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Relationship between maternal transfer of immunity and mother fecundity in an insect

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Trans-generational immune priming (TGIP) corresponds to the plastic adjustment of offspring immunity as a result of maternal immune experience. TGIP is expected to improve mother's fitness by improving offspring individual performance in an environment where parasitism becomes more prevalent. However, it was recently demonstrated that maternal transfer of immunity to the offspring is costly for immune-challenged female insects. Thus, these females might not provide immune protection to all their offspring because of the inherent cost of other fitness-related traits. Females are therefore expected to adjust their investment to individual offspring immune protection in ways that maximize their fitness. In this study, we investigated how bacterially immune-challenged females of the mealworm beetle, *Tenebrio molitor*, provision their eggs with immune protection according to egg production. We found that immune-challenged females provide a variable number of their eggs with internal antibacterial activity along egg-laying bouts. Furthermore, within the first immune-protected egg-laying bout (2–4 days after the maternal immune challenge), the number of eggs protected was strongly dependent on the number of eggs produced. Immune-challenged females might therefore adjust their investment into TGIP and fecundity according of their individual perception of the risk of dying from the infection and the expected parasitic conditions for the offspring.

Keywords: trans-generational immune priming; ecological immunology; insect immunity; maternal effects

1. INTRODUCTION

Maternal effects play a key role in offspring fitness by modulating its phenotype in accordance to the maternal experience of the environment [1]. They can even affect population dynamics when variation in offspring provisioning exists [2]. Trans-generational immune priming (TGIP) is a parental effect on offspring immunity. It is defined as the transmission of an elevated immunocompetence to the offspring following an immune challenge in the parental generation, improving its resistance to further pathogen encounter [3,4]. This transmission of an amplified immunocompetence to offspring is well documented in vertebrates, where it is achieved through maternal transfer of antibodies that confer to the progeny an early protection before the maturation of its own immune system [5]. In invertebrates, this phenomenon has been shown to occur mainly through phenomenological studies. The underlying mechanisms of this transmission remain unknown, but the effects of the TGIP in the progeny can be found across all life-stages of the protected progeny: from oviposition [6,7], during the larval development [8–11] and persisting even until the adult stage [12–14]. TGIP has been shown to confer to the offspring an enhanced protection in the case of persistence of the maternal infection in its environment [3,4,9,10,13].

Maintaining and using immune defences is costly for organisms [15,16]. It is therefore unsurprising that this elevated immunity comes at several costs for the offspring. In the bumble-bee *Bombus terrestris*, the stimulation of the females with a bacterial pathogen decreases the survival from a heterologous parasitic challenge in offspring [12]. In the mealworm beetle *Tenebrio molitor*, the maternal challenge elevates the haemocyte load of adult offspring at the expense of a prolonged developmental time [14]. Because of these costs, the main condition for its adaptiveness in invertebrates is believed to be the persistence of the infection risk encountered by mothers to the next generation. Thus, it is assumed that generation overlap and/or gregarism would favour the evolution of TGIP in response to pathogens that could persist from one host generation to the next in the environment. In this case, maternal infection becomes a reliable cue predicting the risk of infection of the progeny. As females synthesize and transmit effectors and/or elicitors of immunity to their offspring, we could also expect this transmission to be costly for them, in addition to paying the usual costs of immune activation.

After being immune-challenged, the females of the mealworm beetle *T. molitor* can provide their eggs with an antimicrobial activity [7]. This could result either from an imbuement of the eggs with immune substances within the female reproductive tract or from the incorporation of these immune substances into the eggs during ovogenesis: the first would suggest that the eggs are protected from a pathogen intrusion, whereas an internal localization would instead protect the young larvae at hatching. Interestingly, immune-challenged females do not transfer antibacterial activity to all of their eggs

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(C.Z. 2010, personal observation). The variability of this investment could indicate the existence of a cost for the females to egg protection. Recently, immune-challenged females of *T. molitor* were shown to trade-off their immunity with that transferred to their eggs [7], thus, TGIP may respond to the same constraints as other costly maternal investments that affect progeny quality, such as egg provisioning [17–19]. As a result, mothers are expected to differentially invest in the immune protection of their offspring according to the number of offspring produced to maximize fitness in an environment where infection risk for the offspring is high. Furthermore, the maternal transfer of immunity to the eggs is expected to cease with the disappearance of the pathogenic threat.

In this study, we investigated how long the females of *T. molitor* transfer antibacterial activity to their eggs following an immune challenge, and the localization of this protection (whether antibacterial substances are provided internally or on the surface of the eggs). Finally, because of the inherent cost of the maternal transfer of immunity to the offspring and reproduction, we examined the relationship between transfer of immunity to eggs and fecundity in immune-challenged females. To this end, we assessed the antimicrobial activity of all the eggs from the first immune-protected clutch laid by *T. molitor* females following their immune challenge. Here, we make the hypothesis that maternal transfer of immunity to the eggs is constrained by the availability of antibacterial substances produced by their mother. In that case, females may use two different strategies to protect their eggs according to the number of eggs produced. First, an immune-challenged female may transfer immune substances to all her eggs with the risk of providing an insufficient amount of theses immune substances to efficiently protect each egg. Second, an immune-challenged female may not protect all her eggs, but ensure the transfer of a sufficient amount of immune substances to each egg that received the maternal immune protection. Furthermore, because both egg production and egg protection are costly, a negative relationship between the number of eggs laid and the number of eggs protected is expected. In order to accentuate a potential existing trade-off between the two traits, we manipulated the energetic reserves contained in adult females and their size by limiting their food supply during their larval development.

We show that bacterially immune-challenged females of *T. molitor* provide some of their eggs with internal antimicrobial activity, and that this transmission is transient along egg-laying sequences. All the eggs are not protected within one egg-laying sequence, and the number and the proportion of immune-protected eggs is significantly associated with female fecundity.

2. MATERIAL AND METHODS

(a) *Insect cultures, immune challenge and egg collection*

All mealworm beetles used in this study originated from an outbred stock culture maintained in our laboratory in bran flour added with ad libitum access to water and regularly added with proteins (piglet flour), apple and bread. Pupae were then collected from these stock cultures and adults were maintained individually after emergence in a Petri dish supplied with bran flour and a piece of apple and water for ten days, except for experiment 3 where insects were isolated from this stock culture as young (1 cm) larvae then reared in good or poor food conditions (see experiment 3).

(i) *Experiment 1: temporal dynamics of the antibacterial immune response of immune-challenged females and of the transmission of antibacterial activity to their eggs*

We have previously shown that bacterially immune-challenged *T. molitor* females transferred levels of antibacterial activity to their eggs [7]. Here we wanted to assess in more detail how the number of eggs protected by the mothers changes across the 14 days of the antimicrobial response of their haemolymph [20]. To do this, 140 age-controlled (10 days old) virgin adult females were weighted to the nearest 1 mg and immune-challenged by a single injection of 5 µl of Ringer's solution containing non-purified lipopolysaccharides (LPS: 0.5 mg ml^{-1}) extracted from *Escherichia coli* (Sigma: L8274). This commercial LPS may contain peptidoglycan contaminants [21]. Therefore, LPS injection in our experiments may not strictly mimic a Gram-negative bacterial infection, as it may stimulate both the Imd and Toll pathways [22]. Immediately after their immune challenge, females were paired with a virgin and unchallenged male of the same age and allowed to produce eggs in a Petri dish provided with bleach flour and ad libitum food and water under standard laboratory conditions (25°C, 70% RH, 12 L : 12 D). Random couples were killed each 2 days and provided a haemolymph sample and three eggs that were stored at −20°C for later examination of their antibacterial activity. The remaining couples were transferred into a new Petri dish every second day following the maternal immune challenge, until the last remaining couples had their clutches separated into seven egg-laying sequences. When the female or the male died before the haemolymph collection of the female, the couple was removed from the experiment. Thirty couples were thus removed, resulting in 13 couples used in the first egg-laying sequence, 15 in the second, 18 in the third, then 14, 19, 17 and 14 in the last one. The presence or absence of a zone of inhibition in their eggs was recorded.

(ii) *Experiment 2: localization of the antibacterial activity transferred to the eggs*

Antibacterial activity transferred to the eggs may either result from the mother secreting antibacterial factors onto the egg surface and/or into the eggs. Here we wanted to examine these possibilities by testing the antibacterial activity of both the surface and the inside of eggs. To this purpose, 10 virgin females (10 days old) were immune-challenged, paired with a virgin and unchallenged male of the same age and then allowed to lay eggs as described earlier. On the basis of the results of experiment 1, we assessed the antibacterial activity of the eggs laid by each female between day 2 and day 4 following the maternal immune challenge (see §3). Five random eggs per female were used to test for the presence of antibacterial activity on both the surface and the inside of the eggs among the eggs laid. Because antibacterial activity at the surface of one single egg might be difficult to detect [23], the five eggs collected were put together in a microcentrifuge tube containing 20 µl of cold phosphate-buffered saline (PBS: 100 mM) and then gently agitated for 5 min to suspend potential antibacterial factors present on their surface. Eggs were then removed and the suspension was immediately stored at −20°C for later antibacterial test. For internal egg antibacterial activity, the

internal fluid of each previously washed egg was collected using a pulled glass micro-capillary and flushed into a micro-centrifuge tube containing 10 µl of cold PBS and then stored at −20°C until antibacterial test.

(iii) *Experiment 3: testing the trade-off between mother fecundity and number of immune-protected eggs*

In this experiment, insects were either reared on good food or restricted food conditions (poor food). In the good food condition, 1 cm-long larvae were isolated from the stock culture and then supplied with ad libitum bran flour supplemented with proteins (piglet flour), apple and water. In the poor food condition, 1 cm-long larvae isolated from the stock culture were supplied with ad libitum bran flour and water but without protein or apple supplementation. The latter food condition was used to generate adult insects of smaller size than those raised in good food conditions.

On the basis of the results of experiment 1, the influence of female fecundity on the number of immune-protected eggs was examined on the second egg-laying sequence that is between day 2 and day 4 after the maternal immune treatment. Age-controlled females (10 days old), from good ($n = 54$) and poor ($n = 41$) food conditions were weighted to the nearest 1 mg and either injected with LPS solution as describe earlier or with Ringer solution only as procedural control. Females were paired with a virgin unchallenged male from good food condition and allowed to produce eggs along two egg-laying sequences of 2 days each (from day 0 to day 2: past fecundity, and from day 2 to day 4: current fecundity). The number of eggs laid during each egg-laying sequence was counted and those from the second egg-laying sequences were all assessed for their antibacterial activity. We recorded the presence or absence of a zone of inhibition in these eggs, as well as the size of the zone of inhibition, which indicates the amount of antibacterial activity transmitted by the mothers to their eggs. Thirty-three females did not lay eggs and were removed from the experiment. Therefore, the analyses of the data were performed on a total of 62 females (18 good food/Ringer, 17 good food/LPS, 13 poor food/Ringer, 14 poor food/LPS).

(b) *Analysing the antibacterial activity of the haemolymph and the eggs*

Antibacterial activity of the haemolymph of females was measured on zone of inhibition plates seeded with *Arthrobacter globiformis* (Pasteur institute CIP 105365) as described in the study of Moret [8].

To measure the antibacterial activity of the eggs, individual eggs were thawed on ice, suspended in 2 µl of PBS and homogenized using a pestle, except in the second experiment, in which case egg content and egg surface were isolated before freezing. Antibacterial activity of all the samples was measured from 2 µl of extract using the antibacterial assay described in the study of Moret [8].

(c) *Statistics*

For experiment 1, the antimicrobial activity of the female's haemolymph according to time was analysed using a generalized linear model (GLM) fitted with a Poisson distribution corrected for overdispersion (dispersion parameter = 4.12), with the female body mass as covariate. The temporal dynamics of transmission of antibacterial activity to the eggs was analysed as the proportion of eggs found protected according to their egg-laying sequence using a general linearized mixed model (GLMM) fitted with a binomial distribution (presence/absence of protection in the eggs of each laying sequence), with the female's body mass as covariate and female identity as a random factor. Differences between each egg-laying sequence were analysed using a Tukey's post hoc test ($p < 0.05$).

For experiment 3, the body masses of females were normally distributed within each rearing condition, the effect of larval food conditions on female body mass was therefore examined using a Student's *t*-test. Variation in female fecundity was analysed using a GLMM fitted with a Poisson distribution, according to the maternal treatment, female larval food condition and egg-laying sequences as factors. Since there were two egg-laying sequences, female's ID was repeated twice and thus included in the model as a random factor.

The size of the zone of inhibition of each egg according to the number of eggs protected by the females, their larval food condition and their immune treatment was analysed with a GLMM with a Gaussian distribution, with the female's ID as a random factor.

In this experiment 3, we analysed both the number and the proportion of eggs protected by the females in their current clutch (laid between day 2 and day 4) according to this clutch size (current fecundity = number of eggs laid between day 2 and day 4), in order to highlight both the absolute investment in egg protection and the relative investment into egg protection compared with egg production in these females.

Initial data exploration revealed that, within the first immune-protected egg-laying sequence (between day 2 and day 4 after the immune challenge), the relationship between either the number or the proportion of immune-protected eggs and the current number of eggs produced was quadratic. Therefore, the number of eggs protected in this sequence was analysed using a GLM, with a Poisson distribution corrected for overdispersion (dispersion parameter = 1.42). The initial model used female immune treatment and female larval food condition as factors, current fecundity (the number of eggs laid during this sequence) as a quadratic term and past fecundity (number of eggs laid before the production of the first immune-protected egg-laying sequence, between day 0 and day 2 after the immune challenge) as covariates. The proportion of eggs protected by a female during this sequence was analysed using a GLM with a binomial distribution (presence/absence of protection in the eggs) using the same explanatory variables as mentioned earlier.

All the data were analysed using R software [24]. The GLMMs were performed with the add-on R package lme4 [25].

Model selection was achieved using a stepwise backward deletion procedure with Akaike's information criterion (AIC) whereby initial models included all main effects and two-way interactions [26].

3. RESULTS

(a) *Experiment 1: temporal dynamics of the antibacterial immune response of immune-challenged females and of the transmission of antibacterial activity to their eggs*

As expected, the immune challenge elicited an antimicrobial immune response in the haemolymph of the females and affected the antimicrobial activity of their eggs. The antibacterial activity of the females varied over time ($F_{6,104} = 87.3$, $p < 0.001$), was the highest 2 days after the immune challenge and then kept declining to day 14 (figure 1*a*). The proportion of eggs found protected

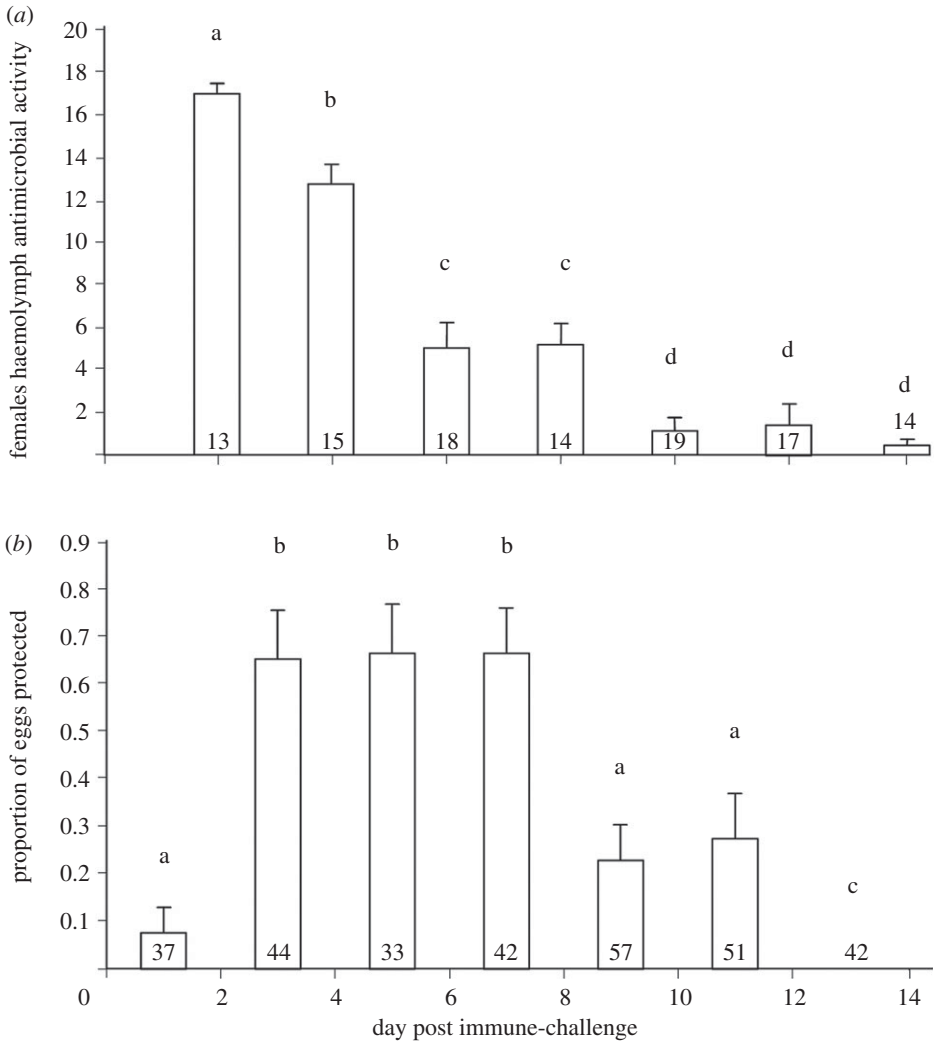

Figure 1. (*a*) Antibacterial activity of the haemolymph of the females (mean diameter of the zone of inhibition in mm ± s.e.) and (*b*) proportion of eggs protected (±s.e.) according to the time following the maternal immune challenge. Time laps with the same letter show no significant difference for the antibacterial activity of the females' haemolymph or in the proportion of eggs protected (Tukey's post hoc test, $p < 0.05$). Numbers inside the bars indicate the number of females or eggs assayed at each egg-laying sequence.

also varies significantly over time ($\chi^2_{6,104} = 59.1$, $p < 0.01$; figure 1*b*). Between day 0 and day 2, the occurrence of a zone of inhibition in the eggs was sparse. We detected a substantial transmission of antibacterial activity to the eggs at day 2, while the antimicrobial activity of the females was declining. The proportion of eggs found protected at each laying sequence remained at the same level between day 4 and day 8. From days 8 to 10 and 10 to 12, the proportion of protected eggs returns to a similar level as that of day 0 to day 2. After 12 days, no eggs were found protected. Even in the most protected egg-laying sequences, the proportion of eggs protected never equalled 100 per cent.

(b) *Experiment 2: localization of the antibacterial activity transferred to the eggs*

We pooled five eggs of each 10 females tested for analyse of antibacterial activity. Antibacterial activity was never found at the surface of these eggs ($n = 10$). We then analysed the internal extracts of each egg separately. All of the eggs showed an internal activity ($n = 50$).

(c) *Experiment 3: testing the trade-off between mother fecundity and number of immune-protected eggs*

Larval food manipulation succeeded in producing adult females of different body masses. Females obtained from poor food conditions were significantly lighter than those obtained from good food conditions (mean ± s.e.: poor = 97.07 ± 22.47 mg; good = 128.20 ± 39.79 mg; $t = 7.19$ d.f. = 60, $p < 0.001$).

Past fecundity and current fecundity were both independent of the maternal immune treatment ($\chi^2_{1,58} = 0.68$, $p = 0.4$) and body mass ($\chi^2_{1,58} = 1.37$, $p = 0.24$). Current fecundity was significantly higher than past fecundity ($\chi^2_{1,58} = 85.28$, $p < 0.001$, current fecundity = 13.56 ± 8.52; past fecundity = 8.21 ± 7.50; $F_{1,60} = 18.66$, $p < 0.001$).

There was no trade-off between the number of eggs protected and the amount of protection allocated per egg in the first protected egg-laying sequence. Instead, the number of eggs protected correlated positively with the amount of protection they received ($F_{1,51} = 6.54$, $p = 0.014$; figure 2). As expected, LPS-treated mothers

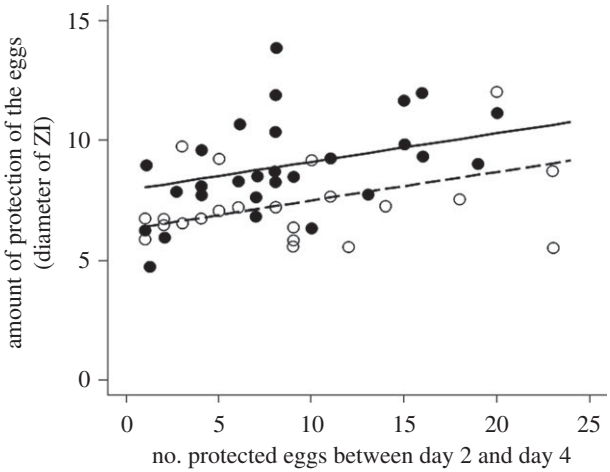

Figure 2. Relationship between the amount of antibacterial activity allocated to each egg by control (dashed line) and LPS-treated females (solid line) between the days 2 and 4 post immune-challenge according to the number of eggs protected in this egg-laying sequence. Open and filled circles represent the mean antibacterial activity of all the eggs protected by each control and LPS-treated mother, respectively.

provide their eggs with higher levels of antimicrobial activity ($F_{1,51} = 14.14$, $p < 0.001$; figure 2). The amount of protection allocated per egg was independent of the larval food condition of females ($F_{1,49} = 0.01$, $p = 0.92$).

There was a significant relationship between the number of eggs protected in the current clutch (between day 2 and day 4 after the immune challenge) and the size of this clutch (current fecundity in interaction with the maternal immune treatment in table 1). In control females, the number of eggs protected correlated positively with current fecundity, whereas for LPS-treated mothers, the relationship was quadratic (figure 3*a*). There was a significant interaction between past fecundity (between day 0 and day 2) and maternal immune treatment on the number of eggs protected between day 2 and day 4 (table 1). In control mothers, the number of eggs protected correlated positively with past fecundity but not in LPS-treated mothers (see electronic supplementary material, figure S1). There was also a significant interaction between the larval food condition of mothers and their past fecundity on the number of eggs currently protected (table 1). In females from good food conditions, the number of eggs protected correlated positively with their past fecundity but not in females from poor food conditions (see electronic supplementary material, figure S2*a*).

Similar to the number of eggs protected, the proportion of protected eggs was significantly associated to current fecundity in interaction with the maternal immune treatment (table 1). In control females, the proportion of eggs protected was not related to current fecundity, whereas for LPS-treated mothers, the relationship was significant and quadratic (figure 3*b*). The proportion of protected eggs was associated to past fecundity in interaction with mother larval food conditions (table 1). In females from good food condition, the proportion of protected eggs correlated positively with their past fecundity but not in females from poor food conditions (see electronic supplementary material, figure S2*b*). However, variation in the proportion of protected eggs could not be explained by past fecundity in interaction with the maternal immune treatment.

4. DISCUSSION

This study provides evidence of a transient maternal transfer of immune protection to the eggs after a bacterially based benign immune challenge of females of the mealworm beetle, *T. molitor*. As previously found in another insect model [6], the maternal transfer of immunity to the eggs of *T. molitor* was achieved through the provision of antibacterial substances inside the eggs rather than an imbuement of the surface of the eggs with immune substances within the female reproductive tract [23,27,28]. During the vitellogenesis, the main components of the eggs are released in the female's haemolymph by the fat body and then recruited inside the eggs (reviewed in [29]). Because the fat body is also the main organ responsible for the synthesis of antimicrobial peptides following an immune challenge [30], this organ may also provide the antimicrobial substances incorporated inside the eggs. Thus, a certain amount of the antimicrobial peptides dedicated to the mother's own defence could be directed to the ovaries and imbued to the eggs.

More importantly, our data reveal that a large number of eggs were not protected, even for the egg-laying sequences where the maternal transfer of immunity was peaking. This result provides further evidence that maternal transfer of immunity in this species is costly and suggests that the immune protection of the eggs is constrained by the availability of antibacterial substances produced by immune-challenged mothers. As a result, immune-challenged females seem to favour the immune protection of a limited number of eggs with a sufficient amount of immune substances per egg to efficiently protect them, instead of supplying equally each egg of the clutch, which might result in an inefficient protection. Indeed, the size of the zone of inhibition of the eggs was repeatable within females ($r = 0.577$ from experiment 3). Furthermore, we did not found any trade-off between the amount of immune protection allocated per egg and the number of eggs protected, as it would be expected if females share their immune resource equally to their eggs. In contrast, we found a positive relationship between these variables.

As female body condition had only a weak effect on the amount of protection allocated per egg, the cost of the maternal transfer of immunity to the eggs may not result from an energy restriction, but rather from a limited amount of antimicrobial peptides that could be transferred to the eggs at a given time. Alternatively, the fact that our model species is able to feed at the adult stage may allow it to compensate for a reduced energy stock at emergence. Therefore, the amount of protection per egg and the number of eggs protected may reflect the quality of the female and/or individual level of investment into TGIP.

As the maternal transfer of immunity is costly for females [7], a negative relationship is expected with other costly fitness traits such as fecundity. However, this relationship may not be necessarily linear [31] as often observed for the relationship between offspring

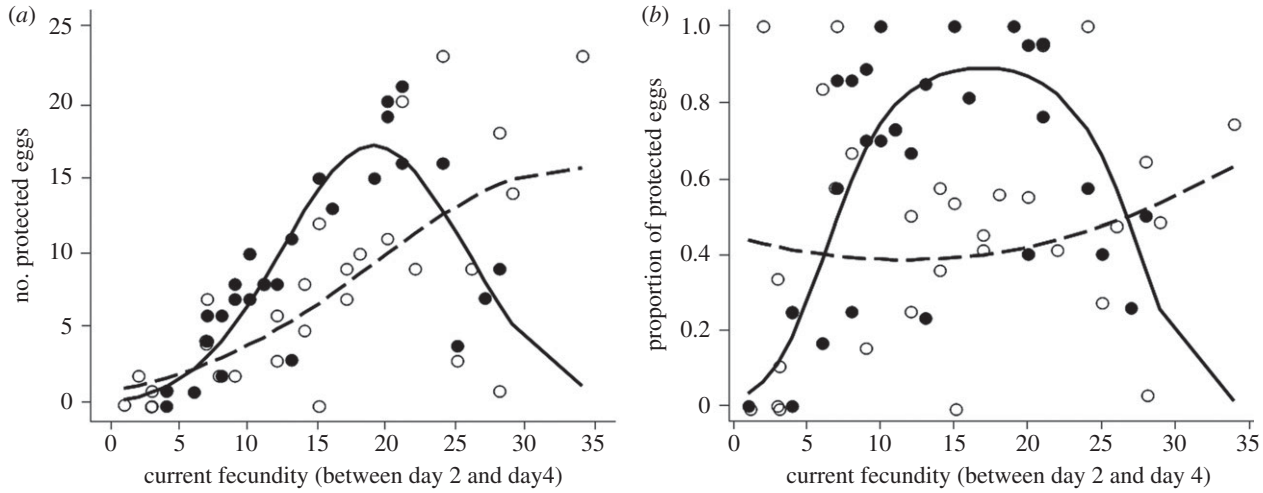

Figure 3. Relationship between the current fecundity (between day 2 and day 4) and (*a*) the number of eggs protected and (*b*) the proportion of eggs protected by control (dashed line) and LPS-treated females (solid line) during this egg-laying sequence. Open and filled circles represent the numbers and proportions of eggs protected by control and LPS-treated mothers, respectively.

Table 1. A summary of the optimal GLM following stepwise-deletion of the number and proportion of eggs protected by females between day 2 and day 4 post immune-challenge, fitted with the effects of the past fecundity (between day 0 and day 2), the current fecundity (between day 2 and day 4), the maternal immune treatment (treatment) and the rearing condition of mothers (condition). Number of eggs protected: $n_{females} = 59$; deviance explained = 77.20%. Proportion of eggs protected: $n_{females} = 59$, deviance explained = 56.91%.

	number of eggs protected		proportion of eggs protected	
source	$LR\chi^2_{1,49}$	p	$LR\chi^2_{1,50}$	p
condition	14.14	<0.001	46.17	<0.001
treatment	2.74	0.098	7.44	0.006
past fecundity	4.07	0.044	97.66	<0.001
current fecundity	2.05	<0.001	36.42	<0.001
current fecundity^2	28.36	<0.001	36.74	<0.001
condition × past fecundity	6.98	0.008	25.73	<0.001
treatment × past fecundity	9.10	0.003	—	—
treatment × current fecundity	11.06	<0.001	19.79	<0.001
treatment × current fecundity^2	13.09	<0.001	20.91	<0.001

size and offspring number in iteroparous species [32]. In *T. molitor*, the number of eggs laid at each reproductive event is highly variable within and between individuals (from experiment 1: mean fecundity ± s.e. between day 0 and day 2 = 12 ± 10.15; day 2 and day 4 = 10.54 ± 6.73; day 4 and day 6 = 9.59 ± 8.8; day 6 and day 8 = 16.28 ± 9.65; day 8 and day 10 = 16.84 ± 6.44; day 10 and day 12 = 15.47 ± 6.84; day 12 and day 14 = 8.93 ± 6.11). Among immune-challenged females, we found a quadratic relationship between their current fecundity and number and proportion of eggs protected. The benefits to the protection of the eggs by challenged females remain to be tested. Assuming that an increase in the eggs immunocompetence would translate in a better resistance to pathogens [33], such a relationship would reveal three main situations in response to the maternal immune challenge, which may have different implications for the fitness of mothers depending whether the infection persists over the maternal generation. First, some females did not invest either in egg production or in egg protection (see left-hand side of the bell-shaped curve in figure 3). The relative success of this clutch will be low irrespective of whether the maternal infection persists or not. These females may have intended to postpone their reproductive effort to the next egg-laying sequences [34]. Alternatively, these females may have laid the eggs that had matured before the immune challenge and were therefore not provided with immune protection, as suggested by the absence of protection in the first egg-laying rank observed in figure 1*b*. Second, some females exhibited an intermediate current fecundity but optimized the protection of their clutch (top of the bell-shaped curve in figure 3). The relative success of this situation will be maximal when the maternal infection is persistent in the next generation. Third, some females exhibited a relatively high fecundity but protected a low number of their eggs (right-hand side of the bell-shaped curve in figure 3). They may gain from producing diverse offspring in a sceptic environment [34], but their relative success will be maximal when the maternal infection does not persist in the next generation. Therefore, the expression of the trade-off between current fecundity and TGIP of

the eggs should be maintained by the variation in the persistence of pathogens between generations.

Because of the trade-off between current egg production and egg protection, TGIP might be expected in iteroparous species rather than in semelparous ones. In line with this, TGIP has been evidenced in iteroparous arthropods [3,4,9–14,35] and not in semelparous ones [36,37]. Iteroparity could allow females to adjust their relative investment into egg protection compared with egg production in accordance to the risk of infection of the progeny and their own risk of dying from the infection. Indeed, iteroparous females may gain from saving immune substances when they are needed for their own defence by delaying investment into egg protection until the pathogenic threat is overcome.

We demonstrate in this study a cost to the inducible transmission of antibacterial activity to the eggs following the immune challenge of the mothers. The existence of such a cost suggests that this transmission might not just be a side effect accompanying the immune reaction of the mothers following an immune challenge, but rather an investment that has been selected.

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