

Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees

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It is widely acknowledged that a mother, and the maternal environment, can have a profound effect on the phenotype of her offspring [1]. For example, immunocompetent vertebrate mothers can pass on immune factors to their otherwise immune naïve offspring [2]. Recently, it has also been demonstrated in invertebrates that maternal contact with pathogens and immune elicitors can have a positive impact on offspring resistance [3] and immune system components [4,5]. Here, using a cross-fostering design, we show that trans-generational immune priming in a social insect, similar to birds [2], takes place through factors in the eggs, with effects persisting into adult worker offspring. Additionally, eggs from immune-challenged mothers are imbued with increased internal antibacterial activity when they are laid.

If maternal experience predicts that of her offspring, trans-generational immune priming dependent on maternal experience has a clear benefit. However, while this may be adaptive in terms of increased offspring protection when infection probability is higher, it may also constrain the options of offspring, due to the costs and benefits associated with maintaining a high degree of immune capacity. This will be of particular relevance for social groups, such as those formed by bumblebees where trans-generational effects on the antibacterial activity of offspring have been demonstrated [5]. The constraints imposed on offspring will greatly depend upon the mode by which immune priming takes place.

We use a cross-fostering design to investigate the route and persistence of trans-generational priming of antibacterial activity in the bumblebee *Bombus terrestris*. Mother queens were removed from hibernation and after seven days were subjected to either a control or a challenge (heat-killed *Arthrobacter globiformis*) injection. The first egg batch was completely removed and eggs measured for antibacterial activity. Mother queens were then allowed to lay a second egg batch, following which all eggs were immediately removed and placed in a clean environment with a surrogate queen. Eggs from challenged/control mothers were given to challenged/control surrogates to create all four possible combinations. Manipulations took place in all combinations, even when the immune status of mother and surrogate was the same (i.e. control/control, challenged/challenged). Surrogates were allowed to raise these eggs to adulthood, at which stage induced antibacterial immune activity was assayed (for detailed methodological information, see Supplemental data published online with this article).

We first checked that our treatment did not impose significant selection on the maternal generation. In line with no selection having taken place, the treatment of queens did not predict their progression

from treatment into the fostering experiment (Binomial logistic regression: z-value = -0.122, n = 178, p = 0.9). Furthermore, first batch traits of egg weight (t-test: t = 1.24, d.f. = 55, p = 0.22; challenged \bar{X} = 2.52 s.e. = 0.057, control \bar{X} = 2.42 s.e. = 0.05), clutch size (t-test on log transformed data: t = 0.74, d.f. = 94, p = 0.46; challenged \bar{X} = 5.94 s.e. = 0.46, control \bar{X} = 5.59 s.e. = 0.65), and time of laying (t-test: t = 1.38, d.f. = 112, p = 0.17; challenged \bar{X} = 26.3 s.e. = 1.17, control \bar{X} = 23.4 s.e. = 1.97) were not significantly influenced by maternal challenge. In adult workers raised from the fostered brood, radial cell length, a proxy for adult size, did not significantly differ between combinations of mother and surrogate (ANOVA: F = 0.25, d.f. = 3,19, p = 0.86; control/control \bar{X} = 2.21 s.e. = 0.08, control/challenged \bar{X} = 2.27 s.e. = 0.10, challenged/control \bar{X} = 2.29 s.e. = 0.06, challenged/challenged \bar{X} = 2.24 s.e. = 0.05).

Maternal challenge had a significant effect on the total antibacterial activity of first batch eggs. Freshly laid eggs of bacterially challenged queens showed higher antibacterial activity than those of control queens (Figure 1; t-test on transformed data [(y + 0.5)^{1.32}]: t = 6.28, d.f. = 30, p < 0.001; challenged \bar{X} = 11.2 s.e. = 0.42, control \bar{X} = 4.98 s.e. = 0.99). This difference could have

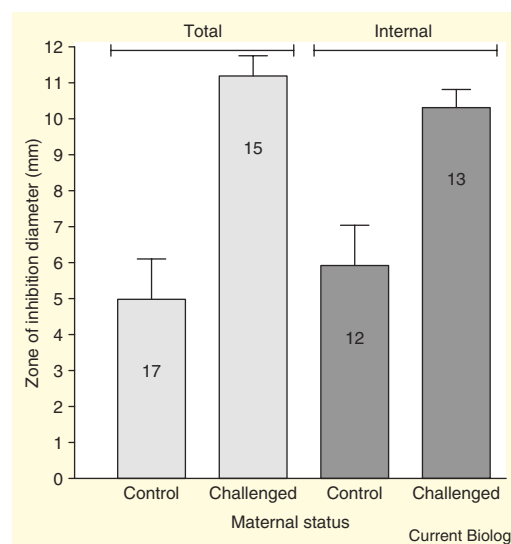


Figure 1. Antibacterial activity of freshly laid eggs from control (saline injected) and bacterially challenged mothers.

Homogenates of whole eggs (pale gray bars) and internal extracts (dark gray bars) were assayed for inhibition of *A. globiformis*. Bars represent colony means (per colony n = 3 for homogenates, n = 2 for internal extracts) + 1 standard error, and numbers inside bars represent the number of mothers assayed.

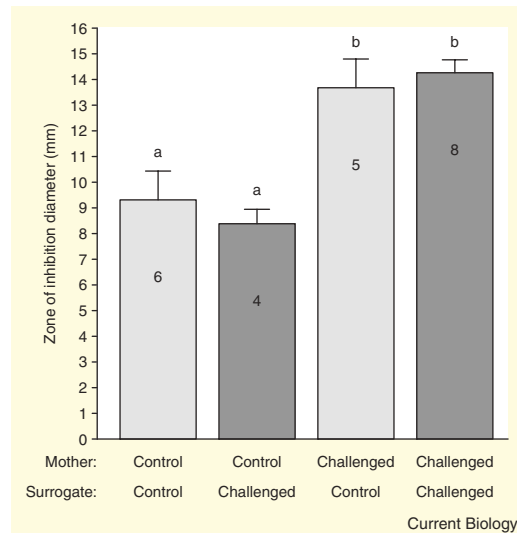


Figure 2. Induced hemolymph antibacterial activity in adult worker offspring.

Workers originated from eggs of control (saline injected) or bacterially challenged mothers and were subsequently fostered and nursed by control or challenged mothers (surrogate). The combination of mother and surrogate is given below each bar. Bars represent colony means (between 1 and 6, average $3.09 \pm \text{s.e. } 0.39$, workers were tested per colony) + 1 standard error, and numbers inside bars represent number of mother/surrogate pairs assayed. Different letters above bars indicate significant differences (Bonferroni corrected pairwise-t-tests: $p < 0.05$).

resulted from queens secreting antibacterial factors onto the egg surface, so internal extracts were therefore subjected to a second analysis. As with whole egg homogenates, internal extracts of eggs from bacterially challenged queens showed higher antibacterial activity (Figure 1; t-test on transformed data $[(y + 0.5)^{1.15}]$: $t = 3.38$, d.f. = 23, $p = 0.003$; challenged $\bar{X} = 10.31$ s.e. = 0.74, control $\bar{X} = 5.92$ s.e. = 1.05). This shows that queens internally imbue their eggs with levels of antibacterial activity that are dependent on their own experience of a challenge.

In the fostering experiment we investigated whether trans-generational immunity in adult offspring is the result of direct effects prior to egg laying or the result of the rearing environment. The combination of mother and surrogate had a significant effect on the induced levels of antibacterial activity in adult workers (Figure 2; ANOVA: $F = 11.74$, d.f. = 3,19, $p < 0.001$; control/control $\bar{X} = 9.31$ s.e. = 1.12, control/challenged $\bar{X} = 8.38$ s.e. = 0.56, challenged/control $\bar{X} = 13.68$ s.e. = 1.12, challenged/challenged $\bar{X} = 14.26$ s.e. = 0.50). This effect is dependent on the status of the mother, with workers originating from eggs of bacterially challenged mothers showing higher levels of activity, irrespective of the status of the surrogate queen (Figure 2; Bonferroni corrected

pairwise-t-tests: $p < 0.05$). These results demonstrate that the trans-generational effects are mediated through factors inside the eggs and that they persist independent of the rearing environment.

Interestingly, the initial imbue of eggs with antibacterial activity can be nicely contrasted with known cases of egg defense. Eggs of many insect species are chemically defended, for example, by external coating with deterrent compounds or by internal deposition of products, such as pyrrolizidine alkaloids [6]. The compounds are primarily used against egg predators and related enemies, but in some cases have been shown to possess antimicrobial activity [7]. However, we here report, to our knowledge, the first case in insects of a transfer to eggs of a level of antibacterial activity that is dependent on maternal immune experience.

Socially living organisms with parental care have two routes by which a trans-generational effect could be manifested. On one hand, indirect priming could take place during rearing, greatly depending on the interactions that take place after egg laying. However, the realization of trans-generational immunity as demonstrated here is functionally analogous to the situation in egg-laying vertebrates [2]. The direct nature of the priming, and also the presence of active antibacterial factors in the

eggs themselves, means that the offspring are probably defended from the outset. This priming will be important in situations where the pathogens faced by a mother are also an immediate threat to offspring, as should be the case for workers in social insect colonies [8].

Supplemental data

Supplemental methodological details are available at <http://www.current-biology.com/cgi/content/full/17/24/R1046/DC1>

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