Genetic variation within social insect colonies reduces parasite load

Stephan Liersch and Paul Schmid-Hempel*

ETH Zürich, Experimental Ecology, ETH-Zentrum NW, CH-8092 Zürich, Switzerland

In colonies of social Hymenoptera (ants, bees and wasps), workers are often not very closely related to each other, because queens mate with several different males (polyandry) or because several functional queens are present (polygyny). Both characteristics increase genetic variation among the queens' reproductive and worker offspring, but the benefits of this increased variation remain obscure. Here we report an experiment where genetically homogeneous and genetically heterogeneous colonies of the bumble bee, *Bombus terrestris*, have been exposed to parasitism under field conditions. Colonies of high or low genetic variation were achieved by adding and removing brood from donor colonies. The results showed a consistent effect in that genetically variable colonies experienced reduced parasite loads, i.e. lower prevalence, intensity and parasite species richness, for a range of protozoa, nematodes, mites or parasitoids affecting the workers. We therefore propose that polyandry and/or polygyny of social insects may be beneficial under parasitism.

Keywords: polyandry; parasitism; polygyny; social insects; mating system; *Bombus terrestris*

1. INTRODUCTION

Honey bees provide some of the most striking examples of multiple mating behaviour with females mating and using sperm from 10–20, or even more, males (Seeley 1985; Oldroyd *et al.* 1995; Moritz *et al.* 1995; Boomsma & Ratnieks 1996; Crozier & Pamilo 1996). Similarly, in many ant species polygyny, i.e. the occurrence of multiple functional queens in the colony, is common, with pleometrosis (foundress associations) and secondary polygyny (the addition of queens to a colony) as the major types (Keller 1993; Bourke & Franks 1995). Both traits require an explanation as polygynous and polyandrous colonies have low degrees of among-worker relatedness. This can lead to within-colony conflicts over resource allocation or over reproductive opportunities among different patri- or matrilineal (Crozier 1979; Boomsma & Grafen 1991; Pamilo 1991; Sundström 1994) and runs counter to straightforward expectations of kin selection theory (Hamilton 1964). Thus, polygyny or promiscuous mating strategies are thought to hamper the evolution and maintenance of sociality by kin selection. In addition, it is to be expected that extra matings are costly in terms of time, energy, or risks of predation (Moritz 1985; Crozier & Pamilo 1996), or that per-capita reproductive output decreases with the presence of additional queens (e.g. Herbers 1984).

A number of hypotheses have therefore been suggested to account for the selective advantages of multiple mating or of multiple queens. For example, the resulting increase in genetic variation could allow the establishment of a more efficient division of labour due to better matched individual work profiles or to reduced worker-queen

conflict over sex allocation to offspring (e.g. Robinson 1992; Crozier & Page 1985; Ratnieks & Boomsma 1995). Multiple mating could also serve to secure a sufficient amount of sperm (e.g. Cole 1983). In contrast, pleiometrosis may have evolved because foundress associations often have higher probability of survivorship than a single foundress (e.g. Bartz & Hölldobler 1982). Secondary polygyny could also be favoured by ecological constraints that make independent founding costly, e.g. a limitation of nest sites or high risks of dispersal (Crozier 1979; Herbers 1993; Bourke & Heinze 1994).

An important class of hypotheses suggests that genetic variation within the colony mitigates against the effects of parasitism (Tooby 1982; Hamilton 1987; Sherman *et al.* 1988; Schmid-Hempel 1994). This would apply to both polyandry and polygyny. Consequently, Keller & Reeve (1994) suggested that both characteristics act 'compensatorily' such that polyandry correlates with monogyny and vice versa. The parasite hypothesis assumes that susceptibility to parasites is genotypically variable and that therefore genetically heterogeneous colonies are less parasitized and are less likely to fail and/or have higher success. Genotypic variation for parasite susceptibility is well documented for the honey bee (e.g. Bamrick 1964; Taber 1982; Rinderer *et al.* 1975; Kulincevic 1986). In the bumble bee *Bombus terrestris* L. evidence for genotype-genotype interactions exists for infectious trypanosome and microsporidian parasites (Shykoff & Schmid-Hempel 1991a; Schmid-Hempel & Schmid-Hempel 1993; P. Schmid-Hempel and R. Loosli, unpublished data) which are prevalent in natural populations (Shykoff & Schmid-Hempel 1991b; Durrer & Schmid-Hempel 1995). However, despite the widespread occurrence of polyandry and polygyny and despite the significance of parasitism for important issues in ecology, evolution and behaviour in

*Author for correspondence (psh@eco.umnw.ethz.ch).

general, empirical tests that would allow the evaluation of the variation-vs-parasite hypothesis are rare and have remained contradictory. For example, Woyciechowski *et al.* (1994) found no evidence of lower levels of infection by the microsporidium *Nosema apis* in multiply mated honey bee colonies. Shykoff & Schmid-Hempel (1991a), on the other hand, demonstrated in the laboratory that transmission of the trypanosome *Crithidia bombi* is reduced in experimentally assembled, genetically heterogeneous groups of bumble bee workers as compared to transmission among full sisters. In addition, certain genotypes were more susceptible to infection. Strong effects of colony genotype on the probability of infection and transmission of *C. bombi* were also confirmed by Schmid-Hempel & Schmid-Hempel (1993).

Bumble bees, *Bombus* spp., are annual social insects. Only the fertilized queens overwinter to start a new colony in spring. As the colony grows in numbers, tasks such as resource collection are taken over by the workers that thus become exposed to parasites. At the end of the colony cycle reproduction occurs when sexuals—females and drones—are produced. Before onset of winter the males die as does the rest of the colony (Alford 1975). Therefore, male bees only provide sperm but do not help in raising brood. Females of several species mate multiply (Hobbs 1967; Foster 1992; Estoup *et al.* 1995). Moreover, in two neotropical species, *B. atratus* and *B. transversalis*, multiple queens are present and can, at least temporarily, form polygynous colonies (Michener 1974).

Bumble bees are also host to a number of viral, bacterial, and protozoan diseases. In addition, nematodes, mites, parasitoids, wax moths and cuckoo bumble bees parasitize individuals, brood or nest provisions (Pouvreau 1973; Pouvreau 1974; Schmid-Hempel *et al.* 1990; MacFarlane *et al.* 1995; Schmid-Hempel 1998). In natural populations, parasite prevalences can be quite substantial (Schmid-Hempel *et al.* 1990; Shykoff & Schmid-Hempel 1991b; Durrer & Schmid-Hempel 1995). Parasite effects range from increased worker mortality, reduction in ovary size and reduced early colony growth (e.g. with the intestinal trypanosome *Crithidia bombi*), to a potentially disastrous devastation of colonies (e.g. by the microsporidian *Nosema bombi*). Earlier studies have provided evidence that colony success is affected by parasitism (Shykoff & Schmid-Hempel 1991c; Müller & Schmid-Hempel 1992, 1993).

Here, we report the results of an experiment that considerably extends earlier studies of the subject and where genetically heterogeneous or genetically homogeneous colonies of the bumble bee *Bombus terrestris* L. were tested under field conditions. This mimicked the effects of polyandry and/or polygyny as compared to monandry/monogyny. The results show a consistent effect of within-colony genetic variation reducing parasitism.

2. MATERIAL AND METHODS

In our experiments, we manipulated the average degree of relatedness among workers within colonies to create either homogeneous (all workers are full sisters) or heterogeneous colonies (not all workers are full sisters) by replacing brood. For this purpose, parasite-free colonies were raised in the laboratory from unrelated queens of *B. terrestris* caught in spring 1996 at various field locations in the Jura mountains. The resulting colo-

nies were allocated at random to one of three groups: heterogeneous ($n=12$ colonies), homogeneous ($n=11$), and donor colonies ($n=9$). The colonies of the first two groups (the experimental colonies) were placed in underground nests in our field site after the first brood had hatched. They could freely forage and develop under natural conditions. Also, the donor colonies were placed in the same manner at the same location in the same field site to ensure similar environmental conditions for their development, although these colonies were kept in flight cages to prevent cross-infections with the experimental colonies. Regular checks assured us that the donor colonies were indeed parasite-free. The field site was located at 600 m elevation in the Jura mountains, northwestern Switzerland, and is a typical bumble bee habitat with many flowering meadows.

The colonies were thus naturally exposed to parasitism in the field and followed over their life cycle. For this, the experimental colonies were checked at night for their general state and development every 2–3 days. On these occasions, a random subsample of approximately 10% of the workers per week were removed and freeze-killed for later dissection and inspection for parasites under the microscope. In our samples, the number of parasite species was not correlated with number of workers checked; therefore, no correction for sampling effort was made. Moreover, in intervals of two weeks, the experimental colonies were also manipulated as follows. First, all workers were removed and counted. These worker counts also served as a basis for the estimation of colony sizes. Then, the amount of brood (the number of pupae) of the colonies of the heterogeneous group was measured, an appropriate part removed and immediately replaced by an equal number of pupae originating from several (2–4) of the donor colonies. This was done so that after the manipulation the proportion of pupae from each donor colony and from the original colony was roughly the same (for example, with three donor colonies, each brood from each of the four colonies made up one quarter of pupae). Hence, the amount of brood remained the same but its composition had changed. As pupal development takes about two weeks, heterogeneous colonies contained a mixture of workers hatched from own and added brood in roughly similar proportions, leading to an estimated average worker relatedness of between $r \approx 0.12$ and $r \approx 0.25$ as compared to $r \approx 0.75$ in the homogeneous colonies. After the brood manipulation, the removed colony workers (minus the share of 10% taken as a sample for parasite checks) were returned to the nest. The added brood was readily accepted and cared for by the workers. We found no difference in background worker mortality between experimental groups, obvious conflicts, or any other aberrant behaviour. The homogeneous colonies were treated in the same manner, i.e. brood was removed but then re-inserted, to control for the effect of manipulation. Newly hatched workers from all broods were thus exposed to parasites affecting the adults in the same way. This procedure was applied four times per colony, and thus the experimental difference was maintained during almost the entire life cycle of the colonies in the field (which is around 6–10 weeks, following the emergence of the first brood). After the fourth manipulation, we continued to count workers and to take subsamples until the queen had died. In addition, heterogeneous and homogeneous colonies were placed pairwise in the field to randomize the effects of micro-habitat variation with respect to treatment.

We compared three measures of presence of parasites infecting the workers of the colony: prevalence of infection (percentage of parasitized workers), parasite load (number of different parasite

infections per average worker), and parasite richness (number of different parasite species found on or in the workers of the colony).

From a total of 23 experimental colonies, we excluded six from the final analysis of parasite load because in these colonies fewer than six workers could be reliably scored for parasites (a cutoff point suggested by previous experience in the system). This left ten heterogeneous and seven homogeneous colonies for the parasite data. However, the data from the excluded colonies showed the very same pattern and thus the reported estimates are conservative. One colony in the heterogeneous group could not be reliably scored for worker numbers and was thus excluded from that part of the analysis (done with the remaining 22 colonies). All analyses were done with SPSS 6.1 and data ln-transformed where necessary to normalize variances.

3. RESULTS

Genetically heterogeneous colonies had significantly lower prevalence, load and richness as expected from the parasite hypothesis (figure 1). Moreover, this pattern was consistent when considered separately for the different parasite categories, although the inevitably smaller numbers did not allow for separate statistical analyses. For example, parasite loads were consistently higher in homogeneous colonies than in heterogeneous colonies for: *Crithidia bombi* (Trypanosoma; 0.242 vs 0.160), *Nosema bombi* (Microsporidia; 0.052 vs 0.017), *Apicystis (Mattesia) bombi* (Gregarina; 0.052 vs 0.036), mites (0.150 vs 0.051), nematodes (0.024 vs 0.013), endoparasitic larvae of

conopid flies (Diptera; 0.042 vs 0.027), and miscellaneous parasites (0.013 vs 0.009) (sign-test with $n=7$, $p=0.01$). This consistency is particularly illuminating, given the many uncontrollable sources of variation involved in a field study. In fact, small differences in environmental conditions for brood in the donor colonies as compared to the experimental colonies may have existed, although we have no such indication from general development and appearance.

During the year of study, which was characterized by a wet and cool summer, the production of sexuals was too scarce to allow for a direct comparison. Therefore, we compared colony size attained, a well established correlate of colony reproductive success (Tschinkel 1993; Schmid-Hempel *et al.* 1993; Schmid-Hempel 1994). Heterogeneous colonies were larger than homogeneous colonies, although only weakly so (Mann-Whitney U -test, $z=1.664$, $p=0.048$ one-tailed, $n=22$ colonies).

4. DISCUSSION

Our results suggest that increased genetic variation within colonies of social insects, such as that resulting from multiple mating by females (polyandry) or the presence of multiple functional queens in the colony (polygyny), is advantageous with respect to parasitism under field conditions. It is furthermore remarkable that smaller parasite loads in variable colonies were found for a wide range of parasite types, irrespective of whether the parasite is transmitted by direct infection within the colony (for example, trypanosomes or microsporidia) or acquired by each individual independently outside (conopid flies). This suggests that transmission efficiency within the colony is not necessarily the important factor for explaining this difference. In fact, the respective hypotheses (e.g. Sherman *et al.* 1988; Schmid-Hempel 1994) do not specify any particular kind of parasitism but simply assume genotypic variation in susceptibility of workers.

We believe that the current study is the first demonstration of this kind. It corroborates the earlier findings of Shykoff & Schmid-Hempel (1991a) with experimentally assembled worker groups of *B. terrestris* in the laboratory that were exposed to the trypanosome *C. bombi*. Additional evidence for the importance of genetic variation comes from the comparison of a variety of ant species. In this case, the average genetic relationship among workers within colonies was found to be positively correlated with the number of parasite species known from the species (Schmid-Hempel 1998). This is as expected if genetic variation within the colony, i.e. lower degrees of average relatedness, reduces parasitism, although such comparative studies have their own advantages and problems (e.g. Read 1988). Similarly, Keller & Reeve (1994) found a negative association between polygyny and polyandry across a number of ant species and interpreted it in terms of the maintenance of sufficient genetic variation within the colony as defence against parasites. A proper comparative analysis of this pattern is still lacking, and Boomsma & Ratnieks (1996) could not confirm the result, but such a correlation would be expected under the variation-as-defence-against-parasites hypothesis.

With respect to polyandry, less attention has been paid to benefits that females gain by increasing variance among

Figure 1. Measures of parasite presence (bars) in the experimental colonies. Shown are (a) parasite prevalence in homogeneous (0.524 ± 0.223 , 95% C.I.) and heterogeneous colonies (0.270 ± 0.138) (t -test with arcsin-transformed values: $t=2.37$, $P=0.032$, d.f. = 15); (b) parasite load in homogeneous (0.575 ± 0.11 , S.E., $n=52$ scored workers) and heterogeneous colonies (0.312 ± 0.07 , $n=80$ workers) (t -test with ln-transformed values: $t=1.83$, $P=0.028$ one-tailed, d.f. = 15); and (c) average parasite richness in homogeneous (2.43 ± 0.43 species, S.E., $n=52$ scored workers) and heterogeneous colonies (1.50 ± 0.17 , $n=80$ workers) (t -test with ln-transformed values: $t=2.24$, $P=0.041$, d.f. = 15). Figures below axis are number of colonies in group.

offspring (e.g. Ridley 1988; Oldroyd *et al.* 1992; Ratnieks & Boomsma 1995) than to the benefits of mating with 'good' males (e.g. Birkhead & Parker 1996). The latter requires females to be able to choose their mates according to desired characteristics, either directly or by cryptic choice. These abilities have been demonstrated in many cases (e.g. Andersson 1994; Olsson *et al.* 1996; Eberhard 1996). Although an increase in offspring variance per se does not necessarily depend on choice, this may be a useful side effect. Nevertheless, multiple mating to increase variance among offspring requires that there be little or no sperm competition or that it be under some female control. In fact, severe sperm competition may result in particular males siring a majority or even all of the offspring despite polyandry (Olsson *et al.* 1996). The two processes differ in their consequences on sexual selection, population structure or the maintenance of genetic variation. It is therefore important to empirically distinguish their relative importances in natural systems.

Curiously enough, the study species used here, *B. terrestris*, is, according to current knowledge, mostly singly mated and monogynous in natural populations (Röseler 1973; Alford 1975; Estoup *et al.* 1995). However, this observation does not contradict or invalidate our findings. Here, we show that polyandry or polygyny would benefit females (queens) through its effects on genetic variation and parasite load. Whether or not polyandry and polygyny are maintained in any given population depends on additional factors such as colony size-specific reproductive success, the costs of additional matings or additional queens, or prevailing parasite pressure (Schmid-Hempel 1994; Bourke & Franks 1995). Nevertheless, the intriguing question arises why queens, despite the benefits demonstrated here, typically mate only once, particularly as the males are usually much more numerous at mating sites. Similarly, why is polygyny, e.g. foundress associations as found in more primitive eusocial bees (Michener 1974), not more common in the group?

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