Short-term benefits, but transgenerational costs of maternal loss in an insect with facultative maternal care

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A lack of parental care is generally assumed to entail substantial fitness costs for offspring that ultimately select for the maintenance of family life across generations. However, it is unknown whether these costs arise when parental care is facultative, thus questioning their fundamental importance in the early evolution of family life. Here, we investigated the short-term, long-term and transgenerational effects of maternal loss in the European earwig Forficula auricularia, an insect with facultative post-hatching maternal care. We showed that maternal loss did not influence the developmental time and survival rate of juveniles, but surprisingly yielded adults of larger body and forceps size, two traits associated with fitness benefits. In a cross-breeding/cross-fostering experiment, we then demonstrated that maternal loss impaired the expression of maternal care in adult offspring. Interestingly, the resulting transgenerational costs were not only mediated by the early-life experience of tending mothers, but also by inherited, parent-of-origin-specific effects expressed in juveniles. Orphaned females abandoned their juveniles for longer and fed them less than maternally-tended females, while foster mothers defended juveniles of orphaned females less well than juveniles of maternally-tended females. Overall, these findings reveal the key importance of transgenerational effects in the early evolution of family life.

1. Introduction

Family life is a common phenomenon in nature and is usually associated with substantial fitness benefits for offspring. These benefits mostly derive from the expression of parenting behaviours [1,2] such as nest construction, brood/juvenile attendance or food provisioning [2,3], and are thus contingent on the parental presence. Consequently, parental loss, e.g. due to clutch desertion or premature mortality, has been predicted to entail severe fitness costs for offspring that may ultimately select for the maintenance of family life across generations. In line with this prediction, short-term costs of parental loss have been reported in a large set of taxonomically diverse species, in which it is typically associated with a reduction in growth and/or survival rates of juveniles [1,4–6]. Importantly, other studies also showed that parental loss can entail long-term and transgenerational costs by hampering the mating success of adult offspring and diminishing the level of care they express towards their own descendants [1,7,8]. For instance in rats, females that had experienced long periods of maternal loss as pups exhibited low levels of care towards their own offspring, which in turn also exhibited lower levels of care as F2 adults [9,10].

Somewhat surprisingly, the long-term and transgenerational effects of parental loss have only been studied in altricial vertebrates, in which juveniles exhibit limited foraging capabilities and thus heavily rely on parental resources [11–13]. However, investigating the occurrence of these effects in precocial invertebrates, in which juveniles exhibit early foraging capabilities and consequently only facultatively rely on parental resources [14,15], could provide crucial information on the early evolution of parental care. Indeed, transgenerational costs could be a key promoter of the maintenance of family life when parental loss has limited (if any)
short-term costs in terms of offspring survival, a scenario that applies to precocial systems and probably prevailed in the early evolution of family life [16]. Furthermore, studying the consequences of parental loss in precocial invertebrates could help to determine whether the tight association between parental care and offspring survival (as is found in altricial vertebrates) is a prerequisite for the expression of transgenerational costs, thus shedding light on the importance of these costs in the multiple forms of family life.

Here, we used a series of two experiments encompassing three generations of individuals to investigate the short-term, long-term and transgenerational effects of maternal loss in the European earwig Forficula auricularia. In this precocial insect, mothers provide care to their eggs over winter and, after hatching, to their mobile juveniles (called nymphs) [17,18]. Post-hatching maternal care lasts for several weeks and takes multiple forms including nymph grooming and food provisioning through regurgitation [19,20]. Although the early foraging capability of nymphs allows them to survive in the absence of a tending mother, maternal loss has been shown to entail short-term costs under suboptimal food quality, as it reduces the survival rate of nymphs until their fourth and last developmental instar [15]. Conversely, a second study showed that maternal loss enhances nymph survival rate when food quantity is limited, a result possibly due to a mother–offspring conflict over restricted food access [21].

In our first experiment, we investigated the short-term effects of maternal loss on the newly produced offspring. To this end, we reared nymphs with or without their mother under ad libitum food conditions and then monitored their development and survival rates until adulthood, as well as measured body size and forceps length in adults (two known fitness-related traits [22,23]). If maternal care was a key component of offspring development and survival, we would expect that maternally-deprived adults emerge earlier due to developmental stress [21], survive less well and exhibit smaller body and forceps sizes compared with maternally-tended adults. In our second experiment, we used the adults produced in the first experiment to determine whether maternal loss had long-term and transgenerational effects on the expression of maternal care. The expression of maternal care is generally known to reflect phenotypic and/or genetic traits of the caring mother, as well as maternally-inherited and paternally-inherited traits expressed by the tended juveniles, e.g. through maternal effects and epigenetic modifications [20,24,25]. We, therefore, conducted a full-factorial cross-breeding/cross-fostering experiment, in which we mated maternally-deprived and -tended females with maternally-deprived and -tended males, and cross-fostered the resulting eggs to a foster mother of the same or a different experimental group. We then measured the reproductive output of these families and determined the level of maternal care expressed by the foster mothers. If maternal loss had long-term negative effects, we would expect maternally-deprived mothers to exhibit a lower reproductive output and to express lower levels of maternal care. If maternal loss had transgenerational effects, we would expect the genetic origin of nymphs to affect the expression of care by foster mothers.

2. Material and methods

(a) Experiment 1: short-term effects of maternal loss

(i) Experimental design

The short-term effects of maternal loss were tested by rearing nymphs from 80 families of the European earwig F. auricularia with or without their mother. These families descended from 80 females and 73 males that were collected in a natural population in Dolcedo, Italy in September 2012 and kept under standard laboratory conditions throughout the experiments (details in [18]). Upon egg-laying, females were isolated in Petri dishes (9 cm diameter). One day after egg hatching, approximately 40 nymphs per brood (original brood size: mean ± s.e. = 61.2 ± 1.3) were set up in a new Petri dish either with their mother (maternally tended, n = 40 families; mean brood size = 39.6 ± 0.29) or without

Figure 1. Experiments investigating (a) short-term as well as (b) long-term and transgenerational effects of maternal loss. Transgenerational effects could be mediated by the experimental background of the genetic mother (gMother) and genetic father (gFather) of the offspring, as well as the one of their foster mother (fMother). Grey individuals have been maternally tended (MT), whereas black ones have been maternally deprived (MD).
their mother (maternally deprived, \( n = 40 \) families; \( 39.3 \pm 0.35 \); figure 1a). Fourteen days later, all tending mothers were removed from the maternally-tended groups to mimic natural family disruption [20]. Both maternally-deprived and -tended nymphs were subsequently transferred to larger Petri dishes (14 cm diameter) to allow their development until adulthood. At adult emergence, males and females of each family were separated to avoid inbreeding and ensure virility [26]. All Petri dishes contained humid sand as ground material, a plastic tube as a shelter and an ad libitum amount of laboratory food, which mainly contained carrots, flower pollen and dry cat food (detailed composition in [17]).

(ii) Measurements of life-history traits in \( F_1 \) individuals

We measured the developmental time and survival rate of \( F_1 \) nymphs at each developmental instar, as well as the eye distance (a proxy of body size) and the forceps length of the resulting adults. Developmental time was defined as the day at which the first nymph within a brood was observed to moult into the next instar (newly moulted individuals stay whitish for one day), a measurement known to predict the developmental time for the whole brood [27]. Survival rates were measured for each developmental instar by counting the number of offspring alive three days after the first moulted individual had been observed. For the 1st instar, nymph survival was measured on day 10 after egg hatching. Finally, the average eye distance and forceps length of two haphazardly chosen male and female adults per family were measured to the nearest 0.001 cm using a camera coupled to a binocular microscope (Leica DFC425, Leica Microsystems Ltd., Heerbrugg, Switzerland) and the software LEICA APPLICATION SUITE 4.5.0. Note that one or two individuals were removed at each nymphal instar to conduct another experiment not presented here. These removed nymphs were excluded from survival rate calculations.

(b) Experiment 2: long-term and transgenerational effects of maternal loss

(i) Experimental design

The long-term and transgenerational effects of maternal loss were tested using three successive steps: (i) cross-breeding maternally-deprived and -tended adults, (ii) cross-fostering the resulting eggs to foster mothers of either the same or a different experimental background than their biological mother, and finally (iii) splitting the resulting families into two groups, one of which stayed with the foster mother, whereas the other was orphaned (figure 1b). The cross-breeding was conducted by pairing 78 females with 78 unrelated males to obtain 19–20 replicates of each of the four possible combinations of maternally-deprived and -tended adults (figure 1b; electronic supplementary material, table S1). These adults were haphazardly chosen among the ones obtained from experiment 1. Note that two families did not produce enough adults and could thus not be used in experiment 2. Maternal pairs were maintained under standard laboratory conditions for two months, after which females were isolated to allow egg laying [21].

The resulting eggs were cross-fostered on average 5.7 ± 0.7 (± s.e.) days after they had been laid. The cross-fostering was conducted to obtain the 16 possible combinations of experimental backgrounds of the parents (maternally-deprived or -tended), i.e. by controlling for the background of the genetic mother (gMother) and the genetic father (gFather) of the transferred eggs, as well as for the background of the recipient mother (then called foster mother, fMother) and the male who was paired with the foster mother (fFather) (figure 1b). Females of the European earwig readily accept foreign eggs [20], thus allowing us to ensure that foster mothers were always unrelated to the eggs they tended. After cross-fostering, all foster females and their adopted eggs were kept under standard laboratory conditions until egg hatching. Note that six of the 78 females were excluded from the cross-fostering, because they either did not produce any eggs or produced them too early/late to conduct a cross-fostering (details in the electronic supplementary material).

Finally, the split-clutch was conducted to determine whether maternal attendance could mask the transgenerational effects of maternal loss on offspring life-history traits. One day after egg hatching, the nymphs of each of the cross-fostered families were attributed to two equally-sized groups: one group was tended by the foster mother for 14 days (then called maternal presence), whereas the other group was raised without a mother (then called maternal absence; figure 1b). At day 14 after egg hatching, the foster females were discarded from the experiment and the nymphs maintained under standard laboratory conditions until they reached adulthood.

(ii) Measurements of maternal care by \( F_1 \) adults and of life-history traits in \( F_2 \) individuals

We measured the long-term and transgenerational effects of maternal loss on the expression of three forms of maternal care (in the maternal-presence groups). The first form was (i) clutch defence, which reflected the females’ willingness to protect their clutch of eggs or nymphs from predator attacks. Note that under natural conditions, mothers typically stay on or in the vicinity of their clutch to defend it against predators such as pseudo-scorpions and male earwigs (J. Meunier 2015, personal observation). It was measured on day 10 after egg-laying (egg defence) and on day 4 after hatching (nymph defence), by standardly poking each female on the pronotum with a glass capillary (one poke per second) and then recording the number of pokes required until she moved more than two body lengths away from her clutch. The second form of maternal care was (ii) clutch desertion, which showed how long females abandoned their clutch after being chased away by a simulated predator attack. Under natural conditions, temporary clutch abandonment can be costly for females, as it allows predators—including conspecifics—to consume the clutch of eggs or nymphs. It was measured by recording the time the female took to return to her clutch of eggs or nymphs after the end of the clutch defence measurement.

Finally, the third form of maternal care was (iii) food provisioning, which revealed how much food earwig mothers provided to their nymphs. In brief, the process involved four standard [17] and successive steps that started at day 6 after hatching. They consisted of: (i) food depriving mothers and nymphs for 24 h, (ii) isolating females for 1 h while offering them ad libitum green-coloured pollen pellets (Hoyer and DEKO BACK), (iii) re-assembling each female with a standardized number of 15 (or all if less than 15 available) of her foster nymphs for 15 h, and finally (iv) calculating the proportion of nymphs with a green-coloured gut. This measurement was not conducted in clutches with less than five nymphs. The nymphs from the maternal-presence groups not used in these tests, as well as all nymphs from the maternal-absence groups, were isolated under the same conditions. Once the green-coloured nymphs had been counted, the maternal-presence group was reassembled and all groups were provided with ad libitum laboratory food and maintained under the standard conditions. Owing to the requirement of at least five surviving nymphs per clutch to reliably measure food provisioning and time constraints associated with the simultaneous measurements of multiple forms of maternal care across clutches, we used 61 (85%) of the 72 cross-fostered clutches to measure clutch defence, 59 (82%) to measure desertion time and 47 (65%) to measure food provisioning (sample sizes in the electronic supplementary material, table S1).

We also explored the transgenerational effects of maternal loss on three egg and four nymphal traits (in both maternal-presence
and -absence groups). The three egg traits were measured on the first clutch produced by each female and consisted of the number, mean weight (per clutch) and hatching success of these eggs. The number of eggs was counted 3 days after the first egg had been observed. Their mean weight was measured at that time by weighing a random sample of 10 eggs to the nearest 0.1 μg. The hatching success was obtained by dividing the number of nymphs one day after hatching by the number of eggs transferred to the foster female. The four nymphal traits included their number, mean initial weight, survival rates, as well as developmental time until adulthood. One day after hatching, the number of nymphs was counted and a haphazard sample of 10 nymphs weighed to the nearest 0.1 μg. Nymph survival rate was measured by dividing the total number of adults that emerged from each type of group by the number of nymphs originally transferred into these groups. Finally, nymph developmental time was defined as the number of days between hatching and the emergence of the first adult in each of the two groups. All weighing was conducted using a micro-scale (PESCALE, MYA5).

(c) Statistical analyses

The short-term effects of maternal loss were analysed using a series of one generalized linear mixed model (GLMM) and three linear mixed models (LMMs). In the first models, nymph survival rate (GLMM) or nymph developmental time (LMM) at each instar was entered as the response variable, while maternal loss (bimodal; maternally-tended or -deprived, respectively MT or MD) and each developmental instar (continuous) were used as explanatory variables. The family identification (ID) was entered as a random effect into these two models, because each family group was measured at each instar. In the other models, the mean eye distance (LMM) or the forceps length corrected for body size (see the electronic supplementary material for calculation; LMM) was entered as the response variable, and maternal loss and sex as explanatory factors. The family ID was also entered as a random effect, because each family group provided values for males and females.

The long-term and transgenerational effects of maternal loss were analysed in another series of linear models (LMs), generalized linear models (GLMs) and LMMs. The first models were fitted using either nymph number (LM), nymph weight at hatching (LM) or food provisioning (measured as proportion of coloured recipient nymphs, GLM) as response variable, and gMother, gFather and fMother as explanatory factors. In the second set of models, egg number (LM), egg weight (LM) or hatching success (GLM) was entered as response variable, and gMother and gFather as explanatory factors. In the last models, the response variable was either clutch defence (log-transformed number of pokes withstood; LMM) or clutch desertion (log-transformed time away from clutch; LMM), and the explanatory factors were gMother, gFather, fMother and the type of clutch (eggs or nymphs). In these LMMs, the ID of the clutch was entered as a random effect, because the measurements were conducted on eggs and nymphs from the same clutches.

Finally, offspring developmental time and survival rate until adulthood were analysed in two separate steps. In the first step, the effects of gMother, gFather and fMother on offspring developmental time (LM) and survival rate (GLM) were tested in the split-clutches tended by a foster mother (i.e. in the maternal-presence groups only). Because fMother was never significant in these tests (electronic supplementary material, table S2), we pooled the two levels of this factor and, in a second step, compared offspring developmental time and survival rates of maternal-presence and -absence groups. To this end, we tested whether gMother, gFather and/or the presence of a tending mother (yes or no) affected either the developmental time (LMM) or the survival of nymphs until adulthood (GLMM). The ID of the original clutch was entered as a random effect into these two models because split-clutches with and without a tending mother were used.

Statistical analyses were conducted using the software R v. 3.1.3. All interactions between explanatory factors were tested in each model, which were then simplified stepwise by removing the non-significant interactions (all p > 0.12). All GLMMs were fitted with a binomial error structure corrected for overdispersion. Proportion data (e.g. survival and developmental rates) were entered into these models using the cbind function. Note that fFather was not entered into any statistical model because there was no specific prediction based on this effect for the different measurements (see also [20]) and this ensured an average of 7.5 replicates for each of the tested combinations (electronic supplementary material, table S1).

3. Results

(a) Experiment 1: short-term effects of maternal loss

Contrary to the predicted short-term costs of maternal loss, we found that maternal loss positively shaped two morphological traits in adult offspring (figure 2). Specifically, maternally-deprived adults had wider eye distances (figure 2a; Likelihood ratio (LR) $\chi^2 = 5.75$, $p = 0.016$) and longer forceps (corrected for eye distance; figure 2b; LR $\chi^2 = 6.83$, $p = 0.009$) than maternally-tended adults. Moreover, eye distance was overall wider for females (mean ± s.e. = 1.42 ± 0.006 mm) than for males (1.39 ± 0.006 mm; LR $\chi^2 = 8.25$, $p = 0.004$) and was not shaped by an interaction between maternal loss and sex (LR $\chi^2 = 1.25$, $p = 0.264$). The statistical model on the corrected forceps length reported no main significant effects of sex (LR $\chi^2 = 0.36$, $p = 0.547$) or of the interaction between maternal loss and sex (LR $\chi^2 = 3.58$, $p = 0.058$).

Maternal loss did not influence nymph survival rate (figure 2c; LR $\chi^2 = 0.05$, $p = 0.826$) and developmental time (figure 2d; LR $\chi^2 = 0.45$, $p = 0.502$) until adulthood. Not surprisingly, the nymphs survival rate decreased with time (LR $\chi^2 = 919.19$, $p < 0.0001$; model estimate ± s.e. = -0.81 ± 0.04), while the nymphs’ developmental time increased with developmental instar (LR $\chi^2 = 6543.81$, $p < 0.0001$; estim. ± s.e. = 17.04 ± 0.30). However, nymph developmental instar did not interact with maternal loss to shape these two measurements (survival rate: LR $\chi^2 = 0.53$, $p = 0.465$; developmental time: LR $\chi^2 = 0.002$, $p = 0.963$).

(b) Experiment 2: transgenerational effects of maternal loss

Maternal loss affected the expression of three forms of maternal care by adult offspring through long-term effects in the caring mother, as well as through inherited, parent-of-origin-specific effects expressed in the nymphs (table 1). First, maternally-deprived foster mothers abandoned their clutch for an overall longer period of time than maternally-tended foster mothers (table 1b and figure 3a), an effect that was independent of the genetic parents of the offspring and of the age of the clutch (i.e. eggs or nymphs, table 1b). Second, the maternally-deprived foster mother provisioned overall less nymphs with food than maternally-tended foster mothers (table 1c and figure 3b). Food provisioning was also shaped by an interaction between the experimental backgrounds of the two genetic parents of the tended nymphs (table 1c). Nymphs of maternally-tended males received more food when they had been produced by maternally-deprived compared
with maternally-tended females (figure 3c; estim. ± s.e. = 1.25 ± 0.59; \( t_{48} = 2.11, p = 0.041 \)), whereas the experimental background of the females had no effect on food provisioning when nymphs were sired by maternally-deprived males (estim. ± s.e. = 2.0.54 ± 0.60; \( t_{48} = -0.90, p = 0.374 \)). Finally, maternally-deprived mothers produced nymphs, but not eggs, that were overall less defended by the caring foster mothers (figure 3d; Interaction in table 1a; nymph stage: estim. ± s.e. = -0.51 ± 0.25; \( t_{67} = -2.04, \ p = 0.045 \); egg stage: estim. ± s.e. = 0.12 ± 0.24; \( t_{67} = 0.52, \ p = 0.606 \)). Note that all but two measurements of maternal care were independent of each other (electronic supplementary material,

Table 1. Long-term and transgenerational effects of maternal loss on the expression of maternal care by adult offspring. (Maternal care was measured towards clutches of eggs or nymphs (clutch type), except for food provisioning, which was only expressed towards nymphs. Significant \( p \)-values are in bold. LR, likelihood ratio. Note that non-significant interactions are reported to allow comparison among models, but their removal did not qualitatively change the results.)

<table>
<thead>
<tr>
<th></th>
<th>(a) clutch defence</th>
<th>(b) clutch desertion time</th>
<th>(c) food provisioning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LR ( \chi^2 )</td>
<td>( p )-value</td>
<td>LR ( \chi^2 )</td>
</tr>
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<td>(&lt;0.0001)</td>
<td>0.64</td>
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<tr>
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<td>1.31</td>
<td>0.253</td>
<td>4.62</td>
</tr>
<tr>
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</tr>
<tr>
<td>gFather</td>
<td>0.85</td>
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<td>0.04</td>
</tr>
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<td>CT : gMother</td>
<td>3.95</td>
<td>(0.047)</td>
<td>0.09</td>
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<td>gMother : gFather</td>
<td>0.46</td>
<td>0.498</td>
<td>0.62</td>
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</table>

Figure 2. Short-term effects of maternal loss on the (a) mean eye distance, (b) corrected forceps length, (c) survival rate, and (d) developmental time of offspring. Nymphs were either maternally tended (MT) or maternally deprived (MD) during the 16 days following their emergence.
Only egg defence and the duration of egg abandonment were overall positively correlated (Spearman correlation test, $r_s = 0.32$, $p = 0.009$).

Maternal loss influenced only one of the four measurements taken on nymphs and none of the three measurements taken on eggs produced by the adult offspring. Nymphs produced by maternally-tended females reached adulthood in $72.13 \pm 0.67$ days (mean ± s.e.), which was significantly longer than the $69.25 \pm 0.51$ days required by nymphs produced by maternally-deprived females (table 2). By contrast, the experimental background of the genetic parents had no effect on the number of eggs (gMother: LR $\chi^2 = 0.08$, fMother: LR $\chi^2 = 0.32$).

Table 2. Transgenerational effects of maternal loss on the reproductive output of the resulting adult offspring. (The significant p-value is in bold.)

<table>
<thead>
<tr>
<th>gMother</th>
<th>gFather</th>
<th>fMother</th>
<th>maternal presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.43</td>
<td>0.33</td>
<td>0.32</td>
<td>0.73</td>
</tr>
<tr>
<td>0.232</td>
<td>0.568</td>
<td>0.570</td>
<td>0.394</td>
</tr>
<tr>
<td>0.312</td>
<td>0.776</td>
<td>&gt;0.01</td>
<td>0.776</td>
</tr>
<tr>
<td>0.013</td>
<td>0.394</td>
<td>0.991</td>
<td>0.655</td>
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<td>1.04</td>
<td>0.33</td>
<td>0.01</td>
<td>0.2</td>
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<tr>
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<td>0.564</td>
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<td>0.394</td>
<td>0.865</td>
<td>0.394</td>
<td>0.655</td>
</tr>
</tbody>
</table>

*Because fMother did not influence post-hatching traits measured in nymphs tended by a foster mother (electronic supplementary material, table S2), this factor was pooled to form a new factor describing the presence or the absence of mothers after hatching, then called ‘maternal presence’.

Table S3). Only egg defence and the duration of egg abandonment were overall positively correlated (Spearman correlation test, $r_s = 0.32$, $p = 0.009$).

Maternal loss influenced only one of the four measurements taken on nymphs and none of the three measurements taken on eggs produced by the adult offspring. Nymphs produced by maternally-tended females reached adulthood in $72.13 \pm 0.67$ days (mean ± s.e.), which was significantly longer than the $69.25 \pm 0.51$ days required by nymphs produced by maternally-deprived females (table 2). By contrast, the experimental background of the genetic parents had no effect on the number of eggs (gMother: LR $\chi^2 = 0.08$, fMother: LR $\chi^2 = 0.32$).

Figure 3. Long-term and transgenerational effects of maternal loss on the expression of (a) clutch abandonment, (b,c) food provisioning, and (d) clutch defence by adult offspring. MT, maternally tended; MD, maternally deprived. Sample sizes are at the bottom of each bar.
influence nymphs’ survival rates and developmental times (table 2) or their survival until adulthood (table 2). Notably, the experimental background of the parents also had no effect on the number and mean weight of nymphs at egg hatching (table 2) or their survival until adulthood (table 2). The experimentally derived number of nymphs and developmental times (table 2).

4. Discussion

Our experiments reveal the occurrence, but contrasting nature of short-term, long-term and transgenerational effects of maternal loss in the precocial insect F. auricularia. Under standard laboratory conditions, maternal loss entailed short-term benefits for adult offspring: maternally-deprived adults had wider eye distances and longer forceps than maternally-tended adults, two effects independent of nymph survival and/or developmental time. On the other hand, maternal loss entailed transgenerational costs. These costs were partly mediated by the experimental background of the caring mothers, as revealed by the longer clutch abandonment and lower food provisioning of maternally-deprived as compared to maternally-tended females. They were also partly mediated by the experimental background of the genetic parents: nymphs produced by maternally-deprived females were less well defended by their foster mother. Note, however, that when sired by maternally-tended males, nymphs produced by maternally-deprived females received more food than nymphs produced by maternally-tended females. Finally, we found a transgenerational effect of maternal loss on offspring developmental time: nymphs of maternally-deprived females reached adulthood earlier than nymphs of maternally-tended females. By contrast, there was no evidence for transgenerational effects of maternal loss on the other life-history traits measured in eggs and F2 nymphs, irrespective of the presence or absence of a tending mother after egg hatching.

Somewhat surprisingly, we found that maternal loss did not affect nymph development and survival, but yielded adult offspring of larger body and forceps sizes, two morphological traits associated with fitness benefits in F. auricularia [22,23]. These apparent short-term benefits of maternal loss obtained under laboratory conditions contrast with the short-term costs typically expected under natural conditions (e.g. [4,28]). Previous studies already revealed that the maternal presence reduces nymph survival when families had restricted food access [21], but increased nymph survival when families had access to low quality food [15]. Hence, the effects of maternal presence under ad libitum, high quality food could reveal an increased expression of sibling rivalry when juveniles have access to maternal resources, as proposed in a recent model [29]. Alternatively, these effects could result from maternal behaviours that benefit offspring under natural conditions, but directly or indirectly hamper their development under laboratory conditions. For instance, mothers often cover food in the vicinity of the nest, presumably to prevent microbial development (J. Kramer & J. Meunier 2015, personal observation). This might have been costly for juveniles in the absence of pathogens, as it might have restricted their access to food. This notwithstanding, both scenarios emphasize that the parental presence can be associated with costs for the tended offspring (see also [21]), which emerge when the (laboratory) conditions do not allow (variation in) the benefits of parental care to be revealed. These findings overall indicate that as long as parental care only has limited effects on offspring development and survival (a scenario that presumably prevailed in the early evolution of family life), it is likely that the emergence and maintenance of parental investment into post-hatching care mostly relies on the benefits of parenting behaviours that enable offspring to better cope with environmental constraints, such as limited food access or the presence of pathogens and predators [23].

Contrary to the above effects, maternal loss lowered the expression of maternal care by adult offspring. Females reared without mothers abandoned their juveniles for longer and provisioned them with less food than females reared with tending mothers. To the best of our knowledge, the long-term, negative effects of maternal loss on the expression of maternal care have only been reported in altricial vertebrates [8–10,13]. In these species, the effects of maternal loss typically result from a disrupted learning process [30] and/or from induced hormonal/neurobiological changes during juvenile development. For instance, temporary maternal deprivation is known to alter the brain development of juvenile rodents and primates, which in turns disturbs hormonal and neurobiological processes in adults and then hampers their expression of parental care (e.g. [31,32]). In altricial insects like honeybees and dung beetles, the absence of brood care is also known to delay the development of sensory and integrative brain centres in juveniles (e.g. antennal lobes and mushroom bodies [33,34]). However, the link between these developmental changes and the expression of care remains unknown. Our results thus call for further studies that test the effects of maternal loss on hormonal/neurobiological traits in juveniles of (precocial) invertebrates. Moreover, they suggest that effects of learning [35] on the expression of parenting behaviours might not be restricted to vertebrates. Finally, the occurrence of negative effects of parental loss in a precocial insect demonstrates that their expression is not restricted to species in which parental care is obligatory for offspring survival (such as altricial vertebrates). Note that the larger body and forceps size of maternally-deprived adults are unlikely to be the main drivers of these long-term negative effects on maternal care, as a previous study showed that large F. auricularia females express more rather than less maternal care (including food provisioning) than small females [17].

Importantly, the effects of maternal loss on the expression of maternal care were not only mediated by parental effects reflecting the experimental background of the caring mothers, but also by effects of the background of the parents that produced the nymphs. On the one hand, nymphs of maternally-tended females moulted into adults at a greater age than nymphs of maternally-deprived females. On the other hand, nymphs produced by maternally-deprived females were less well defended by foster mothers, but—when fertilized by maternally-tended males—received more food from foster mothers than nymphs produced by maternally-tended females. These results are overall in line with studies demonstrating that parent-of-origin effects inherited to and expressed by juveniles are key components of.
parental care [20,24,25,36]. Interestingly, our findings also reveal that these effects can be acquired from the early social environment of the future parents (e.g. through epigenetic modifications [37]), showing the central importance of this phenomenon in the evolution of parental investment into care in a precocial insect. Under our experimental conditions, these effects did not translate into the production of high quality nymphs by maternally-deprived females, as we found no parent-of-origin effect of maternal loss on the mean weight and survival rate of these nymphs. However, the accelerated developmental time of the offspring of maternally-deprived females could be indicative of parent-of-origin effects that increase the level of sibling rivalry among these offspring [15,21]. On a proximate level, the transgenerational effects of maternal loss on nymph defence could be mediated by a parent-of-origin effect on the offspring’s chemical signatures. These signatures are known to mediate behavioural interactions between earwig mothers and nymphs as well as among nymphs, to be flexible over time, and to determine the amount of food provisioned by mothers [38].

To conclude, our study demonstrates that maternal loss during earwig family life may entail short-term benefits regarding adult morphology, but is associated with transgenerational costs that are mainly mediated through the expression of parental care. These contrasting effects stress the importance of encompassing the short- and long-term effects of the parental presence when estimating the costs/benefits ratio of mother–offspring interactions for offspring. More generally, the surprising short-term benefits of maternal loss obtained under laboratory conditions shed light on the importance of (natural) environmental constraints on the net benefits gained by juveniles through parental care and on their role in the evolution of family life [3]. Finally, our data reveal that the early social environment of juveniles is a key determinant of parental investment across generations, as it shapes both the behaviour they later adopt as parents and the behaviours they transmit to their own offspring. Hence, this study overall suggests that environmental constraints and transgenerational effects of parental loss are keystones in the emergence and maintenance of ancestral forms of family life, in which parental care has limited effects on offspring development and survival [16].

Data accessibility. Data are deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.sm6n2.

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