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Evidence for intra-colonial genetic variance in resistance to American foulbrood of honey bees (*Apis mellifera*): further support for the parasite/pathogen hypothesis for the evolution of polyandry

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Abstract Explanations for the evolution of multiple mating by social insect (particularly honey bee) queens have been frequently sought. An important hypothesis is that multiple mating is adaptive because it increases intracolony genetic diversity and thereby reduces the likelihood that parasites or pathogens will catastrophically infect a colony. We tested one assumption of this model: that honey bee worker patriline should differ in disease resistance. We used American foulbrood (caused by the bacterium *Paenibacillus larvae*) as a model pathogen. We found that patrilines within colonies do indeed vary in their resistance to this disease.

Introduction

Multiple mating (polyandry) by social insect queens increases intracolony genetic variability and decreases intracolony relatedness. This has important consequences for such fundamental colony characteristics as sex allocation (Crozier and Pamilo 1996) and the genetic framework within which cooperation and conflict over queen and worker reproduction of males is played out (Boomsma and Ratnieks 1996). Furthermore, polyandry runs counter to kin-selection explanations for the maintenance of sociality in social insects because polyandry reduces intracolony relatedness (Hamilton 1972; Crozier and Pamilo 1996; Strassmann 2001). Thus polyandry is considered the derived condition for social insects.

While most social insect species are monandrous, a few species from several of the major groups are highly polyandrous. The prime examples are yellow jacket wasps *Vespa* (Ross 1986), leafcutter ants *Atta* (Fjerdingstad et al. 1998) and *Acromyrmex* (Reichart and

Wheeler 1996) and honey bees *Apis* (reviewed in Palmer and Oldroyd 2000). The most extreme example of multiple mating is found in *Apis dorsata*, in which queens mate with more than 100 drones (Wattanachaiyingcharoen et al. 2003). Queens receive no immediate benefit such as nuptial gifts or access to territory from such behaviour, despite an apparent (but not well quantified) cost associated with prolonged or repeated mating flights (Moritz 1985). Thus, reasons for the apparent paradox of polyandry have frequently been sought (Palmer and Oldroyd 2000; Strassmann 2001)

There are many hypotheses for the evolution of multiple mating by social insect queens in general and honey bees in particular (e.g. reviewed in Crozier and Page 1985; Palmer and Oldroyd 2000; Crozier and Fjerdingstad 2001) but no universally applicable conclusion has yet been reached (Strassmann 2001). One ‘front-running’ hypothesis is that ‘polyandry increases genetic variation within colonies, thereby reducing the likelihood that parasites or pathogens will diminish the worker population to the point of jeopardizing the colony’s survival or reproduction’ (Sherman et al. 1988). This hypothesis has found empirical support in bumble bees (Baer and Schmid-Hempel 1999) and honey bees (Tarpay 2003), and a comparative analysis of ant species showed a positive correlation between intracolony relatedness and parasite load (Schmid-Hempel and Crozier 1999).

Intracolony genetic variance could reduce costs from parasites and pathogens in at least two ways. The first plausible mechanism (Parasite and pathogen hypothesis 1, PPH-1) is analogous to the generally accepted explanation for the evolution of sexual reproduction, the so-called ‘Red Queen’ hypothesis (Maynard Smith 1971; Jaenike 1978; Lively 1987; Ebert and Hamilton 1996). As with the Red Queen hypothesis, the proposed advantage of polyandry arises from reduced parasite transmission within genetically diverse colonies. Under this scenario, patrilines (full sister workers that share the same father) differ in their resistance to relevant parasites and pathogens due to specific gene-for-gene interactions between host and pathogen (Kraus and Page 1998). In

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Table 1 Predictions under the parasite/pathogen hypothesis (PPH) (Sherman et al. 1988). Under both sub-hypotheses, genetically diverse colonies should show higher overall disease resistance or less severity of disease

Hypothesis	Mechanism of resistance	Variation among patrines in disease presence	Variation among parasites
PPH-1	Specific gene-for-gene	Yes when infected by a single pathogen strain	Highly variable strains showing variation in infectiousness on a uniform host
PPH-2	General resistance based on variation in host behaviour	Unlikely	Less significant than under PPH-1

the case of gene-for-gene interactions between hosts and parasites, a genetically diverse colony could reduce the spread of a parasite/pathogen, whereas the same parasite could proliferate within a genetically uniform colony that consists entirely of susceptible hosts (see Table 1). PPH-1 assumes that characteristics of the parasites and pathogens afflicting colonies in successive generations are unpredictable (Sherman et al. 1988) which 'forces queens to mate with many males because they cannot reliably choose one male carrying resistance to the particular diseases that may afflict their workers, immatures or both' (Sherman et al. 1988). Coevolution between parasites and hosts maintains genetic variance in resistance (Sherman et al. 1998). Thus, under PPH-1, we would expect to see genetically diverse colonies having significant differences among patrines in the incidence of diseases.

The second mechanism (PPH-2) is more general, and suggests that behavioural diversity (such as hygienic behaviour in honey bees) increases colony-level resistance to common pathogens (Sherman et al. 1998). Tasks like corpse removal and cell cleaning undoubtedly contribute to disease resistance at the colony level. Genetically mediated variance in the probability that individuals will perform tasks is well documented (Robinson 1992) and, theoretically at least, this variance can increase colony fitness (Fuchs and Moritz 1999; Moritz and Fuchs 1998; Frank 1999). Under this hypothesis, we would not expect to see variance in disease incidence among patrines. However, we would still expect that genetically diverse colonies would tend to have a lower incidence or less severity of disease than genetically uniform ones (Table 1).

There is now experimental evidence from both bumble bees (Liersch and Schmid-Hempel 1998; Baer and Schmid-Hempel 1999) and honey bees (Tary 2003) that colonies with high genetic diversity have lower rates of infection than colonies with low genetic diversity, suggesting that selective pressures from parasites and pathogens may have contributed to the evolution of polyandry in social insects. Evidence demonstrating genetic variability in pathogen resistance among patrines of honey bee colonies, would give further support for PPH-1.

We evaluated a necessary assumption of PPH-1: that there should be patrilineal differences in host resistance to common diseases (Sherman et al. 1988) using the widespread honey bee pathogen *Paenibacillus larvae*, causative organism of American foulbrood (AFB). Sher-

man et al. (1988) argued that evidence contrary to the requirement that worker patrines differ in resistance to relevant honey bee diseases would indicate that the PPH is an unlikely explanation for the evolution of extreme polyandry in honey bees. Other predictions about patterns of resistance under hypotheses PPH-1 and PPH-2 are provided in Table 1. We evaluate these predictions in the light of our finding that patrilineal differences in disease incidence do occur in honey bees.

Materials and methods

In October 1999, six colonies were established, each headed by sister queens artificially inseminated with semen from one drone from each of six unrelated colonies. On 31 January 2000, in the late afternoon, each queen was confined by a cage to an empty comb and then returned to her colony. The cage allowed free access by nurse workers, but not egress by the queens. The following morning, the queens were released and the combs re-caged to prevent further eggs from being laid in them, but allowing nurse workers access so they could rear the eggs. Four colonies (A–D) had eggs laid in both sides of comb upon which the queen was caged. The other two colonies had either no eggs or very few eggs and were not considered further. Two days later, when larvae were less than 24 h old, which was the time that they were most likely to succumb to infection (Woodrow 1942), one side of each comb was sprayed evenly across the comb with 5 ml of 0.25% saline solution containing 5×10^6 AFB spores per millilitre. These spores had been isolated from a single subcultured bacterial colony cultured on a sheep's blood agar plate containing naladixic acid (Hornitzky et al. 1996). Spores were confirmed as *Paenibacillus larvae* by gram staining and observation at 1,000 \times magnification.

On 9 February 2000, when larvae were 7 days old (i.e. sealed, but before symptoms occur; Bailey and Ball 1991), combs were placed in mesh bags to prevent infected individuals from being removed by nurse workers. The bagged combs were returned to their colonies. On 14 February 2000, when infection was obvious by visual inspection, the experimental comb was removed from each colony, wrapped in aluminium foil and stored at -70°C until it was examined.

The brood on the treated side of the comb was examined for disease signs. The presence of AFB clinical signs was determined in the first instance by visual inspection. A decayed, coffee-coloured pupa was taken as evidence of death caused by *P. larvae*. Where there was doubt over the disease status of an individual, we confirmed our diagnosis by gram staining and microscopic examination (Bailey and Ball 1991).

Patriline identification

Individual pupae (numbers in Table 2) were genetically analysed using microsatellites, according to the methods of Oldroyd et al. (2000), to deduce their patrilineal origin. Preliminary analyses showed that microsatellite loci A14 and A107 (Estoup et al. 1994)

Table 2 Percentage of each patriline infected with AFB and total infection in each colony, and results of contingency table tests of the hypothesis that there is no association between patriline and the incidence of American foulbrood disease in each colony; for all tests $df=5$

Patriline	Colony A	Colony B	Colony C	Colony D
1	12.8	13.6	8.3	0
2	15.4	6.2	4.2	27.3
3	10.3	18.5	33.3	36.4
4	7.7	17.3	8.3	27.3
5	46.2	29.6	25	0
6	7.7	14.8	20.8	9.1
Total	25.2%	15.8%	13.0%	6.5%
infection	($n=155$)	($n=514$)	($n=185$)	($n=170$)
χ^2	12.53	6.53	5.09	5.47
$P(\chi^2)$	0.03	0.26	0.40	0.36

combined were able to uniquely identify all patrilines in all colonies, so these loci were used throughout this study.

Data analysis

The frequency of workers infected was compared among patrilines within each colony using χ^2 contingency tests.

Results and discussion

Intracolony genetic variance for disease resistance is a fundamental requirement for PPH-1. Our results provide support for this hypothesis. Although, no colony showed significant differences in rates of infection among patrilines ($P>0.0125$ in all cases) when analysed using an level of significance of 5% for the whole experiment, all colonies provided some examples of extreme differences among patrilines in rates of infection (Table 2). Furthermore, colony A showed significant patrilineal differences on an individual colony basis (Table 2). Thus, despite our relatively small sample sizes in terms of the number of patrilines and colonies examined, we found differences among patrilines in disease incidence in an unselected (in terms of artificial selection) population of honey bees.

Under PPH-1 we expected (1) intracolony variation in host resistance to common parasites and pathogens, (2) variation in parasite virulence on a standard host and (3) that genetically variable colonies suffer less from disease than genetically uniform colonies (Table 1).

Our experiment provides support for the first expectation, and suggests that further experimentation with different pathogens is warranted. Data supporting expectation (3) have recently been published (Tarpy 2003). Testing expectation (2) should be relatively straightforward. For example, various isolates of AFB could be applied to larvae from an inbred, single-patriline colony. Under the assumptions of PPH-1 we would predict that isolates would vary in their rates of infectivity on the uniform host.

We do not wish to suggest that PPH-1 could be the sole reason for the evolution of extreme polyandry in honey bees. Behavioural mechanisms under PPH-2 may well be

as, or more, important contributors to selective pressures for polyandry. Furthermore, it seems very likely that the foremost selective pressure for polyandry arises from the genetic load imposed by the method of sex determination (Shaskolsky 1976; Page 1980; Kraus and Page 1998). However, our results give additional support to the hypothesis of Sherman et al (1988), suggesting that pressure from parasites may well mean that queens derive fitness benefits from multiple mating, and that further empirical tests of the relationship between patrilineal diversity and disease resistance would be worthwhile.

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