from site A) were sampled at 0900 hours and 1430 hours. Killed bees were taken in the traps to the laboratory. For each colony, individuals were sorted according to their subfamily. We then removed their heads and squeezed the abdomens in order to express the crop contents. The volume of the crop contents was determined to the nearest 5 µl for each bee by drawing the liquid into a 50-µl micropipette, and measuring the length of the liquid column (Sylvester et al. 1983). Per cent (wt/wt) soluble solids of nectar was determined for each bee using a Bausch and Lomb refractometer (Sylvester et al. 1983). Legs with pollen were stored frozen and thawed for approximately two hours before the pellets were scraped off and weighed to the nearest µg. Where only one pellet was available for a particular bee, its weight was doubled.

2. Plant species worked by pollen foragers. Collections were made on September 6 and 7 1990 at 0900 hours. Colonies 193 and 195 were foraging poorly at this time, and their data were not analyzed. Colony 197 at site A was substituted. Initially, bees were sorted into subfamilies, and by whether or not they carried pollen. Bees were then sorted according to the color and texture of their pollen pellets. Foraging bees collected from flowers near the apiaries were used to identify the plant genera of six classes of pollen pellet.

Results

Only those bees carrying pollen or with more than 10 µl of crop contents with more than 10% soluble solids were analyzed. Therefore scout bees, guard bees and unsuccessful foragers were excluded from the data sets. Analysis of variance was used to test hypotheses concerning the mean size of pollen and nectar loads and of nectar concentration of returning foragers according to their subfamily and the time of day (Table 1). There were no significant differences between subfamilies in the concentration or volume of nectar loads carried by nectar foragers. In colony 42, + pollen bearers carried 2.1 times more pollen than *cd* pollen bearers, but no other subfamilial differences in weights of pollen pellets were detectable. Significant interactions between subfamily and time of day were detected for nectar concentration and volume in 3 of 14 analyses. These analyses indicate that differences among subfamilies in the mean volume or concentration of nectar loads within colonies were uncommon if they existed at all. Family by time interactions for nectar concentration and volume in colonies 53, 100 and 195 indicate that some subfamilies may have responded differently to available forage during the course of the day.

Table 1. Probabilities associated with observed subfamilial differences in: mean weight of pollen pellets from bees bearing pollen; mean concentration (% soluble solids) and volume of nectar in bees carrying more than 10 µl nectar, followed by the probability of significant subfamily by time interaction (F × T) for these variables. The probabilities were obtained from a two-way analysis of variance for each colony

Colony	Nectar concentration		Nectar volume		Pollen weight	
	Family	F × T	Family	F × T	Family	F × T
42	0.49	0.60	0.97	0.72	0.00	0.34
53	0.89	0.00	0.28	0.22	0.06	0.61
95	0.82	0.29	0.23	0.22	0.26	0.24
100	0.71	0.05	0.20	0.35	0.79	0.69
193	0.06	0.37	0.91	0.74	0.90	0.89
194	0.51	0.48	0.17	0.75	0.48	0.93
195	0.81	0.07	0.37	0.02	0.34	0.24

Fifteen kinds of pollen pellet were identified over the 2 days of the second experiment. The six most common types were identified to genera (Fig. 1). For each colony, the proportion of pollen foragers in each subfamily that foraged on the six commonest kinds of plants (for that colony) are presented in Fig. 1. In order to test the hypothesis that plant species foraged was independent of subfamily, data for the 2 days were pooled in order to increase counts per cell of the contingency table. In addition, if in a given colony fewer than 10 bees of both subfamilies were found with a particular pollen type, then that pollen type was polled into a category called "other". Therefore all marginal totals of the contingency tables exceeded nine. Fisher's exact test of association for $r \times c$ contingency tables was then computed for each colony (Metha and Patel 1983). This statistic, which is appropriate for tables of order greater than 2×2 (Metha and Patel 1983), computes the exact probability of observing a table with as much association as that actually observed. The Fisher statistic for $r \times c$ tables does not require expected cell frequencies to exceed five and is

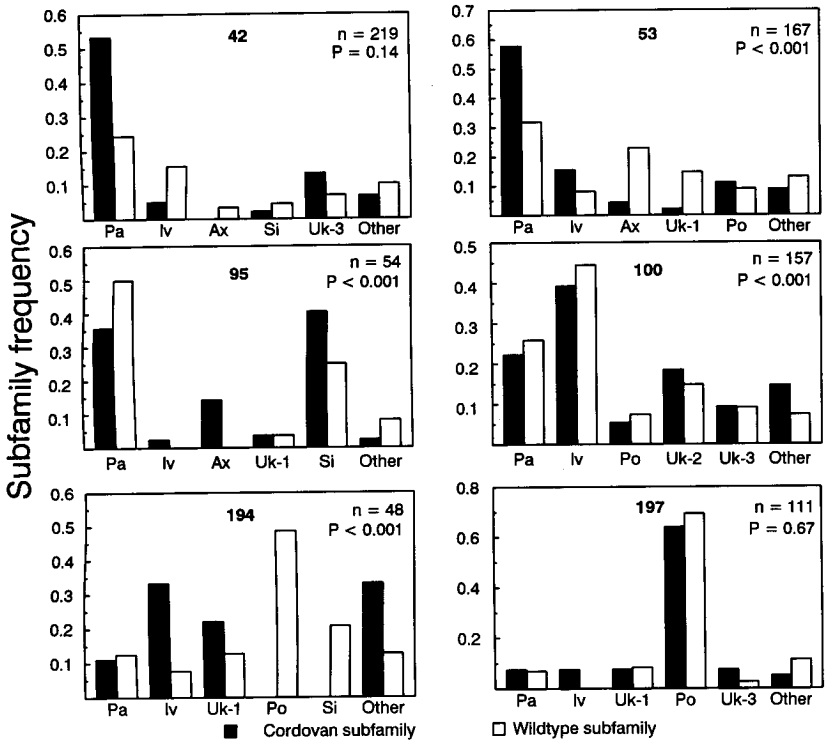

Fig. 1. Proportion of returning pollen-bearing foragers of each subfamily that carried pollen from the plant species specified, in six colonies of honey bees (*bold figures*). *Pa* = *Parthenium* sp., *Iv* = *Iva* sp., *Ax* = *Axonopus* sp., *Po* = *Polygonum* sp., *Si* = *Sida* sp., *Ca* = *Cassia* sp., *Uk* = unknown. The total number (*n*) of pollen bearing bees collected and the probability of observing a $r \times c$ table with as much association as that observed (*P*) are given in the figure. The reported probability is for collapsed tables, in which each pollen category had at least 10 bees. Smaller categories were pooled into the "*other*" categories in the collapsed tables

unaffected by zeroes among the data. In four of six colonies, subfamilies differed significantly in the species they were working. Differences between subfamilies approached significance in an additional colony (42). Differences in the pollen species collected were evident both among and within colonies. For example, all four colonies at site B actively foraged on *Parthenium* sp., but only colony 53 at that site was found to be actively foraging for pollen from *Axonopus* sp. (Fig. 1). There was a complete absence of *Axonopus* foragers in colony 100. Even within colony 53, only one *cd* forager was detected from *Axonopus*. Thus within this group of four colonies and a total of eight subfamilies, only the + subfamily of colony 53 chose to forage actively on *Axonopus*.

Discussion

We were unable to detect subfamilial differences in the concentration or volume of nectar loads for any colony (although colony 193 approached significance for nectar concentration). This suggests that foragers are only marginally affected by genotype in the size or concentration of nectar loads, in agreement with Calderone and Page (1992) who found only minor differences in concentration and volume of nectar loads carried by bees that had undergone artificial selection for pollen and nectar preference. We found only one colony in which subfamilial differences were apparent in the weights carried by pollen foragers. These differences appear to be uncommon, although it is possible that if nectar loads could be identified to plant species, intracolonial differences might have become apparent. Nevertheless, our data (Fig. 1) suggest that striking differences commonly exist among honey bee subfamilies in the plant species worked for pollen (and by extension, probably nectar as well). Figure 1 also shows that our colonies tended to major on 2–3 plant species, and that these varied widely among colonies in the same apiary. Differences between subfamilies in plant species from which pollen is collected may underlie previously reported subfamilial preferences in nectar and pollen foraging (Calderone et al. 1989; Robinson and Page 1989; Oldroyd et al. 1991). They may also be sufficient to explain the small differences in nectar and pollen loads found in this study.

Our data support the hypothesis that there is widespread intra-colonial variance in plant species foraged upon by different subfamilies within colonies. Here we discuss five hypotheses to explain this phenomenon.

Colony fitness is affected by behavioral specialization

Colony fitness may be increased by having a range of genotypes that are more efficient at tackling a range of foraging tasks, for example distance from the colony. Page et al. (1989) developed a simple expression of colony fitness, based on the proportion of two kinds of foraging specialists in a colony. They showed that if specialization increases colony fitness, then polymorphism of alleles which specify specialization will be selected.

Subfamily recognition and size variation affects the accuracy of communication dances

Waddington (1989) showed that when honey bees execute communication dances, they tend to attract recruits of similar size. He speculated that bees of dissimilar size might communicate information on floral resources inaccurately. If this conjecture correctly explains his observations, then a possible consequence is that super-sisters (which would tend to be more similar in size than half-sisters), would tend to recruit super-sisters during communication dances. This preferential passing of information to super-sisters could lead to a separation of pollen types collected by different subfamilies, if floral patches are diverse and are not uniformly spread over the foraging range.

Colony fitness is increased by eclectic foraging

Honey bee nutrition is provided almost exclusively by plant-derived nectar and pollen. Pollens vary in their crude protein content and their nutritional value (e.g. Schmidt and Johnson 1984). Some pollens may be deficient in certain amino acids or vitamins, which make them nutritionally substandard. However, in combination with other pollens, a suitable honey bee diet may result. Honey bee foragers distinguish the nutritional content of pollens poorly if at all (Schmidt 1982), although young pollen-feeding bees may prefer protein-rich pollens to protein-poor pollens (Schmidt and Johnson 1984). They certainly prefer pollens of mixed floral origin rather than single origin (Schmidt 1984). Genetic tendencies to forage on different kinds of plants may lead to much increased colony fitness, by increasing the probability of good colony nutrition and by diluting potentially harmful levels of plant toxins that are present in some pollens (Barker 1978).

Subfamilial differences in pollen preference might be a consequence of selection at epistatic loci

Selection for division of labor in brood care tasks could conceivably result in collateral behavioral polymorphisms in pollen foraging. Further, demonstrated differences in preferences for nectar and pollen foraging (Calderone et al. 1989; Robinson and Page 1989; Oldroyd et al. 1991) could also result in differences in the proportions of pollens collected by different subfamilies.

Different age profiles among subfamilies could affect foraging task

Incomplete mixing of the two kinds of sperm used to inseminate our queens could have resulted in different age profiles between the two subfamilies. As age affects foraging behavior of bees (younger bees are more likely to be recruited by scouts: Lindauer 1953), different age profiles could have produced our results. Some sperm clumping occurs in young naturally mated queens (Taber 1955), probably to the same extent it does in artificially inseminated ones. The effect declines rapidly as queens mature (data of Kerr et al. 1980). Thus colony fitness may be increased by subfamilial specialism caused by mechanical clumping of sperm, rather than direct genetic differences among subfamilies.

Since stability of subfamily relative frequency increases with queen age (Laidlaw and Page 1984; Page et al. 1984; data of Kerr et al. 1980) subfamily relative frequency was likely to be stable in our queens which were all at least 6 months old. Genetic differences therefore seem a much more plausible explanation of our results than mechanical clumping of sperm. We have studied the proportion of subfamilies in returning foragers in two-subfamily colonies (Oldroyd unpublished data; Oldroyd et al. 1991). Regular sampling over 4 months showed that within-day fluctuations exceed between-day fluctuations.

These five hypotheses are not exclusive, and all their processes may contribute to the intracolonial variance repeatedly reported for honey bee foraging behavior. The dance accuracy hypothesis has little appeal since intracolonical variance in body size of honey bees is generally small (Alpatov 1929). However, if foraging specialization does indeed increase colony fitness, then communicating information to similarly specialized individuals would be an essential component of the specialist mechanism.

The other hypotheses suggest that behavioral polymorphism in foraging behavior or for epistatic genes unrelated to foraging behavior can increase colony fitness. These are attractive suggestions, but lack experimental support. An experiment that links increased colony fitness with increased intra-colonial genetic variance is badly needed.

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