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Impact of transgenerational immune priming on the defence of insect eggs against parasitism

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ABSTRACT

Insects are known to prime the immune state of their offspring. However, although the beginning of insect life, the egg stage, is often greatly endangered by parasitism, no knowledge is available regarding whether transgenerational immune priming improves the immune responses of insect eggs to actual parasitoid attacks. Our study revealed suppression of the development of parasitoids in transgenerationally immune-primed *Manduca sexta* eggs and reduced emergence rates of parasitoids from these eggs. The higher defence efficiency of immune-primed *M. sexta* eggs against parasitoids was in agreement with the increased antibacterial activity and phenoloxidase activity of these eggs in response to parasitism compared to the eggs of control parents. Our study showed that immunochallenged insect parents could enable their offspring already in the egg stage to defend more efficiently against parasitic invaders. We discuss whether *M. sexta* benefits from transgenerational immune priming of eggs by limiting the population growth of egg parasitoids.

1. Introduction

The reproductive success of insects depends on numerous factors that affect the parental generation, as well as the offspring. Insect eggs represent a highly vulnerable developmental stage because they are unable to escape from danger. In addition to abiotic stress, insect eggs are exposed to attacks by predators, parasitoids (*e.g.*, Eisner et al., 2000; Fatouros et al., 2008) and fungi (Boos et al., 2014; Ferron, 1978). Furthermore, eggs can be confronted with pathogenic bacteria that are transovarially transmitted from the female into her eggs (Sikorowski et al., 2001) or that are transferred by egg parasitoids into host eggs (Tanada and Kaya, 1993).

When pathogens or parasitoids succeed in invading an insect egg despite maternal protective behaviour (Wong et al., 2013), the invaders must navigate the egg interior, which can contain egg-internal toxins that are supplied by the parents (Blum and Hilker, 2002; Eisner et al., 2002) or by endosymbionts transovarially transmitted from the female to the eggs (Kellner, 2002). Moreover, a growing body of evidence has shown that the immune responses of insect eggs can be activated upon attack (Abdel-latief and Hilker, 2008; Gorman et al., 2004; Jacobs and van der Zee, 2013; Jacobs et al., 2014a). Immune-relevant genes that are upregulated in insect eggs upon attack encode pathogen-recognition proteins, antimicrobial peptides and prophenoloxidase. The latter enzyme is a precursor of phenoloxidase (PO), which catalyses the formation of melanin and generates reactive compounds with broad antibacterial, antifungal, antiviral and even anti-parasitic activity (Zhao et al., 2011). The juvenile stages of an egg parasitoid (eggs, larvae) inside a host egg can become trapped by a melanised capsule that is formed by the host egg cells around the invader (Reed et al., 2007).

The immune activity of insect eggs is not only activated by an actual attack, but it can also be enhanced by parental immune priming. Eggs laid by immunochallenged parents show increased immune activity, even when they do not face a pathogen infection or parasitic attack. Parental immune priming obviously prepares the eggs for possibly impending danger by triggering differential expression of immune-related genes in eggs and/or direct transmission of immune factors into eggs (Freitak et al., 2009, 2014; Sadd and Schmid-Hempel, 2007; Zanchi et al., 2012). The effects of transgenerational priming on offspring immunity have not only been shown in the egg stage of the offspring but also in later (larval, pupal and F1-adult) insect stages (*e.g.*, Moret, 2006; Sadd et al., 2005; Trauer and Hilker, 2013; Zanchi et al., 2011).

While parental immune priming has been shown to increase immune activity of unchallenged eggs, it remains unknown whether parental immune priming can significantly increase the immune responses of insect eggs to an actual attack. Furthermore, knowledge regarding how effectively insect egg immune responses can fend off invaders is lacking. The studies mentioned earlier investigated

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egg immune activity *per se* in particular, rather than studying, *e.g.*, host egg survival, larval hatching or parasitoid emergence rates from eggs exposed to invasion.

Hence, we investigated here the impact of the parental immune state on the immune responses of insect eggs to parasitism. Furthermore, we studied host larval hatching rates and parasitoid emergence rates from host eggs depending on the immune state of the host parents. We chose a host–parasite model system consisting of eggs of *Manduca sexta* and the parasitic wasp *Trichogramma evanescens*, an egg parasitoid that infests a broad range of lepidopteran eggs (Romeis et al., 2005). The number of eggs deposited by a *Trichogramma* female inside a host egg ranges from one parasitoid egg in a small host egg to 30–40 parasitoid eggs in a larger host, such as an *M. sexta* egg (volume of approximately 1.4 mm^3) (Bai et al., 1992). The number of adult parasitoids that emerged from *M. sexta* eggs exposed to *Trichogramma* wasps ranged between 5 and 40 and did not significantly differ between field-collected and lab-parasitised host eggs (Potter and Woods, 2012). Nevertheless, when *Trichogramma* parasitoids oviposited into host eggs laid by healthy *M. sexta* parents, they could successfully develop in only 30% of the host eggs (Abdel-latief and Hilker, 2008). However, it remains unknown whether priming treatment of *M. sexta* parents can improve the immune defence of their eggs against parasitism.

To elucidate whether an immune challenge of *M. sexta* parents had an immune priming effect on the responses of *M. sexta* eggs to parasitism, we challenged the parental generation in its pupal stage with bacterial peptidoglycan (PGN), while the eggs were exposed to parasitoids. We challenged the parental immune system by PGN injection because the binding of bacterial PGN to insect pattern-recognition proteins can result in an increase in phenoloxidase (PO) and antibacterial activity (reviewed by Yu et al., 2002). These immune activities are known to also be inducible in response to parasitic attack that can be associated with bacterial invasion (Carton et al., 2008; Godfray, 1994; Strand and Pech, 1995). Furthermore, oxidation reactions catalysed by PO can form compounds that are toxic to both bacteria and parasitic wasps (Zhao et al., 2011). Thus, standardised injection of distinct amounts of PGN into *M. sexta* pupae allowed to elicit immune reactions that might occur in response to parasitism of pupae and to possibly associated bacterial invasion. In parallel, egg parasitoids can challenge their hosts not only by the feeding activity of parasitoid larvae inside host eggs but also by the bacteria that enter host eggs when parasitoids oviposit into them. Survival of eggs laid by *Nicrophorus* carrion beetles can also be reduced by bacteria present at the oviposition site in the soil where the parents buried vertebrate cadavers (Jacobs et al., 2014b). Activation of the PO cascade and the induction of antimicrobial peptide synthesis have intensively been investigated in different developmental stages of *M. sexta* (reviewed by Kanost et al., 2004; Ragan et al., 2009). Bacterial challenge of *M. sexta* eggs resulted in increased antibacterial activity and the upregulation of genes encoding antimicrobial proteins (Gorman et al., 2004). Parasitism of *M. sexta* eggs elicited immediate upregulation of genes involved in the PO cascade, whereas genes encoding antimicrobial peptides were first downregulated, but some of them were significantly upregulated a couple of days after parasitism began (Abdel-latief and Hilker, 2008).

In detail, we addressed the following questions: (i) Does a parental priming treatment affect *M. sexta* larval hatching rates and parasitoid emergence rates from *M. sexta* eggs? (ii) How does a parental priming treatment affect the development of juvenile *T. evanescens* parasitoids inside *M. sexta* eggs? (iii) Does a parental priming treatment affect the immune parameters (antibacterial and phenoloxidase activity) of *M. sexta* eggs in response to parasitism? We compared these immune parameters in non-parasitised and parasitised eggs of immunochallenged parents with those of eggs laid by control parents.

2. Materials and methods

2.1. Insects

Manduca sexta pupae were obtained from a colony reared in our laboratory on an artificial diet, as described by Trauer and Hilker (2013). Adults were kept in flight cages (50 × 50 × 50 cm) at 24 °C, in 70% r.h. and a 16:8 h L:D cycle; they were fed a 10% honey solution.

Adults of the egg parasitoid *Trichogramma evanescens* were obtained from our laboratory colony, which was kept on eggs of the moth *Sitotroga cerealella*. An original stock population of *T. evanescens* was purchased from AMW Nützlinge (Pfungstadt, Germany). Eggs of *S. cerealella* were continuously purchased from AMW Nützlinge. The parasitoids were reared at 20 °C, in 70% r.h. and 18:6 h L:D cycle.

2.2. Parental priming treatment

For the exposure of the parental generation to priming treatment, both female and male *M. sexta* pupae (21-day-old) from our laboratory were subjected to an injection of 50 µl of bacterial peptidoglycan extracted from *Micrococcus luteus* (PGN, Sigma 53243, 2 µg $µl^{-1}$ in sterile PBS). For controls, male and female pupae were injected with 50 µl of phosphate-buffered saline (PBS, 7 mM KH_2PO_4, 3 mM Na_2HPO_4, 0.13 M NaCl, pH 7.4) or were left untreated (naive). More details on the methods for how the adults were treated were provided by Trauer and Hilker (2013).

We investigated the eggs of three parental treatment groups, which are here referred to as naive (untreated) control parents, PBS-treated control parents and PGN-immunochallenged parents. The emergence rates of adult *M. sexta* from the differently treated pupae were independent of the pupal treatment. The emergence rates were always 100%.

Previous studies showed that these treatments of *M. sexta* pupae affected the immune activity of the adults (Trauer and Hilker, 2013). The PGN treatment of the pupae resulted in a significant increase in the antibacterial activity in the haemolymph of 3-day-old adults (*i.e.*, 5 days after treatment in the pupal stage), while their phenoloxidase activity was not significantly affected by this treatment (Trauer and Hilker, 2013). PBS-control treatment of the pupae did not result in a significant change in antibacterial or phenoloxidase activity in the haemolymph of adults (Trauer and Hilker, 2013).

2.3. Host egg deposition

To collect *M. sexta* eggs, four freshly emerged *M. sexta* females and four males with the same parental treatment were kept in separate flight cages (50 × 50 × 50 cm). The abiotic conditions were the same as those described earlier for rearing. We only used *M. sexta* females and males that emerged 2 days after pupal treatment (or no treatment for naive parents). Eggs that had been laid on the night between the 2nd and 3rd days after adult emergence were used for the experiments. The numbers of four females and four males per cage ensured that a sufficient number of eggs for the experiments were laid overnight (at least 200 eggs per night and cage). We repeated this experimental procedure for egg deposition three to four times for each parental treatment (see Fig. S1) to obtain data that were representative for eggs generated from different parental generations.

Eggs laid by PGN-treated parents are here referred to as immune-primed eggs, whereas non-primed eggs are eggs from naive and PBS-treated parents.

2.4. Exposure of host eggs to parasitoids

Freshly laid eggs (≤14 h old) from each parental generation were split into two groups. One group was exposed to parasitoids, while