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1 **Surrounding pathogens shape maternal egg care but not egg**
2 **production in the European earwig**

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SUMMARY

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

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

INTRODUCTION

28 Pathogens are considered as a major threat in nature, because they often entail large fitness
29 costs in their hosts (Schmid-Hempel 2014). To limit the risks of pathogen exposure and
30 infection, individuals from both vertebrate and invertebrate species have developed a broad
31 spectrum of defences (Siva-jothy et al. 2005; Schmid-Hempel 2014). The most studied
32 defences rely on the immune system of the potential host, which typically detects the
33 presence of pathogens in the organism, prevents their development and ultimately kill and
34 purge them from the host (Siva-jothy et al. 2005; Beckage 2008). However, many non-
35 immunological defences may also help hosts to fight against pathogens (Parker et al. 2011).
36 Among them, hosts have been shown to change some of their behaviours to prevent direct
37 contacts to pathogens (de Roode and Lefèvre 2012). This can be done, for instance, by
38 avoiding infected areas and limiting the consumption of pathogen-infected food (Villani et al.
39 1994; Meyling and Pell 2006; Ormond et al. 2011), and by prophylactically or curatively
40 consuming natural components with antimicrobial properties, a process called self-
41 medication (de Roode et al. 2013; Bos et al. 2015).

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

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

55 Social immunity, however, is not exclusive to eusocial systems and can be found in less
56 derived forms of group living, such as simple family units (Cotter and Kilner 2010a; Meunier
57 2015). In these units, parents generally provide multiple forms of care to their eggs and/or
58 juveniles (Smiseth et al. 2012) and, because eggs and juveniles often exhibit an
59 underdeveloped immune competence (DeVeale et al. 2004; Vogelweith et al. 2017), parental
60 care often includes protection against pathogens. For instance, mothers can protect their eggs
61 against microbial infections by applying antimicrobial secretions to their surface, as reported
62 in blennies, grass gobies and fringed darters (Knouft et al. 2003; Giacomello et al. 2006;
63 Giacomello et al. 2008). Similarly, parents may cover the nest with feces exhibiting
64 antibacterial activity to prevent the development of pathogens next to the juveniles, a
65 phenomenon found in the burying beetle and the European earwig (Rozen et al. 2008; Cotter
66 and Kilner 2010b; Diehl et al. 2015). Finally, females of the beewolf digger wasp *Philanthus*
67 *triangulum* have been shown to transfer symbiotic bacteria to the brood cell cover
68 (Kaltenpoth et al. 2005) and these bacteria to be taken up by the larvae and incorporated into
69 their cocoon to serve as antimicrobial protection (Kroiss et al. 2010). Whereas these maternal
70 behaviours typically protect offspring against pathogens, it remains unclear whether these
71 behaviours are curative/therapeutic (i.e. help fighting against pathogens already in contact
72 with offspring) or prophylactic (i.e. help preventing potential contacts between pathogens and
73 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

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

MATERIAL AND METHODS

117 **Animals and pathogen**

118 All the females used in the following experiments were the first laboratory born generation of
119 individuals collected in 2014 in three independent populations: Vincennes (France; 48° 51' N,
120 2° 26' O), Girona (Spain; 41° 59' N, 2° 49' O) and Montblanc (Spain; 41° 23' N, 1° 10' O). At
121 adult emergence, these females were maintained with unrelated males in groups of 50
122 individuals (balanced sex-ratio) to allow them to mate freely (Meunier et al. 2012). Each group
123 was set up in a plastic container (37 x 22 x 25 cm) grounded with moist sand, containing egg
124 carton as shelters, and maintained under standard conditions (constant 20°C, 10:14 h
125 dark/light cycle, 60% humidity). Three months later, each female was isolated in a Petri dish
126 (9 cm diameter) grounded with moist sand, and subsequently maintained under winter
127 conditions to allow egg production (gradual temperature change from 15°C to 5°C during
128 three weeks, with constant 60% humidity and complete darkness). Ten days later, the
129 temperature was increased to 10°C for two more weeks and finally to 15°C for the rest of the
130 experiments to allow egg development. After egg hatching, each family was kept at room
131 temperature (23°C) until the end of the following experiments. Groups and isolated females
132 were fed twice a week with an *ad libitum* amount of a standard diet mainly composed of
133 pollen, cat food and carrots (see detailed composition in Kramer *et al.*, 2015).

134 The conidia (spores) of the entomopathogenic fungus *M. brunneum* used in the
135 following experiments were obtained from a genotyped strain isolated from soil samples in
136 Switzerland (Reber and Chapuisat 2011). To ensure the virulence of this strain to earwigs, this
137 fungus has been grown on *F. auricularia* for three generations prior to their use in this
138 experiment. Specifically, earwigs were infected in a 1 ml spore solution of *M. brunneum*
139 (10^8 /ml) conidia solved in 0.05% Tween 20 and held in a climate cabinet at 25°C in small Petri
140 dishes (5.5 cm diameter) filled with moist sand. After the death of the exposed individuals,

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

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

150 To investigate whether females avoid laying their eggs in a pathogenic environment, we
151 transferred a random sample of 37 females (n = 11 Girona, n = 18 Montblanc and n = 8
152 Vincennes) to individual Petri dishes (9 cm diameter, grounded with humid sand) divided in
153 two parts of equal size. One side was previously sprinkled with 0.55 ml of a spore solution of
154 *M. brunneum* (10^7 /ml) solved in Tween 20 (0.05%, Roth), whereas the other side (control) was
155 previously sprinkled with the same amount of a spore-free Tween 20 solution. A direct
156 exposure to a solution of 10^7 spores per ml is known to entail a 20% adult mortality after 25
157 days (Kohlmeier et al. 2016). The two sides were separated by a cardboard barrier (8.7 x 1 cm)
158 covered with aluminum foil to avoid any passive diffusion of the applied solutions between
159 the two sides, while allowing females to easily move from one side to the other. Each
160 experimental Petri dish was then checked on a daily basis in order to (1) count the number of
161 days each female stayed on each side until egg laying, (2) record the side on which females
162 deposited their eggs and finally to (3) count the number of times the clutches were moved
163 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.

210 We measured five forms of pre-hatching maternal care: egg gathering, egg defence,
211 clutch abandonment, nest construction and egg grooming. (1) Egg gathering indicates the
212 propensity of a female to gather its eggs after these were experimentally spread out in the
213 nest. This measurement was done five days after egg laying. At that time, we confined each
214 female within its Petri dish, then spread a random selection of 30 eggs (or all, if fewer were
215 available) evenly within a circle (~3 cm diameter, including the site where the eggs were
216 originally laid) and subsequently recorded the seconds between the mother's first movement
217 out of the confinement area and the re-assembly of the eggs within one body length of the
218 female. Note that the eggs not involved in this measurement were kept in a small closed
219 plastic dish in the meantime. (2) Egg defence revealed females' reaction to a simulated
220 predator attack and was measured ten days after egg laying. This measurement was done
221 following a standard method (Thesing et al. 2015), in which females were poked with a glass
222 capillary on the thorax (1 poke / 2 seconds) until they abandoned their clutch (i.e. moved more
223 than two body lengths away from it). The values of egg defence were the number of pokes
224 the females sustained before leaving the eggs. (3) Clutch abandonment was measured
225 subsequently to egg defence and was used to quantify how long a mother abandons her clutch
226 of eggs after a simulated attack. The value of egg abandonment was the time (in seconds)
227 between the moment where each female left its nest due to a simulated predator attack (see
228 above) and the moment where females returned to their clutch and touched at least one egg.
229 (4) Nest construction reflected whether a female invested into the construction of a nest at
230 day 15 after egg laying. Following a method already developed in this species (Meunier et al.
231 2012), we considered females to have constructed a nest when they dug a pit to the ground
232 of the Petri dish and put their eggs into the pit. Finally, (5) egg grooming was recorded 15 days
233 after egg laying, just after checking for females nest construction. At that time, each female

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

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

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

251 Finally, we investigated whether females adjust the number, volume and weight (proxies of
252 egg quality, Koch & Meunier, 2014) of their eggs to the presence (enforced or not) of living
253 pathogens in the environment. To this end, we measured these three traits in the clutches of
254 the 36 females used to address question 1 (n = 11 Girona, n = 18 Montblanc and n = 8
255 Vincennes) and the 86 females that were in the living spores and spore-free control solution
256 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 $((4 \cdot \pi) / 3) \cdot (\text{egg width} / 2)^2 \cdot (\text{egg length} / 2)$. 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

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

RESULTS

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

285 When given a choice, females did not avoid the side covered with pathogens both before
286 oviposition (Mean \pm SE proportion of time spent by a female on the control side: $54.0 \pm 18.0\%$;
287 $t_{48} = 1.52$, $P = 0.136$) and at egg laying (22 and 15 females laid their eggs in the control and
288 infected sides, respectively; exact binomial test: $P = 0.324$). These results were independent
289 of female's population, egg number and of an interaction between these two variables (Table
290 1). Overall, only 1 of the 15 females who laid their eggs on the pathogen side relocated its
291 clutch to the control side, which is comparable to the 1 of the 22 females who relocated its
292 eggs after having laid them on the control side. Note that this general tolerance of females for
293 the spores' side is unlikely to result from the unintentional spread of spores by moving
294 females, since they were equally distributed between the two sides already on the first day
295 (28 and 21 females on the tween and spores sides, respectively; exact binomial test: $P = 0.392$)
296 and the second day (22 and 27 females on the tween and spores sides, respectively; exact
297 binomial test: $P = 0.568$) of the experiment.

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

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

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

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

317 The PCA conducted on the three egg traits provided three orthogonal principal components
318 (PCs), of which we extracted the two first (total variance explained = 97.6 %). The first
319 component (PC1) was highly and positively loaded with egg volume and egg weight (0.959 and
320 0.964, respectively; loading of egg number = -0.42), therefore reflecting egg quality.
321 Conversely, the second component (PC2) was highly and positively loaded with egg number
322 (0.91; loading of egg volume = 0.21; loading of egg weight = 0.19), therefore reflecting egg
323 quantity.

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

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

DISCUSSION

341 Shedding light on which maternal strategies can protect offspring against infection is of central
342 importance to better understand the evolutionary drivers of parental care and more generally,
343 the emergence and maintenance of family life in nature (Royle et al. 2012; Klug and Bonsall
344 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

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

403 Contrary to the previous spore-dependent effects, we demonstrated that earwig
404 females showed no preference for either pathogen-exposed or pathogen-free environments
405 for oviposition, nor did they adjust their egg properties to the presence of pathogens in the
406 nesting area. The reported absence of pathogen-dependent effects in earwigs is uncommon
407 among invertebrates, where most studies show that females either avoid (Meyling and Pell
408 2006; Lam et al. 2010) or prefer (Brütsch et al. 2014; Pontieri et al. 2014) areas covered with
409 entomopathogenic spores for oviposition, as well as either increase (Villani et al. 1994) or
410 reduce (Machtinger et al. 2016) their egg production in presence of entomopathogenic
411 spores. In earwigs, this effect is unlikely to reflect females' inability to detect spore presence
412 (see above). Our results therefore suggest that females detected pathogens but neither chose
413 their egg laying location nor adjusted their investment into egg production accordingly. Two
414 non-mutually exclusive hypotheses could explain this pattern. First, the presence of spores in
415 the environment might not be a major threat for the eggs. This is an unlikely hypothesis, as
416 this generalist entomopathogenic fungus is a well-known threat for eggs in insects (Samuels

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

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

441 **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

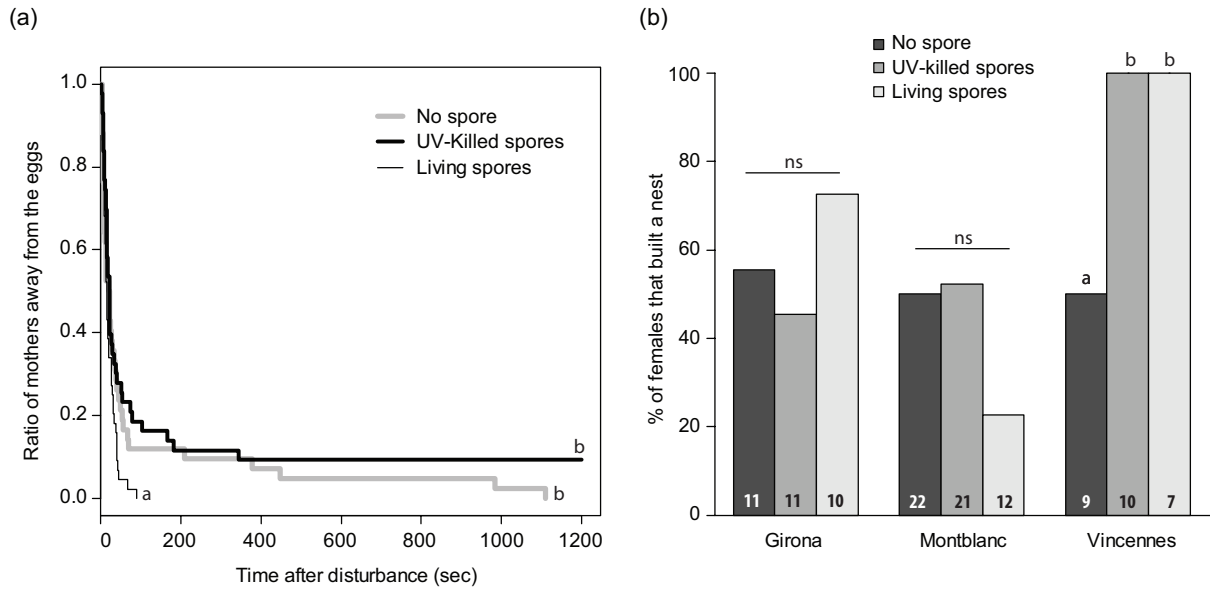
589 **Table 2.** Effects of population and treatment on (a) the duration of clutch abandonment, (b)
 590 nest construction, (c) Egg gathering, (d) egg defence and (e) egg grooming. Significant p-values
 591 are in bold.

	(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		

592 **Table 3.** Effects of the type of experimental arena (females had either the choice or no choice
 593 to lay on a spore-covered area), population and spore presence (if the eggs where laid in the
 594 spore-covered area or not) on egg quality and egg quantity. All interactions have been tested
 595 and were then removed from the statistical models because non-significant. Significant p-
 596 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

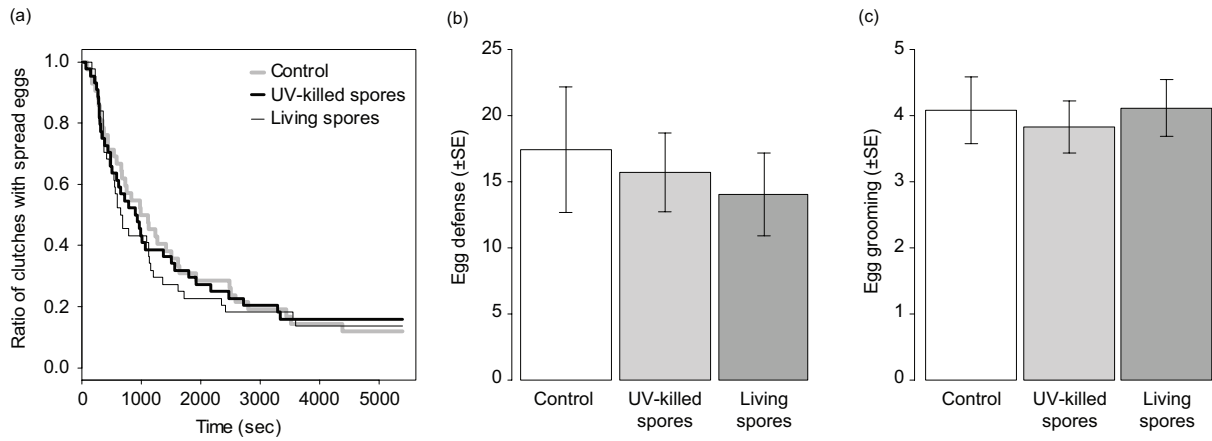
601 as compared to UV-killed spores and no spores at all. (b) Mothers were also more likely to

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

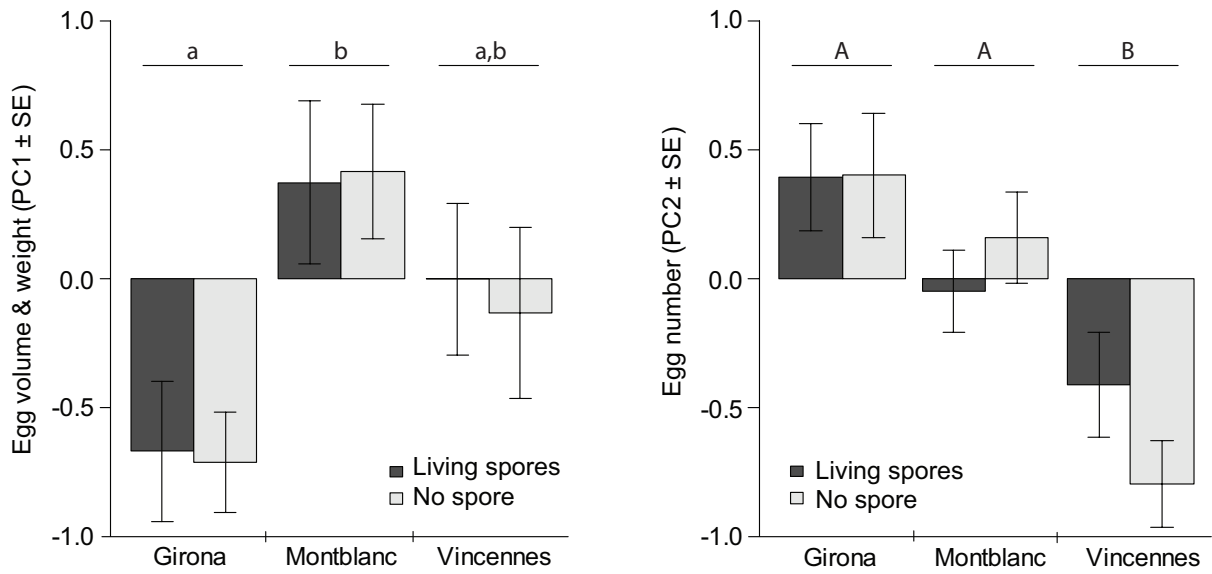
604 of each bar. Different letters correspond to p-values < 0.065.

605



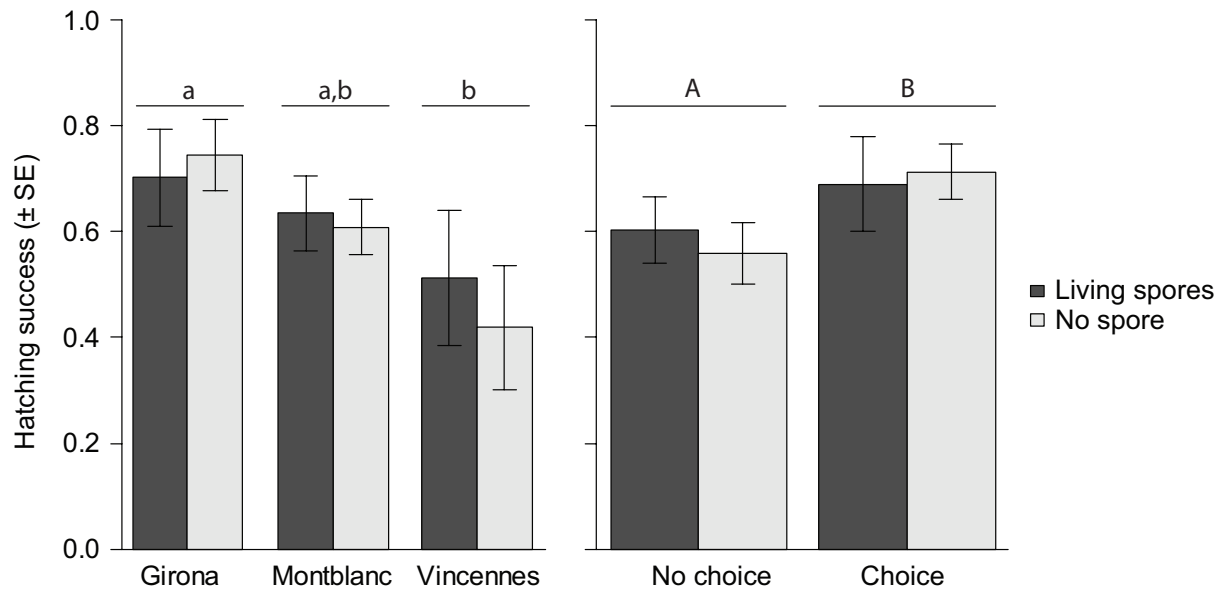
606 **Figure 2.** The presence of either living spores, UV-killed spores or no spores in the nesting
 607 area did not influence three measured forms of maternal care: (a) the speed of egg gathering
 608 after an experimental egg spread, (b) the level of egg defence against a simulated predator
 609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