

Research article

Patriline differences in emergency queen rearing in the honey bee, *Apis mellifera*

N. Châline¹, G. Arnold², C. Papin³ and F.L.W. Ratnieks¹

¹ Laboratory of Apiculture & Social Insects, Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK, e-mail: n.g.chaline@sheffield.ac.uk, f.ratnieks@sheffield.ac.uk

² CNRS UPR 9034, Laboratoire Populations, Génétique et Evolution, 91198 Gif-sur-Yvette, France, e-mail: arnold@pge.cnrs-gif.fr

³ Institut Alfred Fessard, CNRS, 91198 Gif-sur-Yvette, France

Received 10 February 2003; revised 7 March 2003; accepted 17 March 2003.

Summary. In the polyandrous honey bee, *Apis mellifera*, workers can potentially increase their inclusive fitness by rearing full-sister queens. If the mother queen dies suddenly, workers feed a few larvae in worker cells with royal jelly and rear them into queens (emergency queen rearing). Using DNA microsatellite markers we determined the patriline of emergency queens reared in two colonies headed by naturally-mated queens before being made queenless. We found that some patrilines were reared more than others in one colony, but not in the other. These differences between colonies suggest that selective rearing is not always present and this might explain the mixed results of previous nepotism studies in the honey bee.

Key words: *Apis mellifera*, nepotism, kin recognition, queens, microsatellites.

Introduction

Queen honey bees, *Apis mellifera*, mate with many males (Estoup et al., 1994; Arnold et al., 1996; Oldroyd et al., 1997). Within a colony, workers are related to new queens by 0.75 (full-sister) or 0.25 (half-sister). New queens are reared in three contexts: swarming, supersedure of a failing queen, and emergency (if the mother queen dies suddenly). In emergency queen rearing, only a few larvae among the numerous ones available in worker cells are selected by workers to rear as queens. These larvae are then fed royal jelly and the worker cell is transformed into a queen cell. Because of relatedness differences, workers can potentially gain a threefold increase in inclusive fitness if they can cause a full-sister to head a new colony instead of a half-sister (Visscher, 1998). This can lead to reproductive conflict between nestmates if workers can discriminate between half-sister and full-sister

larvae. Arnold et al. (1996, 2000) have shown that the cuticular hydrocarbons of adult workers vary more among patrilines than within patrilines which suggests that there is sufficient variability for workers to discriminate between full and half-sisters, at least for some patrilines. Despite numerous studies, evidence of nepotism in honey bee queen rearing remains controversial and only weak nepotism, or absence of nepotism, has been observed (reviewed in Breed, 1994; Visscher, 1998). Early studies of kin recognition were hindered by a lack of sufficient genetic markers to assess kinship in colonies headed by naturally-mated queens, which have many patrilines. The use of DNA microsatellites now allows the precise assessment of kinship in natural colonies (e.g. Estoup et al., 1994; Palmer and Oldroyd, 2001; Châline et al., 2002) and has greatly facilitated the study of kinship and reproductive conflict in honey bees (e.g. Franck et al., 2002; Tilley and Oldroyd, 1997; Wattanachaiyingcharoen et al., 2002).

Three studies using microsatellites have so far studied nepotism in emergency queen rearing. Tilley and Oldroyd (1997) have shown that in long-established queenless colonies with repeated introductions of young brood, some patrilines were preferentially selected. Osborne and Oldroyd (1999) suggest that this unequal representation of patrilines could be caused by nepotism. On the other hand, Franck et al. (2002) failed to find any patriline differences between worker and queen proportions.

We conducted a similar experiment to that of Tilley and Oldroyd (1997). The patrilines of emergency queens reared after queen removal were determined in two colonies. There was a significant difference between patriline representation in workers and emergency queens in only one colony. These results show that selective queen rearing sometimes occurs and sometimes does not and helps explain why mixed results have been obtained previously.

Material and methods

Sample collection

Two *Apis mellifera* colonies (A and B) with brood and a one-year-old queen kept at the Laboratoire de Neurobiologie Comparée des Invertébrés (LNCI, INRA-CNRS, 91440 Bures-sur-Yvette, France) were studied in 1997. Emergency queen rearing was initiated by removing the queens. The colonies were kept queenless until they contained sealed queen cells. The queen pupae were then collected and placed at -20°C for DNA analyses. At the same time as the queen pupae were removed, we also removed a comb of sealed brood from each colony and placed it in an incubator at 35°C to collect emerging workers. The workers and the queens came from eggs laid at the same time. The mother queens were then reintroduced to their respective colonies. Five weeks later, when the queen had resumed normal egg-laying, we repeated the process above. We repeated the procedure a third time on colony A but the second reintroduction of colony B's queen failed. We did not collect additional samples of workers as Estoup et al. (1994) and Tilley and Oldroyd (1997) have shown that colony kin composition remains stable over short periods.

DNA extraction and microsatellite analyses

DNA was extracted according to Garnery et al. (1990) using proteinase K and phenol. To determine the patrilines of queens and workers, we used three highly polymorphic microsatellite markers (A76, A29 and

B124 identified by Estoup et al., 1994). Polymerase chain reactions followed Estoup et al. (1994). The patriline of each sample was determined using standard procedures (Estoup et al., 1994).

Statistical analyses

We pooled the samples of queen larvae from the different trials for each colony because the sample sizes of some trials were very low (8 and 14). Because the expected values in the contingency tables were low, we analysed the data using Fisher's exact test with an exact method using the program 'Monte Carlo RxC 2.2' developed by W. Engels, University of Wisconsin.

Results

We analysed 136 workers and 66 queens (respectively 14, 28 and 24 for each trial) in colony A and 212 workers and 34 queens (respectively 26 and 8) in colony B. Colony A had 13 patrilines and colony B had 7. There was a significant difference in the patriline composition of queens and workers in colony A ($p = 0.008$, Fisher's exact test), but not in colony B ($p = 0.194$, Fisher's exact test) (Fig. 1). In colony A, one patriline, K, accounted for 19 of the 66 queens (28.8%). If this patriline is removed from the analysis, no significant differences can be found in the distribution of queens and workers of the other patrilines in colony A ($p = 0.281$ Fisher's exact test).

Discussion

Our results show that in emergency queen rearing the proportion of emergency queens and workers can differ in their patriline composition, but that the pattern is not consistent among colonies. It also seems that only a few patrilines are preferred over others, such as patriline K in colony A. Our results confirm, although with slightly different methods, the findings of Tilley and Oldroyd (1997). Contrary to Tilley and Oldroyd (1997), the queen was reintroduced in the test colonies and allowed to re-establish normal egg-laying between samples. Thus, our experiment allowed the different trials to be done in a more natural situation with the queens present in the colonies before each sample.

Our study does not investigate why some patrilines are reared more than others and the actual reason is unclear. It could result from nepotism by rearing workers, or because larvae vary heritably in their attractiveness as potential queens ("royalty alleles"; Osborne and Oldroyd, 1999). However, Osborne and Oldroyd (1999) failed to find any evidence for the "royalty alleles" when rearing queens in related and unrelated colonies. In our study, both possibilities could explain why patriline K, one of 13 patrilines and representing only 8% of the workers, was highly preferred, representing 28% of the queens in colony A. The variability in the expression of preferences between colonies also occurred in Osborne and Oldroyd's (1999) study. This suggests that patriline preference, be it caused by nepotism or "royalty alleles", is a weakly expressed and relatively rare and polymorphic

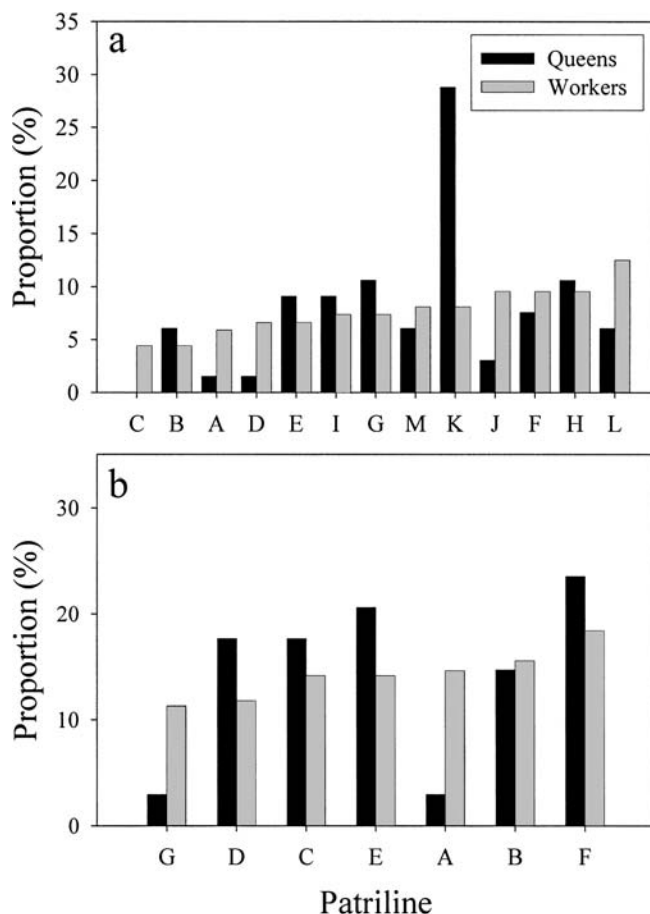


Figure 1. Percentage of each patriline in the worker and queen samples from colony A (a) and B (b).

trait in the honey bee. The number of queens reared from each patriline was not related to its relative size (Fig. 1), which refutes the hypothesis that the patriline with the most workers dominates queen rearing and preferentially rear full-sister larvae (Tilley and Oldroyd, 1997).

Arnold et al. (2000) have shown that, in a colony environment, cuticular hydrocarbon profiles of adult workers from different patrilines often become less distant while some patrilines remain very distinct and this could explain disparities between the different patrilines if a similar pattern occurs in larvae. Such polymorphism between nepotistic and non-nepotistic patrilines can be maintained if kin discrimination is associated with some costs to colony efficiency (Ratnieks and Reeve, 1991) and it would be the same for royalty alleles. Our study adds some evidence for the presence of reproductive competition in queen rearing in the honey bee, at least during emergency queen rearing, but it also shows that much variation occurs among colonies and so may help explain the mixed results in the previous studies.

Acknowledgements

We thank Jean-Francois Odoux for help with the beekeeping, Dominique Huet for assistance in the laboratory. This work was funded by the Institut National de la Recherche Agronomique (INRA) and the Centre National de la Recherche Scientifique (CNRS).

References

- Arnold, G., B. Quenet, J.M. Cornuet, C. Masson, B. DeSchepper, A. Estoup and P. Gasqui, 1996. Kin recognition in honeybees. *Nature* 379: 498–498.
- Arnold, G., B. Quenet and C. Masson, 2000. Influence of social environment on genetically based subfamily signature in the honeybee. *J. Chem. Ecol.* 26: 2321–2333.
- Breed, M.D., C.K. Welch and R. Cruz, 1994. Kin discrimination within honey bee (*Apis mellifera*) colonies – an analysis of the evidence. *Behav. Proc.* 33: 25–39.
- Châline, N., F.L.W. Ratnieks and T. Burke, 2002. Anarchy in the UK: detailed genetic analysis of worker reproduction in a naturally occurring British anarchistic honeybee, *Apis mellifera*, colony using DNA microsatellites. *Mol. Ecol.* 11: 1795–1803.
- Estoup, A., M. Solignac and J.M. Cornuet, 1994. Precise assessment of the number of patrilines and of genetic relatedness in honeybee colonies. *Proc. R. Soc. Lond. B* 258: 1–7.
- Franck, P., M. Solignac, D. Vautrin, J.M. Cornuet, G. Koeniger and N. Koeniger, 2002. Sperm competition and last-male precedence in the honeybee. *Anim. Behav.* 64: 503–509.
- Garner, L., D. Vautrin, J.M. Cornuet and M. Solignac, 1990. Phylogenetic relationships in the genus *Apis* inferred from mitochondrial DNA sequence data. *Apidologie* 22: 87–92.
- Palmer, K.A. and B.P. Oldroyd, 2001. Mating frequency in *Apis florea* revisited (Hymenoptera, Apidae). *Insect. Soc.* 48: 40–43.
- Oldroyd, B.P., M.J. Clifton, S. Wongsiri, T.E. Rinderer, H.A. Sylvester and R.H. Crozier, 1997. Polyandry in the genus *Apis*, particularly *Apis andreniformis*. *Behav. Ecol. Sociobiol.* 40: 17–26.
- Osborne, K.E. and B.P. Oldroyd, 1999. Possible causes of reproductive dominance during emergency queen rearing by honeybees. *Anim. Behav.* 58: 267–272.
- Ratnieks, F.L.W. and H.K. Reeve, 1991. The evolution of queen-rearing nepotism in social Hymenoptera: effects of discrimination costs in swarming species. *J. Evol. Biol.* 4: 93–115.
- Tilley, C.A. and B.P. Oldroyd, 1997. Unequal subfamily proportions among honey bee queen and worker brood. *Anim. Behav.* 54: 1483–1490.
- Vissscher, P.K., 1998. Colony integration and reproductive conflict in honey bees. *Apidologie* 29: 23–45.
- Wattanachaiyingcharoen, W., B.P. Oldroyd, G. Good, L. Halling, F.L.W. Ratnieks and S. Wongsiri, 2002. Lack of worker reproduction in the giant honey bee *Apis dorsata* Fabricius. *Insect. Soc.* 49: 80–85.



To access this journal online:
<http://www.birkhauser.ch>