Foraging activity and immunocompetence in workers of the bumble bee, *Bombus terrestris* L.

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**SUMMARY**

A fundamental assumption of coevolutionary models of host–parasite relations is that resistance is costly. Costs are envisioned as phenotypic or genetic trade-offs in the allocation of limited resources to specific tasks. Using workers of the bumble bee *Bombus terrestris* L., we tested experimentally whether foraging effort, an energetically costly and crucially important task that ensures colony growth, survival and reproduction, is costly in terms of reduced resistance against parasite attack. The experiment showed that indeed workers allowed to forage showed lower levels of immunocompetence, as measured by the degree of encapsulation of a novel antigen, than workers prevented from foraging activity.

1. INTRODUCTION

In host–parasite interactions successful infection and establishment is paramount for the parasite. However, defence is often just one of many concurrent needs that hosts have to fulfil. In addition, because the availability of resources that can be allocated to any one of these needs is generally limited, hosts face the problem of trading-off between self-maintenance, survival, growth and reproduction to maximize fitness (Stearns 1992). Hence, a basic assumption underlying the analysis of host–parasite dynamics and coevolution is that resistance against parasites is costly in terms of resource allocation (see, for example, Keymer & Read 1991). Although evidence is still sparse, negative genetic correlations between resistance and other fitness traits have been found (Simms & Fritz 1990). For example, studies in birds indicate that parental effort may phenotypically reduce immune-mediated parasite resistance (Apanius 1991; Norris et al. 1994).

Insect immunity involves humoral and cellular responses. With the cellular response, haemocytes can attach themselves to large parasites (Gupta 1986). In the process, the foreign object may become completely encapsulated, the cells melanize and the intruder is eventually isolated from host tissue. The ability to encapsulate is known to vary with genotype, degree of genetic relation and nutrition (Carton 1976; Carton et al. 1992; Tanada & Kaya 1993). Here, we ask whether this component of insect immune defence is reduced when some other demanding activity is performed. In particular, we asked whether in the bumble bee *B. terrestris* the ability to encapsulate a novel antigen is negatively related to foraging effort. Bumble bees are social insects where workers do not normally reproduce themselves (but see Owen et al. 1980) but instead forage for the colony. Foraging is linked to reproductive success, because colony growth depends on food provisioning ability and survival of workers, with larger colonies producing more offspring (Pomeroy & Plowright 1982; Müller & Schmid-Hempe1 1992, 1993). On the other hand, flight is an energetically costly activity in bees (Casey & Ellington 1989; Ellington et al. 1990) and this fact alone suggests that foraging must be costly. Whether encapsulation is limited by the same energetic constraints remains to be studied in more detail. But effects of nutrition on haemocyte numbers and the various physiological and metabolic responses to parasitization (Jones & Tauber 1952; Shapiro 1967 ; Hoffmann 1970; Thompson 1993) reflect processes that are likely to be involved in foraging too.

In the field, worker bumble bees are often attacked by parasitic flies (Conopidae, Diptera) that oviposit into the abdomen, with prevalences of over 50% in worker populations investigated in our study areas in Central Europe (Schmid-Hempe1 et al. 1990; Schmid-Hempe1 & Schmid-Hempe1 1991). Because the parasite kills its host within 10–12 days (P. Schmid-Hempe1 et al., unpublished results) an increase in worker mortality rates results in negative effects on colony development and reproductive success (Müller & Schmid-Hempe1 1992, 1993). Therefore, we consider conopid parasitism to be an important selective factor in the natural environment of bumble bees in temperate areas.

2. MATERIALS AND METHODS

Conopid parasitism was used as a model system to analyse the relation between immunocompetence and foraging activity in bumble bees. To experimentally mimic parasitism, we assessed the immunocompetence of workers by implanting an artificial parasite ‘egg’, a piece of nylon of similar physical dimensions to conopid eggs and measured the subsequent encapsulation response after one full day of foraging or non-
foraging activity respectively. This method is similar to that used in the earlier studies of Vinson (1974) and Salt (1980). The artificial parasite egg has the advantage of stimulating host immunity without altering host metabolism to satisfy its requirements. Moreover, there is good evidence that parasites and their hosts are locally co-adapted in their encapsulation response (see, for example, Bouletreau 1986), or that parasitoids may sometimes escape encapsulation altogether (see, for example, Vinson 1974; Salt 1980; Stoltz & Cook 1983). For this reason, immunocompetence, defined as the potential of the host to defend itself against parasitism, is probably best measured by the defence reaction against a novel, ‘passive’ and standardized antigen, rather than by the reaction against a coevolved, interactive, actively evading and variable parasitic organism.

For this experiment, three colonies of _B. terrestris_ were placed inside a building but connected with an entrance tube to a window so that free access to the outside field for the workers existed. In our study, the foraging animals could move in and out of the colony freely and follow their normal daily routine. Bees assigned to the non-foraging treatment were wing-clipped by removing about half of the wing area. Consequently, these bees stayed inside the hive during the same period of time and could follow their in-hive activities. Bees were individually marked at eclosion and used for the tests at 7–10 days of age, their normal age when foraging. Each morning during the duration of the experiment (June–July 1994), bees of the appropriate age classes were collected, randomly assigned to the treatments and wing-clipped as necessary. A piece of nylon (diameter 0.16 mm, length 0.8–1.0 mm) was then implanted between the third and fourth sternite, such that it was fully exposed to the circulating haemolymph. After recovery from the insertion (a few minutes), the workers did resume their normal activity during the day, according to treatment. In the evening, after 13 h of daylight activities, the experimental workers were removed and dissected to extract the implant. The implant was mounted onto a microscopic slide and the degree of encapsulation measured as grey values of passing light by means of a video imaging system. During the experiments, ambient temperatures in the nest and in the field were both around 30 °C. One day (13 h) of foraging versus nest activity was chosen because previous experiments (Schmid-Hempel 1994) showed that longer periods of encapsulation produce similar differences, and because the short-term aspect of the regulation of immunocompetence is of special interest. Statistical analysis was done with the general linear model of SYSTAT and fixed effects.

3. RESULTS AND DISCUSSION

We hypothesized that foraging workers should show a lowered encapsulation response when compared to workers of the same colony that did not forage. Out of all the individuals included in the experiments, it was possible to analyse statistically the data of 77 bees from three colonies. To our satisfaction, the implantation did not visibly affect the behaviour of the bees; regular checks furthermore ensured that workers were indeed foraging or staying inside the nest as intended by the experimental treatment.

For analysis, we first checked whether our measured data for the encapsulation response were normally distributed. Two standard test procedures, plotting the data on normal probability scale, and a Kolmogorov-Smirnov test (modified by Lilliefors, SYSTAT 1991), showed no deviation from normality. This was true for the pooled data as well as for the data of each cell of the subsequent variance analysis (i.e. data grouped by colony and treatment, see table 1: all _p_-values > 0.19, with minimal differences _D_ < 0.22). In addition, the variances across all groups were homogeneous (Bartlett’s-test, _χ_² = 8.54, d.f. = 5, _p_ = 0.13), a fact that was also confirmed by graphical inspection of the studentized residuals plotted against the estimate from the variance analysis model. The use of a parametric variance analysis seemed, therefore, justified. The results from this model showed that there was a clear difference among colonies (see table 1) but that against this background, the degree of encapsulation in foraging workers was indeed significantly lower than in non-foraging workers (see figure 1 and table 1). Although the current sample size is too low, the highly significant colony effect suggests genotypic variation in the encapsulation response. This agrees well with findings from other, laboratory experiments (Schmid-Hempel 1994). We found no relation between body size, implant size and the intensity of the encapsulation response. In addition, ten freshly killed bees were also implanted to control for the effects of reactions not associated with the metabolism of the living animal.
However, these animals never showed any encapsulation and therefore the observed reaction was considered to be a measure of the immunocompetence of the living host.

In a previous experiment with *B. terrestris* (Schmid-Hempel 1994), the wings of the workers were clipped to the extent that they still could fly and forage (10–20% of wing area). The behaviour of the two groups of animals was not different, indicating that wing-clipped bees were working at the same rate but presumably with higher costs. However, the experiment found no difference in the encapsulation response between clipped and unclipped workers. We conclude from this that clipping in itself is unlikely to have caused the observed difference in the present experiment, for example, through stimulation of the immune system by wounding (Pathak 1986).

Our result therefore demonstrates that foraging activity in these insects leads to a decrease in immunocompetence, at least for the samples investigated in this study. The bees differed only in the 13 h period of foraging versus non-foraging, but were otherwise matched with respect to colony, body size, age and developmental conditions. This suggests that the metabolic activity related to foraging by workers competes for self-maintenance functions, such as host defense against parasitism, and that immune system competence can vary on very short timescales.

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