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Janina M C Diehl[1], Joël Meunier[2]

[1] Institute of Organismic and Molecular Evolution, Behavioural Ecology and Social Evolution Group, Johannes-Gutenberg University of Mainz, Mainz, Germany

[2] Institut de Recherche sur la Biologie de l'Insecte (IRBI), UMR CNRS 7261, François-Rabelais University of Tours, Tours, France

Corresponding author: J. Meunier, joel.meunier@univ-tours.fr

SUMMARY

Pathogens are ubiquitous in nature and typically entail major fitness costs in their hosts. These costs can be particularly important when individuals exhibit poor immune defences, as it is often the case during early developmental stages. Hence, selection should favor parental strategies limiting the risks of pathogen exposure and infection in their offspring. In this study, we investigated (1) whether females of the European earwig *Forficula auricularia* avoid areas contaminated with spores of the entomopathogenic fungus *Metarhizium brunneum* prior to and at egg laying, as well as (2) whether spore presence entails an increase in females' investment into both pre-hatching forms of care and clutch quantity and quality. Our results first show that females did not avoid contaminated areas prior to and at egg laying. However, females returned to their eggs faster in presence of living spores compared to UV-killed or no spores. They were also more likely to construct a nest when in presence of both living and UV-killed spores (but only in one studied population). Finally, we found that spore presence did not influence maternal investment into egg grooming, egg gathering and egg defence, as well as into clutch quantity and quality. Overall, our results demonstrate that earwig females do not avoid contaminated environments, but could mitigate the associated costs of pathogen exposure by adjusting their level of egg care. These findings emphasize the importance of pathogens in the evolution of pre-hatching parental care and, more generally, in the emergence and maintenance of family life in nature.

Keywords: Parental care, Social immunity, Subsocial, Dermaptera, Insects

INTRODUCTION

Pathogens are considered as a major threat in nature, because they often entail large fitness costs in their hosts (Schmid-Hempel 2014). To limit the risks of pathogen exposure and infection, individuals from both vertebrate and invertebrate species have developed a broad spectrum of defences (Siva-jothy et al. 2005; Schmid-Hempel 2014). The most studied defences rely on the immune system of the potential host, which typically detects the presence of pathogens in the organism, prevents their development and ultimately kill and purge them from the host (Siva-jothy et al. 2005; Beckage 2008). However, many non-immunological defences may also help hosts to fight against pathogens (Parker et al. 2011). Among them, hosts have been shown to change some of their behaviours to prevent direct contacts to pathogens (de Roode and Lefèvre 2012). This can be done, for instance, by avoiding infected areas and limiting the consumption of pathogen-infected food (Villani et al. 1994; Meyling and Pell 2006; Ormond et al. 2011), and by prophylactically or curatively consuming natural components with antimicrobial properties, a process called self-medication (de Roode et al. 2013; Bos et al. 2015).

Interestingly, defences against pathogens are not only expressed by the hosts themselves (thereafter called personal immune defences) but can also come from surrounding conspecifics and group members (Schmid-Hempel 1998). Over the last decades, these external defences have been mostly studied in colonies of eusocial insects, such as bees, ants and termites (Cremer et al. 2007; Meunier 2015). In these species, this so-called social immunity is often illustrated by allogrooming or hygienic behaviours, during which workers remove parasites from the cuticle of another group member (Reber et al. 2011) or clean the nest from waste materials, such as corpses, left-over food and feces (Bot et al. 2001; Jackson

and Hart 2009; Diez et al. 2012). The expression of social immunity – which can complement personal immune defences – is considered as a central process in the evolution of eusocial species (Meunier 2015), since the obligatory and frequent contacts between their related group members can dramatically increase the risks of pathogen exposure and transmission (Pie et al. 2004; Stroeymeyt et al. 2014).

Social immunity, however, is not exclusive to eusocial systems and can be found in less derived forms of group living, such as simple family units (Cotter and Kilner 2010a; Meunier 2015). In these units, parents generally provide multiple forms of care to their eggs and/or juveniles (Smiseth et al. 2012) and, because eggs and juveniles often exhibit an underdeveloped immune competence (DeVeale et al. 2004; Vogelweith et al. 2017), parental care often includes protection against pathogens. For instance, mothers can protect their eggs against microbial infections by applying antimicrobial secretions to their surface, as reported in blennies, grass gobies and fringed darters (Knouft et al. 2003; Giacomello et al. 2006; Giacomello et al. 2008). Similarly, parents may cover the nest with feces exhibiting antibacterial activity to prevent the development of pathogens next to the juveniles, a phenomenon found in the burying beetle and the European earwig (Rozen et al. 2008; Cotter and Kilner 2010b; Diehl et al. 2015). Finally, females of the beewolf digger wasp *Philanthus triangulum* have been shown to transfer symbiotic bacteria to the brood cell cover (Kaltenpoth et al. 2005) and these bacteria to be taken up by the larvae and incorporated into their cocoon to serve as antimicrobial protection (Kroiss et al. 2010). Whereas these maternal behaviours typically protect offspring against pathogens, it remains unclear whether these behaviours are curative/therapeutic (i.e. help fighting against pathogens already in contact with offspring) or prophylactic (i.e. help preventing potential contacts between pathogens and offspring). Disentangling between these two processes is however crucial to better

74 understand on which level pathogen-induced selection operates in the evolution of parental
75 care.

76 In this study, we investigated whether maternal egg care is a prophylactic process in
77 the European earwig *Forficula auricularia*. Specifically, we tested whether the presence of
78 pathogens in the environment triggers changes in females' investment into pre-hatching egg
79 care (i.e. social immunity) and/or egg production. In this insect species, mothers guard their
80 eggs for several months over winter (Lamb 1976). During egg development (but also during
81 the subsequent development of juveniles), females express a wide range of protective
82 behaviours, such as grooming, clutch displacement and rearrangement, as well as aggressions
83 against predators (Lamb 1976; Meunier and Kölliker 2012; Boos et al. 2014; Koch and Meunier
84 2014; Thesing et al. 2015). Whether mothers can detect the presence of living pathogens in
85 the environment (and not only on the eggs, see Boos et al. 2014) and prophylactically adapt
86 their behaviours remains, however, unknown. Moreover, a recent study revealed that some
87 life history traits could be population specific in earwigs (Ratz et al. 2016), begging the
88 question of whether prophylactic defences against pathogens might differ between
89 populations.

90 We conducted two experiments to test the expression of three prophylactic defences
91 in earwig females originating from three independent populations. First, we tested whether
92 females avoid areas infected with an entomopathogenic fungus to lay their eggs. Second, we
93 investigated whether females modify their investment into egg quantity and quality (i.e. egg
94 size and weight) when the fungus is present in their direct environment. Finally, we tested
95 whether females change their level of pre-hatching care when the nesting area (but not
96 necessarily the eggs) contains this fungus. We used spores of the generalist
97 entomopathogenic fungus *Metarhizium brunneum* (formerly *M. anisopliae*), a natural and

potentially lethal pathogen of *F. auricularia* (Günther and Herter 1974; Kohlmeier et al. 2016) also known to infect and reduce the viability of eggs in many arthropods (Samuels et al. 2002; Gindin et al. 2009; Nozad-Bonab et al. 2017). In the first experiment, we recorded the movement and egg laying behaviours of females preliminary set up in areas covered with a spore solution on one side and with a spore-free solution on the other side. If mothers prophylactically limit the exposure of their eggs to pathogens, we expected females to avoid contact with fungal spores both before and at egg laying. Similarly, females laying their eggs on the spore-exposed side should be more likely to relocate them afterwards. In the second experiment, we measured the level of five forms of egg care behaviours (nest construction, egg gathering, egg defence, egg abandonment and egg grooming) in females previously set up in areas fully covered with either living spores, UV-killed spores or no spores. We predicted that females increase their investment into egg care only in presence of living spores. Finally, we measured the number, mean volume, mean weight and hatching success of the eggs produced in the two experiments. If females can assess the risks of pathogen infection for their eggs (and subsequent juveniles), we expected that females reduce the risks and/or costs of this infection by prophylactically increasing their investment into egg quantity and/or quality when spores were present in the environment. If females manage to mitigate the costs of pathogen exposure, we also predicted egg-hatching success to be independent of pathogen presence.

MATERIAL AND METHODS

Animals and pathogen

All the females used in the following experiments were the first laboratory born generation of individuals collected in 2014 in three independent populations: Vincennes (France; 48° 51′ N, 2° 26′ O), Girona (Spain; 41° 59′ N, 2° 49′ O) and Montblanc (Spain; 41° 23′ N, 1° 10′ O). At adult emergence, these females were maintained with unrelated males in groups of 50 individuals (balanced sex-ratio) to allow them to mate freely (Meunier et al. 2012). Each group was set up in a plastic container (37 x 22 x 25 cm) grounded with moist sand, containing egg carton as shelters, and maintained under standard conditions (constant 20°C, 10:14 h dark/light cycle, 60% humidity). Three months later, each female was isolated in a Petri dish (9 cm diameter) grounded with moist sand, and subsequently maintained under winter conditions to allow egg production (gradual temperature change from 15°C to 5°C during three weeks, with constant 60% humidity and complete darkness). Ten days later, the temperature was increased to 10°C for two more weeks and finally to 15°C for the rest of the experiments to allow egg development. After egg hatching, each family was kept at room temperature (23°C) until the end of the following experiments. Groups and isolated females were fed twice a week with an *ad libitum* amount of a standard diet mainly composed of pollen, cat food and carrots (see detailed composition in Kramer *et al.*, 2015).

The conidia (spores) of the entomopathogenic fungus *M. brunneum* used in the following experiments were obtained from a genotyped strain isolated from soil samples in Switzerland (Reber and Chapuisat 2011). To ensure the virulence of this strain to earwigs, this fungus has been grown on *F. auricularia* for three generations prior to their use in this experiment. Specifically, earwigs were infected in a 1 ml spore solution of *M. brunneum* (10^8/ml) conidia solved in 0.05% Tween 20 and held in a climate cabinet at 25°C in small Petri dishes (5.5 cm diameter) filled with moist sand. After the death of the exposed individuals,

corpses were rinsed three times with 1 ml of concentrated bleach and distilled water to prevent contamination by other microbes. Afterwards, the surface sterilized insects were kept in new Petri dishes sealed with parafilm in the climate cabinet (25°C) until growth of the fungus appeared. The resulting spores were rinsed from the body with distilled water and applied on potato extract glucose growth medium (Roth). Growing fruit bodies were finally collected with a sterile scalpel, diluted in 0.05% Tween 20 solution and then used to infect new individuals. This process has been repeated three times to obtain the spores used in the following experiments.

Question 1: Do females avoid infected sites to lay their eggs?

To investigate whether females avoid laying their eggs in a pathogenic environment, we transferred a random sample of 37 females (n = 11 Girona, n = 18 Montblanc and n = 8 Vincennes) to individual Petri dishes (9 cm diameter, grounded with humid sand) divided in two parts of equal size. One side was previously sprinkled with 0.55 ml of a spore solution of *M. brunneum* (10^7/ml) solved in Tween 20 (0.05%, Roth), whereas the other side (control) was previously sprinkled with the same amount of a spore-free Tween 20 solution. A direct exposure to a solution of 10^7 spores per ml is known to entail a 20% adult mortality after 25 days (Kohlmeier et al. 2016). The two sides were separated by a cardboard barrier (8.7 x 1 cm) covered with aluminum foil to avoid any passive diffusion of the applied solutions between the two sides, while allowing females to easily move from one side to the other. Each experimental Petri dish was then checked on a daily basis in order to (1) count the number of days each female stayed on each side until egg laying, (2) record the side on which females deposited theirs eggs and finally to (3) count the number of times the clutches were moved to the other side during egg development. All these measurements were done blind regarding

164 the treatments (i.e. the experimenter did not know which side of the experimental Petri dish
165 contained the pathogen or the control). Each female was provided with an *ad libitum* amount
166 of standard food until egg laying (detailed food composition in Kramer *et al.*, 2015), as females
167 stop feeding once they produce eggs (Kölliker 2007). Note that the pathogen and control
168 solutions were applied twice (once just before the start of the experiment and once one
169 month later) to make sure that each treatment lasts for the entire experiment.

170 We statistically analyzed whether females avoided pathogenic environments for egg
171 laying using two successive steps. In the first step, we conducted two Generalized Linear
172 Models (GLM) with binomial error distribution to determine whether females' choice for each
173 side (i.e. preference for or avoidance of the contaminated side before and at egg laying)
174 depended on clutch size and/or on their population of origin. In the first GLM, the proportion
175 of days each female spent on the contaminated side before egg laying was entered as
176 response variable (using the *cbind* function in R), while egg number, population (Girona,
177 Montblanc, Vincennes) and the interaction between these two variables were used as
178 explanatory variables. In the second GLM, the side chosen for egg laying (1 = contaminated
179 side, 0 = control side) was entered as response variable, while the two factors described above
180 were also used as explanatory variables. Because neither egg number nor population was
181 significant in these two models (see results), we then pooled the data across populations to
182 conduct the second step. In this second step, we used a one sample student's t-test to analyze
183 whether females spent overall more days on the control compared to contaminated side (i.e.
184 ratio of time on the control side compared to the value 0.5), as well as a binomial test to
185 analyze whether the number of females that had laid eggs on the contaminated side was
186 overall lower than the number of females that had laid eggs on the control side. All statistical

187 analyses (here and below) were done with R v3.4.0 (http://R-project.org) loaded with the
188 packages *car*, *lmerTest*, *survival* and *exactRankTests*.

189 **Question 2: Do females adjust their pre-hatching care to the presence of pathogens?**

190 To investigate whether females adapt their investment into pre-hatching egg care to the
191 presence of pathogens in the environment, we used a random sample of 123 females (n = 32
192 Girona, n = 65 Montblanc, n = 26 Vincennes; all different from the ones used to address
193 question 1) distributed among three treatments. The experiment started by isolating these
194 females in Petri dishes grounded with moist sand and entirely sprinkled with 1.1 ml of one of
195 the three following solutions: (1) *M. brunneum* spore solution (10^7/ml 0.05% Tween 20; n =
196 11 Girona, n = 22 Montblanc and n = 9 Vincennes), (2) UV-killed *M. brunneum* spore solution
197 (10^7/ml 0.05% Tween 20; n = 11 Girona, n = 21 Montblanc and n = 10 Vincennes) or (3) spore-
198 free 0.05% Tween 20 solution (n = 10 Girona, n = 22 Montblanc and n = 7 Vincennes). We used
199 both the UV- killed spores and Tween solution as controls to disentangle the effects of
200 pathogen activity (UV-killed versus living spores) from the effects of pathogen presence
201 (spore-free versus living spores). The UV-killed spore solution was exposed to UV light (254
202 nm) for three minutes in the UV irradiation system Bio-Link 254 (Vilber). The UV treatment
203 was efficient enough to inhibit the growth of *M. brunneum* spores on growth medium (results
204 not shown). To mimic the process conducted in experiment 1, the initial application of each
205 solution in the Petri dish was done right before female isolation and a second application one
206 month later. Note that the presence of spores in the environment did not affect females
207 survival rate, as 6 (12.0%), 7 (13,5%) and 6 (12.2%) females died before egg laying in the
208 treatments with living spores, UV-killed spores and no spores, respectively. No female died
209 after egg laying.

We measured five forms of pre-hatching maternal care: egg gathering, egg defence, clutch abandonment, nest construction and egg grooming. (1) Egg gathering indicates the propensity of a female to gather its eggs after these were experimentally spread out in the nest. This measurement was done five days after egg laying. At that time, we confined each female within its Petri dish, then spread a random selection of 30 eggs (or all, if fewer were available) evenly within a circle (~3 cm diameter, including the site where the eggs were originally laid) and subsequently recorded the seconds between the mother's first movement out of the confinement area and the re-assembly of the eggs within one body length of the female. Note that the eggs not involved in this measurement were kept in a small closed plastic dish in the meantime. (2) Egg defence revealed females' reaction to a simulated predator attack and was measured ten days after egg laying. This measurement was done following a standard method (Thesing et al. 2015), in which females were poked with a glass capillary on the thorax (1 poke / 2 seconds) until they abandoned their clutch (i.e. moved more than two body lengths away from it). The values of egg defence were the number of pokes the females sustained before leaving the eggs. (3) Clutch abandonment was measured subsequently to egg defence and was used to quantify how long a mother abandons her clutch of eggs after a simulated attack. The value of egg abandonment was the time (in seconds) between the moment where each female left its nest due to a simulated predator attack (see above) and the moment where females returned to their clutch and touched at least one egg. (4) Nest construction reflected whether a female invested into the construction of a nest at day 15 after egg laying. Following a method already developed in this species (Meunier et al. 2012), we considered females to have constructed a nest when they dug a pit to the ground of the Petri dish and put their eggs into the pit. Finally, (5) egg grooming was recorded 15 days after egg laying, just after checking for females nest construction. At that time, each female

was observed for 46 minutes in its original Petri dish using a scan sampling method (one scan every 2 minutes, i.e. 23 scans per female). To increase the chance of observing egg grooming (Boos et al. 2014), females involved in this measurement were separated from their clutch during 30 min before the measurements. Because earwigs are nocturnal, all behavioural tests were performed under red-light. All behavioural measurements were also done blindly regarding the treatment.

We used one GLM with binomial error distribution, two General Linear Models (LM) and two Cox proportional hazard regression models to analyze the five measured forms of egg care. In these models, either egg gathering (Cox model), egg defence (LM), clutch abandonment (Cox model), nest construction (1 or 0; GLM) or egg grooming (LM) was used as a response variable, whereas treatment (living spores, UV-killed spores or control), population and their interaction were entered as explanatory factors. Note that the two Cox proportional hazard regression models allowed for censored data - that is, females that did not gather all their eggs or did not return to their clutch at the end of the observation time. When applicable, pairwise comparisons between populations or treatments were tested using Tukey contrasts (for LM and GLM) and the survdiff function in R (for Cox models).

Question 3: Do females adjust clutch quality and quantity to pathogen presence?

Finally, we investigated whether females adjust the number, volume and weight (proxies of egg quality, Koch & Meunier, 2014) of their eggs to the presence (enforced or not) of living pathogens in the environment. To this end, we measured these three traits in the clutches of the 36 females used to address question 1 (n = 11 Girona, n = 18 Montblanc and n = 8 Vincennes) and the 86 females that were in the living spores and spore-free control solution treatments in the second experiment (Question 2; n = 22 Girona, n = 45 Montblanc and n = 19

257 Vincennes). The number of eggs produced was counted three days after the first egg laying,
258 because egg production typically takes three days in this species (Koch & Meunier, 2014). At
259 that time, we also weighed a random subset of 10 eggs per female to the nearest of 10^{-3} mg
260 using a micro scale (Pescale MYA 5), as well as measured the mean volume of the same 10
261 eggs (or all, if fewer were available). The mean egg volume per clutch was obtained by
262 measuring the length and width of each of the ten eggs to the nearest 10^{-3} mm and then using
263 these values with the following formula (Meunier and Chapuisat 2009): egg volume =
264 ((4*pi)/3)*(egg width/2)^2*(egg length/2). All size measurements were done through a
265 binocular microscope (Leica, Type DFC425, Leica Microsystems) with 20.0x magnification that
266 was run using the Leica Application Suite software (Version 4.5.0, Leica Microsystems). We
267 finally estimated the hatching success of each of these 122 clutches by counting the total
268 number of nymphs that eventually hatched.

269 To control for the non-independence of egg number, volume and weight, we first
270 conducted a Principal Component Analyses (PCA) to obtain non-correlated principal
271 components (PC) reflecting single or combinations of egg traits. The resulting and selected PCs
272 were then analyzed separately using LMs in which population, treatment (presence or
273 absence of living spores on the area receiving the eggs) and type of arena (partly or fully
274 covered with spores, i.e. Question 1 or 2, respectively) were entered as fixed factors.
275 Independently of clutch properties, we finally analyzed the egg hatching success using a GLM
276 with binomial error distribution corrected for over dispersion. In this model, the hatching
277 success was used as a response variable (entered with the *cbind* function in R). Moreover,
278 population, whether females laid their eggs in Petri dishes half-covered (i.e. experiment 1) or
279 fully covered (i.e. experiment 2) with living spores, and where the eggs have been laid (spores
280 exposed or control side/Petri dish) were entered as explanatory factors. Each of these models

first included interactions between all factors and was then simplified by removing the non-significant interactions (all p-values > 0.073). When applicable, pairwise comparisons between populations were tested using Tukey contrasts.

RESULTS

Question 1: Do females avoid infected sites to lay their eggs?

When given a choice, females did not avoid the side covered with pathogens both before oviposition (Mean ± SE proportion of time spent by a female on the control side: 54.0 ± 18.0%; t_{48} = 1.52, P = 0.136) and at egg laying (22 and 15 females laid their eggs in the control and infected sides, respectively; exact binomial test: P = 0.324). These results were independent of female's population, egg number and of an interaction between these two variables (Table 1). Overall, only 1 of the 15 females who laid their eggs on the pathogen side relocated its clutch to the control side, which is comparable to the 1 of the 22 females who relocated its eggs after having laid them on the control side. Note that this general tolerance of females for the spores' side is unlikely to result from the unintentional spread of spores by moving females, since they were equally distributed between the two sides already on the first day (28 and 21 females on the tween and spores sides, respectively; exact binomial test: P = 0.392) and the second day (22 and 27 females on the tween and spores sides, respectively; exact binomial test: P = 0.568) of the experiment.

Question 2: Do females adapt their pre-hatching care to the presence of pathogens?

In absence of a choice, the presence of pathogens in the environment altered two of the five measured forms of maternal care: the duration of egg abandonment and nest construction

(Table 2, Figure 1). The duration of egg abandonment was overall shorter in females occupying Petri dishes covered with living spores compared to females in Petri dishes with UV-killed spores (Survdiff; $\chi^2_1 = 6.5$, P = 0.011) or without spores (Survdiff; $\chi^2_1 = 3.4$, P = 0.065), even if this last effect was marginally non-significant. The duration of egg abandonment was, however, comparable between Petri dishes covered with UV-killed spores and not covered by any spores (Survdiff; $\chi^2_1 = 1.1$, P = 0.287). On the other hand, the effect of spore presence on nest construction depended on females' population (Interaction in Table 2b). In particular, females from Vincennes were more likely to build a nest in the Petri dishes covered with living spores (Figure 1b; Tukey contrasts; t=3.63, P = 0.004) and UV-killed spores (t=3.71, P = 0.003) compared to females in Petri dishes without any spores, with no difference between the two latter treatments (t<0.001, P = 1.000). The presence of pathogens had, however, no effect on nest construction in females from Girona (Figure 1b; $F_{2,28} = 0.82$, P = 0.451) and Montblanc ($F_{2,62} = 2.51$, P = 0.090). Finally, the speed of egg gathering, the level of egg defence and the occurrence of egg grooming were all independent of treatment and population (Table 2c, 2d and 2e and Figure 2).

Question 3: Do females adjust clutch quality and quantity to pathogen presence?

The PCA conducted on the three egg traits provided three orthogonal principal components (PCs), of which we extracted the two first (total variance explained = 97.6 %). The first component (PC1) was highly and positively loaded with egg volume and egg weight (0.959 and 0.964, respectively; loading of egg number = -0.42), therefore reflecting egg quality. Conversely, the second component (PC2) was highly and positively loaded with egg number (0.91; loading of egg volume = 0.21; loading of egg weight = 0.19), therefore reflecting egg quantity.

Overall, egg quality (PC1) and egg quantity (PC2) were independent of whether the eggs were laid with or without spores in the nesting area, or whether females laid their eggs in Petri dishes either half-covered or fully covered with living spores (i.e. the type of experimental arena; Table 3, Figure 3). By contrast, PC1 and PC2 were population-specific (Table 3, Figure 3). Egg quality (PC1) was larger in clutches produced by females from Montblanc compared to Girona, with an intermediate level in females from Vincennes (Figure 3). Conversely, egg quantity (PC2) was larger in clutches produced by females from both Girona and Montblanc compared to Vincennes, with no difference between the two first populations (Figure 3).

Hatching success was overall higher when females laid their eggs in Petri dishes half-covered (i.e. experiment 1) compared to fully covered (i.e. experiment 2) with living spores ($F_{1,115} = 4.13$, $P = 0.044$; Figure 4). Hatching success also depended on females' population ($F_{2,115} = 3.35$, $P = 0.039$). The hatching success was higher in clutches produced by females from Girona compared to Vincennes ($Z = -2.53$, $P = 0.030$), but comparable between Girona and Montblanc ($Z = -1.06$, $P = 0.242$) and Vincennes and Montblanc ($Z = -1.41$, $P = 0.331$). The hatching success was, however, independent of the presence of living spores in the nesting area ($F_{1,115} = 0.034$, $P = 0.854$).

DISCUSSION

Shedding light on which maternal strategies can protect offspring against infection is of central importance to better understand the evolutionary drivers of parental care and more generally, the emergence and maintenance of family life in nature (Royle et al. 2012; Klug and Bonsall 2014). In this study, we demonstrated that the presence of pathogens in the environment

345 influenced maternal investment into egg care, but not into egg production in the European
346 earwig *F. auricularia*. Specifically, we found that the duration of egg abandonment (after an
347 experimental disturbance) was shorter when the nesting area was covered with living spores
348 compared to covered with UV-killed spores or no spores at all. Females were also more likely
349 to build a nest in presence compared to absence of both living and UV-killed spores, even if
350 this effect was only present in females from one of the three studied populations. By contrast,
351 the presence of living spores had no effect on egg grooming, egg gathering and egg defence.
352 We also showed that mothers did not avoid contaminated areas at egg laying, and that the
353 presence of living spores did not influence maternal investment into egg number, volume and
354 weight, and did not shape hatching success. Finally, our data confirm that egg properties and
355 hatching success are population-specific in this species (see also Ratz et al. 2016).

356 Altogether, our results show that mothers can detect the presence of spores in the
357 environment and consequently increase their investment into certain forms of egg care. In
358 particular, the presence of pathogens shortened the duration of egg abandonment, favored
359 the construction of a nest (in females from Vincennes), but had no effect on maternal
360 investment into egg gathering, egg defence and egg grooming. The effect of pathogens on the
361 first two behaviours is not surprising, as both can help mothers to protect their eggs against
362 pathogen exposure. Returning to the eggs quickly after disturbance is indeed essential to
363 ensure the frequent physical contact between eggs and mothers that are typically required to
364 remove pathogens from egg surface (Boos et al. 2014). Similarly, nest construction is a form
365 of hygienic behaviour that may help cleaning the nest from pathogen spores by shifting the
366 sands around (Arathi et al. 1999). Conversely, egg gathering and egg defence are two maternal
367 behaviours triggered by predator attacks (Thesing et al. 2015) and therefore unlikely to
368 depend on pathogens presence. What was more surprising, however, is that egg grooming

369 was independent of pathogen presence. This behaviour is typically known to help individuals
370 cleaning external parasites and pathogens in insects (Reber et al. 2011; Meunier 2015) and
371 has been previously show to help earwig mothers cleaning their eggs from non-pathogenic
372 fungal spores (Boos et al. 2014). The apparent discrepancy between our results and the ones
373 from Boos et al. (2014) likely relies on the different quantity of spores covering the eggs on
374 the days of measurement. Boos et al. (2014) directly covered the eggs with a high quantity of
375 fungal spores and then immediately measured egg care – resulting in a high concentration of
376 spores on the tested eggs. In our experiment, the eggs were laid in an area contaminated with
377 fungal spores (i.e. indirect spore exposure) and were then groomed by their mothers for 15
378 days before we measured egg care – therefore resulting in a comparatively lower
379 concentration of spores (if any) on the tested eggs. Overall, these studies thus suggest that
380 egg grooming depends on the quantity of spores present on the eggs and hence, that its anti-
381 pathogenic function is curative rather than prophylactic.

382 Somewhat surprisingly, females exhibited different reactions in presence of UV-
383 treated and living spores: the UV-treatment inhibited the effect of spore presence on the
384 duration of egg abandonment, but not on the likelihood of nest construction. It is generally
385 known that individuals can change their expression of social behaviours when they encounter
386 conspecifics that are infected or non-infected by *Metarhizium* spores, as reported in several
387 eusocial insect species (Walker and Hughes 2009; Reber et al. 2011; Leclerc and Detrain 2016).
388 However, the fact that females can change their level of care to the presence of infectious or
389 non-infectious spores in the environment was – to the best of our knowledge – unknown. The
390 capability to discriminate between infectious and non-infectious spores can be crucial for
391 mothers. It may allow females to prevent their investment into costly protections against non-
392 active threats, and instead favor investment in other fitness related processes, such as the

provisioning of food to current offspring or the accumulation of energy for future reproduction (Royle et al. 2012). On a proximal level, the mediators of this apparent discrimination are unclear. *M. brunneum* spores typically infect hosts by adhering to their cuticle and then germinating to penetrate into their body (Vestergaard et al. 1999; Thomas and Read 2007). We hypothesize that the damages of UV on the spores may not only affect their DNA (as generally known, see Braga et al. 2015), but also their shape, chemical signature and/or adherence capability, which all could either help females discriminating against dead spores or simply make dead spores undetectable to females. Further studies should investigate which of these two scenarios explain the present finding and more generally, whether this apparent discrimination capability applies to other pathogens and hosts.

Contrary to the previous spore-dependent effects, we demonstrated that earwig females showed no preference for either pathogen-exposed or pathogen-free environments for oviposition, nor did they adjust their egg properties to the presence of pathogens in the nesting area. The reported absence of pathogen-dependent effects in earwigs is uncommon among invertebrates, where most studies show that females either avoid (Meyling and Pell 2006; Lam et al. 2010) or prefer (Brütsch et al. 2014; Pontieri et al. 2014) areas covered with entomopathogenic spores for oviposition, as well as either increase (Villani et al. 1994) or reduce (Machtinger et al. 2016) their egg production in presence of entomopathogenic spores. In earwigs, this effect is unlikely to reflect females' inability to detect spore presence (see above). Our results therefore suggest that females detected pathogens but neither chose their egg laying location nor adjusted their investment into egg production accordingly. Two non-mutually exclusive hypotheses could explain this pattern. First, the presence of spores in the environment might not be a major threat for the eggs. This is an unlikely hypothesis, as this generalist entomopathogenic fungus is a well-known threat for eggs in insects (Samuels

et al. 2002; Nozad-Bonab et al. 2017) and our data demonstrate that earwig females react to spore presence by increasing the expression of the egg care specifically directed against pathogens. The second hypothesis is that maternal egg care is efficient enough to prevent the infection of eggs by fungal spores, and thus to relax selection for avoidance behaviour and pathogen-dependent change in egg properties. In line with this hypothesis, a previous study demonstrated that maternal presence mitigates the costs of a direct exposure to mould on hatching success in several earwig species (Klostermeyer 1942; Miller and Zink 2012; Boos et al. 2014) and our results demonstrate that the presence of living spores had no effect on hatching success (in presence of a tending mother). Disentangling the effects of temperature and maternal care efficiency on egg production will be the goal of future studies.

To conclude, our results reveal that mothers can express prophylactic mechanisms to defend their eggs against surrounding pathogens and that these defences occur after egg production. Interestingly, these prophylactic defences come together with other types of anti-pathogenic defences exhibited by earwig mothers, such as the removal of fungal spores from egg surfaces (Boos et al. 2014), the transfer of chemical compounds with antimicrobial properties on the eggs (Boos et al. 2014) and the maintenance of feces with antimicrobial functions into the nest (Diehl et al. 2015; Körner et al. 2016). These results thus overall reveal that both prophylactic and curative/therapeutic defences against microbes can co-occur in a species where nesting material is not subject to a direct competition with microbes - as compared, for instance, to carcasses in burying beetles (Rozen et al. 2008) or feces in dung beetles (Byrne et al. 2013). Active protection against microbial pathogens could thus play a central role in the emergence and maintenance of parental care in nature, as well as explain the taxonomically widespread occurrence of egg attendance in insects and arthropods (Wong et al. 2013).

DATA ACCESSIBILITY. The data set has been deposited on Dryad (doi:10.5061/dryad.pv50q).

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585

586 **Table 1**. Effects of population and egg number on females' likelihood (a) to avoid the
587 pathogen-exposed side of the experimental arena before oviposition or (b) to lay eggs on the
588 control side of the experimental arena.

	(a) Before egg laying			(b) At egg laying		
	LR χ^2	df	P	LR χ^2	df	P
Population	0.92	2	0.633	2.36	2	0.308
Egg number	2.10	1	0.147	0.85	1	0.357
Population : Egg number	0.11	2	0.945	2.68	2	0.262

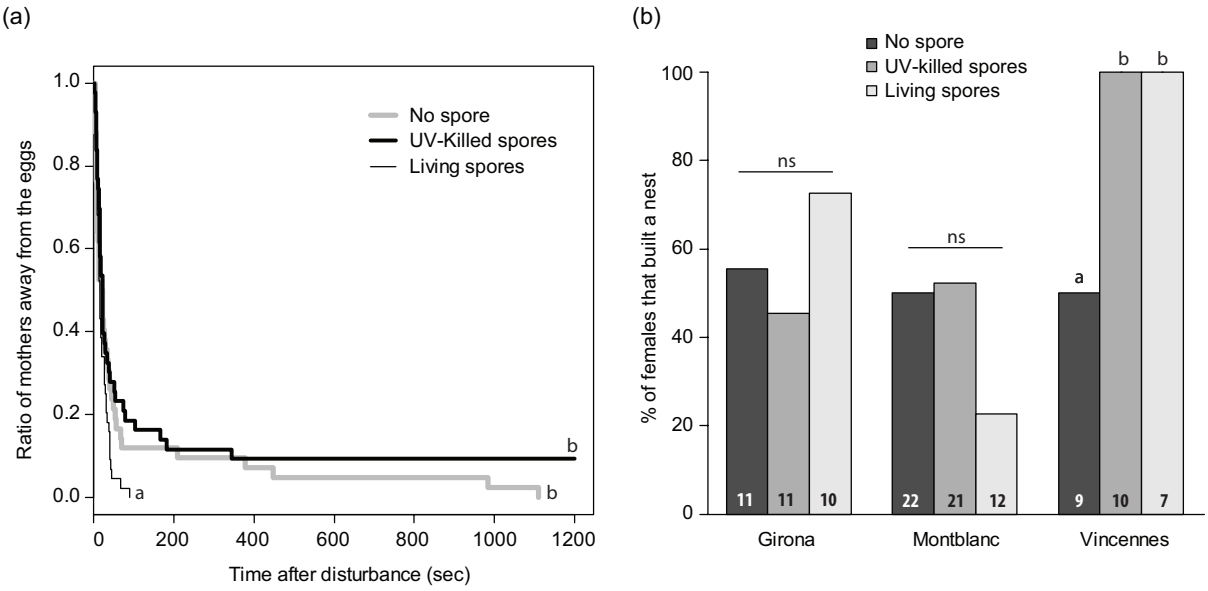
589 **Table 2**. Effects of population and treatment on (a) the duration of clutch abandonment, (b)
590 nest construction, (c) Egg gathering, (d) egg defence and (e) egg grooming. Significant p-values
591 are in bold.

	(a) Clutch abandonment			(b) Nest construction			(c) Egg gathering		
	Chisq	df	P	Chisq	df	P	Chisq	df	P
Population	0.68	2	0.7129	17.25	2	**0.0002**	1.96	2	0.3744
Treatment	7.38	2	**0.0250**	0.78	2	0.6768	0.41	2	0.8143
Population : Treatment	3.73	4	0.4431	16.09	4	**0.0029**	3.85	4	0.4267
Statistical model	Cox model			GLM (binomial)			Cox model		

	(d) Egg defence			(e) Egg grooming		
	F	df	P	F	df	P
Population	1.20	2	0.3061	4.415	2	**0.0143**
Treatment	0.71	2	0.4949	0.163	2	0.8496
Population : Treatment	1.71	4	0.1533	0.884	4	0.4763
Statistical model	LM			LM		

Table 3. Effects of the type of experimental arena (females had either the choice or no choice to lay on a spore-covered area), population and spore presence (if the eggs where laid in the spore-covered area or not) on egg quality and egg quantity. All interactions have been tested and were then removed from the statistical models because non-significant. Significant p-values are in bold.

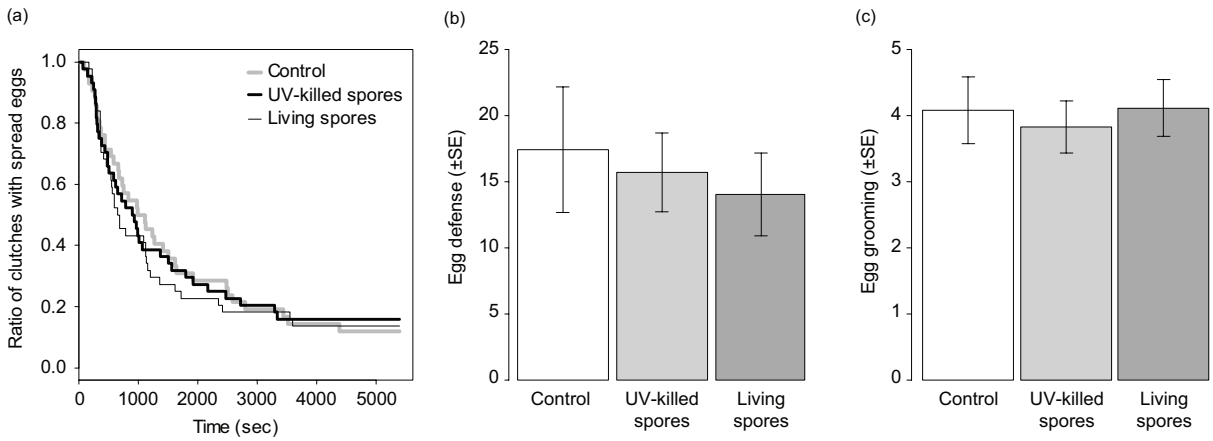
	Egg quality (PC1)			Egg quantity (PC2)		
	F	Df	P	F	Df	P
Experiment	1.58	1	0.212	0.14	1	0.710
Population	6.70	2	**0.008**	9.61	2	**<0.001**
Spores presence	<0.01	1	0.966	0.03	1	0.856



597

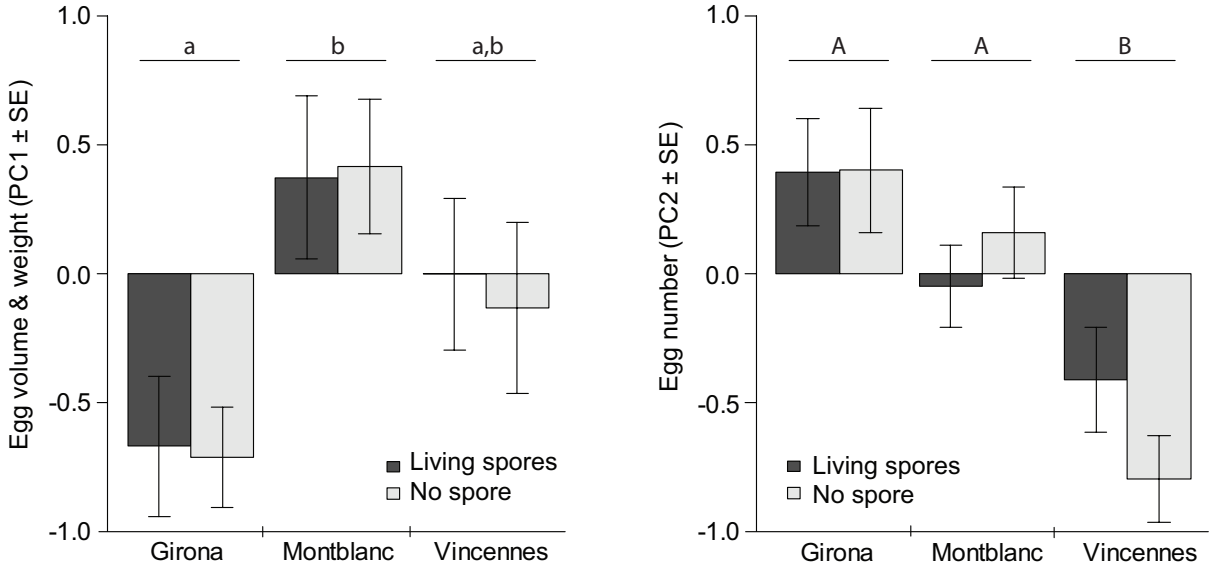
598 **Figure 1**. The duration of clutch abandonment and the likelihood of nest building reflected
599 the presence of either living spores, UV-killed spores or no spores in the nesting area. In
600 particular, (a) Mothers abandoned their clutch of eggs less long in presence of living spores
601 as compared to UV-killed spores and no spores at all. (b) Mothers were also more likely to
602 build a nest in presence of UV-killed and living spores compared to no spore at all, but only
603 when they were originating from the Vincennes population. Sample sizes are at the bottom
604 of each bar. Different letters correspond to p-values < 0.065.

605



606 **Figure 2**. The presence of either living spores, UV-killed spores or no spores in the nesting
607 area did not influence three measured forms of maternal care: (a) the speed of egg gathering
608 after an experimental egg spread, (b) the level of egg defence against a simulated predator
609 attack and (c) the frequency of egg grooming.

610

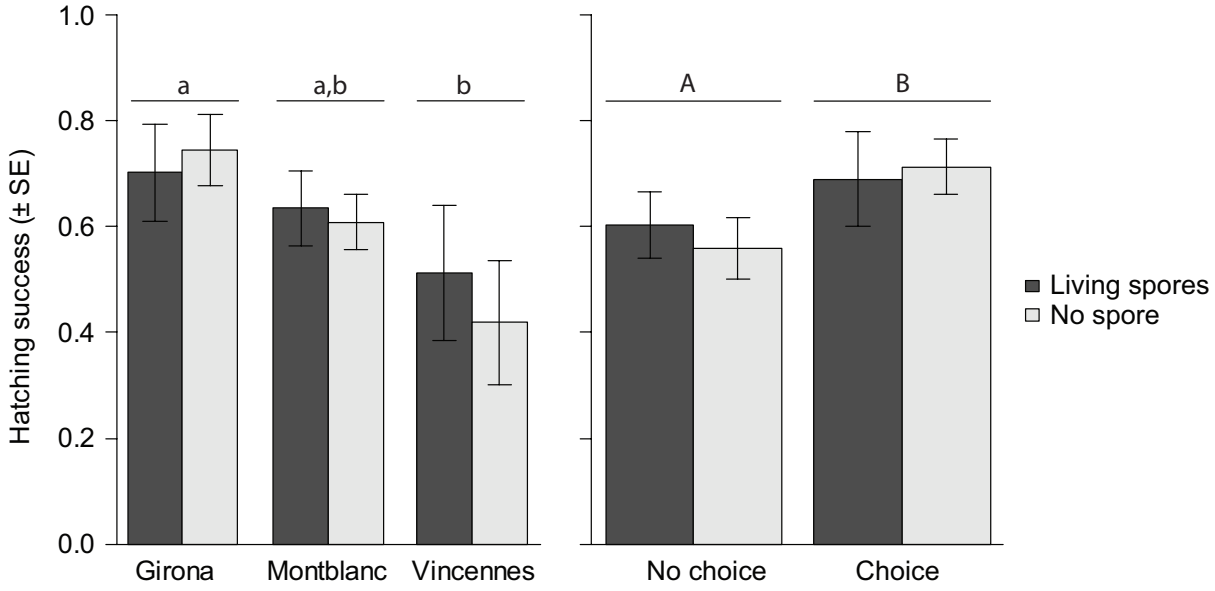


611 **Figure 3**. The population, but not the presence of spores in the nesting area shaped the quality

612 (PC1) and quantity (PC2) of eggs produced. Pairwise comparisons based on Tukey contrasts.

613 Different letters $P < 0.005$.

614



615 **Figure 4**. Effect of population, possibility for females to avoid locations covered with spores

616 (choice or no choice) and of the presence of living spores in their vicinity on hatching success.

617 Pairwise comparisons based on Tukey contrasts. Different letters $P < 0.030$.