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Trans-generational immune priming in a social insect

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Detecting functional homology between invertebrate and vertebrate immunity is of interest in terms of understanding the dynamics and evolution of immune systems. Trans-generational effects on immunity are well known from vertebrates, but their existence in invertebrates remains controversial. Earlier work on invertebrates has interpreted increased offspring resistance to pathogens as trans-generational immune priming. However, interpretation of these earlier studies involves some caveats and thus full evidence for a direct effect of maternal immune experience on offspring immunity is still lacking in invertebrates. Here we show that induced levels of antibacterial activity are higher in the worker offspring of the bumblebee, *Bombus terrestris* L., when their mother queen received a corresponding immune challenge prior to colony founding. This shows trans-generational immune priming in an insect, with ramifications for the evolution of sociality.

Keywords: trans-generational immunity; social insects; antibacterial activity

1. INTRODUCTION

Invertebrates rely on an array of effector systems for immune defence (Schmid-Hempel 2005). Such effectors include inducible production of antimicrobial peptides and the constitutive phenoloxidase cascade leading to melanisation. This invertebrate immune system was traditionally considered to lack immune priming, where an initial immune challenge gives rise to a better secondary response. Among the arguments against immune priming, and particularly immune memory, in invertebrates is that they lack the system of clonal expansion of specific leucocytes. In addition, invertebrates often have relatively short longevity and thus most will have died through aging processes before secondary pathogen exposure occurs (Little & Kraaijeveld 2004). Whilst no mechanism to maintain specific memory in invertebrates is yet known, longevity is no longer an issue when immunity across generations is considered. Especially under low dispersal and minimal environmental change, offspring will be faced with a similar pathogen pressure as their mothers. In this scenario offspring could benefit if their mother bestows appropriate levels of immunity on them. Such an act is termed trans-generational immune priming. In vertebrates this is an accepted phenomenon (Grindstaff *et al.* 2003). In invertebrates evidence is limited, but studies have demonstrated trans-generational resistance to pathogens (Little *et al.* 2003). However, alternative explanations for those findings include selection instead of maternal transfer (Huang & Song 1999). A study showing trans-generational resistance to bacteria in clonal offspring of the crustacean *Daphnia* does not suffer from such potential for selection on the maternal population, but does not directly demonstrate an effect on immune defence *per se* (Little *et al.* 2003). While a previous study on *B. terrestris* demonstrated an effect of nursing sisters on their sexual brother's phenoloxidase activity (Moret & Schmid-Hempel 2001), thus demonstrating immune priming in a social context, no study of invertebrates has directly shown a trans-generational effect on an assayed immune parameter.

Trans-generational immune priming would seem particularly beneficial in insects that invest heavily into parental care and where most offspring remain at the natal site, as in social insects where generations and environments obligatorily overlap. For example, the pathogen community encountered by a queen bumblebee on emergence from hibernation and in initial colony foundation is likely to be related to that encountered by her worker offspring. Given this, we investigated whether immune-challenged queens bestow their future offspring with increased immunity.

2. MATERIAL AND METHODS

(a) *Insects*

All animals used in this study were of the species *Bombus terrestris*. Young queens (gynes) and their unrelated mates were sourced from colonies set up from queens collected in North Western Switzerland in the spring of 2004. At 7–11 days post eclosion these gynes were mated, and 5–7 days later artificially hibernated ($4\pm2\,^{\circ}C$) for 30 ± 3 days. On removal, queens were assigned to one of two treatments (see below). Following the treatments queens were allowed to start colonies. All colonies and individually isolated bees were kept at $28\pm2\,^{\circ}C$ under red light illumination, with pollen and sugar water (ApiInvert®) provided *ad libitum*.

(b) *Maternal challenge*

Queens were assigned to either a control or immune-challenged group. Ten days post hibernation immune-challenged queens received heat-killed bacteria *Arthrobacter globiformis* (DSM No. 20124) suspended in sterile insect saline (10^8 bacterial cells ml^{-1}). At the same time point, control queens received injections of sterile insect saline only. In both groups, queens were chilled on ice and 2 µl injections carried out using a sterile pulled glass micro-capillary inserted between the first and second abdominal tergites.

(c) *Offspring immune responses*

Immune parameters of offspring workers were measured in colonies that had grown to a sufficient size. Ten callow workers were collected from each colony and housed individually. On day five after emergence, five workers from each colony received a 2 µl injection of lipopolysaccharide (LPS, Sigma L-2755, 0.5 mg ml^{-1} in saline ringer solution), and 24 h later their haemolymph was assayed for induced antibacterial activity using a standard zone of inhibition assay (Moret 2001). LPS is known to activate the antibacterial response in insects (Moret & Schmid-Hempel 2001). The five remaining workers of each colony were also bled and their haemolymph measured for constitutive levels of phenoloxidase (PO) (Moret 2001). Due to the timing of worker collection and the number of callow workers available, one of the colonies used in the PO assay was not used in the assay of antimicrobial activity.

3. RESULTS

The treatment received by individuals in the maternal generation was not a significant predictor of colony founding success in a binary logistic model

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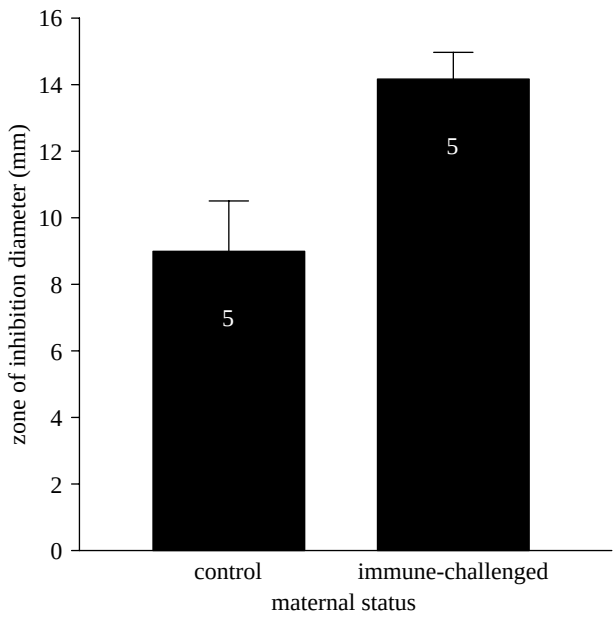

Figure 1. Haemolymph antibacterial activity in worker offspring of control and immune-challenged mothers. Antibacterial activity was higher in workers of queens that were immune challenged prior to colony founding (repeated measures ANOVA: $F_{1,8}=5.72$, $p=0.044$). Bars represent colony means ($n=5$ workers per colony as repeated measures for a colony)+1 standard error and numbers inside bars represent colonies assayed.

(LR$\chi_2=0.98$—change in 2 log-likelihood if term removed from the model—d.f.$=2$, $p=0.952$). This suggests that no selection for colony initiation, and thus immune traits, was imposed by the treatment regime. However, in worker offspring of these founded colonies there was a significant effect of maternal immune challenge on antibacterial activity (figure 1). We found no effect on constitutive levels of phenoloxidase activity (repeated measures ANOVA: $F_{1,9}=0.018$, $p=0.895$).

4. DISCUSSION

Our results show trans-generational immune priming in a social insect. We demonstrate that induced levels of antibacterial activity are higher in the worker offspring of the bumblebee *Bombus terrestris* L. when their mother queen received a corresponding bacterial-based immune challenge prior to colony founding. In contrast to other work showing trans-generational effects on offspring resistance (Huang & Song 1999), our study directly assays immunity, and its findings are clearly not a result of selection on the maternal population. Consequently, our work contributes to the view that increased offspring resistance in other invertebrates (Little *et al.* 2003) results from epigenetic effects on immunity.

An interesting comparison can be made with previous findings in the bumblebee system, where Moret & Schmid-Hempel (2001) found priming of PO activity, but not antibacterial activity in males from colonies in which their sister workers had received prior immune challenges. This difference is despite the similar bacterial-based immune stimulants used in both studies. One plausible explanation for this difference could be the contrasting life-styles of the two castes. Workers leave to forage but return to the natal nest, whereas males do not, dispersing soon after emergence and rarely, if at all, returning (Alford 1975). These differences may result in exposure to differing pathogen communities, with the dispersing males encountering pathogens varying from those present in the natal colony. In such a case, priming of a more general immune effector system, such as that employing the PO cascade (Schmid-Hempel & Ebert 2003), would seem a logical protective solution. By remaining in the natal nest, workers are likely to encounter a similar pathogen community to the mother queen, and thus priming of more specific immune defences would be appropriate. Alternatively, different cues from mothers to offspring, or between siblings, could result in the priming of different immune effector systems. At this time, the nature or timing of the priming cues are not known in this system.

Where mothers and offspring are presented with a similar pathogen pressure, a plastic change in offspring depending on maternal immune experience is likely to be beneficial if immune responses are costly and yet efficient immune responses are necessary. The exact mechanism by which such trans-generational immune effects are achieved is as yet unknown. However, regardless of the mechanism employed, these effects will have implications beyond the mothers and offspring in which they occur. Immune priming, whether within individuals or across generations, is likely to have effects on host–pathogen interactions and therefore disease dynamics (Kurtz 2004; Little & Kraaijeveld 2004). In addition, the finding also adds to the understanding of costs and benefits of social evolution. Resident parasite pressures impose an additional threat to offspring that stay at the natal nest and help. These parasites and pathogens may become adapted to a host genotype, or be transmitted more easily given a dense group of closely related individuals, as is the case in many social insect colonies (Schmid-Hempel 1998). Such a threat would seem to favour the dispersal and self-propagation of individuals over the evolution of sociality, or demand increased maternal immune protection for offspring staying at the natal nest. Given that immune defence is costly (Moret & Schmid-Hempel 2000), this alters the balance of costs and benefits factored into Hamilton's equation on the evolution of social behaviour (Hamilton 1964*a*,*b*).

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