

from a single recessive drone, the resulting two worker groups exhibit some preference for nestmates of their own colour, though all are full sisters¹³.

Use of allozyme markers¹ 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 patrilineages 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 males¹⁴, 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 lines⁸.

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 patrilineages 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 colony¹, 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 system¹¹. 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 queens¹⁵.

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 coopera-

tive 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.

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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 Velthuis⁸ 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 study¹³ 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 all¹⁸.

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.

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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