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Original Article

Maternal care provides antifungal protection to eggs in the European earwig

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Many insects raise their offspring on organic substrates or in the soil where microorganisms are abundant. Microbes may pose a serious threat to offspring development and survival by either decomposing food resources or directly infecting the offspring. Selection to cope with these effects may favor social defenses, for example, through forms of parental care that can limit or eliminate these threats to offspring fitness. In this study, we experimentally tested if maternal egg attendance in the European earwig *Forficula auricularia* has a function as a social defense against mold infection of eggs by manipulating exposure of eggs to mold spores and the presence of the mother in a fully factorial design. Furthermore, we investigated the potential roles of egg grooming behavior and maternal transfer of chemicals as underlying mechanisms. As predicted, the beneficial effect of egg attendance on hatching success was significantly enhanced when eggs were exposed to the mold. Females significantly increased their egg grooming duration in response to mold exposure of her eggs, and the quantity of chemicals (identified as hydrocarbons) was maintained among attended eggs but decreased substantially among unattended eggs. Maternal transfer of chemicals was confirmed in extractions of glass beads that were mingled into attended or unattended clutches. This study shows that maternal egg attendance in the European earwig has a social defense function protecting offspring against mold infection. The maternal egg grooming behavior seems to be key for this effect, probably through both the mechanical removal of spores and the continued application of chemical substances on the egg surface.

Key words: **antimicrobial defense, Dermaptera, egg attendance, egg grooming, insect, *Mucor*, social defence, social evolution.**

INTRODUCTION

Across animal species and taxa, a broad variety of mechanisms are known to help individuals limit their risks of infection by parasites and microbial pathogens. These mechanisms include parasite avoidance, self-grooming, or specific and nonspecific immunological responses (Schmid-Hempel 2003; Schmid-Hempel and Ebert 2003; Cremer and Sixt 2009; Cotter and Kilner 2010a). In addition to individual defenses against infection, recent studies demonstrated that socially mediated collective mechanisms also evolved in group-living organisms. Forms of social immunity are expected because the frequent and intimate contacts between individuals in group-living organisms facilitate disease transmission. An added factor specific to kin groups (e.g., eusocial insect colonies) is that the close genetic relatedness between group members may render them susceptible to the same pathogens (Schmid-Hempel 2003; Cremer et al. 2007). A very common behavioral form of social immunity is allogrooming, during which individuals groom other individuals to remove, for example, pathogenic fungi on the surface of other group members or to apply antimicrobial chemical substances (Rosengaus et al. 1999; Ugelvig et al. 2010; Reber et al. 2011; Tragust et al. 2013). Allogrooming can also mediate pathogen exposure of the grooming individuals which can enhance their own resistance on a later exposure (Ugelvig and Cremer 2007; Konrad et al. 2012).

Whereas mechanisms of social immunity received growing attention in eusocial insects such as ants, bees, or termites (Traniello et al. 2002; Cremer et al. 2007; Ugelvig and Cremer 2007; Reber et al. 2011), there is comparably little research on social defense mechanisms in non-eusocial systems, such as families where parents care for their own offspring. In families, parasites pose a potential threat to offspring fitness, and selection may favor forms of parental care that include mechanisms of social defenses against detrimental effects of parasites on offspring. For example, in birds, females deposit hormones or antibodies to the eggs protecting their offspring from infections during early development (e.g., Grindstaff et al. 2003). Or in some insect species with parental care, parents transfer antimicrobial substances to their offspring's food sources to prevent this food from degradation and, hence, to

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reduce offspring competition with microbes (Trumbo 2012). For example, in the burying beetle *Nicrophorus vespilloides*, parents protect the vertebrate carcass on which they breed from bacterial degradation by using secretions from their anal glands (Rozen et al. 2008; Cotter and Kilner 2010b; Arce et al. 2012). In the beewolf digger wasps *Philanthus triangulum*, females embalm the food provisioned to their offspring (paralyzed honeybees) with hydrocarbons that prevent water condensation and thereby inhibit mold growth (Herzner and Strohm 2007). Furthermore, females provide secretions to the brood cell cover containing symbiotic bacteria from their antennal glands. In many cases, these bacteria are then taken up by the larvae and later incorporated into their cocoon as a protection against fungal or bacterial infections (Kroiss et al. 2010).

A widespread form of parental care in insects is egg attendance (Trumbo 2012; Wong and Kölliker 2012; see Royle et al. 2012). Whereas parental egg attendance is generally known to protect eggs against inter- or intraspecific predation (Cocroft 1999; Zink 2003; Miller and Zink 2012; Trumbo 2012; Wong and Kölliker 2012), little is known about its protective function against egg infection by fungi and bacteria (Costa 2006; Trumbo 2012). Fungi and bacteria may be key ecological agents of selection for parental egg attendance in insects, as many species raise their clutch of eggs on organic substrates or in burrows/tunnels in the soil where they are continuously in contact with bacteria, fungi, and mold (Costa 2006; Cremer and Sixt 2009; Reber and Chapuisat 2012; Trumbo 2012). These microbes all pose potentially serious threats not only indirectly by decomposing food sources (see above) but also directly by infecting the eggs and/or impacting embryonic development and survival.

An antifungal/parasitic function of egg attendance (in particular egg grooming) by parents or workers has been suggested in several insect species (e.g., Costa 2006; Trumbo 2012), but only few experiments have been carried out to address this question directly using an experimental approach. In the ant *Formica selysi*, workers increase their egg grooming behavior in response to exposure to pathogens (Reber et al. 2011), and earwig (Dermaptera) females exhibit a characteristic egg grooming behavior when they tend their clutch (Costa 2006). In the ring-legged earwig *Euborellia annulipes* (Klostermeyer 1942) and the maritime earwig *Anisolabis maritima* (Miller and Zink 2012), unattended eggs were shown to be more often infected by mold than attended eggs. However, because mold exposure was not experimentally manipulated, these latter results did not allow disentangling whether egg attendance was causal in reducing mold infection, or whether it enhanced egg survival through another function, leading to higher egg mortality in unattended clutches and enhanced opportunistic mold growth on already dead eggs. Buxton and Madge (1974) carried out an experiment to test if maternal egg grooming in the European earwig *Forficula auricularia* is to mechanically remove mold spores. Their mechanical treatment of eggs with a paintbrush reduced mold infection, but it is uncertain if the brush treatment is a meaningful imitation of the mechanical effect of maternal egg grooming. Also, it remains to be tested whether females use other forms of protection, for example, by applying specific chemicals on the egg surface (Herzner and Strohm 2007; Matsuura et al. 2007; Tragust et al. 2013) to limit the risks of microbial infections.

In this study, we used a combination of behavioral experiments and chemical extraction and quantification methods to test the antimicrobial function of maternal care in the European earwig (*F. auricularia*) and to investigate potential underlying behavioral and chemical mechanisms. The European earwig is ideal to address this question because 1) females attend their clutches in burrows in the soil with the corresponding exposure of eggs to soil fungi and other microorganisms, 2) females show a characteristic egg-grooming behavior, 3) previous studies suggest maternal care is required for successful egg hatching (see above), and 4) many of the life history traits of this species have been well studied (Lamb and Wellington 1975; Lamb 1976; Costa 2006; Kölliker 2007; Meunier and Kölliker 2012; Meunier et al. 2012). By conducting a full-factorial experiment in which we manipulated the presence/absence of the attending mother, as well as the exposure of eggs to mold spores, we tested the predictions that 1) the beneficial effect of maternal egg attendance on offspring fitness (in terms of hatching success and hatchling body weight) is enhanced when the eggs are exposed to mold spores, 2) females respond to spore exposure of their eggs by increasing the duration of maternal egg grooming, and 3) mothers transfer chemical compounds to the eggs that may directly or indirectly protect the eggs against mold growth.

MATERIALS AND METHODS

Origin of the tested individuals

The *F. auricularia* adults used in our experiments were from the fifth generation of a large population reared in the laboratory and originating from a population that was collected in the field in Dolcedo (Italy) in May 2009 (Meunier et al. 2012). The laboratory population was maintained, each generation, by breeding newly produced adults distributed over plastic containers (37 × 22 × 25 cm), in which groups of 24 virgin females were allowed to mate with 24 unrelated males. During the adult stages, these populations were maintained under a 14:10 h light:dark cycle, at constant 20 °C and 70% relative humidity, and were fed twice a week with the standard food used for the laboratory populations (consisting of Agar-Agar, carrots, bird and dry cat food, wheat germ, cooked egg yolk, ascorbic and sorbic acid) (Meunier et al. 2012). When the first female was observed laying eggs in its original plastic container, all females were isolated in individual Petri dishes containing humid sand as a substrate. Water was added as necessary to keep the sand humid. The dishes were maintained in complete darkness, first under 10 °C for 2 weeks for initiation of oviposition, and then at 15 °C and 70% humidity afterward for egg laying and until hatching (Meunier et al. 2012). Females had no access to food from egg laying to hatching (Kölliker 2007; Meunier et al. 2012).

Origin and identification of the tested mold

The mold used to manipulate the presence of spores on eggs (see below) was initially collected in the containers where we held the earwigs. The mold mainly grew on earwig food or frass. We then cultivated the mold on our standard earwig food under 10:14 h light:dark cycle, 20/15 °C, and 70% relative humidity. The mold spores involved in this experiment originated from 5-day-old cultures, and they were transferred for exposure to filter papers by harvesting the spores of the mold sweeping a Pasteur pipette first over the mold and then rubbing the spores from the pipette onto the filter papers. After an initial morphological identification of 14 sporulated isolates, the reliability of this identification was confirmed using molecular methods (see Supplementary Materials). It is well known that humidity affects mold growth (e.g., Mari et al. 2000), which is why we carried out a preliminary study where the effects of mold exposure and maternal attendance were tested

under 2 different levels of humidity (i.e., very high and relatively low amounts of water added to the sand substrate). Because humidity had no significant effect on how mold exposure affected hatching success (n = 101; P = 0.609; Boos S, Kölliker M, unpublished data), we carried out the main study under the regular laboratory conditions.

Experiment 1: effects of maternal egg attendance with and without mold exposure

The effects of maternal egg attendance and egg mold exposure on hatching success and offspring quality (hatchling body weight) was tested in 50 clutches, each split in 2 halves, using a split-clutch design (Figure 1). Two factors were manipulated: 1) the presence/absence of the attending mother (within [split]-clutch treatment) and 2) the exposure to mold spores/sham treatment of the eggs (between-clutch treatment). The clutches were randomly assigned to the spore exposure treatment or control on the second day after oviposition, resulting in 25 clutches where the eggs were later exposed to mold spores and 25 clutches where the eggs were sham treated. The mean (± standard deviation [SD]) clutch size was 66.84 (± 7.16) eggs. Each clutch was then split into 2 sets of approximately 31 eggs each (on average 31.80 ± 2.26 [mean ± SD] eggs). The eggs of each egg set were then transferred into a new Petri dish with humid sand as substrate as before, and either with mother (maternal presence treatment) or without mother (maternal absence treatment). The experimental groups were then maintained under standardized laboratory conditions (see above).

The mold spore exposure treatments took place 8 days after oviposition. The split clutches were each separately isolated for 60 min for exposure treatment during which the eggs of each set were moved gently 3 times over a filter paper (Whatman, Sigma-Aldrich, Buchs, Switzerland; diameter 4.25 cm) with a spatula. The filter papers were either untreated (control) or mold spores were previously spread over the filter paper (spore exposure treatment). To confirm successful transfer of spores onto the eggs and to ensure that the treatment did not cause damage to the eggs, we carefully checked pictures of each set of eggs taken with a Leica MZ 12.5 stereomicroscope after treatment (magnitude ×1.0). Spores/sporangia were easily visible as black speckles on the white surface of the eggs (Boos S, personal observations). After the exposure treatment, each set of eggs was transferred to a new Petri dish (diameter: 6 cm) either with or without their mother (according to the maternal presence treatment).

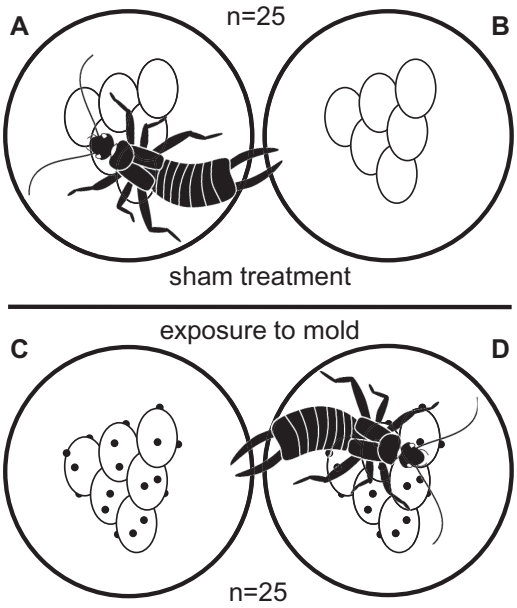

Figure 1
Graphical illustration of the experimental design. Each clutch was split into 2 sets of approximately 31 eggs each (A and B or C and D), which were then assigned 1 of 4 treatments: (A) maternal attendance and no application of spores, (B) no maternal attendance and no application of spores, (C) no maternal attendance and application of spores, and (D) maternal attendance and application of spores. Black dots in (C) and (D) symbolize mold spores/sporangia. *n* refers to the number of replicates per treatment.

Directly after this exposure treatment, we recorded female behavior under infrared light using a Sony HDR SR8E Video camera (mode: SD/LP) for 75 min. From the recordings, the total time spent egg grooming was quantified by playing the movie sequences with the software QuickTime player (Version 10.0) with an 8-fold time lapse. The first 2 min of each video were discarded as habituation phase. The egg grooming measurements were highly repeatable (r = 0.99 using 10 randomly chosen females scored twice; Lessells and Boag 1987).

After filming, the eggs and females (if any, depending on treatment) were returned into their Petri dishes and held under standard laboratory conditions until hatching (generally occurring 2 weeks later). One day after the first egg hatched, the total hatching success and offspring quality per clutch were assessed by counting the number of hatched nymphs and weighing all nymphs to the nearest 0.01 mg using a Mettler Toledo MT5 high precision balance, respectively.

Experiment 2: effect of maternal presence on chemical compounds on egg surface

This series of 2 experiments aimed at testing whether mothers transfer chemical compounds to their attended eggs. In the first, we investigated the influence of maternal egg attendance on the quantity of chemical compounds present on the surface of eggs using a split-clutch experiment. Similarly to experiment 1, the 2-day-old clutches of 20 females were split into 2 sets of approximately 29 eggs each (on average 29.25 ± 3.30 eggs) and then transferred into a new Petri dish either with their mother (maternal presence treatment) or without mother (maternal absence treatment). Changes in the quantity of chemical compounds present on the respective eggs were then tested by randomly sampling 5 pairs (i.e., sets of eggs with and without their own mother) on day 2, 8, 14, and 20 after setup and then extracting the chemical compounds present on the eggs. The difference in the chemical profile of eggs attended by females and unattended eggs is indicative for maternally transferred chemical substances.

To confirm maternal transfer, we ran an additional experiment in which 10 glass beads (diameter 1.25–1.65 mm, Roth, Arlesheim, Switzerland) were mixed with the eggs directly after oviposition in each of 5 clutches (i.e., in 10 split-clutches with and without mother). The females in all cases accepted the glass beads and showed similar tending behavior as toward eggs (Boos S, personal observations; Worthington 1926; Butnariu et al. 2013). The chemicals on the surface of the glass beads were extracted 20 days after setup. The presence of chemical compounds on the glass beads in the maternal presence treatment and their absence in the maternal absence treatment would demonstrate the transfer of chemical compounds from the females to their eggs. We conducted the same handling and extraction in the maternal absence treatment to control for potential contaminations from other eggs.

Chemical extractions of the eggs

Eggs (or glass beads) were extracted for 6 min in 200 µL extraction solution consisting of 1.25 ng/µL of an internal standard (*n*-octadecane, $C_{18}H_{38}$, Sigma-Aldrich, Buchs, Switzerland) in *n*-heptane (C_7H_{16}, Roth, Arlesheim, Switzerland). Hundred microliters of each extract was stored at −28 °C until analysis. Chemical analysis was carried out with an Agilent7890 gas chromatograph coupled to an Agilent5975C inert XC MSD mass spectrometer. Two-microliter aliquots of each extraction sample were injected using splitless mode on a DB5 column (HP-5ms: length: 30 m, inner diameter: 0.25 mm, film thickness: 0.25 µm, Agilent Technologies, Basel, Switzerland) with a SSL injector temperature held constant at 250 °C and a helium flow rate of 1 mL/s. GC oven temperature started at 70 °C and was held for 2 min. The temperature was then ramped at different rates over the course of the run: It was initially raised at 15 °C/min to 232 °C where it was held for 11 min. In a next step, temperature increased at 5 °C/min to 263 °C and then at 15 °C/min to 300 °C where the temperature was finally held for 7 min.

We focused on a qualitative identification of compounds to identify the chemical class of the found compounds and the most abundant peaks by comparing the mass spectra of the peaks with the mass spectral library NIST2008.

Statistical analysis

The statistical analyses were conducted using R (Version 2.15.2). The influence of the maternal presence and spore exposure treatments on hatching success (calculated through the *cbind(x,y)* function in *R*, in which *x* was the number of nymphs at hatching and *y* the number of eggs that did not hatch [total eggs − nymphs at hatching]) and the mean weight of nymphs were analyzed using 2 generalized linear mixed models (GLMMs), with binomial error distribution (linked with a logit transformation) and with Gaussian error distribution (linked with identity), respectively. In these models, maternal presence, spore exposure, and their interactions were entered as fixed factors, and the clutch identity (ID) as a random factor to accommodate the statistical dependencies arising from the split-clutch design (using glmmPQL; package car Version 2.0-16). Two clutches were excluded from the analysis of hatching success because the mothers ate the eggs. Egg grooming behavior was analyzed using a linear model with the exposure treatment as fixed factor. The change of the chemical compounds on the egg surface over time and the influence of maternal presence on this pattern were also analyzed using a GLMM (with Gaussian error distribution and an identity link), in which the maternal presence treatment was entered as a fixed factor, the day of extraction as covariate, and the clutch ID as a random factor. Glass beads were analyzed with a linear model with the maternal presence treatment as a fixed factor. The quantities of the chemical compounds on the egg surface and glass beads were cubic root transformed for analyses.

RESULTS

Experiment 1: effects of maternal egg attendance with and without mold exposure

As predicted, if maternal egg attendance has an antifungal function, the beneficial effect of maternal presence on hatching success was significantly enhanced when the eggs were exposed to spores. This is shown by a significant effect of the interaction between the maternal presence and the spore exposure treatment on hatching success (Figure 2A; $\chi^2_1 = 55.34$, $P < 0.001$; main effect maternal presence treatment: $\chi^2_1 = 138.08$, $P < 0.001$; main effect spore exposure treatment: $\chi^2_1 = 59.85$, $P < 0.001$). This interaction arose because spore exposure significantly decreased hatching success in unattended clutches ($\chi^2_1 = 130.21$, $P < 0.001$) but had

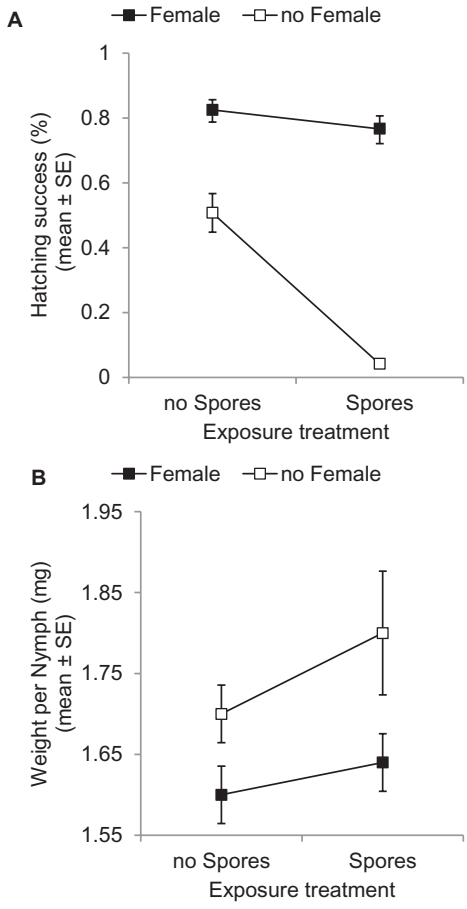

Figure 2
Effects of mold spore exposure of eggs and maternal presence on hatching success (A) and mean hatchling body weight (B). Shown are means and standard errors. The results are in relation to the spore exposure treatment. Filled symbols are for the treatments where the mother was present, open symbols for the treatments where the mother was absent (see variable legend).

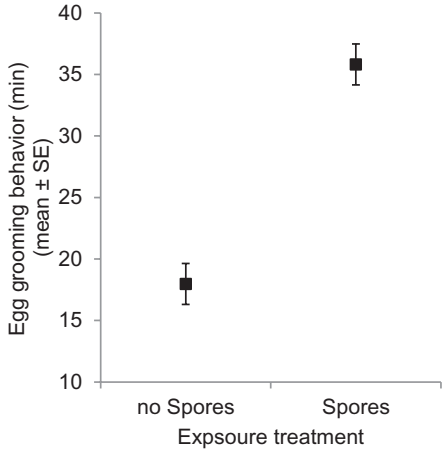

Figure 3
Effect of mold spore exposure on the duration of maternal egg grooming. Quantification of egg grooming was made over the course of a 75-min observation period. Shown are means and standard errors.

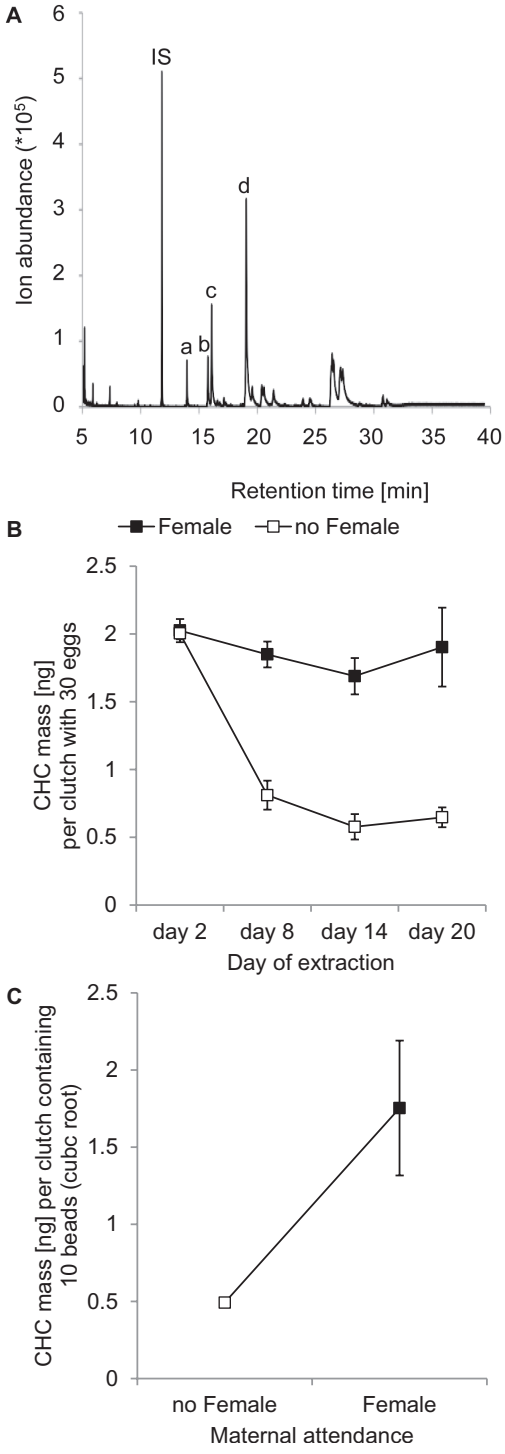

Figure 4
Maternal transfer of chemical compounds to the egg surface. (A) Exemplary chromatogram (total ions) of the extract from an attended clutch. Most of the visible peaks were hydrocarbons. The major peaks were identified as (a) heneicosane ($C_{21}H_{44}$), (b) Z-9-tricosene ($C_{23}H_{46}$), (c) tricosane ($C_{23}H_{48}$), and (d) Z-12-pentacosene ($C_{25}H_{50}$). IS indicates the internal standard *n*-octadecane ($C_{18}H_{38}$). (B) Change of total hydrocarbon quantities over time in clutches attended by their mother (filled symbols), and in unattended clutches (open symbols). (C) Total hydrocarbon quantities on glass beads mingled into maternally attended clutches and unattended clutches. In (B) and (C), means and standard errors are shown.

no significant effect on hatching success in clutches attended by a female ($\chi^2_1 = 1.51$, $P = 0.22$).

Nymph weight at hatching was significantly lower in clutches attended by females than in unattended clutches (Figure 2B; $\chi^2_1 = 4.34$, $P = 0.037$) but was not significantly influenced by spore exposure ($\chi^2_1 = 1.63$, $P = 0.202$) or by an interaction between the maternal presence and the spore exposure treatment ($\chi^2_1 = 0.45$, $P = 0.504$). There was no significant association between nymph weight and clutch size ($\chi^2_1 = 1.16$, $P = 0.281$).

As predicted, if females respond to spores on their eggs, tending females spent significantly more time egg grooming in the spore exposure treatment than in the unexposed control (Figure 3; *t*-test; $t_{48} = 7.59$, $P < 0.001$).

Experiment 2: effect of maternal presence on chemical compounds on egg surface

The chemical compounds extracted from the egg surface (Figure 4A) were mostly hydrocarbons. If these hydrocarbons were at least partly transferred by the female, we expected their quantity to be maintained in the presence of a tending mother and to decrease over time in the absence of a tending mother. This expected pattern was supported by our data. The summed quantity of hydrocarbons did not change significantly in the maternal presence treatment (Figure 4B; $\chi^2_1 = 0.53$, $P = 0.467$) but decreased strongly and significantly over time in the maternal absence treatment (Figure 4B; $\chi^2_1 = 34.92$, $P < 0.001$). The difference in slopes between treatments was significant (interaction effect: $\chi^2_1 = 20.74$, $P < 0.001$; main effect maternal presence: $\chi^2_1 = 3.50$, $P = 0.061$; main effect time: $\chi^2_1 = 35.18$, $P < 0.001$).

Maternal transfer of the hydrocarbons on the egg surface was confirmed in the glass beads experiment. Extracts from glass beads that were mixed into maternally attended clutches contained significantly higher quantities of hydrocarbons than glass beads mixed into unattended clutches (Figure 4C; *t*-test; $t_8 = 2.88$, $P = 0.021$).

DISCUSSION

Egg attendance is a widespread form of parental care among invertebrates (Tallamy 1984; Costa 2006; Trumbo 2012; Wong et al. 2013) and is known to enhance the fitness of eggs through protection against inter- or intraspecific predation (e.g., Pollard 1984; Tallamy 1984; Machado and Oliveira 2002; Zink 2003; Miller et al. 2011; Miller and Zink 2012). The potential role of maternal egg attendance as a form of social defense against parasites/microbes, although proposed repeatedly, was only rarely tested experimentally. Here, we demonstrated in the European earwig that 1) maternal egg attendance strongly and significantly reduced the detrimental effects of spore exposure of eggs on hatching success, 2) spore exposure of eggs significantly increased the duration of maternal egg grooming, and 3) maternal egg attendance lead to a larger overall amount of chemical compounds (hydrocarbons) on the eggs, apparently through continued maternal transfer. Furthermore, we also found 4) that hatchling body weight was lower in attended than unattended clutches but that this effect was independent of mold exposure.

We showed that the presence of the mother particularly enhanced hatching success under mold exposure, demonstrating an antifungal social defense function of female egg attendance. In fact, exposed eggs without tending mother had very low average hatching success of only 4% (compared with 77% with a tending

mother; Figure 2A), which shows the detrimental effects of mold on offspring fitness. This result, together with the observation that mold occurs commonly in the soil where organic matter is decomposed (e.g., Mari et al. 2000; Gherbawy et al. 2009), suggests a role for soil fungi as agents of selection contributing to the maintenance of maternal egg attendance in earwigs. Our results do not allow direct inference about the role of soil microbes (i.e., mold) in the evolutionary origin of maternal care. It is conceivable that the susceptible eggshell of Dermapterans is ancestral (in conjunction with the secondary loss of an ovipositor among the Neopteran lineage) and maternal egg attendance evolved as a parental care adaptation to protect susceptible eggs from environmental hazards (Zeh et al. 1989) such as mold. However, the current dependence of the eggs on maternal social defenses may not adequately reflect the susceptibility of eggs when this form of maternal care originated. Given maternal protection, it may have secondarily evolved to become more susceptible to infection (see Trumbo 2012) in which case the current benefits of maternal egg attendance would overestimate the benefits characterizing an ancestral state (Smiseth et al. 2012).

Our results clearly show that mothers enhanced offspring fitness under mold exposure of eggs in terms of hatching success. The results on hatchling body weight, a potential measure of offspring quality because heavier nymphs have a survival advantage in cannibalistic interactions (Dobler and Kölliker 2011), were less straightforward. Nymphs hatching from attended clutches were significantly lighter than those hatching from unattended clutches (an effect not significantly influenced by mold exposure). There are several possible explanations for this result. For example, only the highest quality eggs may have hatched in the absence of a mother resulting in less numerous but heavier individuals. This hypothesis would imply that mothers helped during the hatching process, a potential additional function of egg attendance, which has not yet been examined in earwigs. Alternatively, it is conceivable that early hatched nymphs cannibalized later hatched ones (i.e., sibling cannibalism occurs readily in *F. auricularia*; see Dobler and Kölliker 2010), but in this experiment, no cannibalism was observed during the short time window over which hatching of a clutch occurs (approximately 24 h: Boos S, unpublished data). Finally, the effect may be due to a difference in water balance between unattended and attended eggs in which case variation in fresh weight among hatchlings would not necessarily relate to a measure of quality. We showed that mothers transfer hydrocarbons to the egg surface, and it is well known that hydrocarbons on the cuticle of insects are key for water homeostasis (Blomquist and Bagnères 2010). Thus, the low quantities of hydrocarbons on the unattended eggs (Figure 4B,C) might have facilitated passive water absorption by the embryos from the substrate and surrounding air leading to their heavier fresh weight at hatching (Chauvin et al. 1991).

For our spore exposure experiments, we collected the spores from mold growing on the food and frass of the earwigs in our laboratory population. Morphological and DNA barcoding identification confirmed that this mold was part of the genus *Mucor* (see Supplementary Figure 1). *Mucor* is an ubiquitous microbial genus occurring broadly also in the soil (e.g., Mari et al. 2000; Gherbawy et al. 2009; Reber and Chapuisat 2012), has a saprophytic lifestyle, and gains nutrition from the decomposition of organic matter. Furthermore, the *Mucorales* have also been identified in arthropod habitats such as in nests of the Indian and European paper wasp (*Ropalidia maginata*; Jayaprakash and Ebenezer 2010, *Polistes dominulus*; Madden et al. 2012). Given the broad occurrence in the soil (including reports from soil samples in Italy; Mari et al. 2000) and unspecific/opportunistic nature of how this fungus infects or decomposes organic matter, we expect earwigs to be commonly exposed to this widespread mold under natural conditions and hypothesize that the defense shown by earwig females is probably rather unspecific (see below). Further experiments should however test the specificity of maternal egg-grooming behavior and the maternally transferred chemical compounds with regard to different kinds of soil microbes (Reber and Chapuisat 2012).

In terms of underlying mechanisms of maternal defenses, our results suggest roles for the egg grooming behavior and the application of hydrocarbons to the egg surface. As expected, earwig mothers spent significantly more time grooming their eggs when eggs were exposed to mold spores. Thus, females detected the presence of the fungal spores and flexibly responded by a 2-fold increase in their grooming duration. It is noteworthy that after mold application and the observation of egg grooming behavior, eggs were always free of mold (Boos S, personal observations), suggesting that earwig mothers removed the mold spores mechanically by egg grooming. Maternal egg grooming correspondingly has a function that is analogous to allogrooming in ant and termite colonies (Matsuura et al. 2007; Ugelvig and Cremer 2007; Tragust et al. 2013).

The duration of egg grooming in the absence of fungal spores was still quite substantial (18 min out of 75-min observation). It is conceivable that this grooming may at least partly reflect a response to other microbes that were present on the eggs. Alternatively, egg grooming may have additional functions that explain this baseline level of egg grooming such as the application of hydrocarbons for egg water homeostasis (see above).

Whether females transferred chemical compounds during egg attendance was tested by comparing the chemical profiles of heptane extracts between attended and unattended eggs, and between glass beads mingled into attended and unattended clutches. In both cases, all the identified chemical compounds were hydrocarbons. Their total extracted quantity decreased quickly in unattended clutches, whereas it was maintained in attended clutches. This effect lead to a significantly higher quantity of hydrocarbons on attended eggs within 6 days, an effect increasing in magnitude over the course of time. This is evidence that earwig mothers progressively transfer hydrocarbons to their eggs during egg attendance, maintaining a constant amount on the egg surface, whereas unattended eggs lost a substantial fraction over the course of a few days. It seems likely that the chemicals were provided with the saliva via egg grooming, but we cannot exclude other ways of application such as the spraying of eggs with secretions from their dorsal abdominal glands (Eisner 1960; Eisner et al. 2000; Gasch et al. 2013). Irrespective of the transfer mechanism, the hydrocarbons may contribute to egg protection against mold through the indirect mechanism proposed by Strohm and Linsenmair (2001) whereby the application of hydrocarbons prevents water condensation on the surface resulting in a suboptimal microclimate for mold germination and growth.

Based on our extraction and analytical methods, we did not find chemical compounds with known antibiotic/antifungal properties and we did not test for peptides, lysozymes (Matsuura et al. 2007; Rozen et al. 2008; Cotter and Kilner 2010b), or symbiotic bacteria (Kroiss et al. 2010) that may protect eggs against mold infection. A recent study demonstrated experimentally the antimicrobial activity of the general defensive secretions of *F. auricularia*, arguing that the contained benzoquinones play an important role (Gasch et al. 2013). One could also speculate that some bacteria surrounding the

earwig eggs and potentially delivered by the mother may produce antimicrobial peptides to protect eggs from fungus infection (analogous to the processes in the paper wasp *Polistes dominulus*; Madden et al. 2012 or the beewolf digger wasps *P. triangulum*; Strohm and Linsenmair 2001). Clearly, a full understanding of the chemical defense in a maternal care context will require further research on transferred compounds and their antimicrobial properties.

To conclude, we demonstrated that maternal egg attendance includes forms of social defenses against the infection and decomposition of eggs by mold in the European earwig *F. auricularia*. Our results overall suggest that egg grooming is a maternal behavior that may generally serve both a mechanical removal of egg spores by the females' mouthparts and the application of chemicals through transferred saliva (Costa 2006). The former may be a mechanism to quickly defend against an immediate threat (i.e., spores already present on the eggs), and the latter possibly a longer term protection to prevent or reduce scope for spores to adhere to or germinate on the eggs. Further research is needed to disentangle the role of mechanical and chemical maternal defenses as well as their specificity. It is expected that maternal (social) defenses as described here in *F. auricularia* should be widespread in arthropods that breed in the soil or on organic material because of the high expected prevalence of egg exposure to soil microbes that decompose organic material (such as eggs) (Costa 2006; Rozen et al. 2008; Arce et al. 2012; Reber and Chapuisat 2012; Trumbo 2012). Thus, the antifungal/antimicrobial function of egg attendance may be key to explain why egg attendance is such a widespread form of parental care in insects/arthropods.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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