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1 **Negative association between parental care and sibling cooperation in earwigs: a new**
2 **perspective on the early evolution of family life?**

3 **Running title:** Earwig sibling cooperation and parental care

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Abstract

11 The evolution of family life requires net fitness benefits for offspring, which are commonly
12 assumed to mainly derive from parental care. However, an additional source of benefits for
13 offspring is often overlooked: cooperative interactions among juvenile siblings. In this study,
14 we examined how sibling cooperation and parental care could jointly contribute to the early
15 evolution of family life. Specifically, we tested whether the level of food transferred among
16 siblings (sibling cooperation) in the European earwig *Forficula auricularia* (1) depends on the
17 level of maternal food provisioning (parental care), and (2) is translated into offspring survival,
18 as well as female investment into future reproduction. We show that higher levels of sibling
19 food transfer were associated with lower levels of maternal food provisioning, possibly
20 reflecting a compensatory relationship between sibling cooperation and maternal care.
21 Furthermore, the level of sibling food transfer did not influence offspring survival, but was
22 associated with negative effects on the production of the second and terminal clutch by the
23 tending mothers. These findings indicate that sibling cooperation could mitigate the
24 detrimental effects on offspring survival that result from being tended by low quality mothers.
25 More generally, they are in line with the hypothesis that sibling cooperation is an ancestral
26 behavior that can be retained to compensate for insufficient levels of parental investment.

27 **Keywords:** social evolution; precocial species; insects; maternal care; sibling rivalry; *Forficula*
28 *auricularia*

Introduction

29 The evolution of social life requires that the benefits individuals gain through group living
30 outweigh its inherent costs (Alexander, 1974; Bourke, 2011). These costs typically arise from
31 a higher risk of pathogen transmission (Schmid-Hempel, 1998; Altizer *et al.*, 2003), as well as
32 from an increased intensity of competition for limited resources and reproduction (Mock &
33 Parker, 1997; Krause & Ruxton, 2002; Roulin & Dreiss, 2012). Conversely, the benefits of social
34 life are usually attributed to social interactions among group members that can, for example,
35 enhance predator defense and foraging efficiency (Krause & Ruxton, 2002; Royle *et al.*, 2012).
36 The basic challenge in understanding the evolution of social life is thus to unravel the nature
37 and functional interactions of mechanisms underlying the net benefits of group living
38 (Alexander, 1974; Bourke, 2011).

39 Our current knowledge of the mechanisms that shape social evolution mostly stems
40 from studies on the highly derived social systems of mammals, birds and eusocial insects (e.g.
41 Wilson, 1971; Royle *et al.*, 2012), that are characterized by obligatory and often permanent
42 forms of group living. However, only little attention has been paid to the study of less derived
43 stages of social evolution, such as those found in species exhibiting facultative and/or
44 temporary forms of family life with parental care. Investigating the interplay of evolutionary
45 mechanisms that underlie the net benefits of group living in such species is crucial to expand
46 our understanding of the emergence of social life from an ancestral, solitary state (Smiseth *et*
47 *al.*, 2003; Falk *et al.*, 2014; Meunier, 2015), which is considered to be one of the major
48 transitions in the evolution of life (Szathmáry & Maynard Smith, 1995; Bourke, 2011).

49 Over the last decades, offspring benefits of family life were traditionally attributed to
50 interactions between parents and offspring in the form of parental care (reviewed in Royle *et*

51 *al.*, 2012). However, an often overlooked source of benefits is sibling cooperation (Forbes,
52 2007; Roulin & Dreiss, 2012), which is promoted by the additional indirect fitness benefits of
53 assisting genetically related individuals (Hamilton, 1964; West *et al.*, 2002). Cooperation
54 among adult siblings is common in nature, such as in cooperatively breeding vertebrates
55 (Clutton-Brock, 2002; Koenig & Dickinson, eds, 2004) or in colonies of eusocial insects (Wilson,
56 1971), but an increasing number of studies also reports cooperation among juvenile siblings.
57 For instance, offspring express mutual cleaning in the Mississippi Kite *Ictinia mississippiensis*
58 ("allo-preening; Botelho *et al.*, 1993) and the ambrosia beetle *Xyleborinus saxesenii*
59 (Biedermann & Taborsky, 2011), as well as share food in the Common Barn-owl *Tyto alba*
60 (Roulin *et al.*, 2012), the huntsman spider *Delena cancerides* (Yip & Rayor, 2013) and the
61 European earwig *Forficula auricularia* (Falk *et al.*, 2014).

62 Although both sibling cooperation and parental care may provide substantial benefits
63 to juveniles during family life, it remains surprisingly unexplored how these behaviors are
64 related when they co-occur. Assessing the modality of their co-occurrence would allow to
65 determine their independent or joined roles, as well as their respective importance in the
66 evolutionary transition from solitary to group living (Falk *et al.*, 2014). The association
67 between sibling cooperation and parental care - if any - could either be complementary or
68 compensatory. In the first case (here termed complementarity hypothesis), the level of sibling
69 cooperation is predicted to be positively correlated with the level of parental care. This
70 scenario could, for example, be based on a higher propensity of siblings to cooperate with
71 each other when the level of parental care is high, which in turn should reduce offspring
72 competition and conflict (Roulin & Dreiss, 2012) that are otherwise predicted to hamper
73 cooperation (Frank, 1998). Such a positive correlation could be expected in altricial species,
74 in which offspring exclusively rely on parental resources. In the second case (here termed

75 compensation hypothesis), the level of sibling cooperation is expected to be negatively
76 associated with the level of parental care. This scenario likely applies to precocial species,
77 which exhibit a non-derived and non-obligatory form of family life. In these species, offspring
78 do not exclusively rely on parental resources, but instead either have direct access to the
79 resources used as nest material (e.g. carrion and dung) or are mobile and capable of
80 independent resource acquisition in the vicinity of the nest site. Consequently, offspring
81 competition over parental resources could be reduced and offspring could benefit from
82 sharing independently acquired resources with siblings (Falk *et al.*, 2014), particularly when
83 parental investment is insufficient. Under such circumstances, resource transfer among
84 siblings could even release parents (at least partly) from offspring provisioning.

85 In this study, we examined whether food transfer among siblings (a form of sibling
86 cooperation) and food provisioning by parents (a form of parental care) are complementary,
87 compensatory or independent behaviors in the European earwig *Forficula auricularia* L. In this
88 precocial insect species, mothers care for their mobile offspring (called nymphs) for several
89 weeks after hatching (Lamb, 1976a). During this period, maternal care includes the protection
90 and grooming of nymphs as well as their provisioning with food through regurgitation (Lamb,
91 1976b; Staerke & Kölliker, 2008). However, maternal presence and post-hatching care are
92 not obligatory for offspring survival (Lamb, 1976b; Kölliker, 2007), as nymphs do not only
93 acquire food through maternal provisioning, but forage independently soon after hatching
94 (Lamb, 1976b; Wong & Kölliker, 2012) and share food with their siblings (Falk *et al.*, 2014).
95 Within earwig families, this food transfer among siblings has been shown to be predominantly
96 mediated by active allo-coprophy, a form of sibling cooperation defined by a socially-
97 induced increase in feces production by donor nymphs and the subsequent consumption of
98 these feces by recipient siblings (Falk *et al.*, 2014).

99 To unravel the relation between parental care and sibling cooperation in earwig
100 families, we measured the co-expression of maternal food provisioning and sibling food
101 transfer. Because group size can be an important parameter in family interactions that is
102 classically assumed to affect the intensity of competition among group members (Alexander,
103 1974; Shen *et al.*, 2014) and has been linked to differences in mortality and developmental
104 rates in European earwigs (Kölliker, 2007; Meunier & Kölliker, 2012b), we first tested (1)
105 whether group size (offspring number) shaped the expression of sibling food sharing and
106 maternal food provisioning. We then investigated (2) the nature and direction of the potential
107 association between the two types of food transfer. Finally, we tested whether the level of
108 sibling food transfer (3) affects offspring fitness and/or (4) reflects the quality of the tending
109 mothers. To these ends, we first investigated whether levels of food transfer were associated
110 with changes in offspring development and survival, and then with the number of eggs
111 produced by mothers in their following (and final) reproductive attempt.

Methods

112 **STUDY ANIMALS AND LABORATORY REARING**

113 The adult female earwigs used in our experiment descended from 160 individuals collected
114 in a natural population in Dolcedo, Italy in September 2012. These field-caught earwigs had
115 been maintained in the laboratory under standard rearing conditions (detailed in Meunier *et*
116 *al.*, 2012; Koch & Meunier, 2014) for one generation. After emergence, F1-adults were
117 maintained in large plastic containers (37 x 22 x 25 cm) for three months to allow uncontrolled
118 mating in sex-balanced groups of adults with different genetic origins. The following

119 experiment involved a random sample of 54 of these F1 females and their subsequent first
120 clutch of offspring.

121 **GENERAL EXPERIMENTAL SETUP**

122 We successively measured the levels of sibling food transfer and maternal food provisioning
123 in 54 clutches (see experimental details in Fig. 1). Four days after egg hatching, mothers were
124 isolated from their clutches and the nymphs randomly attributed to either of two groups of
125 equal size termed SFT- and MFP-groups. The SFT-groups were used to measure the level of
126 sibling food transfer (details below), whereas the MFP-groups were used to measure the level
127 of maternal food provisioning (details below). Because the mothers were involved in both
128 types of measurements, 28 clutches were used to first measure sibling food transfer (days
129 four to five) and then maternal food provisioning (days eight to nine), whereas the order of
130 tests was reversed in the other 26 clutches (Fig. 1). In between the two measurements (i.e.
131 from day five to day eight), the two groups of nymphs were maintained separately and
132 received an *ad libitum* amount of uncolored pollen pellets as food source (naturally yellow
133 colored flower pollen formed into pellets; Hochland Bio-Blütenpollen, Hoyer GmbH, Polling,
134 Germany). During the same period, mothers were isolated and likewise fed with uncolored
135 pollen pellets.

136 Once sibling food transfer and maternal food provisioning had been measured, we
137 investigated the association between these measurements and offspring fitness and/or
138 female quality by measuring nymph development and survival, as well as maternal
139 investment in their 2nd clutch. To this end, nymphs from SFT- and MFP-groups were
140 reassembled with their mother on day nine. Five days later, the mothers were isolated to

141 mimic natural family disruption and allow the production of a second clutch (Meunier *et al.*,
142 2012), whereas the nymphs were maintained in groups until adult emergence. Nymph
143 development time was recorded by checking daily for the emergence of the first second-instar
144 nymph in each clutch, and the proportion of nymphs that survived until adulthood was
145 assessed by counting the number of nymphs that molted into adults and then dividing this
146 number by the number of nymphs that initially entered the experiment. Finally, maternal
147 investment in their 2nd clutch - if any – was measured by counting (1) the number of days
148 between their isolation for 2nd clutch production and oviposition, (2) the number of 2nd clutch
149 eggs present three days after the first egg laying had been observed (egg laying takes up to
150 three days), as well as (3) the number of 2nd clutch nymphs present one day after the first
151 hatching had been observed (egg hatching generally occurs over a single day).

152 Groups of nymphs (and, when not isolated separately, their mother) were maintained
153 in medium sized Petri dishes (9 x 2 cm) until the end of family life (on day 14) and subsequently
154 in large Petri dishes (13.8 x 2 cm) until adult emergence. Each Petri dish contained humid sand
155 as ground material and a plastic tube as shelter. During their isolation from day five to day
156 eight, mothers were maintained in small Petri dishes (5.5 x 1.2 cm) inlaid with a moist paper
157 towel. Mothers and nymphs received *ad libitum* uncolored pollen pellets as food source from
158 hatching to day three. Conversely, nymph and mothers were provided with an *ad libitum*
159 amount of artificial diet twice a week from day nine until the end of the experiment (food
160 composition detailed in the supplementary material). Note that we used pollen instead of this
161 artificial diet during sibling food transfer and maternal food provisioning tests because of its
162 better dyeability (see below). Groups of nymphs and mothers were always food deprived one
163 day prior to the sibling food transfer and maternal food provisioning tests to increase foraging

164 and solicitation behaviors (of the nymphs) on the following day (Staerke & Kölliker, 2008;
165 Falk *et al.*, 2014).

166 **MEASURING SIBLING FOOD TRANSFERT AND MATERNAL FOOD PROVISIONING**

167 The measurements of sibling food transfer and maternal food provisioning were
168 implemented by taking advantage of an exceptional property of *F. auricularia* nymphs:
169 ingested colored food is visible through the partially transparent cuticle of first-instar nymphs
170 and can thus be used as a marker of food transfer between family members (Staerke &
171 Kölliker, 2008; Falk *et al.*, 2014). In brief, sibling food transfer was measured by (1) providing
172 half of the nymphs of the SFT-group with green-colored food, then (2) reassembling these
173 colored (donor-) nymphs with their uncolored remaining siblings (recipients) and their
174 mother, (3) allowing family interactions overnight and finally (4) counting the number of
175 newly colored recipient nymphs. To this end, we first divided each SFT-group into two sub-
176 groups of equal size (Fig. 1). All nymphs of one of the sub-groups were marked by clipping off
177 the distal third of the right cercus (Wong & Kölliker, 2013). This marking had no influence on
178 the proportion of newly green-colored nymphs in the sibling food transfer tests (Wilcoxon
179 rank sum test; $W = 288.5$, $P = 0.967$). After marking, we randomly selected either the marked
180 or the unmarked subgroup and transferred it for one hour to a small Petri dish containing an
181 *ad libitum* amount of green-colored food (donor sub-group; naturally yellow colored pollen
182 mixed with a blue food dye; Dekoback, Online Ideen GmbH, Germany). Meanwhile, the other
183 (recipient) sub-group was food-deprived whereas the mother was provided separately with
184 uncolored pollen. The nymphs of the donor and recipient sub-group were then assembled
185 overnight with their mother in a medium-sized Petri dish. Fifteen hours after the setup, we

186 counted all nymphs in their respective sub-groups and determined the number of newly
187 green-colored nymphs in the recipient sub-group under a stereo-microscope. We fed
188 mothers, and thus allowed for maternal provisioning during sibling food transfer tests,
189 because this ensured a more direct link between our measures of maternal food provisioning
190 and sibling food transfer. Note that mothers were isolated and fed with uncolored pollen
191 pellets between day five and eight to ensure that the feces of mothers first involved in the
192 maternal food provisioning measurement (see below) had lost their coloration before sibling
193 food transfer measurements.

194 We refrained from feeding a fixed number of donor nymphs across clutches of
195 different size, because this would have artificially increased competition for colored feces in
196 large clutches. Instead, always feeding half of the nymphs of the SFT-group ensured that the
197 *per capita* availability of feces for recipient nymphs was *a priori* independent of clutch size.
198 Because the higher absolute amount of feces available during sibling food transfer tests in
199 larger clutches could potentially promote competition among multiple recipients and thus
200 influence the distribution of feces (see also discussion), we additionally assessed the intensity
201 of coloration in a random subset of 31 clutches by differentiating between strongly and
202 weakly colored nymphs. Strongly colored nymphs generally exhibit a homogenous coloration
203 of their entire body that is visible to the naked eye, whereas weakly colored nymphs only
204 show a light coloration of their gut that can often only be seen on their ventral side and when
205 using binoculars. If competition for a constant *per capita* amount of feces increases with
206 clutch size, we would expect an increase of sibling food transfer with clutch size accompanied
207 by an increase in the proportion of nymphs that received only little food from their siblings
208 and thus were only weakly colored.

209 Maternal food provisioning was measured by (1) providing the mother with green-
210 colored food, then (2) reassembling the fed mother with the nymphs of the MFP-group, (3)
211 allowing them to interact overnight and finally (4) counting the number of nymphs that
212 ingested the colored food provided by the mother. Specifically, maternal food provisioning
213 was measured using the entire MFP-group, in which half of the nymphs were marked by
214 clipping their cercus to ensure that marking could not hamper comparisons between
215 maternal food provisioning and sibling food transfer. Marking the nymphs did not affect
216 maternal provisioning (Wilcoxon signed rank test; $V = 191.5$, $P = 0.962$). After the nymphs had
217 been marked, the mother had access to colored food for one hour, whereas all the nymphs
218 were food deprived. Subsequently, the nymphs of the MFP-group and their mother were
219 assembled overnight in a medium-sized Petri dish to allow food transfer between individuals.
220 Note that the number of recipient nymphs during the maternal food provisioning test was
221 large (i.e. twice the number of recipient nymphs used in the sibling food transfer test) to
222 account for the higher absolute amount of food that mothers can potentially provide to their
223 offspring (Mas & Kölliker, 2011; Meunier & Kölliker, 2012a; Meunier *et al.*, 2012). Fifteen
224 hours after the setup, we counted the number of marked and unmarked green-colored
225 nymphs. Overall, the scoring of nymphal coloration was performed by one single observer
226 and – in case of the second food transfer test - blindly regarding the level of food transfer
227 measured during the first food transfer test in the same clutch.

228 **STATISTICAL ANALYSES**

229 All statistical analyses were performed using the software R 3.0.1 (<http://www.r-project.org/>)
230 complemented with the packages “car” and “MASS”. We first tested the overall importance

231 of sibling food transfer (SFT) and maternal food provisioning (MFP) on the gain in coloration
232 of recipient nymphs using a generalized linear mixed model (GLMM) with a binomial error
233 distribution corrected for overdispersion. In this model, the proportion of colored recipient
234 nymphs was entered as response variable (via the “cbind”-function in R), while the type of
235 test (SFT or MFP; categorical), the order of the tests (SFT/MFP or MFP/SFT; categorical), clutch
236 size (continuous) and all interactions among these three factors were entered as explanatory
237 variables. Because each clutch was used to measure both sibling food transfer and maternal
238 food provisioning, clutch-ID was entered as a random factor into the model. To control
239 whether differences in the number of recipient nymphs involved in the sibling food transfer
240 and maternal food provisioning tests drove the results of the above model, we conducted an
241 additional linear mixed model (LMM), in which we used the same set of explanatory and
242 random variables but entered the absolute number of colored recipient nymphs as response
243 variable. The potential influence of clutch size on the distribution of food among multiple
244 recipient nymphs during sibling food transfer tests was analyzed in a generalized linear model
245 (GLM) with binomial error distribution corrected for overdispersion. In this model, we entered
246 the proportion of weakly colored nymphs among all colored recipients as response and clutch
247 size (continuous) as explanatory variable.

248 We then analyzed whether the level of sibling food transfer was positively
249 (complementarity hypothesis) or negatively (compensation hypothesis) associated with the
250 level of maternal food provisioning in each clutch. To this end, we tested the correlation
251 between the deviations from the predicted levels of sibling food transfer and of maternal food
252 provisioning using a Pearson product-moment correlation. These deviations were defined as
253 the residuals of sibling food transfer and maternal food provisioning from the first model, i.e.
254 the parts of the proportions of colored recipient nymphs in sibling food transfer and maternal

255 food provisioning tests, respectively, that were not explained by clutch size and order of
256 testing. Note that we back-transformed the residuals to their original (i.e. proportional) scale
257 to facilitate their interpretation in the figures.

258 Finally, we tested whether deviations from the predicted level of sibling food transfer
259 (see above for definition) were linked to offspring fitness and/or maternal investment in the
260 2nd clutch. We calculated a series of four linear models and three generalized linear models
261 with binomial error structure corrected for overdispersion. In the linear models (LMs), the
262 deviations from sibling food transfer were entered as an explanatory variable, and the
263 development time of nymphs, the time from isolation to 2nd clutch production, the number
264 of eggs or the number of nymphs in the 2nd clutch as continuous responses. In the generalized
265 linear models (GLMs), we entered the proportion of 1st clutch-nymphs that survived until
266 adulthood (continuous), the occurrence of 2nd clutch production (bimodal) or the hatching
267 success of the 2nd clutch (continuous) as response variable. Note that we also tested the effect
268 of deviations from the predicted level of maternal food provisioning on the above mentioned
269 measures of offspring fitness and maternal investment in the 2nd clutch. The results of the
270 corresponding analyses, which resemble the results based on the deviation from the
271 predicted level of sibling food transfer but in opposite directions, are given in Table S1.

272 Statistical models were simplified stepwise by removing non-significant interaction
273 terms ($P > 0.05$). The significance-level for the analyses of maternal 2nd clutch production and
274 fitness traits in offspring was adjusted to correct for multiple testing using the MFDR (Mean
275 False Discovery Rate) approach to $\alpha c = 0.029$ according to $\alpha c = \frac{n+1}{n \times 2} \times 0.05$ where n denotes
276 the number of tests. Our analyses involved 48 of the 54 clutches initially set up. Among the 6
277 clutches not used in the analyses, (1) three were excluded because nymphs of the donor-
278 group either escaped or were cannibalized by their siblings, which potentially could have

279 biased our measure of food transfer, (2) two clutches showed an exceptionally high
280 proportion of donor nymphs that failed to feed on the green colored food prior to the sibling
281 food transfer test (50 and 70%, respectively vs. $4 \pm 8\%$ (Mean \pm SD) in the remaining clutches)
282 and finally (3) one clutch was excluded because the mother still produced green-colored feces
283 prior to the sibling food transfer test, which prevented reliable measurements of sibling food
284 transfer. As a result, the analyzed data set comprised 24 of the 28 clutches that were
285 subjected to sibling food transfer measurements on day four, and 24 of the 26 clutches used
286 to measure sibling food transfer on day eight. A total of 41 out of 48 (85.4%) mothers
287 produced a 2nd clutch and were used to analyze the time from isolation to 2nd clutch
288 production, as well as the hatching success of the 2nd clutch.

Results

289 Overall, food transfer among nymphs occurred more frequently than maternal provisioning
290 (94% vs. 67% of all clutches; $\chi_1^2 = 9.45$, $P = 0.002$). The proportion of newly-colored recipient
291 nymphs varied substantially between families and ranged from 0 to 100% after both types of
292 food transfer tests, with a median value of 75% of recipient nymphs newly colored in the
293 sibling food transfer test and 22% in the maternal food provisioning tests (Wilcoxon signed
294 rank test, $V_{47} = 169$, $P < 0.001$; Fig. S1). These values are comparable to the proportions
295 presented in previous studies (Meunier & Kölliker, 2012a; Meunier *et al.*, 2012; Falk *et al.*,
296 2014), indicating that they are unlikely to only reflect the different initial numbers of recipient
297 nymphs during the two types of food transfer tests, as well as the limited contacts between
298 mother and nymphs during the experiment.

299 An interaction between clutch size and the type of food transfer shaped the
300 proportion of recipient nymphs that became colored during the food transfer tests (Table 1A,
301 Fig. 2A). Specifically, the proportion of colored nymphs was positively associated with clutch
302 size in the sibling food transfer tests (Estimate \pm SE: 0.035 ± 0.015 , $t_{46} = 2.43$, $P = 0.019$), but
303 not in the maternal food provisioning tests (Estimate \pm SE: -0.016 ± 0.019 , $t_{46} = -0.86$, $P =$
304 0.386). The contrasting influence of clutch size on maternal food provisioning and sibling food
305 transfer was also present when analyzing the absolute number of newly colored recipient
306 nymphs (Table 1B, Fig. 2B). Accordingly, the number of nymphs that had received food
307 increased with clutch size in the case of sibling food transfer (Estimate \pm SE: 0.240 ± 0.028 , t_{46}
308 $= 8.635$, $p < 0.001$), but not in the case of maternal food provisioning (Estimate \pm SE: $0.086 \pm$
309 0.073 , $t_{46} = 1.18$, $p = 0.243$). Contrary to the expectation that competition for a constant per
310 capita amount of feces increases with clutch size, the ratio of nymphs that were weakly
311 colored after sibling food transfer tests did not depend on clutch size ($\chi_1^2 = 0.44$, $P = 0.501$).
312 Independent of other effects, the proportion and absolute number of nymphs that received
313 food from family members was higher on day eight (Mean \pm SE; proportion = 0.55 ± 0.05 ;
314 absolute = 7.75 ± 0.82) than on day four (Proportion = 0.42 ± 0.05 ; absolute = 5.67 ± 0.69)
315 after hatching (Table 1A and 1B, Fig. S2), presumably reflecting the increased nutritional
316 needs of older nymphs (Wong & Kölliker, 2012).

317 The level of sibling food transfer was negatively associated with the level of maternal
318 food provisioning after taking the influences of clutch size and the day of the respective food
319 transfer test into account ($\rho = -0.306$, $S_{46} = 24062$, $P = 0.035$, Fig. 3). This result is in line with
320 the hypothesis of a compensatory relationship between sibling cooperation and parental
321 care.

322 Finally, the level of food transfer among siblings was correlated with the expression of
323 fitness-relevant traits in mothers, but not in their offspring. Specifically, higher than predicted
324 levels of sibling food transfer were associated with increased delays in the mother's
325 production of a 2nd clutch (Table 2A, Fig. 4A) and reduced numbers of 2nd clutch eggs (Table
326 2A, Fig. 4B). They were however not linked to the occurrence of 2nd clutch production (Table
327 2A), the hatching success of the 2nd clutch eggs (Table 2A) or the number of resulting nymphs
328 (Table 2A). Higher than predicted levels of sibling food transfer were neither associated with
329 the development time of 1st clutch offspring (Table 2B), nor with the probability of their
330 survival until adulthood (Table 2B).

Discussion

331 A growing number of studies have demonstrated that the benefits of family life for offspring
332 do not only derive from parental care, but can also arise from cooperative interactions with
333 juvenile siblings (e.g. Botelho *et al.*, 1993; Biedermann & Taborsky, 2011; Roulin *et al.*, 2012;
334 Yip & Rayor, 2013; Falk *et al.*, 2014). In addition to challenging the so far almost exclusive
335 focus on parental care as the predominant mechanism promoting the emergence of family
336 life (e.g. Clutton-Brock, 1991; Royle *et al.*, 2012), these findings prompted the question
337 whether sibling cooperation and parental care might have jointly shaped the evolutionary
338 transition from solitary to social life.

339 In this study, we showed that, in the European earwig, the level of sibling food transfer
340 (a form of sibling cooperation characterized by a socially induced, kin-directed production of
341 feces that can be consumed by other group members) - but not of maternal food provisioning
342 (a form of parental care) - increased with group size. Notably, this increase was not associated

343 with a change in food distribution among offspring, a result expected under the assumption
344 that recipient nymphs do not compete more intensively for donor feces in larger clutches.
345 Furthermore, higher than predicted levels of sibling food transfer (with regards to clutch size
346 and the day of measurement) were associated with lower than predicted levels of maternal
347 food provisioning and *vice versa*. This finding is in line with the hypothesis of a compensatory
348 relationship between sibling food transfer and maternal food provisioning. Finally, higher
349 than predicted levels of sibling food transfer were associated with a delayed production and
350 reduced size of the mothers' 2nd clutch, but not with the development time and the survival
351 of 1st clutch nymphs until adulthood, the hatching success of the 2nd clutch eggs and the
352 number of resulting nymphs.

353 A compensatory association between sibling food transfer and maternal food
354 provisioning during family life suggests that the benefits of parental care and sibling
355 cooperation can be entangled and could have jointly promoted the early evolution of group
356 living. Parental provisioning of offspring is a derived form of care that has been proposed to
357 emerge from an ancestral state resembling that of contemporary precocial species (Gardner
358 & Smiseth, 2010). In this state, benefits of food sharing and/or other forms of sibling
359 cooperation might have played an essential role in maintaining family life, while
360 simultaneously setting the stage for the evolution of parental provisioning, e.g. by providing
361 the offspring with an additional incentive to re-aggregate after independent foraging trips
362 which in turn could enable the parent(s) to (mass-)provision their offspring more effectively.
363 The coexistence of maternal food provisioning and sibling food transfer in earwig families
364 suggests that sibling food transfer might currently be maintained in families of the European
365 earwig to compensate for low levels or even complete lack of maternal food provisioning
366 (Mas & Kölliker, 2011; Meunier & Kölliker, 2012b; Meunier *et al.*, 2012). The overall higher

367 prevalence of sibling food transfer as compared to maternal food provisioning is in line with
368 this scenario, as it suggests that sibling food transfer also occurred in families in which the
369 mother did not provision (this study), or was experimentally prevented from provisioning her
370 offspring (Falk *et al.*, 2014). Conversely, sibling food transfer might have evolved secondarily
371 to compensate for low levels of maternal food provisioning. This alternative scenario is
372 however unlikely since the evolution of parental provisioning also drives the evolution of
373 increased levels of sibling competition (Smiseth *et al.*, 2007; Gardner & Smiseth, 2010), which
374 in turn should impede the evolution of sibling cooperation (Frank, 1998; West *et al.*, 2001).
375 As a consequence, some forms of sibling cooperation, especially if they involve the exchange
376 of resources acquired independently from parents, could be lost when the evolution of
377 parental provisioning progresses.

378 While sibling food transfer reflects a form of cooperation by donor nymphs (Falk *et al.*,
379 2014), both cooperative as well as competitive behaviors could mediate the distribution of
380 the publicly available feces among recipient nymphs. Cooperation is generally less likely to
381 occur if competition between interacting individuals is high (Frank, 1998; West *et al.*, 2001).
382 Accordingly, the incentive of offspring to share food should be inversely related to the severity
383 of sibling competition, which in turn is classically assumed to increase with group size
384 (Alexander, 1974; but see Shen *et al.*, 2014). Contrary to this prediction, our results showed
385 that the level of sibling food transfer increased with group size. This increase of sibling food
386 transfer could reflect an increased propensity of donor nymphs to transfer food to their
387 siblings in larger clutches. Such an association could be expected if the higher number of
388 potential donors in larger clutches ensures that the likelihood of reciprocally receiving food
389 in times of need is increased. This in turn would lower individual costs of food sharing.

390 Alternatively or additionally, the association of sibling food transfer with group size
391 could reflect increased competition of recipient nymphs in larger clutches. In this situation,
392 the *per capita* amount of feces cooperatively produced by donor nymphs would be
393 independent of group size and the increase of sibling food transfer with group size would be
394 solely based on increasing scramble competition among recipients for the publicly available
395 feces. Such an increase of sibling competition with clutch size could for example be expected
396 if maternal investment in individual offspring decreases with increasing clutch size. In line
397 with the hypothesis of increased competition in larger clutches, sibling rivalry has been shown
398 to increase with group size in earwigs (Kölliker, 2007; Meunier & Kölliker, 2012b). However,
399 contrary to this hypothesis, we found the level of maternal food provisioning to be
400 independent of clutch size. Likewise, the proportion of recipients that received only little food
401 from their siblings did not increase with clutch size, indicating that competition did not lead
402 to a more skewed distribution of food in larger clutches. Finally, the increase of sibling food
403 transfer with clutch size could be independent of changes in nymphal behavior with clutch
404 size, and instead simply reflect the increased absolute amount of donor-feces available to the
405 recipients in larger clutches and/or secondary transfer of colored food among recipient
406 nymphs. These hypotheses are however unlikely to be the sole drivers of our results, as the
407 absolute number of recipient nymphs linearly increased with clutch size (and the increased
408 amount of feces is thus accounted for in our sibling food transfer measurement) and because
409 each individual cannot produce a larger amount of feces than the amount of resources it
410 previously ingurgitated (i.e. a nymph's feces production cannot feed more than one sibling
411 until satiety). The mechanism(s) underlying the increase of sibling food transfer with clutch
412 size will be explored in further studies.

413 The benefits of sibling cooperation have been proposed to be an important driver of
414 the evolution of family life (Falk *et al.*, 2014). However, we found that higher than predicted
415 levels of sibling food transfer were neither linked to offspring survival until adulthood nor
416 associated to their development time. One potential explanation for this apparent lack of
417 fitness benefits for offspring is that sharing food with siblings does not augment the overall
418 benefits of maternal care (Kölliker, 2007), but rather only allows nymphs to compensate for
419 the detrimental effects of low levels of maternal food provisioning. In line with this
420 hypothesis, we found that higher than predicted levels of sibling food transfer were
421 associated with lower than predicted levels of maternal food provisioning. Moreover, Falk et
422 al. (2014) observed lower levels of sibling food transfer when nymphs had the possibility to
423 freely interact with their mother, suggesting that nymphs could prefer maternal food
424 provisioning over sibling food transfer due to the higher quality of the maternally-provided
425 food. Alternatively, the absence of differences in survival could also reflect a limited
426 importance of sibling food transfer (and maternal food provisioning) for nymphal survival
427 under laboratory conditions. This could be the case since these conditions allow self-foraging
428 in the absence of the risk of predation and consequently relax nymphal dependence on
429 resources obtained by other family members.

430 Sibling cooperation by definition entails benefits for offspring, but cooperative
431 interactions among their offspring could also benefit parents, for example by reducing
432 offspring demand and hence allowing parents to reduce investment in parental care.
433 However, earwig mothers tending clutches with higher than predicted levels of sibling food
434 transfer did not produce larger 2nd clutches, despite the fact that they simultaneously showed
435 low levels of maternal food provisioning. Interestingly, these mothers even produced fewer
436 2nd clutch eggs and delayed the production of their 2nd clutch longer than mothers tending

437 nymphs that showed lower levels of sibling food transfer. Hence, mothers likely do not
438 selectively retain resources for the production of their 2nd clutch when their 1st clutch
439 offspring shows high levels of sibling food transfer. Instead, the combination of low levels of
440 maternal food provisioning during 1st clutch family life and the small size and delayed
441 production of the 2nd clutch suggests that variation in maternal investment into offspring care
442 and future reproduction reflect differences in intrinsic female quality (Reznick *et al.*, 2000;
443 Koch & Meunier, 2014). Whether and how such differences in female (and nymph) quality
444 affect sibling food transfer will be investigated in further studies.

445 To conclude, our study reveals that maternal care and sibling cooperation are
446 interdependent processes that together shape food acquisition by offspring in the European
447 earwig *F. auricularia*. Our results are in line with a compensatory relationship between sibling
448 cooperation and maternal care and thus suggest that sibling cooperation is an ancestral
449 behavior that can persist to mitigate the detrimental effects of low levels of parental care.
450 More generally, our findings stress the importance of sibling cooperation among juvenile
451 offspring in the early evolution of social life, especially if the cooperative interactions involve
452 the transfer of resources acquired independently of parents.

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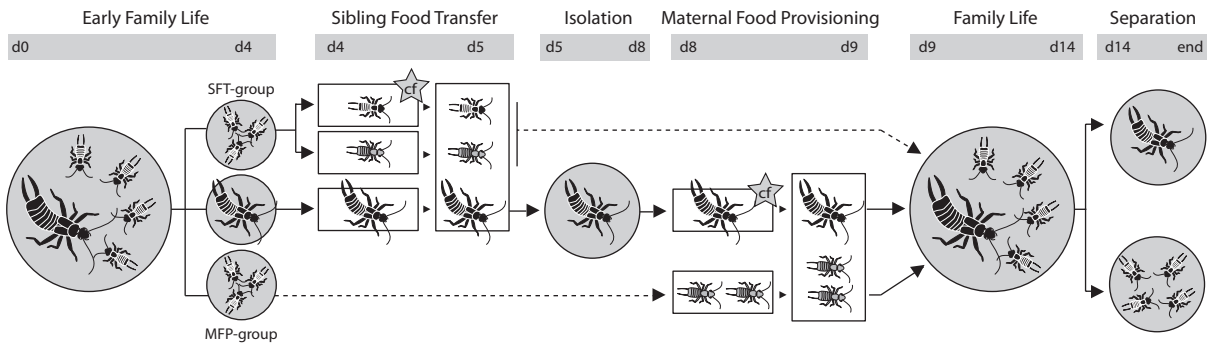
539 **Table 1:** Effects of type of test (sibling food sharing or maternal food provisioning), clutch size
 540 and day of food-transfer test on (A) the proportion and (B) the number of nymphs that
 541 received food from family members. Significant P-values are in bold print.

	(A) Proportion of nymphs colored		(B) Number of nymphs colored	
	Wald χ_1^2	p	Wald χ_1^2	p
Type of food-transfer (TFT)	44.44	< 0.0001	2.72	0.0992
Clutch size (CS)	< 0.01	0.9533	19.46	< 0.0001
Day of food-transfer test	8.19	0.0042	5.95	0.0147
TFT:CS	6.85	0.0089	5.33	0.0209

542

543 **Table 2:** Effects of the deviation from the predicted level of sibling food transfer on either (A)
 544 traits of mothers and nymphs in the second clutch or (B) traits of nymphs in the first clutch.
 545 Statistical values were obtained from linear models (LM) or generalized linear models (GLM).
 546 P-values that remained significant after correction for multiple testing are in bold.

	Deviation from predicted sibling food transfer				
	Model	n	Estimate ± SE	t	P
(A) Second clutch					
Occurrence of 2 nd clutch production	GLM	48	-4.06 ± 2.50	-1.63	0.111
Days between isolation and egg laying	LM	41	8.99 ± 3.63	2.48	0.018
Egg number	LM	48	-27.27 ± 10.57	- 2.58	0.013
Hatching success	GLM	41	1.07 ± 1.13	0.95	0.347
Nymph number	LM	48	-8.62 ± 11.67	-0.74	0.464
(B) First clutch					
Nymph development time	LM	48	-1.16 ± 0.57	- 2.05	0.047
Nymph survival until adulthood	GLM	48	0.13 ± 0.23	0.57	0.574



547

548 **Figure 1:** Experimental setup. White boxes indicate measurements of sibling food transfer

549 and maternal food provisioning. Sibling food transfer was measured by providing the SFT-

550 group of nymphs (called donor nymphs) with colored food (indicated with “cf”), then

551 reassembling these newly colored nymphs with a group of food deprived siblings (called

552 recipient nymphs; grey individuals) and their mother, allowing family interactions overnight

553 and finally counting the number of recipient nymphs that ingested the colored food provided

554 by the donor nymphs. Conversely, maternal food provisioning was measured by providing the

555 mother with colored food, then reassembling the fed mother with the MFP-group of nymphs

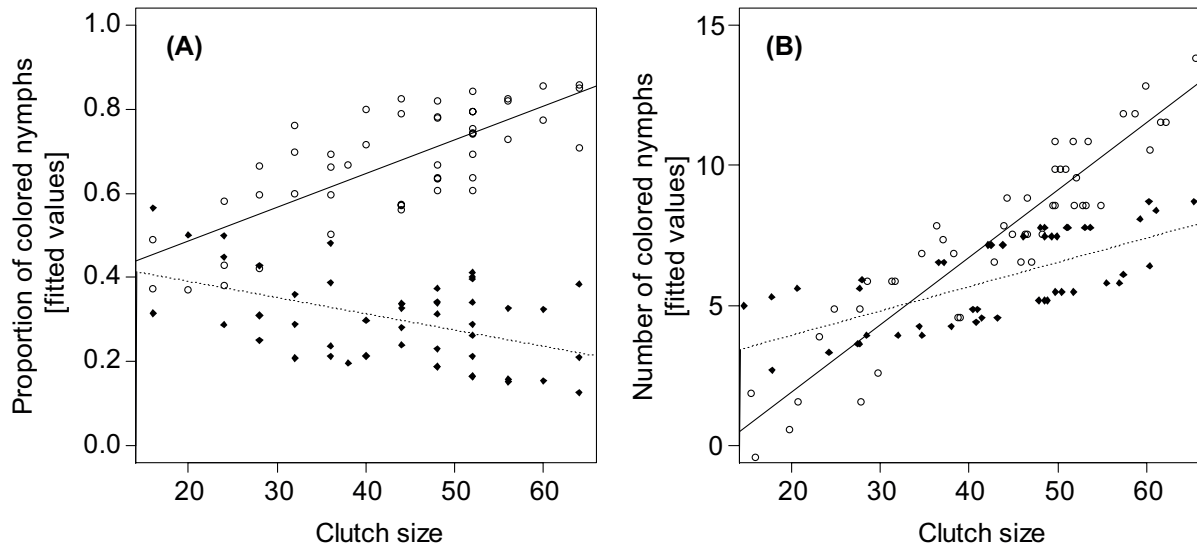
556 previously set aside, allowing them to interact overnight and finally counting the number of

557 nymphs that ingested the colored food provided by the mother. Note that the order of food

558 transfer test was reversed in half of the tested families.

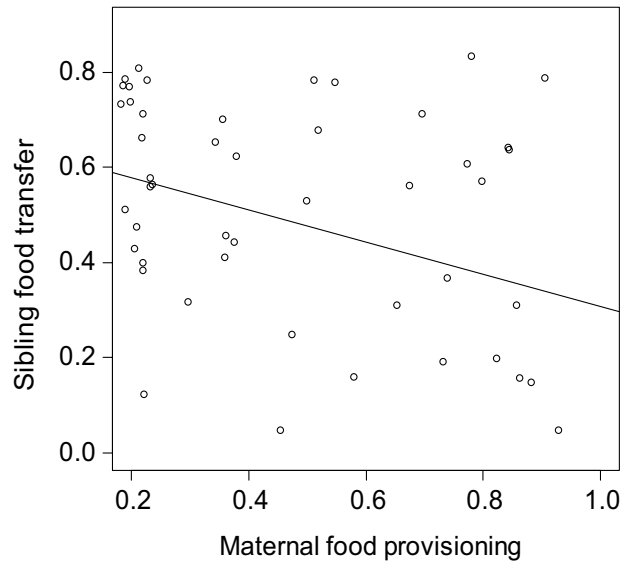
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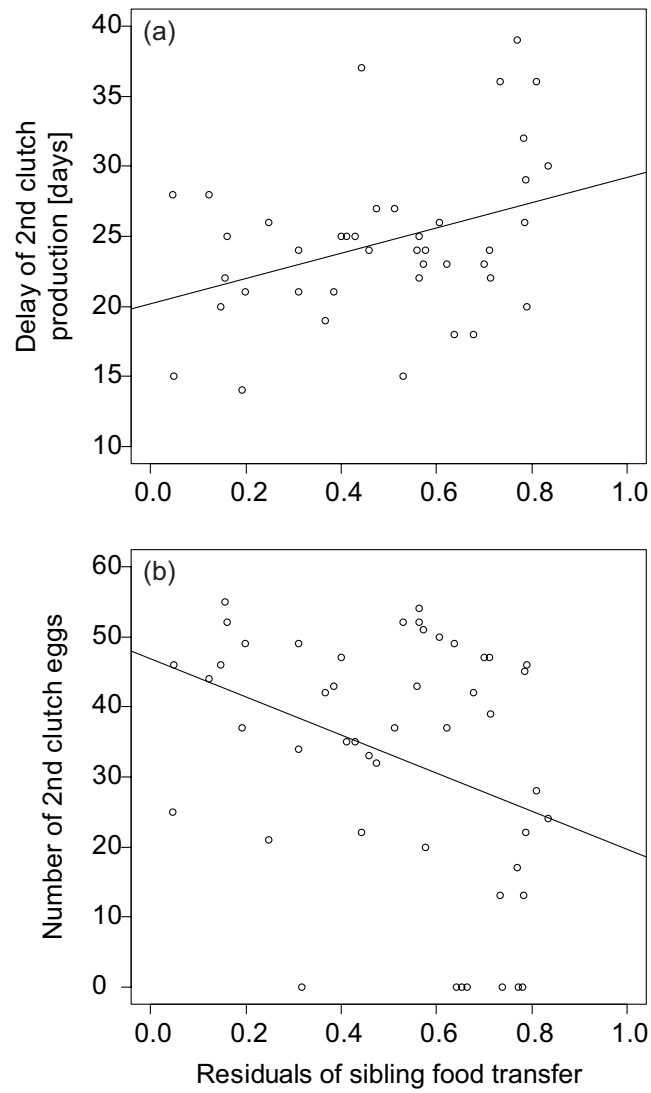
560 **Figure 2:** Influence of clutch size on the proportion (A) and number (B) of nymphs that
 561 received food during maternal food provisioning (filled squares) and sibling food transfer
 562 (open circles).

563



564 **Figure 3:** Correlation of the residuals of sibling food transfer and maternal food provisioning
565 after taking the influences of clutch size and the day of the respective food transfer test into
566 account (see Results).

567



568 **Figure 4:** Correlation of the residuals of sibling food transfer with (A) the duration from
 569 maternal isolation until 2nd clutch production, and (B) the number of eggs in the 2nd clutch.