Friend or foe? The apparent benefits of gregarine (Apicomplexa: Sporozoa) infection in the European earwig

Francisco Arcila, Joël Meunier

Friend or foe? The apparent benefits of gregarine (Apicomplexa: Sporozoa) infection in the European earwig

Francisco Arcila[a], Joël Meunier[b,*]

[a] *Institute of Organismic and Molecular Evolutionary Biology, Johannes-Gutenberg University of Mainz, Mainz, Germany*

[b] *Institut de Recherche sur la Biologie de l'Insecte, UMR 7261, CNRS, Université de Tours, Tours, France*

*Corresponding author. Joël Meunier. *E-mail address*: joel.meunier@univ-tours.fr

ABSTRACT

Studying the costs and benefits of host-parasite interactions is of central importance to shed light on the evolutionary drivers of host life history traits. Although gregarines (Apicomplexa: Sporozoa) are one of the most frequent parasites in the gut of invertebrates, the diversity of its potential impacts on a host remains poorly explored. In this study, we addressed this gap in knowledge by investigating the prevalence of natural infections by the gregarine *Gregarina ovata* and testing how these infections shape a large set of morphological, behavioural and physiological traits in the European earwig *Forficula auricularia*. Our results first show that *G. ovata* was present in 76.8% of 573 field-sampled earwigs, and that its prevalence was both higher in males compared with females and increased between July and September. The load of *G. ovata* in the infected individuals was higher in males than females, but this sex difference vanished during the season. Our experiments then surprisingly revealed apparent benefits of *G. ovata* infections. Food-deprived hosts survived longer when they exhibited high compared with low gregarine loads. Moreover, the presence of gregarines was associated with a reduced phenoloxidase activity, indicating a lower immune resistance or a higher immune tolerance of the infected hosts. By contrast, we found no effect of *G. ovata* presence and number on earwigs' development (eye distance, forceps length), activity, food consumption or resistance against a fungal pathogen. Overall, our findings suggest that *G. ovata* could be involved in a mutualistic relationship with the European earwig. Given the ubiquitous presence of gregarines among invertebrates, our data also suggest that this common member of insect gut flora could have a broad and positive role in the life history of many host species.

Keywords: Dermaptera, Symbiosis, Parasitism, Mutualism, Insect

1. Introduction

Invertebrates typically host a great number of organisms in their gut, among which gregarines (Apicomplexa: Sporozoa) are probably the most frequently reported across terrestrial, marine and freshwater species (Bollatti and Ceballos, 2014; Criado-Fornelio et al., 2017). Gregarines are unicellular organisms that may reach a length of 10 mm (Manwell, 1961). They typically live freely (as trophozoites) in the epithelial cells of the midgut, attached to the intestinal wall cells or in the gaeca's lumen of the intestines of their host (Valigurová et al., 2007), where their density can be extremely high and completely fill a host gut (Klingenberg et al., 1997). Gregarines reproduce within their host gut either asexually through fissions (subclass: Eugregarinida) or sexually by schizogony (subclass: Schizogregarinida) and typically reach new hosts after those ingest the infective oocysts released in the environment with the feces of the current hosts (Clopton, 2002).

Over the last decades, a large number of studies showed that the presence of gregarines typically comes with a broad range of costs for the hosts. For instance, gregarine infection reduces body weight and food consumption in the grasshopper *Atractomorpha crenula* (Johny et al., 2000), increases susceptibility to a fungal pathogen in the cockroach *Blattella germanica* (Lopes and Alves, 2005) and hampers larval and pupal development in the mosquito *Aedes triseriatus* (Beier, 1983) and the red flour beetle *Tribolium castaneum* (Gigliolli et al., 2016). In several damselfly species, the presence of gregarines also alters survival, egg production, mating decisions, oviposition strategies and wing ornaments (Córdoba-Aguilar et al., 2003; Córdoba-Aguilar and Munguía-Steyer, 2013; Suhonen et al., 2017), while it reduces the capability of male odonates to defend their territory (Siva-Jothy and Plaistow, 1999). Finally, the presence of

gregarines can also have broad impacts on social organisation, as it diminishes the foraging activity of infected workers and reduces colony productivity in the wasp *Polybia occidentalis* (Bouwma et al., 2005).

However, a few other works provide results that are at odds with those findings and show that the presence of gregarines can come with no costs or even provide apparent benefits for the host. For instance, a series of works demonstrate that gregarines do not shape growth, development time, adult size, resistance to starvation or adult survival in the water strider *Gerris buenoi* and the pseudoscorpion *Victorwithius similis* (Klingenberg et al., 1997; Bollatti and Ceballos, 2014). Conversely, the level of gregarine infection was positively associated with adult survival rates under food stress in the odonate *Enallagma boreale* (Hecker et al., 2002), with the speed of host development in the cat flea *Ctenocephalides felis* (Alarcón et al., 2017) and with the successful development of larvae under food stress in the mealworm beetle *Tenebrio molitor* (Harry, 1967; Rodriguez et al., 2007). Gregarine infection during the larval stage was also found to reduce parasite load in adults of the flour beetle *Tribolium confusum* (Thomas and Rudolf, 2010).

Whereas these works overall suggest that gregarines can have contrasting and host-specific effects, most of these studies only focus on a limited number of host traits, possibly hampering our understanding of the diversity of impacts gregarine infections can have in a single host species. In this study, we addressed this gap in knowledge by testing and discussing the impacts of gregarine presence and number on a large set of morphological, behavioural and physiological traits in the European earwig *Forficula auricularia* (Dermaptera: Forficulidae). In this insect species, adults live in large mixed-sex groups from early summer to early/late autumn

(Weiß et al., 2014; Ratz et al., 2016), during which they encounter multiple mating partners (Sandrin et al., 2015). Females isolate in early autumn/winter to produce a clutch of eggs. During the subsequent weeks (i.e. all over winter), these mothers typically stop their foraging activities (Berleur et al., 2001; Kölliker, 2007; Tourneur and Meunier, 2020) to provide extensive forms of care to their eggs and then to their resulting juveniles (Gingras and Tourneur, 2001; Kölliker, 2007; Meunier et al., 2012; Boos et al., 2014; Thesing et al., 2015; Diehl and Meunier, 2018). Although the presence of gregarines – and particularly *Gregarina ovata* – is known in the European earwig since Dufour's first taxonomical description of the gregarines (Fig. 1; Dufour, 1828), the outcomes of this infection remain surprisingly poorly studied in this species. To date, the few available works only report contrasting and debated effects showing that gregarines come with reductions in adults size and male forceps length (Brindley and Potts, 1910; Wheeler, 1910; Ollason, 1970) or that it has no effect on the development of male testes and forceps length (Diakonov, 1925).

We conducted a series of field measurements and laboratory experiments to obtain an overview of *G. ovata* prevalence in the studied population, and to study its effects on earwigs' body size, length of sexual ornaments, general activity, energy intake and protection against a fungal pathogen. In particular, we (i) measured the prevalence and load of *G. ovata* trophozoites (its free-living stage) in the guts of males and females sampled in a natural population. We then tested (ii) whether this infection came with reduced investments during development in adults' body size and forceps length, two traits associated with reproductive success in this species (Walker and Fell, 2001; Körner et al., 2017). Using two laboratory experiments, we also investigated the impact of gregarine infection on hosts' activity and energy intake by testing (iii)

whether infected hosts exhibit reduced general activity and (iv) a weak level of food consumption, respectively. Note that we could have also expected that gregarine infection leads to an increase in the level of feces production by the host to favour its own transmission to new hosts. We then conducted two additional laboratory experiments to test (v) whether gregarine infection increases host investment into phenoloxidase activity, a classical component of the immune defence in insects and earwigs (Cerenius and Söderhäll, 2004; Vogelweith et al., 2017a, 2017b), and to test (vi) whether gregarine infection improves resistance against exposure to the common entomopathogenic fungus *Metarhizium brunneum*. To overall address whether the potential impacts of gregarine infection on their hosts are sex-specific (Ilvonen et al., 2016), we conducted all these experiments using both male and female earwigs.

2. Materials and methods

2.1. Earwig field sampling, gregarine counting and identification

The 573 *F. auricularia* adults (288 males and 285 females) used in the following experiments were field sampled either in July 2015 (192 males and 188 females) or in September 2015 (96 males and 97 females) in a natural population located in Finthen, Germany (49º 59' 48''N, 8º 10' 03''W). Except when it is mentioned otherwise, we conducted each experiment on the day of earwig field sampling. We measured the number of gregarines present in their gut within the next 2 days following field sampling (i.e. immediately at the end of each experiment) by first anaesthetizing each individual with CO_2, and then extracting and dissecting its gut to count the number of gregarine trophozoites – its free-living stage. This counting was done using

a binocular scope (Leica S8APO, Germany). The counting of gregarine trophozoites was always conducted without the observer knowing the experimental treatments.

2.2. Identification of gregarine species

The gregarine species found in *F. auricularia* has been identified as *G. ovata* (Dufour, 1828) according to the extended gregarine morphometric sets described by Clopton (2002) involving the length of deutomerite, length of protomerite, maximum width of deutomerite, width of deutomerite at equatorial axis and the standardized indices between these measurements for associated primite and satellite trophozoites (for complete terminology and measurement descriptions, see Clopton (2002) and (Clopton et al. (2008)). Identification was carried out using a total of 24 random mature gametocysts extracted from 24 random earwigs sampled in July and September 2015. The morphometric measurements were done to the nearest 0.001 cm using a camera coupled to a binocular (Leica DFC425, Leica Microsystems Ltd., Heerbrugg, Switzerland) and the digital measurement software Leica Application Suite 4.5.0.

2.3. Earwig morphology

To investigate the link between gregarine infection and earwig morphology, we measured the minimum eye distance (a proxy of body size; Kramer et al., 2017) and the mean forceps length of the 573 field-sampled *F. auricularia* adults. These measurements were done to the nearest 0.001 mm using a camera coupled to a binocular (Leica DFC425, Leica Microsystems Ltd., Heerbrugg, Switzerland) and the software Leica Application Suite 4.5.0. We obtained the mean forceps length by measuring the distance between the distal tip and the basal outer edges of the right and left forceps of each individual, and then by averaging these two values (Thesing et al.,

2015). Earwig males and females can be easily discriminated using the shape of their forceps, which is curved and straight, respectively (Albouy and Caussanel, 1990). All the 573 field-sampled individuals were used for these morphometric measurements and each individual was then randomly distributed in one of the four following experiments.

2.4. Experiment 1: General activity

To test whether gregarine infection leads to reduced host activity, we measured the general activity of 50 males and 50 females field-sampled in July 2015 using a multi-chamber setup (Modlmeier and Foitzik, 2011). In this setup, each individual was placed in the centre of a cleaned plastic arena consisting of a central chamber connected to eight additional chambers through individual corridors (length 32 mm) regularly disposed around the central chamber. Every chamber had a 29 mm diameter and was empty. The number of cells visited by each individual was counted during the 10 min following their introduction into the setup and the resulting value was used as a proxy to determine an individual's activity (Modlmeier and Foitzik, 2011). All these measurements were done under a red light, as earwigs are nocturnal.

2.5. Experiment 2: Food consumption

To test whether gregarine infection affects food consumption, we conducted a 2x2 full-factorial experiment using another set of adults field-sampled in July 2015. In this experiment, 99 field-sampled individuals (49 males and 50 females) were immediately isolated in Petri dishes (10 cm diameter) and then provided either with an ad libitum amount of artificial diet (24 males and 25 females; details of food composition in Kramer et al., 2015) or without any food source (25 males and 25 females). One hour later, each individual was transferred to a new Petri dish

grounded with a humid filter paper (Macherey-Nagel NN 616, diameter 90 mm), in which individuals were allowed to produce faecal pellets (Falk et al., 2014). Twenty-four hours later, we counted the number of faecal pellets present on the filter paper, which is a known proxy for the amount of food previously consumed by an earwig (Körner et al., 2016). During the 24 h of isolation, the individuals were kept under 60% humidity, 12:12 h light:dark cycle at 20:18°C, respectively.

2.6. Experiment 3: Immune activity

To investigate whether a host's immune activity reflects its gregarine infection, we measured the level of phenol- and pro-phenoloxidase enzyme activity in 181 individuals (93 males and 88 females) field-sampled in July 2015. Phenoloxidase activity is a key component of insect immunity and typically mediates the melanisation of foreign objects and the release of cytotoxic agents through the activation of its inactive precursor, pro-phenoloxidase (Cerenius and Söderhäll, 2004). Our measurement of total-PO (i.e. the sum of prophenoloxidase and phenoloxidase) activity followed a standard protocol (Vogelweith et al., 2017a; Körner et al., 2018), in which each field-sampled individual was first CO_2-anaesthetized, then pricked with a clean needle between the seventh and eighth dorsal tergite and 1-2 µl of their hemolymph were finally extracted through the wound using a glass capillary. The extracted hemolymph was immediately mixed with a Cacodylate buffer solution (0.01 M sodium cacodylate, 0.005 M CaCl2; pH 6.5) in an Eppendorf tube placed on ice and then maintained at -20°C. Several days later, the frozen hemolymph solutions were thawed on ice and 15 µl were subsequently added to a well of a 96-well plate chilled on ice, together with 96 µl of distilled water, 10 µl of cacodylate buffer and 12 µl of α-Chymotrypsin (Sigma C-7762). The plate was then maintained for 10 min at room

temperature, after which we added 42 µl of L-Dopa (Sigma D-9628) to transform the inactivated pro-phenoloxidase enzyme into the activated phenoloxidase. The plate was finally placed into a spectrophotometer (Multiscan FC, Thermofisher Scientific) maintained at 30°C, in which a total of 800 measurements (one per 10 s) were done at 492 nm. The total-PO activity was defined as the slope of the enzymatic reaction curve during the linear phase of the reaction (Vmax value: change in absorbance units/min) and measured using the R-based program PO-CALC (Kohlmeier et al., 2015).

2.7. Experiment 4: Resistance against pathogens

We tested whether gregarine infection enhances a host's resistance against an experimental exposure to a fungal pathogen using the 194 (96 males and 98 females) individuals field-sampled in September 2015. We manipulated pathogen exposure using a method previously developed by Kohlmeier et al. (2016), in which each individual is first isolated for 10 min for acclimatization and then dipped into a 2 ml Eppendorf tube previously filled with 200 µl of either a conidiospores solution of *Metarhizium brunneum* diluted in 0.05% Tween 20 (10^7 spores/ml, *n* = 47 males and 49 females) or a control spore-free suspension of 0.05% Tween 20 (*n* = 49 males and 49 females; Sigma P-1379). We subsequently transferred pathogen-exposed and control-exposed individuals into separate Petri dishes, in which we checked their survival on a daily basis for 30 days. All these individuals had no access to a food source during those 30 days. We counted the number of gregarines present in each individual either a few hours (maximum 24 h) after their death or at day 30 if they survived until then. Petri dishes were of 5 cm diameter, were grounded with humid sand and maintained at 25°C. *Metarhizium brunneum* is known to infect and reduce the survival of a wide range of insects (including earwigs) in nature, and was

obtained from a strain isolated from soil samples in Switzerland and previously genotyped for identification (Reber and Chapuisat, 2011). The survival rate was recorded without the observer knowing the type of exposure (i.e. spores or control suspension).

2.8. Statistical analyses

We first tested the link between gregarine infection and earwigs' eye distance, sex and mean forceps length using a Generalized Linear Model (GLM) fitted with a binomial error distribution and a General Linear Model (LM). In these models, we entered the presence/absence of *G. ovata* trophozoites (1 or 0) or the log-transformed number of *G. ovata* trophozoites present in the infected individuals (continuous) as a response variable, respectively. Because the mean forceps length of adults was positively correlated with their minimum eye distance and the strength of this correlation was sex-specific (Fig. 2), we used a 'corrected forceps length' in the statistical models. To obtain this corrected value, we used the residuals of two polynomial LMs (one per sex, see Fig. 2), in which the mean forceps length was the response variable, and body size was the explanatory variable. The resulting 'corrected forceps length' therefore shows whether individuals had longer (or shorter) forceps than the value predicted by both their sex and eye distance.

The data from experiments 1, 2 and 3 were tested using a series of six LMs, in which either the number of faecal pellets (Experiment 1), the number of visited cells (Experiment 2) or the phenoloxidase activity (log(+0.001)-transformed, Experiment 3) was used as a response variable. In these models, the sex of the individual and either the presence/absence of *G. ovata* trophozoites or the log-transformed number of *G. ovata* trophozoites in the infected individuals were entered as explanatory variables. The presence/absence of an additional food source and

the interaction between this factor and the other ones were also used as explanatory factors in the two models on feces production.

The survival rate of individuals involved in Experiment 4 was analysed using Cox proportional hazard regression model allowing for censored data (adults still alive 30 days after exposure). In this model, the sex, exposure (spore or control) and log-transformed number of *G. ovata* trophozoites were entered as explanatory variables. Note that in the control-exposed treatment, only three individuals (two females and one male) turned out to be without any *G. ovata* trophozoites (compared with 16 in the pathogen-exposed treatments). To limit the critical statistical bias generated by this unbalanced data set, we excluded from the analyses all 19 individuals that had no *G. ovata* trophozoites across the different treatments. The statistical analyses were thus overall based on 174 individuals that all contained at least one *G. ovata* trophozoite in the gut, i.e. 90.16% of the original data set.

We conducted all statistical analyses using the software R v3.6.0 (https://www.r-project.org) loaded with the packages *car, polynom, survival* and *emmeans*. Every model was first computed with interactions among all explanatory variables and then simplified stepwise based on the Akaike Information Critera (AIC) (all the removed interactions had *P* values >0.09). When necessary, we conducted pairwise comparisons between interacting factors using model contrasts corrected for multiple testing following the Tukey Honestly Significance Difference (HSD) method (package *emmeans*). Note that some non-significant interactions are still present in Table 1 to facilitate models comparisons and to provide support for the discussion, but that their removal from the models does not lead to qualitative changes in the results.

2.9. Data availability

All data are available at the public repository Zenodo (DOI 10.5281/zenodo.3701364).

3. Results

Gregarina ovata trophozoites were present in 440 (76.7%) of the 573 field-sampled individuals. The infected individuals contained 52.8 ± 4.5 (mean ± SE) trophozoites per gut (median = 23), with a number ranging from 1 to 950 (Fig. 3A). The likelihood of finding at least one *G. ovata* trophozoite in an earwig gut was overall higher in adults that were field-sampled in September compared with July (Fig. 3B, Table 1), and overall higher in males compared with females (Fig. 3C, Table 1). Interestingly, the infected males had more *G. ovata* trophozoites than the infected females when sampled in July (Table 1, Model contrast, $P = 0.0006$), whereas this effect disappeared in individuals sampled in September (Fig. 3D, Model contrast, $P = 0.1467$).

3.1. No link between gregarine infection and earwig morphology

The minimum eye distance of the field-sampled earwig ranged from 1.19 to 1.55 in males and 1.24 to 1.55 in females. Similarly, the forceps length ranged from 3.02 to 7.04 mm in males (corrected forceps length from -1.21 to 2.24) and from 2.77 to 3.77 mm in females (corrected forceps length from -0.71 to 0.38). Neither the minimum eye distance nor the corrected forceps length was linked to the presence and number of *G. ovata* trophozoites in the earwig gut (Table 1).

3.2. No link between gregarine infection and earwigs' general activity (Experiment 1)

Males and females visited from two to 45 cells (mean ± SE = 23.08 ± 0.82) over the 10 min of observation. This general activity was independent of both the presence and the number of *G.*

ovata trophozoites in their gut (Fig. 4A and 4B, Table 2A) and independent of the sex of the tested individuals (Table 2A).

3.3. No link between gregarine infection and food consumption (Experiment 2)

Males and females produced up to 51 faecal pellets (mean ± SE = 11.65 ± 0.86). The number of faecal pellets was overall higher when individuals had access to a food source (14.39 ± 1.30) compared with no food source (8.96 ± 1.01; Table 2B). However, this number was independent of both the presence and the number of *G. ovata* trophozoites in their gut (Figs. 4C and 4D, Table 2B), independent of the sex of the tested individuals, as well as independent of interaction between food supplementation and gregarine presence/number (Table 2B).

3.4. Gregarine infection shapes earwigs' immune activity (Experiment 3)

The total-PO activity was higher in the absence compared with the presence of *G. ovata* trophozoites (Fig. 4E and 4F, Table 2C). However, we did not detect an effect of the number of gregarines in the infected individuals, nor an effect of the sex of the tested individuals (Table 2C).

3.5. No link between gregarine infection and earwigs' resistance against a fungal pathogen, but gregarine infection overall delays earwigs' deaths (Experiment 4)

Overall, 47 of the 174 (27.0%) tested individuals died (13 females and 34 males) during this experiment, out of which 36 were exposed to the suspension with *M. brunneum* spores. The speed of death over 30 days was overall faster in the exposed compared with control treatment (Fig. 5A, Table 2D) confirming that the tested pathogen increases host mortality, faster in females compared with males (Fig. 5B, Table 2D), and faster in individuals containing a small compared with a large number of *G. ovata* trophozoites (Fig. 5C, Table 2D). By contrast, the likelihood of

surviving until Day 30 was independent of the interaction between gregarine number and pathogen exposure (Table 2D).

4. Discussion

In this study, we aimed to shed light on the prevalence of *G. ovata* infection in earwig males and females and to investigate the costs and benefits of this infection for these hosts. Our results first reveal that *G. ovata* was present in 76.8% of the earwigs sampled in a natural population. This prevalence was overall higher in males compared with females and increased between July and September. Interestingly, the load of *G. ovata* in the infected individuals was higher in males compared with females, but this sex difference was only present in July. Our series of laboratory experiments then showed a surprising association between the presence of *G. ovata* and earwigs' immune activity, as the hosts' total-PO activity was overall lower in the presence compared with the absence of *G. ovata* (but independent of gregarine loads in the infected individuals). We also found that *G. ovata* provides apparent benefits to its host in terms of survival, as (food-deprived) earwigs survived overall longer when their gut contained large compared with small numbers of *G. ovata.* Our experiments, however, failed to show any association between *G. ovata* infection and earwigs' eye distance or forceps length (revealing that if the infection occurred during development, it did not shape these two morphological traits in the resulting adults), as well as any association between *G. ovata* infection and general activity, food consumption or resistance against a fungal pathogen.

Our data first show that the prevalence of gregarine infection was overall higher in males compared with females. The presence of sex-specific differences in gregarine infection has been

extensively investigated in damselflies and dragonflies, where most studies show no differences between sexes in terms of gregarine prevalence and load (Ilvonen et al., 2016), and only a few of them report sex-specific levels of infection in favour of either males or females (Locklin and Vodopich, 2009; Córdoba-Aguilar and Munguía-Steyer, 2013). There are several explanations as to why males may generally exhibit a higher susceptibility to gregarine infection compared with females. Firstly, males exhibit a low investment in their protection against pathogens because they typically live for a shorter time than females and invest most of their energy into mating effort (Stoehr and Kokko, 2006; Zuk, 2009; Córdoba-Aguilar and Munguía-Steyer, 2013). This explanation is unlikely to apply to earwigs. Even if males exhibit a comparatively shorter lifespan (Tourneur and Meunier, 2020), they are known to exhibit higher levels of immune defence and could thus be more tolerant to infection compared with females (Rantala et al., 2007; Vogelweith et al., 2017a). Another potential explanation is that earwigs express sex-specific behaviours that increase (or decrease) the risk of gregarine infection. For instance, one sex could be more likely to hunt prey already infected by gregarines, as reported in *Calopteryx virgo* (Åbro, 1996), or to reduce contacts with infected conspecifics, as induced by the presence of territories in *Libellula pulchella* (Marden and Cobb, 2004). The European earwig is an omnivorous and highly gregarious species, where individuals live in large mixed-sex groups and show frequent and tight behavioural interactions (Dobler and Kölliker, 2010; Weiß et al., 2014). Whether earwig males are more likely than females to consume prey infected by gregarines (e.g. through cannibalism) and/or are more gregarious than females should be investigated in future research.

Our data then show that the intensity of gregarine infection increased between July and September in females whereas it remained stable and high in males over the same period. Sex

differences in the dynamics of parasite development are common in animals (Zuk and McKean, 1996; vom Steeg and Klein, 2016). This effect may result from sex-specific (direct or indirect) protection against infections in the hosts (Taylor and Kimbrell, 2007; Duneau et al., 2017) and/or specific adaptations of the parasites to the sex of the host (Duneau and Ebert, 2012). Earwig males and females are known to present several differences in terms of morphology and physiology (Rantala et al., 2007; Körner et al., 2017; Vogelweith et al., 2017a), but the impacts of these differences on gregarine development remain to be explored. Notwithstanding the drivers of this sex difference, it is important to note that gregarines eventually managed to reach the same quantity in females and males, which indicates that these drivers only have a seasonal effect on gregarine development in its host's gut.

Independent of host sex, we found that the level of total-PO activity was overall lower in earwigs containing at least one *G. ovata* trophozoite in their gut compared with earwigs without any trophozoites. These findings contrast with results showing that gregarine presence is associated neither with total-PO activity nor encapsulation response in damselflies (Córdoba-Aguilar et al., 2006; Contreras-Garduño et al., 2008; Honkavaara et al., 2009). In earwigs, our findings reflect either that gregarines only manage to establish in guts of the hosts exhibiting low immune activity, or that gregarines inactivate this line of host immune defence. The inactivation of the host immune defence is used by many pathogens to break host resistance against their development (Akira et al., 2006), but its use by gregarines remains unknown. Note that if this second hypothesis holds true, our results suggest that gregarines do not inactivate other lines of the host's immune defences, as we found that gregarines do not alter an earwig's resistance against a fungal pathogen. An alternative hypothesis to explain our results is that earwigs actively

reduce their immune activity to accept gregarines. This could be because gregarines provide essential benefits to the hosts (e.g. extended survival, see below) or because they offer additional immune protection to the hosts (which can consequently reduce their investment into total-PO). Disentangling these hypotheses will require us to manipulate the presence or absence of gregarines in earwigs and test the resulting effects on their total PO-activity.

Interestingly, the earwigs involved in the experiment testing their resistance against a fungal pathogen survived longer when they had a large compared with a weak load of gregarines and this pattern was independent of fungal exposure. Because these earwigs had no access to a food source during this experiment, this apparent benefit of gregarine infection suggests either that starved earwigs exhibiting the highest longevity also happen to be the most tolerant to gregarines, or that gregarines actively extend the survival of their starved host. The first possibility is unlikely to explain our results, as our results report no association between the level of gregarine infection and several morphological, behavioural and immunological proxies of earwig quality (see tests of these proxies in Kramer and Meunier, 2016; Ratz et al., 2016). On the other hand, the fact that gregarines would actively extend the survival of their starved host is somewhat surprising, as gregarines typically derive the nutrients ingested by their hosts to feed themselves and pursue their own metabolic process (Schawang and Janovy, 2001; Schreurs and Janovy, 2008; Randall et al., 2013). These apparent benefits nevertheless open a possibility for the occurrence of a mutualistic relationship between *G. ovata* and *F. auricularia* shaping earwig survival, and call for future experiments exploring the nutritional benefits of gregarine infection. These nutritional benefits could be particularly important in the European earwig, as females experience long periods of food deprivation over winter (Gingras and Tourneur, 2001; Kölliker,

2007; Tourneur and Meunier, 2020) during which they also provide extensive and energetically costly forms of care to their eggs (Koch and Meunier, 2014; Diehl and Meunier, 2018; Van Meyel et al., 2019). Assuming that gregarines help to resist against starvation, having a large stock of gregarines in the gut before egg production (as we observed in September) might, therefore, ensure female survival over the coming periods of starvation and high energy demand. Testing this hypothesis will require the manipulation of both the starvation level of the host and its *G. ovata* load, identifying the nutrients possibly exchanged between both organisms and finally investigating the importance of these exchanges in the host's reproductive success.

Our findings overall question whether the observed (absence of) effects are due to the ability of earwigs to limit gregarine burden (resistance) and/or to limit the harm caused by this burden (tolerance). Disentangling these two processes can have a profound impact on our understanding of this host-parasite system (Råberg et al., 2007), since the evolution of resistance is expected to reduce parasite prevalence in the host population, whereas tolerance should have a neutral or positive effect on the prevalence of the parasite (Boots, 2008; Råberg et al., 2009). Resistance should thus ultimately lead to antagonistic coevolution between host and parasite (Woolhouse et al., 2002), whereas tolerance opens the possibility of the emergence of mutualistic relationships between the two organisms (Boots, 2008; Råberg et al., 2009). Although no gregarine species is currently known to be involved in this last type of relationship, the apparent benefits of gregarines on the survival of (starved) earwigs call for further experimental exploration of this hypothesis. Given the ubiquitous presence of gregarines among invertebrates (Bollatti and Ceballos, 2014; Criado-Fornelio et al., 2017), our data more generally suggest that

this common member of the insect gut flora could have a positive yet currently neglected role in the life history of many host species (Harry, 1967; Hecker et al., 2002; Rodriguez et al., 2007).

To conclude, our study reveals that *G. ovata* is a frequent member of the earwig gut microbiota, that its prevalence is sex-specific and changes over time, and that its presence is associated with several advantages and no apparent costs for adult earwigs. In particular, the presence and/or the number of *G. ovata* trophozoites was associated with longer survival (in starved individuals) and a reduced investment into phenoloxidase activity, whereas it was independent of earwigs' eye distance, forceps length, general activity, food consumption and resistance against a fungal pathogen. These findings overall call for further experimental studies exploring the underlying mechanisms driving this sex-specific pattern of infection, and investigating the possibility of a (currently neglected) mutualistic relationship between *G. ovata* and *F. auricularia*.

Acknowledgements

We thank Franck Dedeine, David Duneau, Jean-Claude Tourneur and Sophie Van Meyel for their comments on this manuscript. We also thank all members of "Team Earwig" at the University of Mainz, Germany, for their help with the different measurements conducted in this study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. FA dedicates this work to the memory of Wilson Arcila, beloved Father.

References

Åbro, A., 1996. Gregarine infection of adult *Calopteryx virgo* L. (Odonata: Zygoptera). J. Nat. Hist. 30, 855–859. https://doi.org/10.1080/00222939600770481

Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen Recognition and Innate Immunity. Cell 124, 783–801. https://doi.org/10.1016/j.cell.2006.02.015

Alarcón, M.E., Jara-F, A., Briones, R.C., Dubey, A.K., Slamovits, C.H., 2017. Gregarine infection accelerates larval development of the cat flea *Ctenocephalides felis* (Bouché). Parasitology 144, 419–425. https://doi.org/10.1017/S0031182016002122

Albouy, V., Caussanel, C., 1990. Dermaptères ou perce-oreilles. Fédération Francaise des Sociétés de Sciences Naturelles, Paris.

Beier, J.C., 1983. Effects of gregarine parasites on the development of *Dirofilaria immitis* in *Aedes triseriatus* (Diptera: Culicidae). J. Med. Entomol. 20, 70–75. https://doi.org/10.1093/jmedent/20.1.70

Berleur, G., Gingras, J., Tourneur, J.-C., 2001. Influence of diet on development and oviposition of *Forficula auricularia* (Dermaptera: Forficulidae). Can. Entomol. 133, 705–708.

Bollatti, F., Ceballos, A., 2014. Effect of gregarines (Apicomplexa: Sporozoa) on survival and weight loss of *Victorwithius similis* Beier, 1959 (Arachnida: Pseudoscorpiones). J. Invertebr. Pathol. 117, 13–18. https://doi.org/10.1016/j.jip.2014.01.002

Boos, S., Meunier, J., Pichon, S., Kölliker, M., 2014. Maternal care provides antifungal protection to eggs in the European earwig. Behav. Ecol. 25, 754–761. https://doi.org/10.1093/beheco/aru046

Boots, M., 2008. Fight or learn to live with the consequences? Trends Ecol. Evol. 23, 248–250. https://doi.org/10.1016/j.tree.2008.01.006

Bouwma, A.M., Howard, K.J., Jeanne, R.L., 2005. Parasitism in a social wasp: Effect of gregarines on foraging behavior, colony productivity, and adult mortality. Behav. Ecol. Sociobiol. 59, 222–233. https://doi.org/10.1007/s00265-005-0028-5

Brindley, H.H., Potts, F.A., 1910. The effect of parasitic castration in insects. Am. Assoc. Adv. Sci. 32, 836–837.

Cerenius, L., Söderhäll, K., 2004. The prophenoloxidase-activating system in invertebrates. Immunol. Rev. 198, 116–26.

Clopton, R.E., 2002. The Gregarines: A generic level review, in: Lee, J.J., Leedale, G., Patterson, D., Bradbury, P.C. (Eds.), Illustrated Guide to the Protozoa. Society of Protozoologists,

Lawrence, Kansas, pp. 205–288.

Clopton, R.E., Cook, T.J., Cook, J.L., 2008. *Gregarina tropica* n. sp.(Apicomplexa: Eugregarinorida: Gregarinicae: Gregarinidae) parasitizing the brown-winged earwig, *Vostox brunneipennis* (Dermaptera: Comp. Parasitol. 75, 215–227. https://doi.org/10.1654/4359.1

Contreras-Garduño, J., Córdoba-Aguilar, A., Peralta-Vázquez, H., Jiménez-Cortés, J.G., Luna-González, A., Campa-Córdova, A.I., 2008. Differences in immune ability do not correlate with parasitic burden in two Zygoptera species (Calopterygidae, Coenagrionidae). Odonatologica 37, 111–118.

Córdoba-Aguilar, A., Contreras-Garduño, J., Peralta-Vázquez, H., Luna-González, A., Campa-Córdova, A.I., Ascencio, F., 2006. Sexual comparisons in immune ability, survival and parasite intensity in two damselfly species. J. Insect Physiol. 52, 861–869. https://doi.org/10.1016/j.jinsphys.2006.05.008

Córdoba-Aguilar, A., Munguía-Steyer, R., 2013. The sicker sex: Understanding male biases in parasitic infection, resource allocation and fitness. PLoS One 8. https://doi.org/10.1371/journal.pone.0076246

Córdoba-Aguilar, A., Salamanca-Ocaña, J.C., Lopezaraiza, M., 2003. Female reproductive decisions and parasite burden in a calopterygid damselfly (Insecta: Odonata). Anim. Behav. 66, 81–87. https://doi.org/10.1006/anbe.2003.2198

Criado-Fornelio, A., Verdú-Expósito, C., Martin-Pérez, T., Heredero-Bermejo, I., Pérez-Serrano, J., Guàrdia-Valle, L., Panisello-Panisello, M., 2017. A survey for gregarines (Protozoa: Apicomplexa) in arthropods in Spain. Parasitol. Res. 116, 99–110. https://doi.org/10.1007/s00436-016-5266-0

Diakonov, D.M., 1925. Experimental and biometrical investigations on dimorphic variability in Forficula. J. Genet. 15, 201–232.

Diehl, J.M., Meunier, J., 2018. Surrounding pathogens shape maternal egg care but not egg production in the European earwig. Behav. Ecol. 29, 128–136. https://doi.org/10.1093/beheco/arx140

Dobler, R., Kölliker, M., 2010. Kin-selected siblicide and cannibalism in the European earwig. Behav. Ecol. 21, 257–263. https://doi.org/10.1093/beheco/arp184

Dufour, L.M., 1828. Note sur la gregarine, nouveau genre de ver qui vit en troupeau dans les intestins des divers insectes. Ann Sci Nat Série I, 366–369.

Duneau, D., Ebert, D., 2012. Host Sexual Dimorphism and Parasite Adaptation. PLoS Biol. 10, e1001271. https://doi.org/10.1371/journal.pbio.1001271

Duneau, D.F., Kondolf, H.C., Im, J.H., Ortiz, G.A., Chow, C., Fox, M.A., Eugénio, A.T., Revah, J.,

Buchon, N., Lazzaro, B.P., 2017. The Toll pathway underlies host sexual dimorphism in resistance to both Gram-negative and Gram-positive bacteria in mated *Drosophila*. BMC Biol. 15, 1–17. https://doi.org/10.1186/s12915-017-0466-3

Falk, J., Wong, J.W.Y., Kölliker, M., Meunier, J., 2014. Sibling cooperation in earwig families provides insights into the early evolution of social life. Am. Nat. 183, 547–557. https://doi.org/10.1086/675364

Gigliolli, A.A.S., Julio, A.H.F., Conte, H., 2016. The life cycle of *Gregarina cuneata* in the midgut of *Tribolium castaneum* and the effects of parasitism on the development of insects. Bull. Entomol. Res. 106, 258–267. https://doi.org/10.1017/S0007485315001121

Gingras, J., Tourneur, J.-C., 2001. Timing of adult mortality, oviposition, and hatching during the underground phase of *Forficula auricularia* (Dermaptera: Forficulidae). Can. Entomol. 133, 269–278.

Harry, O.G., 1967. The effect of a eugregarine *Gregarina polymorpha* (Hammerschmidt) on the mealworm larva of *Tenebrio molitor* (L.). J. Protozool. 14, 539–548.

Hecker, K.R., Forbes, M.R., Léonard, N.J., 2002. Parasitism of damselflies (*Enallagma boreale*) by gregarines: sex biases and relations to adult survivorship. Can. J. Zool. 80, 162–168. https://doi.org/10.1139/z01-213

Honkavaara, J., Rantala, M.J., Suhonen, J., 2009. Mating status, immune defence, and multi-parasite burden in the damselfly *Coenagrion armatum*. Entomol. Exp. Appl. 132, 165–171. https://doi.org/10.1111/j.1570-7458.2009.00877.x

Ilvonen, J.J., Kaunisto, K.M., Suhonen, J., 2016. Are sexes equally parasitized in damselflies and dragonflies? Oikos 125, 315–325. https://doi.org/10.1111/oik.02437

Johny, S., Muralirangan, M.C., Sanjayan, K.P., 2000. Parasitization potential of two cephaline gregarines, *Leidyana subramanii* Pushkala and Muralirangan and *Retractocephalus dhawanii* sp. n. on the tobacco grasshopper, *Atractomorpha crenulata*. J. Orthoptera Res. 9, 67–70. https://doi.org/10.2307/3503635

Klingenberg, C.P., Barrington Leigh, R.H., Keddie, B.A., Spence, J.R., 1997. Influence of gut parasites on growth performance in the water strider *Gerris buenoi* (Hemiptera: Gerridae). Ecography (Cop.). 20, 29–36. https://doi.org/10.1111/j.1600-0587.1997.tb00344.x

Koch, L.K., Meunier, J., 2014. Mother and offspring fitness in an insect with maternal care: phenotypic trade-offs between egg number, egg mass and egg care. BMC Evol. Biol. 14, 125. https://doi.org/10.1186/1471-2148-14-125

Kohlmeier, P., Dreyer, H., Meunier, J., 2015. PO-CALC: A novel tool to correct common inconsistencies in the measurement of phenoloxidase activity. J. Insect Physiol. 75, 80–84. https://doi.org/10.1016/j.jinsphys.2015.02.015

Kohlmeier, P., Holländer, K., Meunier, J., 2016. Survival after pathogen exposure in group-living insects: don't forget the stress of social isolation! J. Evol. Biol. 29, 1867–1872. https://doi.org/10.1111/jeb.12916

Kölliker, M., 2007. Benefits and costs of earwig (*Forficula auricularia*) family life. Behav. Ecol. Sociobiol. 61, 1489–1497. https://doi.org/10.1007/s00265-007-0381-7

Körner, M., Diehl, J.M., Meunier, J., 2016. Growing up with feces: benefits of allo-coprophagy in families of the European earwig. Behav. Ecol. 27, 1775–1781. https://doi.org/10.1093/beheco/arw113

Körner, M., Foitzik, S., Meunier, J., 2018. Extended winters entail long-term costs for insect offspring reared in an overwinter burrow. J. Therm. Biol. 74, 116–122. https://doi.org/10.1016/j.jtherbio.2018.03.021

Körner, M., Vogelweith, F., Foitzik, S., Meunier, J., 2017. Condition-dependent trade-off between weapon size and immunity in males of the European earwig. Sci. Rep. 7, 7988. https://doi.org/10.1038/s41598-017-08339-6

Kramer, J., Körner, M., Diehl, J.M., Scheiner, C., Yüksel-Dadak, A., Christl, T., Kohlmeier, P., Meunier, J., 2017. When earwig mothers do not care to share: Parent-offspring competition and the evolution of family life. Funct. Ecol. 31, 2098–2107. https://doi.org/10.1111/1365-2435.12915

Kramer, J., Meunier, J., 2016. Maternal condition determines offspring behavior toward family members in the European earwig. Behav. Ecol. 27, 494–500. https://doi.org/10.1093/beheco/arv181

Kramer, J., Thesing, J., Meunier, J., 2015. Negative association between parental care and sibling cooperation in earwigs: a new perspective on the early evolution of family life? J. Evol. Biol. 28, 1299–1308. https://doi.org/10.1111/jeb.12655

Locklin, J.L., Vodopich, D.S., 2009. Bidirectional gender biases of gregarine parasitism in two coexisting dragonflies (Anisoptera: Libellulidae). Odonatologica 38, 133–140.

Lopes, R.B., Alves, S.B., 2005. Effect of *Gregarina* sp. parasitism on the susceptibility of *Blattella germanica* to some control agents. J. Invertebr. Pathol. 88, 261–264. https://doi.org/10.1016/j.jip.2005.01.010

Manwell, R.D., 1961. Introduction to Protozoology. St. Martin's Press, New York.

Marden, J.H., Cobb, J.R., 2004. Territorial and mating success of dragonflies that vary in muscle power output and presence of gregarine gut parasites. Anim. Behav. 68, 857–865. https://doi.org/10.1016/j.anbehav.2003.09.019

Meunier, J., Wong, J.W.Y., Gómez, Y., Kuttler, S., Röllin, L., Stucki, D., Kölliker, M., 2012. One

clutch or two clutches? Fitness correlates of coexisting alternative female life-histories in the European earwig. Evol. Ecol. 26, 669–682. https://doi.org/10.1007/s10682-011-9510-x

Modlmeier, A.P., Foitzik, S., 2011. Productivity increases with variation in aggression among group members in Temnothorax ants. Behav. Ecol. 22, 1026–1032. https://doi.org/10.1093/beheco/arr086

Ollason, J.G., 1970. Studies on structural variation in the common earwig *Forficula auricularia* L. PhD thesis. University of Durham, United Kingdom.

Råberg, L., Graham, A.L., Read, A.F., 2009. Decomposing health: tolerance and resistance to parasites in animals. Philos. Trans. R. Soc. B Biol. Sci. 364, 37–49. https://doi.org/10.1098/rstb.2008.0184

Råberg, L., Sim, D., Read, A.F., 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science (80-.). 318, 812–814. https://doi.org/10.1126/science.1148526

Randall, J., Cable, J., Guschina, I.A., Harwood, J.L., Lello, J., 2013. Endemic infection reduces transmission potential of an epidemic parasite during co-infection. Proc. R. Soc. B Biol. Sci. 280. https://doi.org/10.1098/rspb.2013.1500

Rantala, M.J., Roff, D.A., Rantala, L.M., 2007. Forceps size and immune function in the earwig *Forficula auricularia* L. Biol. J. Linn. Soc. 90, 509–516. https://doi.org/10.1111/j.1095-8312.2007.00741.x

Ratz, T., Kramer, J., Veuille, M., Meunier, J., 2016. The population determines whether and how life-history traits vary between reproductive events in an insect with maternal care. Oecologia 182, 443–452. https://doi.org/10.1007/s00442-016-3685-3

Reber, A., Chapuisat, M., 2011. Diversity, prevalence and virulence of fungal entomopathogens in colonies of the ant *Formica selysi*. Insectes Soc. 59, 231–239. https://doi.org/10.1007/s00040-011-0209-3

Rodriguez, Y., Omoto, C.K., Gomulkiewicz, R., 2007. Individual and population effects of eugregarine, *Gregarina niphandrodes* (Eugregarinida: Gregarinidae), on *Tenebrio molitor* (Coleoptera: Tenebrionidae). Environ. Entomol. 36, 689–693. https://doi.org/10.1093/ee/36.4.689

Sandrin, L., Meunier, J., Raveh, S., Walser, J.-C., Kölliker, M., 2015. Multiple paternity and mating group size in the European earwig, *Forficula auricularia*. Ecol. Entomol. 40, 159–166. https://doi.org/10.1111/een.12171

Schawang, J.E., Janovy, J., 2001. The response of *Gregarina niphandrodes* (Apicomplexa: Eugregarinida: Septatina) to host starvation in *Tenebrio molitor* (Coleoptera: Tenebrionidae) adults. J. Parasitol. 87, 600. https://doi.org/10.2307/3285099

Schreurs, J., Janovy, J., 2008. Gregarines on a diet: The effects of host starvation on *Gregarina confusa* Janovy et al., 2007 (Apicomplexa: Eugregarinida) in *Tribolium destructor* Uyttenboogaart, 1933 (Coleoptera: Tenebrionidae) larvae. J. Parasitol. 94, 567. https://doi.org/10.1645/ge-1325r.1

Siva-Jothy, M.T., Plaistow, S.J., 1999. A fitness cost of eugregarine parasitism in a damselfly. Ecol. Entomol. 24, 465–470. https://doi.org/10.1046/j.1365-2311.1999.00222.x

Stoehr, A.M., Kokko, H., 2006. Sexual dimorphism in immunocompetence: What does life-history theory predict? Behav. Ecol. 17, 751–756. https://doi.org/10.1093/beheco/ark018

Suhonen, J., Ilvonen, S., Dunn, D.W., Dunn, J., Härmä, O., Ilvonen, J.J., Kaunisto, K.M., Krams, I., 2017. Parasitism affects variation in a male damselfly sexual ornament. Ethol. Ecol. Evol. 30, 1–11. https://doi.org/10.1080/03949370.2017.1354920

Taylor, K., Kimbrell, D.A., 2007. Host immune response and differential survival of the sexes in Drosophila. Fly (Austin). 1, 197–204. https://doi.org/10.4161/fly.5082

Thesing, J., Kramer, J., Koch, L.K., Meunier, J., 2015. Short-term benefits, but transgenerational costs of maternal loss in an insect with facultative maternal care. Proc. R. Soc. B Biol. Sci. 282, 20151617. https://doi.org/10.1098/rspb.2015.1617

Thomas, A.M., Rudolf, V.H.W., 2010. Challenges of metamorphosis in invertebrate hosts: Maintaining parasite resistance across life-history stages. Ecol. Entomol. 35, 200–205. https://doi.org/10.1111/j.1365-2311.2009.01169.x

Tourneur, J., Meunier, J., 2020. Variations in seasonal (not mean) temperatures drive rapid adaptations to novel environments at a continent-scale. Ecology. https://doi.org/10.1002/ecy.2973

Valigurová, A., Hofmannová, L., Koudela, B., Vávra, J., 2007. An ultrastructural comparison of the attachment sites between *Gregarina steini* and *Cryptosporidium muris*. J. Eukaryot. Microbiol. 54, 495–510. https://doi.org/10.1111/j.1550-7408.2007.00291.x

Van Meyel, S., Devers, S., Meunier, J., 2019. Love them all: mothers provide care to foreign eggs in the European earwig *Forficula auricularia*. Behav. Ecol. 30, 756–762. https://doi.org/10.1093/beheco/arz012

Vogelweith, F., Foitzik, S., Meunier, J., 2017a. Age, sex, mating status, but not social isolation interact to shape basal immunity in a group-living insect. J. Insect Physiol. 103, 64–70. https://doi.org/10.1016/j.jinsphys.2017.10.007

Vogelweith, F., Körner, M., Foitzik, S., Meunier, J., 2017b. Age, pathogen exposure, but not maternal care shape offspring immunity in an insect with facultative family life. BMC Evol. Biol. 17, 69. https://doi.org/10.1186/s12862-017-0926-y

vom Steeg, L.G., Klein, S.L., 2016. SeXX matters in infectious disease pathogenesis. PLoS Pathog. 12, 1–6. https://doi.org/10.1371/journal.ppat.1005374

Walker, K.A., Fell, R.D., 2001. Courtship roles of male and female European earwigs, *Forficula auricularia* L. (Dermaptera : Forficulidae), and sexual use of forceps. J. Insect Behav. 14, 1–17.

Weiß, C., Kramer, J., Holländer, K., Meunier, J., 2014. Influences of relatedness, food deprivation, and sex on adult behaviors in the group-living insect *Forficula auricularia*. Ethology 120, 923–932. https://doi.org/10.1111/eth.12261

Wheeler, W.M., 1910. The effects of parasitic and other kinds of castration in insects. J. Exp. Zool. 8, 377–438. https://doi.org/10.1126/science.32.832.836

Woolhouse, M.E.J., Webster, J.P., Domingo, E., Charlesworth, B., Levin, B.R., 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nat. Genet. 32, 569–577. https://doi.org/10.1038/ng1202-569

Zuk, M., 2009. The sicker sex. PLoS Pathog. 5, 1–3. https://doi.org/10.1371/journal.ppat.1000267

Zuk, M., McKean, K.A., 1996. Sex differences in parasite infections: Patterns and processes. Int. J. Parasitol. 26, 1009–1024. https://doi.org/10.1016/S0020-7519(96)00086-0

Fig. 1. *Gregarina ovata* in the European earwig. (A) An adult male of the European earwig. (B) Gregarine trophozoites can be observed through the gut wall of a dissected adult. Earwig's head is on the left and the arrow shows the trophozoites in the foregut. (C) Gregarine trophozoites in an open gut.

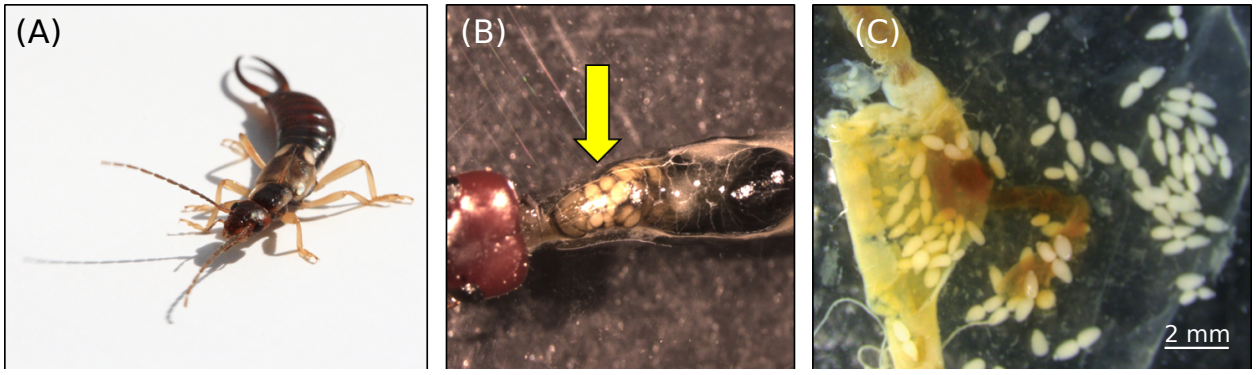

Fig. 2. Overall correlations between forceps length and minimum eye distance in earwig males (blue triangles) and females (orange dots). The forceps length of the host was positively associated to its body size, but the strength of this association depended on the sex (Polynomial Linear Model with the eye distance used at degree 15; eye distance: $F_{23,533} = 13.09$, $P < 0.0001$; sex: $F_{9,533} = 68.24$, $P < 0.0001$; interaction between eye distance and sex: $F_{15,533} = 8.09$, $P < 0.0001$, adjusted $R^2 = 0.55$). Specifically, the association was both steeper and more convex in males compared with females. To control for this sex-specific association, we thus used a 'corrected' mean forceps length in the corresponding statistical models, which was obtained by extracting the residuals of two polynomial LMs conducted either in the male or in the female dataset.

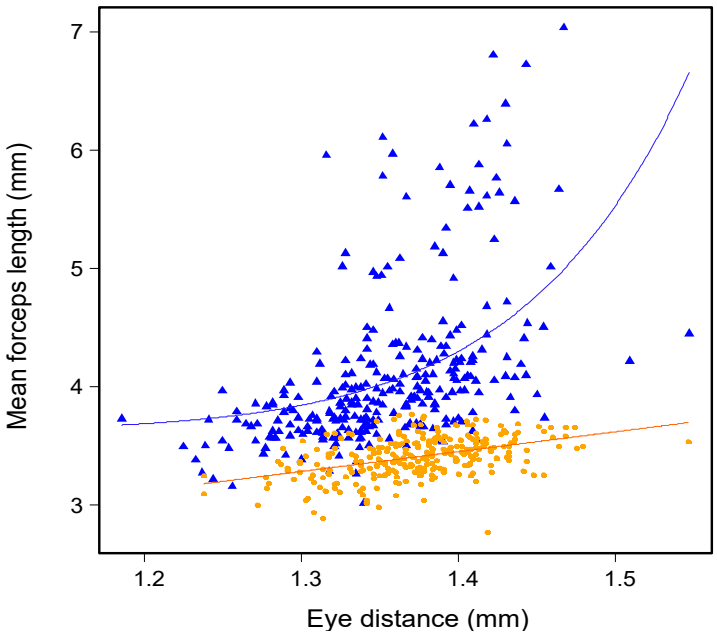

Fig. 3. Overview of *Gregarina ovata* prevalence and loads in the tested population of *Forficula auricularia*.* $P < 0.05$, *** $P < 0.001$.

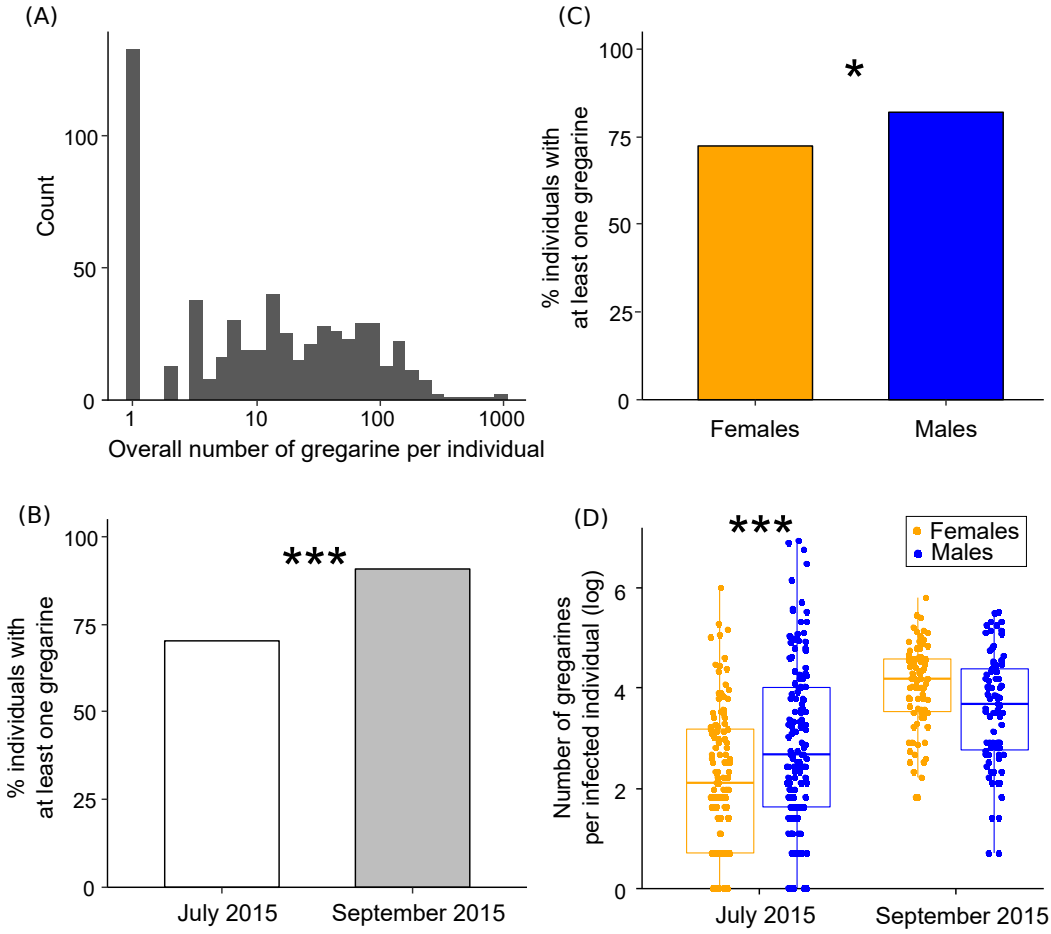

Fig. 4. Links between *Gregarina ovata* infection and the (A, B) general activity, (C, D) food consumption and (E, F) immune activity of *Forficula auricularia*. The total-phenoloxidase activity was log+0.001 transformed. * $P < 0.05$

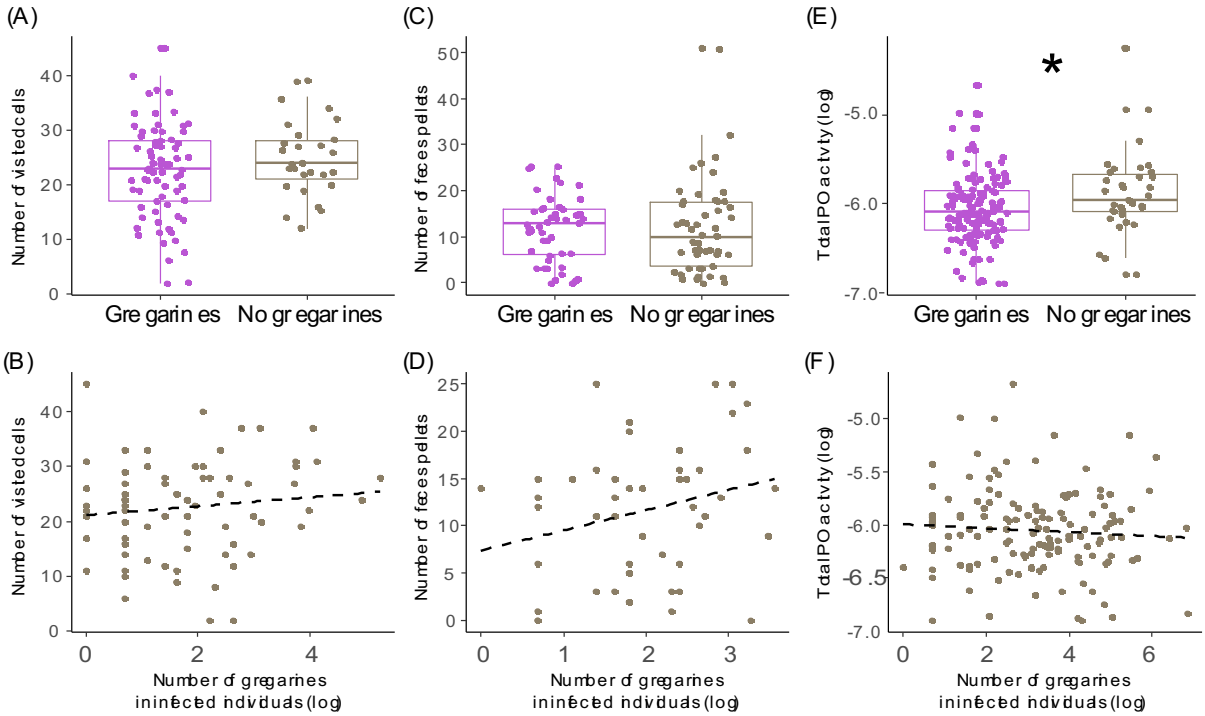

Fig. 5. Effects of (A) pathogen exposure, (B) sex and (C) *Gregarina ovata* loads on the survival of starved earwigs. The curves in C are representative of two cohorts of individuals having either comparatively high (90 trophozoites) or low (18 trophozoites) loads of gregarines. These values are, respectively, the third and first quartiles of the trophozoite numbers counted per individual in this experiment. Each figure encompasses all the tested earwigs and shows differences between levels of the given factor. *** $P < 0.001$.

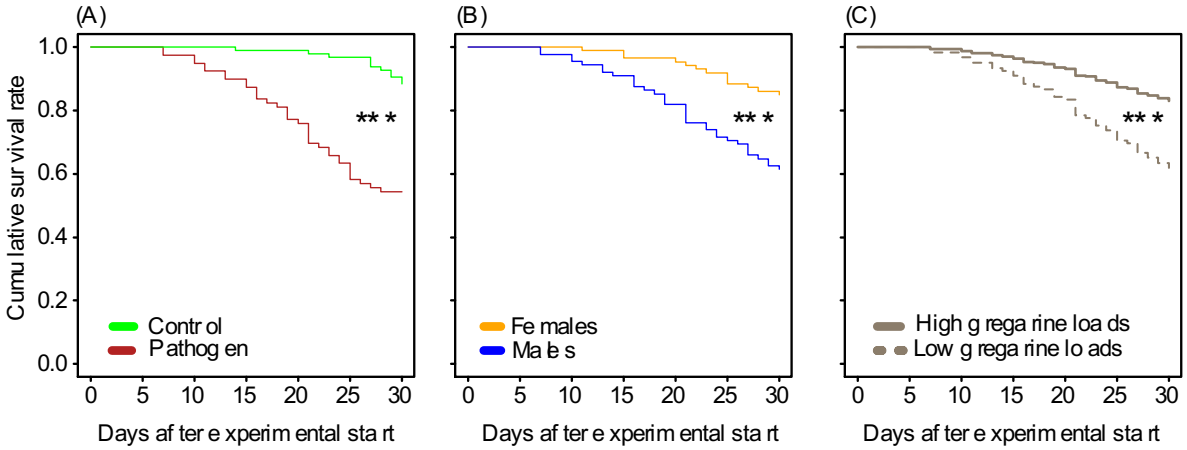

Table 1. Links between the (a) presence and (b) number of gregarine trophozoites in an earwig gut and its corrected forceps length, eye distance, sex and its date of field sampling. Significant *P* values are in bold. LR = Likelihood Ratio.

	Gregarine presence		Gregarine number	
	LR $\chi^2_{(1)}$	*P*	$F_{(1,429)}$	*P*
Corrected forceps length	0.36	0.5486	0.50	0.4785
Eye distance	0.01	0.9218	0.10	0.7485
Sex	6.62	**0.0101**	2.81	0.0942
Sampling date	7.76	**<0.0001**	89.72	**<0.0001**
Sex : Sampling date	1.47	0.2256	17.11	**<0.0001**

Table 2. Effect of gregarine infection, host sex and other parameters on earwigs' (A) general activity, (B) feces production, (C) total-Phenoloxidase activity and (D) survival of a fungal pathogen. LR = Likelihood ratio. Significant *P* values are in bold.

	Gregarine presence		Gregarine number	
Experiment 1: General activity				
Gregarine	$F_{(1,97)} = 1.34$	$P = 0.2495$	$F_{(1,70)} = 0.42$	$P = 0.5174$
Sex	$F_{(1,97)} = 2.80$	$P = 0.0976$	$F_{(1,70)} = 3.62$	$P = 0.0612$
Experiment 2: Feces production				
Gregarine	$F_{(1,94)} = 0.06$	$P = 0.8094$	$F_{(1,42)} = 2.13$	$P = 0.1520$
Sex	$F_{(1,94)} = 1.15$	$P = 0.2855$	$F_{(1,42)} = 3.84$	$P = 0.0569$
Food access	$F_{(1,94)} = 10.92$	**$P = 0.0013$**	$F_{(1,42)} = 3.76$	$P = 0.0593$
Gregarine : Food access	$F_{(1,94)} = 0.80$	$P = 0.3740$	$F_{(1,42)} = 0.29$	$P = 0.5943$
Experiment 3: Total-PO activity				
Gregarine	$F_{(1,172)} = 4.26$	**$P = 0.0406$**	$F_{(1,137)} = 0.58$	$P = 0.4495$
Sex	$F_{(1,172)} = 0.58$	$P = 0.4492$	$F_{(1,137)} = 0.20$	$P = 0.6522$
Experiment 4: Survival to a fungal pathogen				
Gregarine	-	-	LR $\chi^2_{(1)} = 13.57$	**$P = 0.0002$**
Sex	-	-	LR $\chi^2_{(1)} = 10.88$	**$P = 0.0009$**
Fungal exposure	-	-	LR $\chi^2_{(1)} = 35.36$	**$P < 0.0001$**
Gregarine : Fungal exposure	-	-	LR $\chi^2_{(1)} = 0.15$	$P = 0.6970$