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EARWIG MOTHERS CAN BOOST OFFSPRING'S DEFENCE AGAINST PATHOGENS DURING POSTOVIPOSITION CARE

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Abstract

An important function of parental care is to ensure the survival of offspring against pathogens. In insects, it was long believed that this function was limited in scope and time and consisted mainly of parental behaviours reducing contact between offspring and pathogens. However, recent data show that insect parents can also improve offspring's defence against pathogens by transferring their acquired immune experience to the eggs during oogenesis. Here, we investigated whether this parental protection could be extended to include postoviposition care. We addressed this question in the European earwig, *Forficula auricularia*, an insect in which mothers provide extensive forms of care to their eggs and juveniles. We immune-challenged mothers with dead bacterium *Serratia marcescens* or control solutions while they were caring for their eggs and then monitored the survival rate of these mothers and their resulting adult offspring after a new infection by the same live pathogen. In line with our prediction, we found that both the mothers that were immune-challenged after oviposition and the adult offspring reared by these immune-challenged mothers had the highest survival after infection. However, what triggered this higher survival rate was the wounding of the mothers and not their previous exposure to dead bacteria. Our data also show that these higher survival rates were not associated with changes in the expression of maternal egg care or offspring quality. Overall, our findings shed light on a novel benefit of postoviposition parental care in insects. More generally, they support the recent claim that access to social immunity by offspring may play a key role in the early emergence and maintenance of family life in animals.

Keywords: European earwig, *Forficula auricularia*, immune memory, immune priming

Insect, parental care, TGIP

Introduction

Parental care is ubiquitous in animals (Smiseth et al., 2012). From mammals to birds, arachnids, molluscs and insects, parental care typically ensures normal development and survival of juveniles in the face of various environmental hazards (Klug & Bonsall, 2014). Among these, pathogens are often critical (Schmid-Hempel, 2021; Vale et al., 2018). In vertebrates, parental protection against pathogens can take a variety of forms that are expressed throughout offspring development. They can help to both limit offspring's exposure to current or future pathogens (e.g. nest construction) and strengthen their immune defences against potential future infections (e.g. transfer of immunity; Bush & Clayton, 2018; Jacquin et al., 2012; Knouft et al., 2003). In insects, this protection has long been believed to be less diverse (Meunier et al., 2022), mostly occurring before oviposition or hatching and only helping the offspring to limit contact with pathogens. For example, some insect mothers add self-secreted substances with antimicrobial properties to the nest environment (Diehl et al., 2015; Rozen et al., 2008) or clean the eggshell and surface of the juveniles to rid them of external microorganisms (Boos et al., 2014; Smiseth et al., 2012).

Over the past decade, a growing body of evidence has shown that insect parents may not only limit offspring contact with pathogens but also have long-term effects on the defence of their offspring against pathogens (Roth et al., 2018). For instance, these studies show that the immune protection acquired by insect parents after an initial immune challenge (i.e. immune priming; Contreras-Garduño et al., 2016; Dhinaut et al., 2018; Prigot-Maurice et al., 2021) can be transferred to the eggs during oogenesis (i.e. transgenerational immune priming; Eggert et al., 2014; Tetreau et al., 2019) and this transfer can later enhance the protection of newly hatched juvenile and adult offspring against this or other pathogens (Pigeault et al., 2016; Rutkowski et al., 2023). However, this parental transfer still has a relatively narrow window of action because it occurs before oviposition, and it is unclear whether insect mothers can pass on their acquired immune protection later during egg and offspring development (Sadd & Schmid-Hempel, 2007). This postoviposition transfer could occur in species that exhibit parental care, which is a behaviour that can last for several seasons up to several years in some insects (Machado & Trumbo, 2018; Meunier et al., 2022). In these species, it could help parents to reduce the potential mismatch between the pathogens encountered by the parents before oviposition and during egg development and those encountered by the resulting offspring throughout their development (Roth et al., 2018; Sadd & Schmid-Hempel, 2009). Parental care is known to mediate the transfer of various substances from parents to offspring (including eggs), and some of these substances can shape

the immune response of juveniles (Meunier et al., 2022; Onchuru et al., 2018; Tetreau et al., 2019). Finally, the ability of parents to transfer immune experience acquired after egg laying (or after the birth of offspring) to their young has been reported in mammals and birds (Jacquin et al., 2012; Sparks et al., 2020), opening up scope for further investigation in other taxonomic groups (Sadd & Schmid-Hempel, 2007), such as insects (Duarte et al., 2016; Ratz et al., 2021; Steiger et al., 2011).

In this study, we used the European earwig, *Forficula auricularia*, to test whether mothers can improve their own defence against pathogens following an immune challenge and whether they can transfer this enhanced protection to their offspring during postoviposition care. In this insect, mothers usually lay their eggs in early winter. They then care for the eggs over the following 2 months of winter and for the newly hatched juveniles (called nymphs) for 2 weeks in early spring (Meunier, 2024). Earwig maternal care can take a variety of forms, including nest building, grooming and application of chemical substances on eggs and nymphs, clutch displacement, food provisioning and expression of fierce behaviour against intruders (Merleau et al., 2022; Staerke & Kölliker, 2008; Thesing et al., 2015; Tourneur et al., 2022; Van Meyel et al., 2019). Earwig mothers also protect their eggs and juveniles against pathogens. For instance, they actively remove fungal spores from the eggshell (Boos et al., 2014), clean and rebuild their nest in the presence of active pathogens in the environment (Diehl & Meunier, 2018) and line the nest with their faeces, which contain antimicrobial compounds, to prevent microbial growth (Diehl et al., 2015). All these forms of care are essential for the survival of the eggs because the clutch will die from mould within a few days in the absence of a mother (Boos et al., 2014; Van Meyel et al., 2019). However, this is not the case for nymphs, which can survive just as well in the absence as in the presence of a mother after hatching (Kölliker, 2007). An increasing number of studies in this species have shown that age, sex, mating status, rearing conditions, morphology and social environment can all influence the immunity and survival of nymphs and adults following pathogen infection (Coulm & Meunier, 2021; Kohlmeier et al., 2016; Körner et al., 2018; Vogelweith, Foitzik, et al., 2017; Vogelweith, Körner, et al., 2017). However, it is not known whether the survival of mothers following pathogen infection can be enhanced after an initial immune challenge and whether mothers can transfer this enhanced protection to their offspring during postoviposition care.

To address these questions, we immune-challenged earwig mothers with the Gram-negative bacteria *Serratia marcescens* during postoviposition egg care and then evaluated the survival rate of these mothers and their adult offspring after a new injection of the same

pathogen (homologous challenge) at a median lethal dose. To induce the immune challenges, we either injected the mothers with a solution of heat-killed *S. marcescens* at two concentrations, wounded them by injecting a sterile buffer solution of phosphate-buffered saline (PBS) or left them unmanipulated (no injection or wounding) as a control group. We then investigated whether immune-challenged mothers boost their offspring's defence against pathogens by altering their expression of care and/or whether this boost could reflect changes in the overall quality of their offspring. To this end, we measured four forms of maternal egg care and four proxies for egg and offspring quality in the tested clutches. If an immune challenge improves the survival of mothers following a subsequent infection and if these mothers transfer the resulting protection to their offspring during postoviposition care, we expected that both mothers and their adult offspring would survive the median lethal dose injection better if the mother had been immune challenged than if she had not been immune challenged. If this transfer was due to changes in the expression of care and altered offspring quality, we expected the maternal immune challenges to modify the expression of maternal egg care and improve the resulting clutch quality.

METHODS

Insect Rearing

Our experiment involved a random subset of 220 of 2000 females collected in July 2021 from peach orchards near Valence, France (Lat 44.9772790, Long 4.9286990). These females belonged to *F. auricularia* sp. A (González-Miguéns et al., 2020). Immediately after field sampling, 2000 females were placed with field-sampled males from the same population in plastic containers (balanced sex ratio) lined with moistened sand and containing egg cardboard to provide shelter for individual earwigs. This set-up allowed for uncontrolled mating (Sandrin et al., 2015). Each container was then maintained in cycles of 12 h of light at 20 °C followed by 12 h of darkness at 18 °C. They received an ad libitum amount of artificial food that was changed once a week and consisted mainly of a mixture of pollen, cat food and bird seed (Kramer et al., 2015).

Five months later, we isolated each of the 2000 females to mimic their natural reproductive behaviour and stimulate oviposition (Kölliker, 2007). To do this, we transferred them to individual petri dishes (55 mm × 12 mm) lined with moistened sand and then kept them in constant darkness at 10 °C until oviposition and egg hatching. Of these 2000 isolated females, we immune-challenged a random subset of 220, of which we used 120 to measure their survival to a new infection and 100 to successively measure maternal egg care, egg

development and survival of offspring to a new infection (Fig. 1). Between the day of isolation and oviposition and then from egg hatching onwards, all females (and the resulting offspring) were fed an ad libitum amount of artificial food once a week. We removed food between oviposition and hatching because mothers typically stop their foraging activity during this period (Kölliker, 2007). After hatching, mothers and juveniles were maintained under a 12:12 h light:dark cycle at 20:18 °C.

Bacterial Strain and Culture Conditions

The bacteria used in this study were *S. marcescens*, which are generalist, opportunistic entomopathogens commonly found in soil and water and are often used to test for the presence of immune priming and transgenerational immune priming in insects (Medina Gomez et al., 2018; Mikonranta et al., 2014; Rosengaus et al., 2013). This strain was isolated in the laboratory from honey bees, *Apis mellifera*, and then routinely cultivated at 30 °C on lysogeny broth (LB; 10 g/litre tryptone, 10 g/litre NaCL and 5 g/litre yeast extract) agar plates. Before the experiments, a single colony was transferred to 10 ml of LB and incubated overnight at 30 °C under constant shaking (180 rpm) to obtain exponentially growing *S. marcescens*. We then diluted the culture by introducing it into fresh LB at a final optical density at 600 nm of 0.1 (10^8 colony-forming unit [CFU]/ml) and incubated the mixture for 4 h at 30 °C under constant shaking. Bacteria were then washed twice ($6000 \times g$ for 3 min) with 1 ml of sterile PBS before use.

Maternal Immune Challenge

Forty-seven days after oviposition (i.e. approximately 10 days before the expected date of egg hatching; Tourneur & Meunier, 2020), we randomly assigned 220 females to four immune challenge treatments. We injected them with either 50 nl of a solution of heat-killed *S. marcescens* diluted in sterile PBS at a concentration of (1) 2×10^5 CFU/ml or (2) 2×10^7 CFU/ml, (3) with 50 nl of sterile PBS or (4) did not inject them at all. The sample size per treatment is shown in Table 1. There was no difference in the number of eggs produced per female between the four treatments ($F_{3,209} = 1.25$, $P = 0.292$). We killed *S. marcescens* using a water bath at 65 °C for 30 min (Prigot-Maurice et al., 2021), and we verified the absence of growth by spreading the bacteria on LB agar plates (30 °C) and observing the petri dishes for 4 days. Each female was injected in the ventral abdominal segment using a Drummond Nanoject (3-000-207). After the immune challenge, each female was immediately returned to

her eggs. In total, we carried out three blocks of immune challenges at 1-week intervals, each using different bacterial cultures and sterile PBS solutions.

Survival of Mothers after Injection of the Live Pathogen

We tested the ability of immune-challenged mothers to survive a subsequent infection by injecting live *S. marcescens* into 120 of the 220 mothers (from two blocks of immune challenges) and then monitoring their survival for 21 days (Fig. 1). Seven days after the immune challenge, we injected each female with 50 nl of a solution of live *S. marcescens* at a concentration of 2×10^7 CFU/ml. These mothers were randomly distributed across the four immune challenge treatments as follows: 28 females previously injected with 2×10^5 CFU/ml of heat-killed *S. marcescens* ($N=14$ and 14 in the first and second blocks, respectively), 29 females previously injected with 2×10^7 CFU/ml of heat-killed *S. marcescens* ($N = 13$ and 16), 33 females previously injected with PBS ($N = 18$ and 15) and 30 control females ($N = 15$ and 15). The injection of live *S. marcescens* was performed as detailed above. After this injection, each female was returned to her eggs and maintained at 19°C to allow egg development and hatching. We then recorded the survival of each female daily for the following 21 days. None of the mothers tested died between the immune challenge treatment and the injection of live *S. marcescens*.

Maternal Egg Care

We tested whether maternal immune challenge alters the expression of four forms of egg care (egg collection rate, egg defence, delay in maternal return after clutch abandonment and mother–egg contacts) in the 100 mothers not used for survival analyses (Fig. 1). We first measured the egg collection rate either 24 h ($N = 62$ mothers) or 48 h ($N = 58$ of the previous 62 mothers) after maternal immune challenge (13 and 18 mothers per treatment and time point). This behaviour reflects the propensity of an earwig mother to gather her eggs after they have been experimentally spread out (Merleau et al., 2022). For each clutch, we carefully removed the mother from her original petri dish, scattered 20 of her eggs (all eggs in the very few cases where there were fewer than 20) within a 4 cm circle and then returned each mother to her petri dish. Two hours later, we recorded whether each mother gathered the entire clutch, i.e. whether all eggs were within one egg distance from each other. When clutches contained more than 20 eggs, the remaining eggs were maintained in a separate petri dish lined with moist sand during these 2 h and then returned to the original clutch (Merleau et al.,

2022).

We then measured the level of clutch defence 24 h after maternal immune challenge. This behaviour reflects the willingness of earwig mothers to protect their eggs from a simulated predator attack (Thesing et al., 2015). In a standard manner, we poked each mother by hand on the pronotum with a glass capillary (Hirschmann ringcaps 1/2/3/4/5 μ l; 1 poke/s) and recorded the number of pokes required until the mother moved more than one body length away from her initial position. All females were close to their eggs at the start of the measurement. As a third form of egg care, we measured the delay of maternal return after clutch abandonment. This behaviour reflects how long earwig mothers are willing to abandon their clutch of eggs after a simulated predator attack (Van Meyel et al., 2019). It was measured by recording the time the mother took to return to her clutch just after the end of the egg defence measurement. This delay was recorded for a maximum of 10 min. Finally, we measured the number of mother–egg contacts 72 h after immune challenge for 30 min. These numbers were obtained using a scan-sampling technique in which we recorded every minute (for 30 min) whether mothers were interacting with their eggs (mother–egg contacts), defined as mothers actively touching their eggs with their antennae or mouthparts (Merleau et al., 2022).

For all behavioural measurements, the observer did not know the immune challenge treatment of the mother tested. Observations were made by a single observer (MB). We conducted all these behavioural measurements in 30 mothers from each of the 2×10^7 CFU/ml, 2×10^5 CFU/ml, PBS and control treatment groups (Table 1). At the end of the behavioural measurements, each mother was maintained under standard conditions (see above).

Egg and Offspring Quality

We then tested whether maternal immune challenge after oviposition affected egg and offspring quality using the 100 clutches involved in the maternal egg care measurements (Table 1). We measured four proxies for these qualities, namely the likelihood of complete clutch failure, egg development time, egg hatching rate and mean weight of the resulting nymphs. We first defined complete clutch failure as a binary value reflecting either that all eggs disappeared from the clutch (1) or at least one nymph emerged from the clutch (0). Egg disappearance is typically due to filial egg cannibalism in this species (Van Meyel & Meunier, 2020). Second, we defined egg development time as the number of days between oviposition and egg hatching (if any). Third, we defined egg hatching rate by comparing the number of

newly produced nymphs 1 day after egg hatching, because all eggs typically hatch within a day (Koch & Meunier, 2014), with the number of eggs initially present. Finally, we measured the mean weight of the newly produced nymphs by weighing a random group of 10 nymphs per family (or all nymphs if fewer than 10 were available) 1 day after the first egg had hatched (all nymphs typically hatch within a day). The weighing was conducted to the nearest 0.01 mg using a microbalance (OHAUS Discovery DV215CD).

Survival of Adult Offspring after Injection of Live Pathogen

Having tested whether mothers were better able to survive a pathogen infection after a previous immune challenge, we finally tested whether they were able to transfer this enhanced protection to their offspring during postoviposition care. We used a total of 88 F1 adult offspring produced by a random subset of 37 of the 100 immune challenged females (1–4 offspring per family, mean = 2.4) involved in the measurement of egg care and offspring quality (Table 1, Fig. 1). Approximately 30 days after their emergence, we injected these adult offspring with 50 nl of a solution of live *S. marcescens* at a concentration of 2×10^6 CFU/ml. This provided us with (1) 33 F1 adults from mothers previously injected with 2×10^7 CFU/ml of heat-killed *S. marcescens*, (2) 12 F1 adults from mothers previously injected with 2×10^5 CFU/ml of heat-killed *S. marcescens*, (3) 21 F1 adults from mothers previously injected with PBS and (4) 22 F1 adults from control mothers. (The number of males and females per treatment is reported in Table 1.) We then recorded the survival of each adult daily for the following 21 days. Note that differences in sample size between treatments do not reflect differences in mortality but are due to a blinded sampling process. The injection of live *S. marcescens* followed the method detailed above.

Statistical Analysis

All statistical analyses were performed with R v4.3.0 (R Core Team, 2023) loaded with the packages DHARMA (Hartig, 2020), car (Fox & Weisberg, 2019), emmeans (Lenth, 2022), survival (Therneau, 2020a) and coxme (Therneau, 2020b). We analysed the survival of immune-challenged mothers and their descendants using one Cox proportional hazard regression model and one mixed-effects Cox proportional hazard regression model, respectively, allowing for censored data, i.e. individuals alive 21 days after the lethal injection. In these two models, the type of immune challenge (control, PBS, 2×10^5 CFU/ml of heat-killed *S. marcescens* or 2×10^7 ml of heat-killed *S. marcescens*) was entered as an

explanatory factor. Because some offspring originate from the same mothers, the ID of the mother of origin of each offspring was entered as a random effect into the model on offspring survival. The model for maternal survival also included the set of injections (first or second) and the interactions between these factors as explanatory factors, whereas the model for F1 adult survival also included the sex of the offspring (male or female) and the interactions between these factors as explanatory factors. We then analysed egg care using two linear models (LMs), one generalized LM (GLM) and one Cox proportional hazard regression model, allowing for censored data (this time, to account for females that did not return to their eggs at the end of the observation time). In these four models, the response variable was either clutch defence (LM), number of mother–egg contacts (LM), egg gathering (GLM with a binomial error distribution) or delay of maternal return after clutch abandonment (Cox model). The explanatory variable was always the type of maternal immune challenge, to which we added the day of measurement after challenge (24 h or 48 h) and the interaction between these two variables in the model on egg gathering. Finally, we analysed the fate of eggs using four models, in which we entered the type of maternal immune challenge as the explanatory variable and the likelihood of complete clutch failure (GLM with a binomial error distribution), egg development time (LM), egg hatching rate (GLM with a binomial error distribution corrected for overdispersion) and mean weight of nymphs (LM) as the response variables. The egg hatching rate was entered as an odd ratio using the `cbind` function in R and was based on the number of juveniles produced and the number of eggs that did not result in juveniles (Thesing et al., 2015). Overall, we checked that all model assumptions were met using the DHARMA R package. We conducted post hoc pairwise comparisons on the LM, GLM and Cox models using the estimated marginal means of the models, and we corrected *P* values for multiple testing using the Tukey method, as implemented in the *emmeans* R package

Ethical Note

The study involved 220 females and 88 of their adult offspring. All individuals were handled with care. The 220 females were a subsample of 2000 females collected in the field and then maintained under laboratory conditions. The females (and offspring) not used in this study were involved in other experiments (not reported here). No animal ethics committee approval was required.

RESULTS

Survival of Mothers after Injection of Live Pathogen

The survival of mothers injected with live *S. marcescens* depended on their previous immune challenge (Fig. 2a; likelihood ratio $\chi^2_3 = 23.80$, $P = 0.0001$). However, the pattern observed did not support better protection after exposure to dead *S. marcescens*. Mothers previously injected with PBS survived longer than those previously injected with 2×10^5 (pairwise comparisons using log-rank test; $P = 0.011$) or 2×10^7 CFU/ml of heat-killed *S. marcescens* ($P < 0.0001$) and control mothers ($P = 0.023$). Moreover, control mothers survived better than mothers that were previously injected with 2×10^7 CFU/ml of heat-killed *S. marcescens* ($P = 0.038$). There was no difference in survival rate between mothers previously injected with 2×10^5 and 2×10^7 CFU/ml of heat-killed *S. marcescens* ($P = 0.226$) and between mothers previously injected with 2×10^5 CFU/ml of heat-killed *S. marcescens* and control mothers ($P = 0.525$). Overall, mothers had a better survival rate in the second than in the first series of injections (LR $\chi^2_1 = 4.64$, $P = 0.021$), but this effect was similar for each injection treatment (interaction between the set of injections and the type of immune challenge: LR $\chi^2_3 = 6.61$, $P = 0.085$).

Survival of Adult Offspring after Injection of Live Pathogen

The survival of F1 adults injected with live *S. marcescens* also depended on the immune challenge of their mothers after oviposition (Fig. 2b; LR $\chi^2_3 = 13.13$, $P = 0.004$). As with their mothers, F1 adults survived overall longer when their mothers were previously injected (after oviposition) with PBS compared with F1 adults with mothers were previously injected 2×10^5 (pairwise comparisons using log-rank test; $P = 0.016$) or 2×10^7 CFU/ml of heat-killed *S. marcescens* ($P = 0.033$) and control mothers ($P = 0.048$). By contrast, there was no difference in survival between F1 adults from control mothers and those from mothers previously injected with 2×10^7 ($P = 1.000$) or 2×10^5 ($P = 0.826$) CFU/ml of heat-killed *S. marcescens* and between F1 adults from mothers previously injected with 2×10^7 CFU/ml of heat-killed *S. marcescens* and those from mothers previously injected with 2×10^5 CFU/ml of heat-killed *S. marcescens* ($P = 0.797$). The survival rate of F1 adults injected with live *S. marcescens* was also independent of sex (LR $\chi^2_1 = 0.23$, $P = 0.635$) and the interaction between sex and maternal immune challenge (LR $\chi^2_3 = 3.26$, $P = 0.353$).

Effect of Maternal Immune Challenge on Egg Care and the Fate of Eggs

The immune challenge of mothers did not affect their expression of egg care (Fig. 3). It did

not affect egg gathering (Fig. 3a; LR $\chi^2_3 = 0.70$, $P = 0.872$), clutch defence (Fig. 3b; $F_{3,96} = 0.36$, $P = 0.779$), delay of maternal return after clutch abandonment (Fig. 3c; $\chi^2_3 = 1.27$, $P = 0.735$) and number of mother–egg contacts (Fig. 3d; $F_{3,96} = 0.29$, $P = 0.831$). Egg gathering was also independent of the day of measurement (i.e. 24 h or 48 h after maternal immune challenge; LR $\chi^2_1 = 0.25$, $P = 0.620$) and interaction between the day of measurement and the type of injection (LR $\chi^2_3 = 5.47$, $P = 0.141$).

Similarly, the immune challenge of mothers did not affect the fate of their eggs. It did not influence the likelihood of complete clutch failure (LR $\chi^2_3 = 2.55$, $P = 0.466$), egg development time ($F_{3,76} = 0.77$, $P = 0.513$), hatching rate (when at least one nymph was present; LR $\chi^2_3 = 0.95$, $P = 0.813$) and mean weight of newly hatched nymphs ($F_{3,78} = 0.16$, $P = 0.924$).

DISCUSSION

Protecting offspring from the risk of infection is a key function of parental care. A growing body of research in insects shows that an important way of fulfilling this function is by transferring the acquired immune experience of the parents to the eggs during oogenesis (Eggert et al., 2014; Roth et al., 2018; Tetreau et al., 2019). Our data support the existence and transfer of such acquired immune experience in the European earwig but reveal that this transfer may occur much later in the development of offspring through postoviposition egg and/or juvenile care. In particular, we showed that earwig mothers acquired better protection against *S. marcescens* after previous wounding (and PBS injection) than after exposure to heat-killed *S. marcescens* or no previous immune challenge. We also showed that this enhanced defence acquired after oviposition was passed on to their offspring. The eggs cared for by treated mothers produced adult offspring that survived the injection of live *S. marcescens* better if their mother was wounded (and PBS injected) than if she was not wounded or if she was injected with heat-killed *S. marcescens*. This apparent increase in maternal and offspring's defence against pathogens was not associated with changes in the expression of the four measured forms of maternal egg care or with changes in egg and offspring quality.

We first found that wounding (together with a PBS injection) made earwig mothers less susceptible to subsequent infection by the pathogen *S. marcescens*. In insects, wounding is typically known to activate several channels of the cellular and humoral immune response (Krautz et al., 2014; Peuß et al., 2015). The resulting immune investment can be inexpensive

for the host (Ardia et al., 2012; Freitak et al., 2003; Rantala & Roff, 2006), which can thus maintain it for up to several weeks to quickly fight off pathogens that would enter through the wound opening (Krautz et al., 2014). Our data are consistent with the existence of such a low-cost, sustained immune response to wounding in earwig females, although further research is needed to quantify and compare the costs of the immune response to wounding and microbial injection. Interestingly, they also emphasize that this response becomes expensive when wounding is accompanied by contact with (dead) pathogens. This is because challenging the immune system of earwig mothers by wounding and injecting killed *S. marcescens* made them more susceptible to subsequent infection. Taken together, these data may suggest that the development of an immune response against a heat-killed pathogen is more costly than after a simple injury in earwig mothers (Ardia et al., 2012; Bonneaud et al., 2003) and that this cost can be high enough to reduce the mother's ability to fight a subsequent infection. Future studies are now required to determine which components of the earwig immune system are both activated by wounding and involved in the defence against *S. marcescens* and to determine whether they may be involved in acquired defence against other pathogens in earwigs (Acuña Hidalgo & Armitage, 2022; Contreras-Garduño et al., 2016; Dhinaut et al., 2018; Pham et al., 2007; Roth et al., 2009).

We showed that not only do mothers survive a pathogen infection better after a previous wounding but they also pass this enhanced protection on to their offspring during postoviposition care. Adult offspring were less susceptible to *S. marcescens* infection if they had been reared by mothers that had been wounded (and PBS injected) rather than not wounded or immune-challenged with dead *S. marcescens*. These results reveal not only that insect parents can improve their offspring's defence against infection by ensuring pathogen avoidance and transferring immune factors during oogenesis (Eggert et al., 2014; Tetreau et al., 2019) but also that they can do so much later in development. Contrary to a previous finding in the bumble bee *Bombus terrestris* (Sadd & Schmid-Hempel, 2007), our results highlight that insect parents may have a much wider range of actions in terms of timing and mode of action to boost offspring defence against pathogens. The apparent discrepancy between the earwig and bumble bee data may be because the fitness of queens is only little dependent on the postoviposition care they provide (workers are the ones that usually get involved in egg care), but future studies are needed to test this hypothesis. More generally, our results emphasize that remaining within a family group can have major long-term benefits for earwig juveniles, even if they are mobile, are capable of searching for food independently and can survive in the absence of a mother (Kölliker, 2007; Thesing et al., 2015). Given that

all these characteristics were probably common to all animal species at the beginning of the evolution of family life (Kramer & Meunier, 2019), our findings support the recent claim that access to social immunity could play a major role in the early emergence and then consolidation of family life in nature (Biedermann et al., 2021; Meunier, 2015).

Although our study shows that mothers can strengthen their offspring's defences against pathogens after egg laying, the mechanisms at play during this transmission remain unclear. One potential mechanism is that injured mothers alter their investment in care, resulting in offspring that are better equipped to fight *S. marcescens* infection. Our results do not support this possibility, at least in terms of egg care in the days following maternal treatment. Wounded earwig mothers showed the same level of egg care and their offspring were of comparable quality to other mothers. This altered expression of care would also be inconsistent with data from a recent study in the burying beetle *Nicrophorus vespilloides* (Ratz et al., 2021), which showed that infected beetle mothers do not change their expression of posthatching care (possibly due to a terminal investment) and that infection does not change the quality of their offspring. Nevertheless, we cannot rule out the possibility that injured earwig mothers alter the expression of other forms of egg care or posthatching care towards juveniles and/or that their adult offspring may be of a different quality. Future studies should thus explore these possibilities. Another alternative is that earwig mothers have enhanced their offspring's defence against *S. marcescens* by transferring immune effectors such as mRNA, antimicrobial peptides, vitellogenin and pattern recognition receptor and other signals (e.g. bacterial fragments and microRNAs) to their eggs and/or nymphs during care (Jacobs et al., 2014; Knorr et al., 2015; Salmela et al., 2015). This has been proposed to mediate the transfer of immunity to the eggs still in the reproductive tract of insect females (Tetreau et al., 2019) and could apply to earwigs because mothers groom the eggs and have frequent mouth-to-mouth contact with their newly hatched juveniles (Mas & Kölliker, 2011; Van Meyel et al., 2019). Moreover, it is known that their grooming allows the transfer of cuticular hydrocarbons and microbial communities from mothers to the eggshell (Boos et al., 2014; Greer et al., 2020), mouth-to-mouth contacts mediate the transfer of gut contents to juveniles (Staerke & Kölliker, 2008) and their presence alters the microbiota of both juveniles and the resulting adults (Cheutin et al., 2024). To verify the occurrence of these transfers, it is now necessary to identify the physiological, cellular and molecular immune processes associated with the survival rate of adult earwigs following infection with *S. marcescens* to quantify the potential cost of their production to mothers and, finally, to determine which maternal behaviours allow the transfer of which immune effectors (if any) to

the offspring (Shikano et al., 2015; Zanchi et al., 2011, 2012).

Conclusion

Our study demonstrates that earwig mothers are better able to survive infection by *S. marcescens* if they have been previously wounded (and injected with PBS) and that this enhanced defence can be transmitted to their offspring during postoviposition care. Our data suggest that this transfer is unlikely to be due to short-term changes in the expression of maternal care by wounded females and the production of better-quality offspring by these females. Further studies are now needed to test the impact of wounding on nymph care and investigate the immunological basis of these effects (Körner et al., 2020; Vogelweith, Körner, et al., 2017). On a more general level, these findings shed light on another advantage for offspring to remain with their parents in a species where parental care is facultative, which provides support to the recent claim that access to social immunity could be an important promoter of social evolution (Biedermann et al., 2021; Körner et al., 2020; Meunier, 2015; Van Meyel et al., 2018). Finally, our results call for future studies to elucidate the immune pathways that are potentially involved in the enhanced survival of infected mothers and offspring and thus to determine whether the reported results are related to immune priming and transgenerational immune priming (Roth et al., 2018; Tetreau et al., 2019) or reflect other forms of maternal investment that directly or indirectly influence how earwigs defend against pathogens.

Data Availability

Both data sets and R script are provided in the Supplementary material.

Declaration of Interest

The authors declare no conflict of interest.

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Supplementary Material

Supplementary material associated with this article can be found, in the online version.

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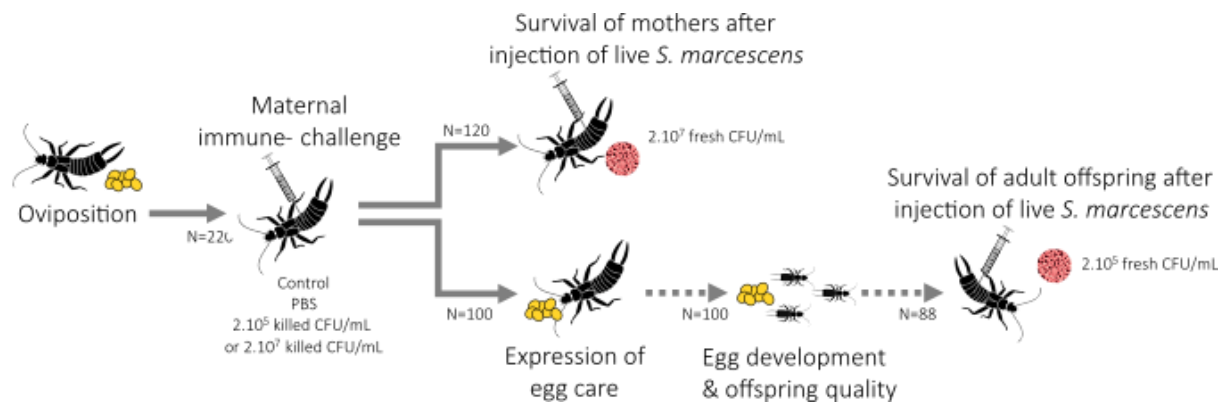


Figure 1. Overview of the experimental design. CFU: colony-forming unit; PBS: phosphate-buffered saline.

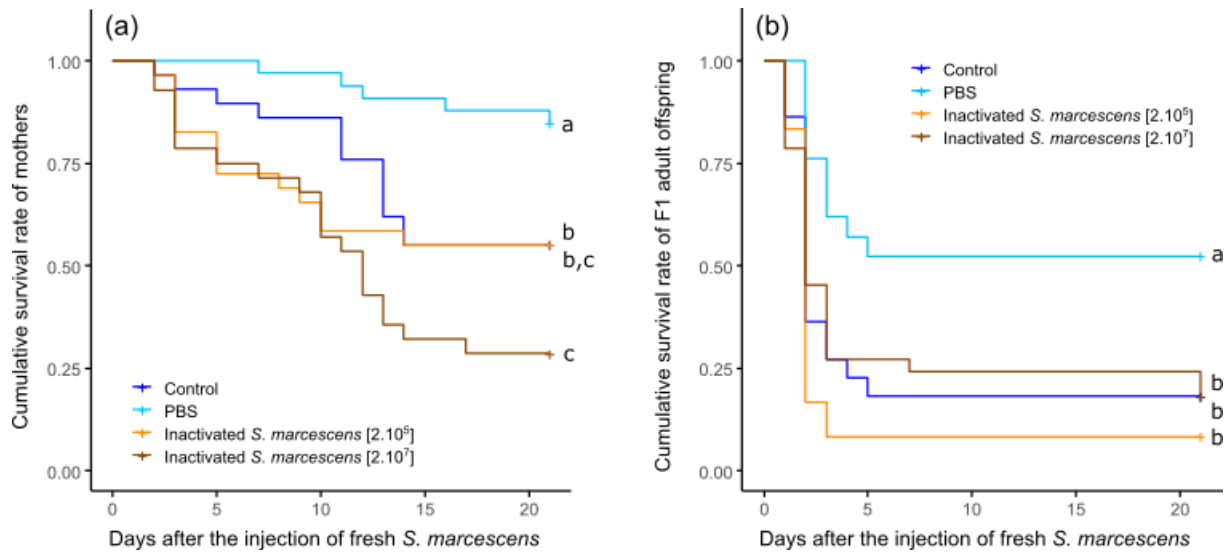


Figure 2. Effect of a previous immune challenge on the survival of (a) mothers and (b) adult offspring after the injection of live *Serratia marcescens* bacteria. Mothers were either not injected (control), injected with a phosphate-buffered saline (PBS) solution or injected with a solution of dead *S. marcescens* at a concentration of 2×10^5 or 2×10^7 CFU/ml. Different letters correspond to $P < 0.05$. CFU: colony-forming unit.

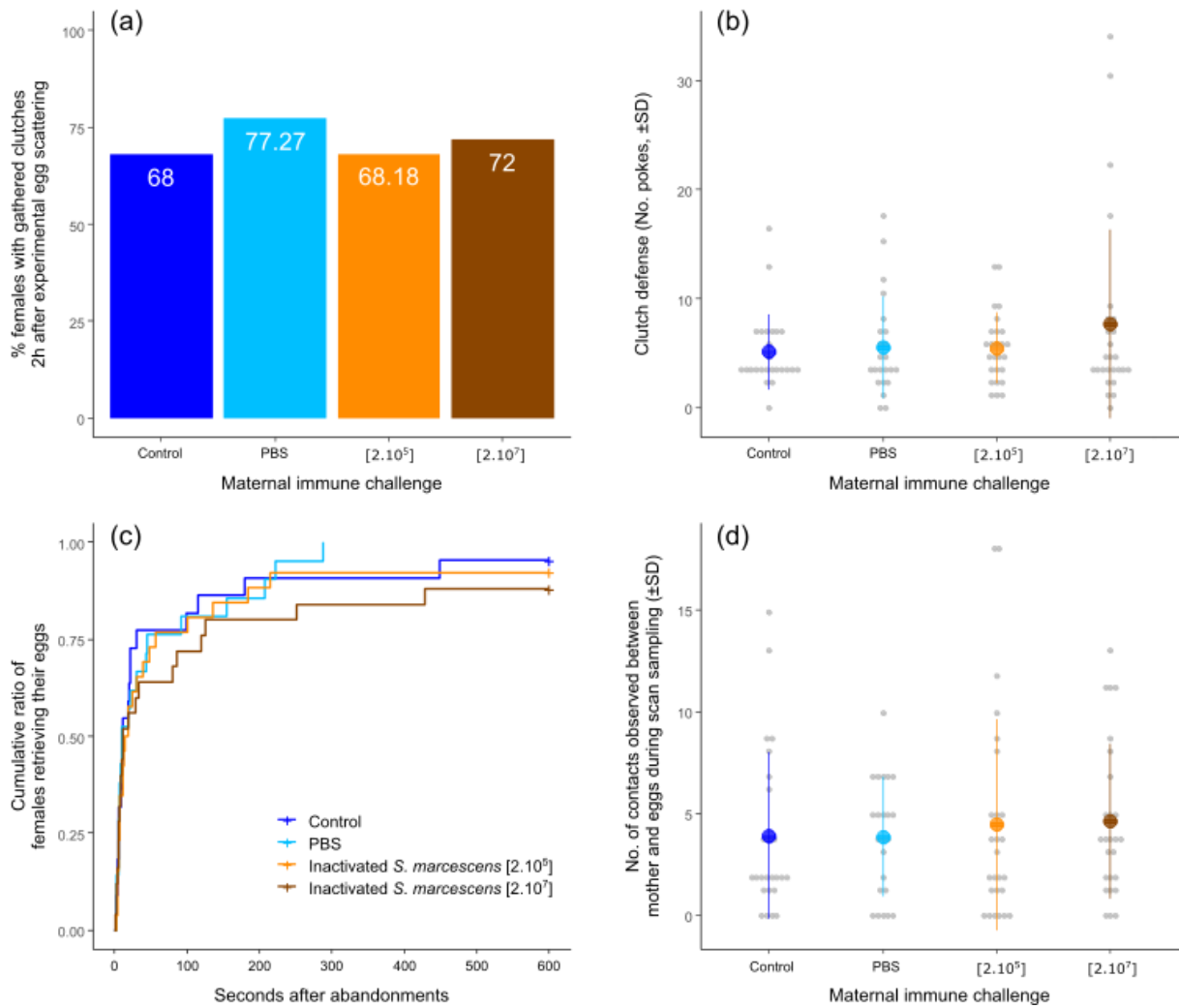


Figure 3. Effect of maternal immune challenge on the expression of four forms of egg care. (a) The percentage of females that gathered eggs after they were experimentally scattered; (b) clutch defence (the number of pokes required until the mother moved more than one body length away from her initial position); (c) the cumulative ratio of females retrieving their eggs after a simulated predator attack; and (d) the number of mother–egg contacts during scan sampling. Mothers were either not injected (control), injected with a phosphate-buffered saline (PBS) solution or injected with a solution of dead *Serratia marcescens* at a concentration of 2×10^5 or 2×10^7 CFU/ml. Grey dots are raw values, coloured dots are means and whiskers are SDs. CFU: colony-forming unit.

Table 1

Number of individuals used in the different steps of the experiment.

Immune challenge treatment	Number of mothers receiving immune challenge treatment	Number of	Number of	Offspring used to measure survival after injection	
		mothers used to measure survival after injection	mothers used to measure egg care and egg development	Number of offspring	(Females, males, families)
Control	55	30	25	22	(13, 9, 10)
PBS	56	33	23	21	(10, 11, 8)
2×10^5 CFU/ml	54	28	26	12	(8, 4, 5)
2×10^7 CFU/ml	55	29	26	33	(22, 11, 14)
Total	220	120	100	88	

CFU: colony-forming unit.