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A distinct infection cost associated with trans-generational priming of antibacterial immunity in bumble-bees

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The adaptive value of facultative maternal adjustment of offspring immunity, or trans-generational immune-priming, will depend on the ecological background. In particular, where there is a mismatch between the immune adjustment and offspring environment, the immunological link between mothers and offspring may be disadvantageous owing to the presence of associated costs. Costs to an individual of responding to an immune challenge are extensively documented. However, in addition to parents, the relevant costs for trans-generational immune-priming also pertain to offspring, but as yet it is unknown what costs offspring will bear. In bumble-bees, higher antibacterial activity has been shown as a trans-generational effect when mothers receive a bacterial-based immune challenge prior to colony founding. Here we show that while naive offspring from immune-challenged mothers do not show evidence for a direct energy-related survival cost, they do show increased susceptibility to a parasite distinctly unrelated to the maternal challenge. The presence of costs associated with trans-generational immune-priming will shape the evolution of this trait depending on the ecological setting.

Keywords: social insects; *Bombus terrestris*; trans-generational immunity; immune costs

1. INTRODUCTION

A successful immune system is undoubtedly an important factor in determining an individual's evolutionary fitness. However, natural variation in immunity is widespread, and underlying costs have been advocated as creating at least some of this variation (Schmid-Hempel 2003). The costs of individual investment into immunity have been empirically tested, also quite extensively in invertebrates, and include energetic trade-offs, demonstrated by decreased immunity on nutrient limitation (Siva-Jothy & Thompson 2002), decreased survival (Moret & Schmid-Hempel 2000) and self-harm caused by an activated immune system (Sadd & Siva-Jothy 2006). Owing to the costs and benefits of immunity, optimal investment into traits relating to immune defence will vary over environments, depending on the parasite pressure (Sadd & Schmid-Hempel 2009).

A particular case of investment into immunity is trans-generational immune-priming, with maternal immune experience influencing levels of immunity in offspring. Enhancement of offspring immunity or resistance as a result of maternal immune experience has been demonstrated in both invertebrates (Little *et al.* 2003; Sadd *et al.* 2005; Moret 2006; Sadd & Schmid-Hempel 2007) and vertebrates (Grindstaff *et al.* 2003). Intuitively, it would appear that a trait such as facultatively adjusted immune investment in offspring is not universally beneficial because of its facultative nature. In fact, in the absence of associated costs, we might expect primed levels of immunity across all offspring, independent of maternal experience. Unfortunately, studies addressing costs associated with trans-generational immune-priming are rare, and restricted to the case of negative effects on further antibody production or increased intracellular infections in offspring, in the case of a massive passive antibody transfer from vertebrate mothers (Grindstaff *et al.* 2003).

Owing to the link between mother and offspring, trans-generational immune-priming poses many intriguing questions relating to immune costs for evolutionary ecologists. The evolution of the trait, based on the fitness consequences of its presence, will depend on the presence of associated costs and the relationship between maternal and offspring environment (figure 1). In the presence of costs, trans-generational immune-priming is only likely to evolve where there is a high probability of offspring encountering the same parasite environment as their mothers. This scenario is plausible for social insects, where worker offspring stay at the natal nest. In line with this, higher induced antibacterial activity has been shown as a trans-generational effect in the bumble-bee *Bombus terrestris* L. when mothers receive a bacterial-based immune challenge prior to colony founding (Sadd *et al.* 2005). While it is clear that the mother is likely to carry the usual immune-activation costs (Moret & Schmid-Hempel 2000), it is less clear what the consequences will be for offspring.

In this study, we investigated costs associated with observed trans-generational priming of antibacterial activity in offspring of the bumble-bee, *B. terrestris*. In one group of offspring we measured survival on starvation. This assay has been previously used to investigate immune-related costs in this system (Moret & Schmid-Hempel 2000), and relates to the energy use of an organism. Increased energetic demand by a trait will materialize in a decreased survival under food limitation. A further cost may materialize in trade-offs within the defence system itself, be these between different immune pathways or investment in defence between separate body compartments (e.g. haemolymph and gut). To test for this possibility, we experimentally infected offspring with *Crithidia bombi* (Trypanosomatidae). The haemocoelic bacterial-based immune challenge leading to trans-generational immune-priming of antibacterial immunity in offspring, is distinct in type and location from infection by the parasite *C. bombi* that resides in the gut of bumble-bees.

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maternal parasite environment	offspring parasite environment /presence of cost: A cost	A no cost	B cost	B no cost
A	+[a]	+	–	=
B	–	=	+[a]	+

Figure 1. Schematic showing the influence of associated costs and similarity between maternal and offspring environments (parasite A or B) on the fitness implications for offspring of trans-generational immune-priming. For the sake of simplicity, parasite environment can refer to either different parasite pressures, or parasite types. [a]It is assumed that for trans-generational immune-priming to have evolved at all, there must be a net positive influence when the maternal and offspring environments match (benefits > costs).

2. MATERIAL AND METHODS

(a) *Insects, bacteria and parasites*

Healthy young queens and males were sourced from colonies of queens collected in Spring 2007 (Switzerland). Queens were mated 9 ± 2 days post-eclosion, and 4 days later artificially hibernated ($4 \pm 2^{\circ}$C) for 69 ± 2 days. Otherwise, bees were kept at $28 \pm 2^{\circ}$C under red light, with pollen and sugar water (ApiInvert) provided ad libitum, unless the experiment demanded otherwise.

The bacteria, *Arthrobacter globiformis* (DSM No. 20124), was prepared as in Sadd & Schmid-Hempel (2007).

The *C. bombi* isolate used (07.128) was sourced from a queen collected in 2007 (Switzerland). This isolate was maintained in *B. terrestris* workers. For experimental infections, faeces were collected from these workers, parasite cells counted and adjusted to 1000 cells μl^{-1} in 50 per cent sugar water.

(b) *Maternal challenge*

Queens were assigned to one of the three groups. Seven days post-hibernation queens were injected with either sterile insect ringer (control) or heat-killed *A. globiformis* (challenged) (see Sadd & Schmid-Hempel 2007). A third group was kept naive to act as surrogates to control offspring-rearing conditions.

(c) *Fostering of brood*

Eggs of treatment queens were immediately transferred to a surrogate naive sister queen to control the rearing conditions of offspring. Eggs of the surrogates were constantly removed. On adult emergence, fostered offspring were individually isolated.

(d) *Survival on food-deprivation*

Survival assays of workers (two to five offspring per mother, mean = 3) were begun between 08.30 and 09.30. Five days after emergence workers were placed in fresh boxes without sugar water or pollen. Survival was monitored every hour.

(e) Crithidia *experimental infections*

Experimental infections (four offspring per mother) occurred between 14.30 and 15.30. Workers were starved for 2.5 h, and then presented with 10 µl of *Crithidia* solution (1000 cells μl^{-1}) to take up *per os*. After 7 days the intensity of infection was measured by dissecting the gut, and counts adjusted to number of parasite cells per bee.

(f) *Induction and measurement of offspring antibacterial activity*

Induced antibacterial activity against *A. globiformis* was measured as in Sadd & Schmid-Hempel (2007), with samples measured in triplicate (one to three offspring per mother, mean = 1.7).

(g) *Analyses*

The influence of maternal challenge (treatment) on offspring *Crithidia* infection intensity, survival time and wing radial cell length (a token for adult size measured in all assayed offspring) were all analysed with analysis of variance (ANOVA) using lme in R 2.6 (R Development Core Team 2007). Response variables were appropriately transformed where necessary. Treatment was included as a fixed factor and mother as a random factor nested within treatment. Offspring genotype (maternal origin × paternal origin) and wing radial cell length (when not the response variable) were also included in further models, but these models did not fit the data significantly better than the simple model with treatment alone. For offspring antibacterial activity, because of a lack of replicates for some mothers, average activity was calculated for each mother and the influence of treatment analysed with a *t*-test.

3. RESULTS

Following the treatment of mothers, the production and entry of offspring into the subsequent assays was not significantly influenced by treatment (binomial logistic regression: z-value = 0.094, $n = 38$, $p = 0.925$). Furthermore, offspring genotypes present (maternal origin × paternal origin) were equally distributed across the two treatments.

We measured antibacterial immune activity to ensure that this study population showed the same trans-generational effects as had been reported in earlier studies (Sadd *et al.* 2005; Sadd & Schmid-Hempel 2007). Indeed, the antibacterial activity we measured by the diameter of zones of inhibition was greater in offspring from challenged mothers (mean ± s.e. = 12.95 ± 0.37 mm) than those from control mothers (mean ± s.e. = 10.76 ± 0.21 mm) (t-test on transformed data ($y^{0.5}$): $t = 5.25$, d.f. = 8, $p = 0.0008$).

We found no significant difference in the survival on starvation of offspring from immune-challenged (mean ± s.e. = 9.2 ± 0.72 h) or control (mean ± s.e. = 8.8 ± 0.5 h) mothers (ANOVA on transformed data ($y^{0.5}$): $F_{1,8} = 0.02$, $p = 0.905$). In our setup, a further lack of evidence for trans-generational immune-priming of offspring having energetic consequences for traits outside the immune system comes from the fact that wing radial cell length of all offspring did not significantly differ between treatments (ANOVA: $F_{1,8} = 0.002$, $p = 0.964$; control: mean ± s.e. = 2.264 ± 0.048 mm; challenged: mean ± s.e. = 2.265 ± 0.028 mm).

Indicative of a cost associated with trans-generational immune-priming, maternal treatment had a significant effect on the level of *Crithidia* infection found in offspring workers. Workers originating from bacterially challenged mothers showed higher infection levels (mean ± s.e. = 911 850 ± 290 759 cells per bee) than those workers from control mothers (mean ± s.e. = 246 350 ± 114 166 cells per bee) (ANOVA on transformed data ($y^{0.35}$): $F_{1,8} = 6.52$, $p = 0.034$, figure 2).

4. DISCUSSION

While we did not uncover direct survival costs associated with trans-generational immune-priming, we demonstrate that costs materialize in other defence components, leading to increased susceptibility against *C. bombi*. This increased susceptibility will have an impact on offspring, given the serious fitness consequences of *Crithidia* infection (Brown *et al.* 2000).

The basis of the demonstrated cost could stem from two potential sources, both of which have some

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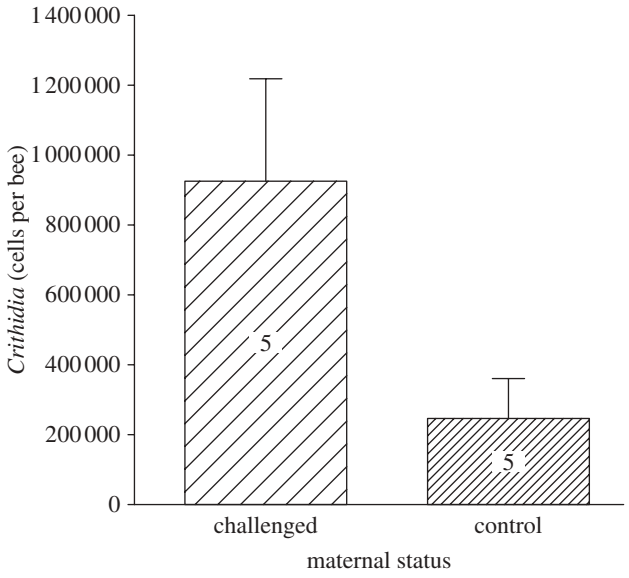

Figure 2. Offspring with trans-generationally primed antibacterial activity show increased susceptibility to a parasite distinct from the maternal challenge. The infection intensities of the trypanosome parasite *Crithidia bombi* (parasite cells per bee) in worker offspring originating from control (saline injected) or bacterially challenged mothers. Bars represent the colony means (four workers were tested per colony)+1 s.e. and numbers inside bars represent number of colonies assayed.

support from costs investigated at the individual level. First, the cost could result from trade-offs between different immune components. The distinct priming immune challenge and parasite exposures could be combated by different immune components. Mallon *et al*. (2003) showed that bumble-bee colonies showing specific responses towards *C. bombi* have lower encapsulation ability, and other studies with invertebrates have shown evolutionary trade-offs between immune traits (Cotter *et al*. 2004). An alternative to a trade-off between different components of the immune system is a trade-off between spatially separate physiological compartments. As described earlier, the priming is of antibacterial activity within the haemocoel, while *Crithidia* infects the gut. This spatial trade-off concept has been used to explain negative correlations between gut parasite burden and an immune measure following an acute immune challenge in the haemocoel in damselflies (Siva-Jothy *et al*. 2001).

The presence of costs associated with trans-generational immune-priming will mean that if offspring encounter a parasite environment distinct from their mother, trans-generational immune-priming may have a negative impact on offspring fitness (figure 1). This will mean that specific prerequisites are required for the evolution of trans-generational immune-priming. These include factors influencing the probability that offspring encounter the same parasite environment as their mothers (e.g. dispersal, spatial heterogeneity of environment, temporal change in environment). Once it is in place, the costs associated with trans-generational immune-priming are likely to select for offspring traits that reduce the mismatch between their environment and the prior maternal environment. The presence of direct and lasting trans-generational effects (Sadd & Schmid-Hempel 2007), coupled with costs, would in particular favour offspring behaviour that ensures stability between maternal and offspring environment. For example, such offspring behaviours might include staying at the natal nest, which during the evolutionary development of social life would lead to the predominance of conditions that are conducive to the maintenance and evolution of sociality (Emlen 1994).

The cost of trans-generational immune-priming that we demonstrate shows that costs can occur in our specific setup. However, this does not preclude the existence of other costs in other situations. In natural environments, the investment by mothers into trans-generational immune-priming is likely to be based on a more complex parasite environment. Thus, trans-generational immunity and its consequences observed in offspring, would be the outcome of decisions based on a diverse parasite community, costs to the mother of allocation to offspring, and the costs and benefits to offspring.

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