Reprinted from Nature, Vol. 346, No. 6286, pp. 706–708, 23rd August, 1990

Nepotism in the honey bee

SIR—Reports that honey-bee *(Apis mellifera)* workers discriminate nepotistically among nestmate patrilines, by Page *et al.*[1] and others (for example, refs 2–8), have generated considerable interest. But Queller *et al.*[9] recently found that *Polistes annularis* wasps, under natural conditions, do not preferentially join more closely related natal nestmates during foundation of their spring colonies. Similarly, within colonies of the polygynous carpenter ant *Camponotus planatus,* workers fail to distinguish their mother queen, sister workers or sister virgin queens from those of other naturally cohabiting nestmate matrilines (N.F.C. and S. Cover, unpublished results). The discrepancy between the results obtained in wasps and ants and in the honey bee could reflect a unique kin-recognition ability of the latter. Alternatively, we suggest that the composition of experimental colonies containing artificially low numbers of patrilines, of artificially high phenotypic distinctiveness, may yield nepotism as an artefact[10].

Apparent kin recognition can easily arise as the accidental by-product of different discrimination mechanisms based on genetically correlated cues (such as species or mate recognition)[11]. Social insects are acutely sensitive to foreign odours normally used to identify and reject non-nestmates from the colony. Honey-bee workers in colonies of artificial phenotypic heterogeneity may perceive their peculiar nestmates as smelling more like foreigners than like ordinary nestmates. Discrimination in such behaviours as worker interactions, swarming and queen brood rearing may result, not from a preference for one's own patriline, but from a bias against the other, though not reaching the threshold necessary for rejection[7,10]. Strange-smelling larvae may in addition elicit discrimination that is normally directed against diseased brood, though again below the threshold for removal; such behaviour is known to be important for disease resistance in honey bees[12].

Various experimental methods have been used to form genetically mixed colonies of honey bees, all of which combine a diversity of phenotypes which may not typically coexist. In early studies, colonies containing multiple kin groups were formed by adopting brood between foreign nests, of known or assumed relatedness[2,3]. An apparent improvement was introduced by artificially inseminating a queen with drone sperm bearing heritable colour markers, yielding half-sibships visually distinguishable to the investigators (for example, refs 4–8). Because bees recognize relatives by olfaction, the colours themselves were usually assumed not to affect recognition responses. But marker genotypes have been shown to alter the bees' odour phenotypes as well. When a queen heterozygous for a recessive marker is inseminated with sperm from a single recessive drone, the resulting two worker groups exhibit some preference for nestmates of their own colour, though all are full sisters[13].

Use of allozyme markers[1] rather than colours does not eliminate the problem without evidence that the variation between genotypes at other loci is comparable to that among offspring of a naturally mated queen. Particularly if the marker-carrying lines used were originally drawn from different populations, they may well differ in a variety of metabolic traits that exaggerate their olfactory discriminability. In addition, the number of patrilines in these experiments has been limited to two or three, the number of genetic markers available. In nature, honey-bee queens reportedly mate with 7–17 males[14], presenting workers with a more daunting discriminative task. One study indicates that the nepotistic behaviour observed in two-patriline colonies disappears in colonies containing seven or eight lines[8].

The degree of discrimination observed within honey-bee colonies is generally weak, never reaching a 2:1 preference for full sisters. Yet workers that are able to distinguish among partrilines should maximize their inclusive fitness by aiding full sisters exclusively, particularly when rearing queen larvae — assuming genotypic equivalence in reproductive value. (Feeble full-sister larvae should not be exclusively preferred to vigorous half-sisters. But variation in brood vigour would never lead multiple worker lines to prefer full sisters; rather, each line should prefer the same larvae.) Selection for the collective efficiency of all lineages has been suggested as a brake on kin-group selfishness within the colony[1], but it is unnecessary to invoke adaptation on the colony level to account for the pattern. Biases are expected to be weak when kin recognition is not itself under selection, but is a by-product of another genetic discrimination system[11]. By contrast, stronger patrilineal differences are reported in studies of genetic influence on honey-bee division of labour, a phenomenon which has also been documented in colonies containing naturally mated queens[15].

Finally, kin recognition can also be induced within ant colonies of artificial phenotypic heterogeneity. When *Camponotus floridanus* workers originating from distant locales (Tallahassee and the Florida Keys) are experimentally adopted into one nest, they detect and persistently investigate nestmates from the other population, with a bias of 1.12:1 (ref. 16). Consistent with the hypothesis that discrimination represents not nepotism, but a side-effect of inter-colony hostility, workers use their recognition ability to bias weak aggression toward alien nestmates, but fail to prefer sisters in cooperative food exchanges and grooming. Given the apparent absence of kin recognition in natural colonies of ants and wasps, and the inconclusiveness of the evidence for honey bees, we would argue that adaptive nepotism among nestmates has still not been demonstrated in any social insect.

NORMAN F. CARLIN
PETER C. FRUMHOFF
Museum of Comparative Zoology Laboratories,
Harvard University,
Cambridge, Massachusetts 02138, USA

PAGE *ET AL.* REPLY—Carlin and Frumhoff suggest that a reduced number of subfamilies ('partrilines' derived from different fathers) may lead to atypical behaviour, citing the study of Hogendoorn and Velthuis[8] as evidence. Hogendoorn and Velthuis established colonies that had two distinguishable subfamilies, yellow and black, and other colonies that had seven or eight subfamilies, one yellow and six or seven black. They then observed the feeding and 'aggressive biting' interactions of individuals within these colonies to determine if food was being preferentially exchanged among members of the same subfamily and if aggressive biting occurred between individuals of different subfamilies.

We calculated biases directly from the marginal totals of their contingency tables; in all four trials of two subfamilies and three of four trials of seven and eight subfamily colonies, the biases were in the direction expected if preferential kin discrimination was occurring. The bias was statistically significant for one trial ($P < 0.01$) of an eight-subfamily colony while the other three trials lacked sufficient statistical power to test any effect because sample sizes were too small (8, 22 and 3 observations of yellow–yellow worker interactions, respectively). We believe that Carlin and Frumhoff's interpretation of ref. 8 to show a lack of discrimination in worker interactions is incorrect.

The suggestion that the use of genetic markers affects recognition directly or by linkage with genes that affect recognition, is important and plausible. But Frumhoff in his unpublished study[13] did not resolve whether the single, recessive gene marker used, or genes linked to that marker, affected recognition. This kind of linkage effect does not influence the reported kin-recognition studies because the markers and their associated linkage groups are distributed randomly within each subfamily; subfamily composition is determined by the paternal genomes. Linkage is not an issue for subfamily recognition because all paternal genes are linked, a

consequence of haplodiploidy.

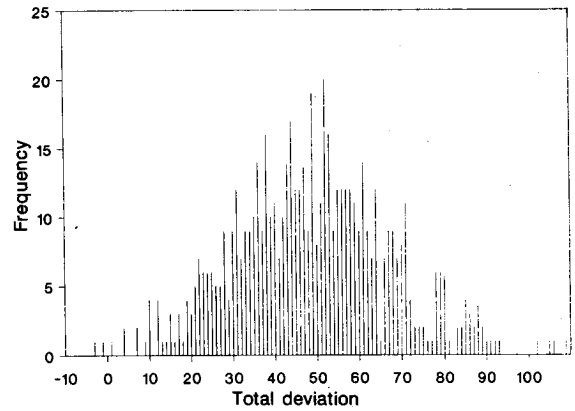
The real issue associated with genetic markers and kin-recognition studies is whether the markers themselves can affect recognition. Colour and allozyme markers occur naturally and are polymorphic in most populations. Perhaps the most significant argument against Carlin and Frumhoff is that studies using allozymes, single-gene recessive mutations and polygenic integument colour yield the same kind of result as studies that use naturally mated queens and no markers at all[18].

Genetic manipulation may result in greater genotypic diversity in colonies than would occur under natural conditions, though there is no evidence for this in honey bees. Greater phenotypic diversity may increase the experimental resolution of phenomena that occur at very low levels in colonies with less diversity, rather than introduce artefacts. These low-level effects that have been so frequently reported are themselves intriguing evolutionary puzzles.

Experimental evidence does exist to support the conclusion that worker honey bees can recognize and discriminate among nestmates from different subfamilies under experimental conditions. Although everyone acknowledges the limitations of their experiments, there is no evidence that these limitations introduce experimental artefacts.

ROBERT E. PAGE JR
Department of Entomology,
University of California,
Davis, California 95616, USA
MICHAEL D. BREED
Department of Environmental, Population and Organismic Biology,
University of Colorado,
Boulder, Colorado 80309, USA
WAYNE M. GETZ
Department of Entomology,
University of California,
Berkeley, California 94720, USA

SIR—Page *et al.*[1] recently claimed that honey bees working on queen cells bias the rearing of queens in favour of supersisters rather than half-sisters. To test the statistical significance of the finding, Page *et al.* identified "nepotistic" subfamilies (those with highest ratio of adults on queen cells relative to larvae), and summed deviations between expected queen frequency (based on larval frequency) and observed queen frequency of these subfamilies for 30 trials. Page *et al.* then used a computer simulation to generate a population with subfamily frequencies equal to estimates from pooled experimental larvae and queen frequencies, drawing experiment-sized samples for larvae, queens and adults. The simulated nepotistic subfamily was determined as described, and deviation in frequency of these subfamilies between queens and larvae were calculated for 30 simulated trials and summed. The probability of obtaining simulated total deviations as extreme as that observed was estimated as the per cent of 1,000 simulations in which the total deviation exceeded 60.



Simulation was used to generate 600 data sets each of 3 trials of 10 colonies of 3 subfamilies at equal frequency. The frequency distribution of simulations which produced each deviation in 'observed' and expected numbers of queens for nepotistic subfamilies is plotted. Page *et al.* observed a deviation of 60 queens for their experimental data. The figure shows that this is well within the range expected by chance. Data sets were also generated for colonies with differing subfamily relative frequency. Results obtained were similar to those in figure.

The simulation performed by Page *et al.* supports the nepotism hypothesis, but this probably stems from the interaction of two experimental and two simulation deficiencies. The experimental deficiencies are the small sample sizes and that 6 of the 10 experimental queens were heterozygous for MDH, necessitating allocation of subfamily affinity on the basis of probability. These shortcomings mean that subfamily frequencies were estimated with high sampling error.

The simulation deficiencies are: first, for the real data, the wrong subfamily will be designated nepotistic when, through sampling error, its relative frequency is underestimated in larvae, causing large deviations between observed and expected frequencies in queens. Incorrect subfamilies will be chosen less often in the simulations, because all genotypes are known, and the sample size is larger. Second, worker and queen samples are pooled to provide estimates of simulated subfamily frequencies. (Expected values for the observed data were calculated from larval frequencies alone.) The weighted average is inevitably intermediate between queen and larval frequencies, thereby reducing the magnitude of deviations between frequencies in the simulated queen and larval samples. Thus the total deviation will appear smaller for the simulated data than the observed. If identical procedures are used for the observed data (queen frequencies are predicted from pooled larval and queen frequencies), then the total number of excess queens is −2, indicating no nepotism.

We generated 600 random 'data' sets drawn from a population of three subfamilies at equal frequency and analysed them using the procedure of Page *et al.* (see figure). Of our data sets, 26 per cent had deviations exceeding 60. We then "estimated the probability of getting a deviation that was as great or greater than our results", using the Page *et al.* simulation. Of our data sets, 84 per cent produced a 'significant' deviation. Thus even data drawn from a totally non-nepotistic population can exhibit 'statistical significance' with these procedures.

There is no evidence in the data of Page *et al.* that subfamily relative frequencies differ significantly between workers and queens, which they should if nepotistic rearing occurred. It is suggested that nepotistic subfamilies are genetically predisposed to rear queens. But nepotistic subfamily identity varies within colonies, suggesting that genetic determination of nepotism does not exist.

BENJAMIN P. OLDROYD
THOMAS E. RINDERER
Agricultural Research Service,
US Department of Agriculture,
Baton Rouge, Louisiana 70820, USA
STEVEN M. BUCO
Statistical Resources,
7332 Highland Road,
Baton Rouge, Louisiana 70808, USA

PAGE AND ROBINSON REPLY—The ability of worker honey bees to discriminate among queen larvae on the basis of kinship has been controversial since two papers were published in 1984, when R.E.P. and Erickson reported[19] that workers could distinguish between nestmate and non-nestmate larvae while Breed, Velthuis and G.E.R.[2] failed to find evidence for discrimination. These and other subsequent studies (for example, refs 3, 4) can certainly be criticized on the basis of some artificiality introduced by the experimental methods, criticisms usually stated by the authors themselves (see ref. 17).

Oldroyd *et al.* correctly point out that our demonstration[1] of nepotistic queen rearing is the result of a sampling bias in the Monte-Carlo simulation we used. We have now reanalysed some of our original data using more conventional statistical methods that are free of the biases present in the simulation model. Full details are available on request from R.E.P.

Behavioural heterogeneity within honey-bee colonies is in part a consequence of colony genetic structure. In our study, this was most dramatically demonstrated by the difference in the subfamily composition of the adult workers sampled on queen cells (presumed to be engaged in

queen care) and the composition of samples of worker larvae (presumed to be the pool from which larvae were drawn for workers to select among as queens, or remove). This heterogeneity probably results from patterns of sperm use by queens resulting from the incomplete mixing of sperm from their many mates, or from behavioural biases that are a consequence of the genotypes of workers. The effect of genotype is demonstrated by comparing the subfamily representation of adult workers sampled on queen cells versus those sampled on worker brood. Differences in these distributions are significant in 4 of 10 colonies and highly significant for all colonies combined (our unpublished data, available from R.E.P.).

Worker larval samples differ from queen samples, but these differences are only marginally significant. Both queen and worker larval samples fluctuate significantly from trial to trial within colonies, but these fluctuations are different, suggesting that some additional nonrandom process is affecting the subfamily representation of queens. We suggest that this process involves discrimination during queen rearing.

Our results show that honey-bee colonies have a genotypic and behavioural structure that could favour nepotism during queen rearing, provided that larvae are genotypically labelled, and workers have kinship-related information about larval labels. These necessary elements have all been reported elsewhere[17]. But a conclusive demonstration of nepotistic queen rearing remains elusive.

ROBERT E. PAGE JR
Department of Entomology,
University of California,
Davis, California 95616, USA
GENE E. ROBINSON
Department of Entomology,
University of Illinois,
Urbana, Illinois 61801, USA

1. Page, R.E., Robinson G.E. & Fondrk, M.K. *Nature* **338**, 576–579 (1989).
2. Breed, M.D., Velthuis, H.H.W. & Robinson, G.E. *A. ent. Soc. Am* **77**, 737–739 (1984).
3. Visscher, P.K. *Behav. Ecol. Sociobiol.* **18**, 453–460 (1986).
4. Noonan, K.C. *Ethology* **73**, 295–306 (1986).
5. Getz, W.M., Bruckner, D. & Parisian, T.R. *Behav. ecol. Sociobiol.* **10**, 265–270 (1982).
6. Evers, C & Seeley, T. *Anim. Behav.* **34**, 924–925 (1986).
7. Frumhoff, P.C. & Schneider, S. *Anim. Behav.* **35**, 255–262 (1987).
8. Hogendoorn, K. & Velthuis, H.H.W. *Naturwissenschaften* **75**, 412–413 (1988).
9. Queller, D.C., Hughes, C.R. & Strassmann, J.E. *Nature* **344**, 388 (1990).
10. Carlin, N.F. *Neth. J. Zool.* **39**, 86–100 (1989).
11. Grafen, A. *Anim. Behav.* **39**, 42–54 (1990).
12. Rothenbuhler, W.C. *Am. Zool.* **4**, 111–123 (1964).
13. Frumhoff, P.C. thesis (Univ. California, Davis, 1987).
14. Winston, M. *The Biology of the Honey Bee.* (Harvard Univ. Press, Cambridge, 1987).
15. Calderone, N.W., Robinson, G.E. & Page, R.E. *Experientia* **45**, 765–767 (1989).
16. Carlin, N.F., Hölldobler, B. & Gladstein, D.S. *Behav. ecol. Sociobiol.* **20**, 219–227 (1987).
17. Page, R.E. & Breed, M.D. *Trends ecol. Evol.* **2**, 272–275 (1987).
18. Breed, M.D., Butler, L. & Stillerr, T.M. *Proc. natn. Acad. Sci. U.S.A.* **82**, 30058–3061 (1985).
19. Page, R.E. & Erickson, E.H. *A. ent. Soc. Am.* **77**, 578–580 (1984)