Maternal care and interactions within and between families: How the environment and chemical communication shape family life in a social insect

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SUMMARY

The evolution of parental care represents an important step in the evolution of sociality and is widespread across different species and different taxa. Parental care is a trait that shows a wide diversity regarding duration and forms of care within and between species and is an important field of research both in evolutionary biology and behavioral ecology. However, its importance in these fields has only been recognized relatively recently. Environmental effects influence condition and individuals will choose distinct behavioral strategies to maximize their fitness. Condition can be communicated through chemicals and such condition-dependent cues can be used by conspecifics to adjust their own behavior. The focus of my dissertation was the investigation of environmental effects and chemical signaling on maternal care and within and between family interactions. I used the European earwig (*Forficula auricularia*) as a model system for my experimental work.

As an introductory chapter, I have written a review article about the evolution of parental care in insects (Chapter 1). Here, I summarized present hypotheses about the roles of ecology and life history from the literature and combined them with new suggestions regarding the influence of social interactions on parental care.

In my first experiment (Chapter 2), I investigated the influence of maternal nutritional condition on mother-offspring interactions. Female condition was manipulated through a high-food and a low-food treatment. I could show that the period and amount of maternal food provisioning was dependent on the condition of the female. Females in poor condition provided food to fewer nymphs and for a shorter period of time compared to females in good condition. Offspring attendance remained at a constantly high level independent of female condition and was maintained by both the female and the nymphs, suggesting strong benefits of living in a (family) group.

In my second experiment (Chapter 3), I investigated effects of nymph condition and food availability on brood mixing in *F. auricularia*. Females provide care for foreign nymphs that join their brood. This however increases brood size and thus competition between the offspring. Previous work showed that cannibalism is directed primarily against unrelated nymphs under conditions of low food availability. My results showed that the brood mixing dynamics are influenced by the condition of the nymphs, but are independent of the food availability in the environment. The overall degree of brood mixing was high, suggesting again benefits of living in groups.

Furthermore, my third experiment (Chapter 4) tested the presence of family specific cuticular hydrocarbon profiles. Insects mainly use cuticular hydrocarbons as means of communication and

individual recognition. Previous results from our group show that there are significant negative effects of inbreeding, which makes kin recognition important in this species. I could show that cuticular hydrocarbon profiles are indeed family specific and that the earwigs have the potential to use them to discriminate kin from non-kin. This allows to direct social behaviors to the appropriate individuals and to avoid inbreeding and the associated fitness losses.

In a fourth experiment (Chapter 5), I tested the effect of maternal condition-dependent cues on nymph selfishness and survival. In species where parental care is provided, offspring could use cues of parental condition to adjust their begging behavior and their selfishness by varying the degree of sibling competition. My results show that maternal condition cues influence offspring survival depending on the time of breeding, and further suggest that offspring use these maternal condition-dependent cues to adjust their degree of selfishness, which changes during the breeding season. Together with former evidence on maternal sensitivities to condition-dependent nymph chemical cues, my study shows context-dependent reciprocal information exchange about condition between earwig mothers and their offspring, mediated by cuticular hydrocarbons.

My last experiment (Chapter 6) investigated the effects of high and low food availability during the juvenile and early adult development and its influences on development, maternal care and egg production. Females of the European earwig represent two distinct phenotypes. One type produces only one clutch, the other type produces two clutches during its life. Previous experiments have shown that the phenotype is not purely inherited genetically, but likely to be condition-dependent. My results revealed that especially restriction in the late juvenile development has negative effects on development and the probability of second clutch production. Environmental conditions experienced early in an individual’s lifetime can have detrimental effects once individuals become adult and need to be considered to understand individual variation in reproductive success and life-history trade-offs.

All in all my results show that behavioral strategies are driven by the condition of the individuals involved. I demonstrated that condition is reflected in the cuticular hydrocarbon profiles of the earwigs. Such condition-dependent chemical cues allow individuals to adjust their behavior according to their own state and to the state of interacting conspecifics. I showed how the environment and chemical communication shape family life in a social insect and revealed how this affects not only maternal care and social interactions within and between families, but also reproductive success of individuals. Thus, my work shows how individual condition affects not only parent-offspring interactions, but also major life history traits like reproductive success.

GENERAL INTRODUCTION

The evolution of parental care is considered an important step in the evolution of sociality. Parental care is widespread across the animal kingdom and it is defined as any parental trait that enhances the fitness of a parent's offspring, and that is likely to have originated and/or is currently maintained for this function (Royle et al. 2012). Ecological factors such as harsh environments, ephemeral food sources or predation pressure were suggested as prime movers for the evolution of parental care (Wilson 1975), but see Chapter 1). Forms of care range from the provisioning of gametes over egg and offspring attendance to highly elaborated variations of food provisioning and even care for mature offspring (Smiseth et al. 2012). Parental care can be performed exclusively by females like in most mammals or arthropods (Clutton-Brock 1991; Trumbo 1996), exclusively by males like in most fishes (Gross & Sargent 1985), or by both sexes combined like in most birds (Cockburn 2006). But there are also a substantial number of exceptions to these trends. Care can be obligate if offspring are not able to survive without at least some parental effort (e.g. birds and most mammals but also in some insects like the giant water bug (Smith 1997)). Contrary, in facultative care species offspring benefit from parental care, but are not fully dependent on it after hatching, e.g. (Krafft et al. 1986; Smiseth & Moore 2002; Kölliker 2007). Insects show a broad diversity in extent and forms of care (see Chapter 1) and thus offer a unique system to study to evolution of parental care.

However, parental care often comes at a cost of survival or future reproduction to the parent, who provides it. This cost-benefit-ratio differs between parents and offspring due to asymmetries in genetic relatedness, leading to a conflict between the parents and the offspring ('parent-offspring conflict') (Trivers 1974). A focal offspring is 100% related to itself, but only 50% related to a full-sibling and even less to a half-sibling. Thus, personal fitness (direct fitness) weighs more than the fitness of its present and future siblings (indirect fitness) (Hamilton 1964; Trivers 1974). Caring parents, on the other hand, are 50% related to all of their offspring, and the focal offspring has equal weight compared with all other offspring in terms of inclusive fitness (Trivers 1974). Each offspring begs for resources to assure its own survival. But the benefits of the parental investment should not be exceeded by its costs.

The environment influences the condition and accordingly the behavioral strategies that individuals will choose to maximize their own fitness. Especially food availability in the environment directly affects the pool of resources, which can be invested into reproduction and parental care. Thus, the cost-benefit-ratio of parental care depends on the condition of the parents and the offspring. For example, in different species of mammals, female nutritional condition influences the period of lactation (Lee 1984; Lycett et al. 1998; Laurien-Kehnen & Trillmich 2004; Wheatley et al. 2006). Poor nutritional condition can lead to shorter periods of lactation in some species (Lee 1984; Wheatley et al. 2006), but in others it will lead to an increase in lactation period (Lycett et al. 1998; Laurien-Kehnen & Trillmich 2004). The expected directionality of the effect of female condition on parental care cannot easily be generalized because it depends on a species' life history and how variation in current condition affects future reproductive success (Lee et al. 1991).

Condition is also influenced by the social environment, for example through cooperation or conflict between individuals. Within families, social interactions occur between parents and offspring or among siblings. This social environment forms part of an individual's ecology and generates new selection pressures, for example through selection on parental food provisioning and offspring begging signals, or through conflicts of interest within families (Trivers 1974). Caring parents are a social environment to which offspring should adapt, and offspring are a social environment to which parents should adapt, and these novel selection pressures should lead to parent and offspring adaptations to family life and the co-adaptation of their traits (Kölliker et al. 2012). Depending on the density of the population and the mobility of parents and offspring, social interactions might also occur between unrelated individuals origination from different families. The costs of parental care can lead to brood parasitic strategies by the parents (Moczek & Cochrane 2006) or by the offspring, if they are mobile (Kölliker & Vancassel 2007). Kin recognition mechanisms are expected to evolve, if these social interactions are beneficial for one, but costly for the other side.

In insects, kin recognition is typically based on chemical cues present on the waxy layer covering their cuticle (Singer 1998; Howard & Blomquist 2005; Blomquist & Bagnères 2010). This waxy layer is mainly composed of cuticular hydrocarbons (CHC), which form a chemical signature that can vary qualitatively and/or quantitatively among species, families and even individuals (Howard & Blomquist 2005). Moreover, CHC were shown previously to also reflect the nutritional condition (Mas et al. 2009) or the social environment of individuals (Meunier et al. 2011). CHC can thus be important cues determining the direction of social interactions within and between families.

The aim of my dissertation was to investigate how the environment and chemical communication influence maternal care and social interactions within and between families, and thus shape family life in a social insect.

The model organism

I conducted my experimental work with the European earwig (*Forficula auricularia* L.; Dermaptera: Forficulidae). This insect model system is especially suited for the investigation of parental care, parent-offspring conflict, family dynamics, and chemical signaling for several reasons. Female earwigs provide maternal care for their offspring (nymphs) in terms of food provisioning and protection against predation, but care is only facultative and nymphs can survive without the mother after hatching (Lamb 1975; 1976b; 1976a; Kölliker & Vancassel 2007). This flexibility of the system provided me the broad necessary scope for experimental manipulation of family life (e.g., presence or absence of maternal care) compared to obligate care systems like mammals or birds, where young offspring are fully dependent on the parents for survival. Furthermore, female earwigs produce one or two clutches in their lifetime (Meunier et al. 2012), indicating scope for mother–offspring conflict over the timing of termination of care (Kölliker 2007). Previous work in *F. auricularia* showed that offspring pheromone signals influence maternal care (Mas et al. 2009; Mas & Kölliker 2011a) and the timing of second clutch production in females (Mas & Kölliker 2011b). Finally, it was also shown that under experimental food restriction, maternal presence can become costly for the offspring, reducing nymph survival until adulthood (Meunier & Kölliker 2012).

Thesis outline

Chapter 1

In this review article, I summarized and discussed empirical evidence from insects for influences of ecology, life history and the social environments on the evolution of parental care. The focus was on the question how ecological factors may interact with animal life histories, and how life histories can be both, evolutionary cause and effect of parental care. Subsequently, I elaborated on how the social environment can influence parental care via interactions within and between families, discussed how family interactions can affect potential benefits and costs associated with parental care, and explored how parent and offspring strategies may evolve as a consequence of these socially mediated modifications of selection on parents and offspring (published as Wong et al. 2013).

Chapter 2

In my first experiment, I investigated the effect on female condition on maternal care. Condition effects on offspring have been broadly studied, e.g., (Kölliker et al. 2005; 2006; Mas et al. 2009), but the effect of parental condition on parent–offspring interactions is less well understood, in particular in species where care is facultative and offspring have the option to beg for food from the parents or to self-forage. In my study, I manipulated female condition in *F. auricularia* and tested the effect of female condition on nymph self-foraging, maternal food provisioning and maternal attendance. Mother-offspring aggregation remained at a high level throughout the experiment suggesting benefits of living in a (family) group, which lead to my second experiment (published as Wong & Kölliker 2012).

Chapter 3

Since parental care is costly for parents in terms of future reproduction, it seems maladaptive to care for unrelated offspring. One exception might be if the additional offspring provide direct benefits to the own offspring. Females of the European earwig provide care for foreign nymphs and do not seem to discriminate between own and foreign offspring (Kölliker & Vancassel 2007). My second experiment investigated brood mixing dynamics and the effects of nymph condition and environmental food availability. The overall degree of brood mixing was high, suggesting again benefits of living in groups (published as Wong & Kölliker 2013). In my third experiment, I investigated whether living in social groups influences the chemical signature of earwigs and if this has consequences on kin recognition cues, which could affect both nymph cannibalism and inbreeding in adults.

Chapter 4

F. auricularia shows gregarious behavior and group living throughout most of its life cycle. The juvenile stages are highly gregarious and also the adults are always in close physical contact with each other, giving scope for social transfer of CHC between individuals. In my third experiment, I investigated whether the CHC profiles of nymphs and adult individuals of *F. auricularia* reflect their maternal origin, by taking chemical extractions from individuals originating from different families. My results show that the CHC profiles in *F. auricularia* display information about the genetic origin and (social) environment. (Wong, Meunier, Lucas & Kölliker, submitted). Previous work showed that also the nymph condition is reflected in their chemical signature, which influences maternal care and mother-offspring interactions (Mas et al. 2009; Mas & Kölliker 2011a). In turn, condition-dependent chemical signals from the mother could influence nymph behavior. This was the focus of my next experiment.

Chapter 5

The evolution of parent-offspring communication has primarily been studied from the perspective of the parents responding to begging signals conveying information about offspring condition (Godfray 1991; 1995; Mock et al. 2011). However, offspring should also be sensitive to cues of parental condition to adjust their own behavior and parents even may have evolved signals to convey information about their condition. In this study, I experimentally tested if cues of maternal condition affect offspring survival, mediated by sibling cannibalism. I also extracted and analyzed the females' cuticular hydrocarbons (Wong & Kölliker, submitted). Maternal CHC-profiles were condition-dependent, which was shown for nymphs before (Mas et al. 2009), and thus hydrocarbons are likely used for reciprocal information exchange. The effect of maternal condition on reproduction was examined in my last experiment.

Chapter 6

Food availability and the environment shape condition and the amount of resources available for reproduction. In my previous experiments, I already found condition-dependent effects on maternal food provisioning and brood mixing dynamics. Condition does also influence the fitness, development and reproduction of individuals, and individuals might evolve condition-dependent strategies to maximize their fitness. Food availability during the juvenile development can influence adult condition later on in life. My last experiment investigated the effects of food availability during the juvenile and early adult development on survival, maternal care and reproductive success. I found that food restriction during the juvenile development has negative effects on survival and adult body weight. Moreover, restriction in the late juvenile development lead to smaller clutch size, lower probability of second clutch production and reduced hatching success in the second clutch. Thus, environmental conditions experienced early can have detrimental effects once individuals become adult and need to be considered to understand individual variation in reproductive success and life-history trade-offs (Wong & Kölliker, in prep).

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CHAPTER 1

The evolution of parental care in insects: The roles of ecology, life history and the social environment

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Abstract

1. Parental care increases the fitness of offspring at a cost to the parents in terms of residual reproductive success. This trade-off may be affected by ecology, life history and the social environment, which raises the question as to how these factors contribute to the evolution of parental care. Here, previous hypotheses concerning the evolution of parental care in insects are summarized and discussed and the underlying empirical evidence is reviewed.

2. Ecological factors such as harsh environments, ephemeral food sources or predation pressure are broadly accepted as evolutionary drivers of parental care. The most consistent evidence supports a role for natural enemies such as predators, microbes and cannibalistic conspecifics. Also, the importance of ecological factors may interact with the life history (parity) of a species, either as a pre-adaptation facilitating the evolution of parental care or as a consequence of enhanced parental investment under parental care. Yet, only limited experimental research has been carried out to test the combined influence of ecology and life history in the evolution of parental care.

3. Several forms of care can mediate the transition from solitary to family living, which entails the emergence of a novel – social – environment that generates new selection pressures from interactions within and between families. In this context, we review examples of studies on communal breeding, brood parasitism, parent–offspring conflict and co-adaptation, and discuss how these social interactions may in turn be influenced by ecological factors such as food availability or population density.

4. Insects are uniquely suitable for experimental and comparative research on the complex interplay between ecology, life history, and the social environment.

Introduction

Parental care is considered a prime example for an altruistic trait that evolved to enhance the fitness of the recipients of care (offspring) at the expense to the donor of care (parents) (Royle et al. 2012). The costs of decreased parental residual reproductive success associated with parental care have to be outweighed by the parents' indirect benefit in terms of an increase in offspring fitness (Hamilton 1964, Smiseth et al. 2012). This kin selected indirect fitness benefit to the parents is typically associated with genetic conflicts between parents and offspring over the level of parental investment, because in sexually reproducing species, parents and offspring are genetically not identical (Parent-offspring conflict; Trivers 1974). There has been strong research emphasis on the importance of close genetic relatedness in the evolution of parental care, which resulted in a large number of theoretical and empirical studies (see Alonzo & Klug 2012) The results of these studies are mixed probably at least partly because the effect of kinship on the evolution of parental care depends critically also on variation between individuals and factors affecting the fitness benefits and costs of care, such as ecological conditions, the life history of individuals, conflicts between the sexes and the social environment in which parents provide care (Alonzo & Klug 2012).

Wilson (1975) proposed specific hypotheses for how ecological factors may influence the evolution of parental care. He predicted that parental care should predominately evolve under stable structured habitats, unusually stressful physical environments, high predation pressure and scarce or specialized food sources. It was not until recently that the importance of ecological factors, for the evolutionary origin of parental care, were rigorously investigated in a series of mathematical models. Klug & Bonsall (2010) showed that parental care can evolve from an ancestral state of no care under a wide range of combinations of ecological conditions and life histories (e.g., egg, juvenile, and adult mortality rates, adult reproductive rate, egg maturation rate, and the duration of the juvenile stage). The authors compared the evolution of parental care in a constant versus a variable environment. They found that in a variable environment, the selection of parental care and the forms of care depend on the interaction between environmental variability, the life history traits affected by such variability and the specific costs of care (Bonsall & Klug 2011). For example, Bonsall & Klug (2011) demonstrated that environmental variability reduces selection for parental care when the costs of care are associated with both reduced parental survival and reproductive rate, but favors parental care if the only cost of care is a reduced parental survival rate. Whereas recent theoretical developments support the idea that ecological agents of selection in combination with pre-existing life histories are important, they also revealed that ecological agents on their own are usually not sufficient to allow the emergence of parental care (Klug & Bonsall 2010; Klug et al. 2012), leaving scope for other important factors. One of them is the social environment, which results from interactions between the two parents (Smiseth & Moore 2004), between parents and offspring (Mas et al. 2009) or among siblings (Ohba et al. 2006). Such tight interactions are indeed known to shape the benefits/costs ratio of care and, hence, possibly to influence the strength of natural selection on parental care once a basic level of care has evolved (Royle et al. 2002; Smiseth et al. 2012).

Our general aim in this review is to summarize and discuss empirical evidence from insects for influences of ecology, life history and the social environments on the evolution of parental care. A great diversity in the forms of parental care has been reported across taxa (Tallamy & Wood 1986; Clutton-Brock 1991; see Royle et al. 2012 for a recent review). Besides birds and mammals, insects are a promising, albeit often understudied system to investigate the evolution of parental care because it presents a particularly wide diversity in the forms, the duration and the intensity of care (Trumbo 2012) (see Fig. 1 for examples). Table 1 illustrates several well-studied examples of the variety of forms of parental care in non-eusocial insects and gives information about the sex of the caregiver. Our review starts by discussing the empirical support for different ecological factors that favor the emergence of parental care. We pay particular attention to how ecological factors may interact with animal life histories (in particular semelparity versus iteroparity) and conclude that it remains unclear whether life histories are evolutionary cause or effect of parental care (or a combination of the two). We then elaborate on how the social environment can influence parental care via interactions within and between families. We discuss how family interactions can affect potential benefits and costs associated with parental care, and how parent and offspring strategies may evolve as a consequence of these socially mediated modifications of selection on parents and offspring. Finally, we discuss our perspective on areas of further research on the evolution of parental care and conclude that insects with their broad diversity in extent and forms of care offer a unique opportunity to conduct this kind of research.

Throughout this review, we follow the definition of parental care by Royle et al. (2012), where parental care is defined as ‘any parental trait that enhances the fitness of a parent’s offspring, and that is likely to have originated and/or is currently maintained for this function’. Because we are interested in parental care *per se*, we decided to not include eusocial insects (e.g., Isoptera, Hymenoptera) in this review because maternal care (i.e., from the queen to the brood) is commonly expressed only relatively shortly during colony foundation (Bourke & Franks 1995; Queller & Strassmann,1998; Boomsma 2009). We limit our discussion to the evolution parental care per se without addressing the question why often female uniparental care, instead of male uniparental or biparental care evolved. We correspondingly provide examples from these different modes of care without discussing selection on male versus female parental care, which was previously discussed for example in Tallamy (2001) and Trumbo (2012). For excellent former reviews on parental care in invertebrates (including insects as well) and on general social living in non-eusocial insects, we refer the interested reader to Trumbo (2012; see also Tallamy 1989, 2001) and Costa (2006), respectively.

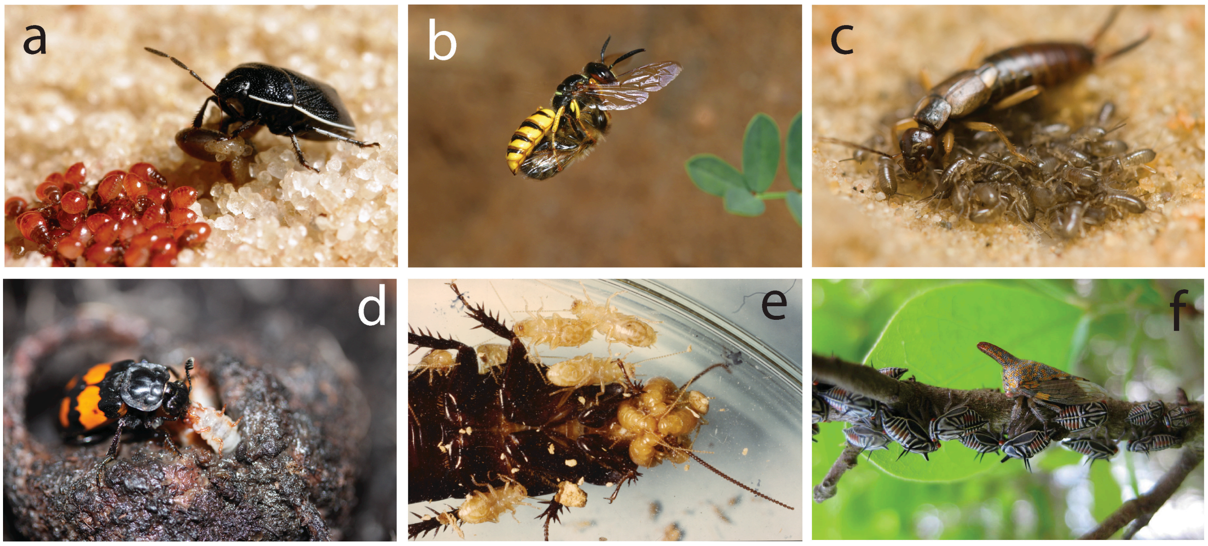

Figure 1. A selection of insect species that provide parental care. (a) A female burrower bug (*Sehirus cinctus*) provisioning mint nutlets to her offspring (photograph: Patrick Alexander). (b) A female European beewolf (*Philanthus triangulum*) carrying a paralysed honeybee in flight to her nest (photograph: Gudrun Herzner). (c) A female of the European earwig (*Forficula auricularia*) with her first-instar nymphs (photograph: Joël Meunier). (d) A burying beetle *Nicrophorus vespilloides* providing food to its larvae via regurgitation (photograph: Per Smiseth). (e) Fourth-instar nymphs of the wood-feeding cockroach *Salganea taiwanensis* feeding on the stomodeal fluids of the female (view from below) (photograph: Kiyoto Maekawa). (f) A female treehopper (*Platycotis vittata*) with her brood of fourth- and fifth-instar offspring (photograph: Jennifer Hamel).

Table 1. Forms of parental care in insects. This table show a summary of well-studied and taxonomically diverse examples in which the benefits of parental care have been shown. Blank cells represent missing information.

Order/Family/species	Care giver	Form of parental care							References
BLATTODEA									
Blaberidae									
Blaberus craniifer	F		EB						Nalepa & Bell (1997)
Byrsotria fumigata	F		EB						Nalepa & Bell (1997)
Diploptera punctata	F			V					Roth & Willis (1957) Nalepa & Bell (1997)
Geoscapheus spp.	F		EB						Nalepa & Bell (1997)
Lanxoblatta emarginata	F				OA				van Baaren *et al*. (2003)
Macropanesthia spp.	F		EB						Nalepa & Bell (1997)
Nauphoeta cinera	F		EB						Nalepa & Bell (1997)
Neogeoscapheus spp.	F		EB						Nalepa & Bell (1997)
Parapanesthia spp.	F		EB						Nalepa & Bell (1997)
Perisphaerus spp.	F					OB			Roth (1981)
Phortioeca nimbata	F				OA				van Baaren *et al*. (2003)
Rhyparobia maderae	F		EB						Nalepa & Bell (1997)
Salganea spp.	B				OA		FP_2		Nalepa & Bell (1997); Maekawa *et al*. (2008)
Salganea taiwanensis	B						FP_2		Maekawa *et al*. (2008)
Schultesia lampyridiformis	F				OA				van Baaren *et al*. (2003)
Thanatophyllum akinetum	F				OA				Nalepa & Bell (1997); van Baaren *et al*. (2003)
Thorax porcellana	F					OB			Nalepa & Bell (1997)
Blattellidae									
Blattella germanica	F		EB						Roth & Willis (1957); Nalepa & Bell (1997)
Blattella vaga	F		EB						Roth & Willis (1957); Nalepa & Bell (1997)
Cryptoceridae									
Cryptocercus kyebangensis	B				OA		FP_2		Park *et al*. (2002)
Cryptocercus punctulatus	B				OA		FP_2	NI	Seelinger & Seelinger (1983); Nalepa (1990)
Cryptocercus spp.	B	EA			OA		FP_2		Nalepa & Bell (1997); Maekawa *et al*. (2008)
COLEOPTERA									
Curculionidae									
Monarthrum spp.	F				OA		FP_1		Kirkendall *et al*. (1997)
Trypodendron lineatum	F				OA				Kirkendall *et al*. (1997)
Xyleborus spp.	F	EA			OA		FP		Kirkendall *et al*. (1997)
Passalidae									
All species	B				OA		FP_2		Schuster & Schuster (1997)
Scarabaeidae									
Onthophagus taurus	F						FP_1		Moczek (1998)
Silphidae									
Nicrophorus spp.	B				OA		FP_2		Scott (1990); Trumbo (1990)
Ptomascopus morio	F				OA				Trumbo *et al*. (2001); Suzuki & Nagano (2006)
Staphylinidae									
Bledius spectabilis	F	EA			OA		FP_2		Wyatt (1986)
DERMAPTERA									
Anisolabididae									
Anisolabis maritima	F	EA			OA		FP_2		Bennett (1904); Suzuki (2010)
Euborellia annulipes	F	EA			OA		FP_2		Rankin *et al*. (1995)
Euborellia plebeja	F	EA							Kamimura (2003)
Forficulidae									
Anechura bipunctata	F	EA			OA				Vancassel (1984)
Anechura harmandi	F	EA			OA		FP_3		Kohno (1997); Suzuki *et al*. (2005)
Forficula auricularia	F	EA			OA		FP_2		Weyrauch (1927); Lamb (1976a); Staerkle & Kölliker (2008)
Forficula decipiens	F	EA			OA				(M. Kölliker, unpublished)
Forficula lesnei	F	EA			OA		FP_2		Timmins (1995)
Labiduridae									
Labidura riparia	F	EA			OA		FP_2		Radl & Linsenmair (1991)
Spongiphoridae									
Chaetospania borneensis	F			V					Kocarek (2009)
Pygidicranidae									
Tagalina papua	F	EA			OA				Matzke & Klass (2005)

Table 1. Continued

Order/Family/species	S	Form of parental care				References
EMBIOPTERA						
Anisembiidae						
Anisembia texana	F	EA		OA		Choe (1994); Edgerly (1997)
Clothodidae						
Antiluparia urichi	F	EA		OA		Edgerly (1997)
Oligotomidae						
Oligotoma humbertiana	F	EA				Edgerly (1997)
HEMIPTERA						
Acanthosomatidae						
Elasmucha ferrugata	F	EA		OA		Kaitala & Mappes (1997)
Elasmucha fieberi	F	EA		OA		Melber & Schmidt (1975); Kaitala & Mappes (1997)
Elasmucha grisea	F	EA		OA		Melber & Schmidt (1975); Kaitala & Mappes (1997)
Belostomatidae						
All sp Belostomatinae	M		EB			Smith (1997); Estévez & Ribeiro (2011)
All sp Lethocerinae	M	EA				Smith (1997); Estévez & Ribeiro (2011)
Cydnidae						
Adomerus triguttulus	F	EA		OA	FP$_2$	Nakahira (1994)
Canthophorus niveimarginatus	F	EA			FP$_2$	Filippi *et al*. (2008)
Parastrachia japonensis	F	EA		OA	FP$_2$	Filippi-Tsukamoto *et al*. (1995b); Hironaka *et al*. (2005)
Sehirus cinctus	F	EA		OA	FP$_2$	Sites & McPherson (1982); Kight (1997)
Membracidae						
Polyglypta dispar	F	EA		OA		Eberhard (1986)
Publilia concava	F	EA		OA		Bristow (1983); Zink (2003b, 2005)
Publilia reticulata	F	EA		OA		Bristow (1983)
Pyrgauchenia tristaniopsis	F	EA				Stegmann & Linsenmair (2002)
Umbonia crassicornis	F			OA		Cocroft (1996)
Reduviidae						
Rhinocoris carmelita	F	EA				Thomas & Manica (2005)
Rhinocoris tristis	M/F	EA				Beal & Tallamy (2006)
Tingidae						
Gargaphia solani	F	EA		OA		Tallamy & Denno (1981)
Leptobyrsa decora	F	EA		OA		Loeb & Bell (2006)
HYMENOPTERA						
Bethylidae						
Goniozus nephantidis	F	EA		OA		Hardy & Blackburn (1991)
Megachilidae						
Osmia lignaria	F				FP$_1$	Torchio & Tepedino (1980)
Sphecidae						
Ammophila aureonotata	F				FP$_1$	Evans (1959)
Ammophila harti	F				FP$_2$	Evans (1959)
Ammophila juncea	F				FP$_1$	Evans (1959)
Ammophila nigricans	F				FP$_1$	Evans (1959)
Ammophila placida	F				FP$_1$	Evans (1959)
Ammophila procera	F				FP$_1$	Evans (1959)
Ammophila pubescens	F				FP$_2$	Evans (1959); Field & Brace (2004)
Ammophila sabulosa	F				FP$_1$	Field (1989)
Philanthus triangulum	F				FP$_1$	Strohm & Linsenmair (2001); Herzner & Strohm (2007)
ORTHOPTERA						
Gryllidae						
Anurogryllus muticus	F	EA		OA	FP$_2$	West & Alexander (1963)

B = biparental; EA = egg attendance; EB = egg brooding; F = female; FP = food provisioning, FP$_1$ = mass provisioning, FP$_2$ = progressive provisioning, FP$_3$ = matriphagy; M = male; NI = care after nutritional independence; OA = offspring attendance; OB = offspring brooding; V = viviparity.

Ecology, life history and insect parental care

In the following part we will explore different previously proposed hypotheses for how ecological factors and variation in life history may shape the evolution of parental care in insects. To this end, we first describe how ecological agents of selection are theoretically related to different forms of care as hypothesized early by Wilson (1971; 1975) and illustrate the evidence and its limits across insect taxa. Although the different ecological factors in reality probably rarely operate in isolation, we discuss them as separate, albeit not mutually exclusive, hypotheses for ecological factors that favor the evolution of parental care (Wilson, 1975).

Do harsh environmental conditions drive the evolution insect parental care?

Whereas adaptations increasing egg development under harsh environmental conditions, such as heat stress, desiccation or high humidity, may include protection of the eggs themselves (e.g., a resistant egg shell), parental egg attendance provides an alternative route for resisting these factors. Care is expected to be superior to direct adaptations by the eggs if the parent suffers substantially less from the challenging condition than the eggs and/or the cost of the protective adaptation is higher than the cost of care for parents (i.e., the costs of care to the parents are exceeded by the benefits to the eggs). An added benefit of adaptation through parental care is that a caring parent can flexibly adjust its caring behavior when necessary, whereas a resistant egg shell would be a fixed trait (see for example Field & Brace 2004).

Several studies provide direct or indirect empirical support for this hypothesis by reporting the benefits of maternal care under specific physical environmental constraints. For example, females of the terrestrial staphylinid beetle *Bledius spectabilis* live in the inhospitable habitat of the intertidal saltmarsh, wherein their burrows experience daily floods by the tide (Wyatt 1986). To prevent flooding of their nest and anoxia of their eggs, females provide care in the form of closing the entrance of their burrow during high-tide and reopening it at low tide (the latter being vital for respiration in the anaerobic soil). In the shield bug *Parastrachia japonensis* or the European earwig (*Forficula auricularia*), females attend their eggs and were reported to move them to a new nest site, if the physical conditions become unfavorable due to flood or desiccation (Weyrauch 1927; Filippi-Tsukamoto et al. 1995a). Male belostomatid water bugs like *Belostoma flumineum* engage in brooding behavior by keeping eggs wet, frequently exposing them to atmospheric air, and maintaining an intermittent flow of water over them by stroking them with the hind legs (Smith 1976; Estévez & Ribeiro 2011). If eggs were detached from the males they failed to hatch. An extreme form of care that may occur under very low food availability is matriphagy. In the hump earwig (*Anechura harmandi*), an obligatory matriphagous species, first instar nymphs kill and eat their mother before dispersing from the nest (Kohno 1997; Suzuki et al. 2005). Hump earwig mothers do not seem to attempt escape from cannibalism by their nymphs and even do not produce a second clutch when being experimentally isolated from their nymphs. Thus, matriphagy provides important benefits to offspring while the costs for the female seem very low due to the low chances of future reproduction (Suzuki et al. 2005). Also, anatomical/morphological adaptations by parents may enhance offspring fitness under harsh physical conditions. For instance, the brood sac of lecithotrophic and matrotrophic viviparous cockroaches like *Rhyparobia maderae* or *Diploptera punctata* protects the developing offspring from heat, cold, moisture, desiccation, anoxia and osmotic stress within the female body (Nalepa & Bell 1997).

In these examples it seems likely that harsh environments contributed to the described parental adaptations. Nevertheless, harsh conditions do not necessarily favor the evolution of parental care because they usually not only increase the potential benefits of parental care to offspring, but they may also induce parent-offspring competition for limited resources or enhance the costs to the parents to provide care under such aggravated conditions. Irrespective of the type of ecological harshness, it generally holds that if the costs of care exceed the associated benefits, care will not be selected for despite the potentially large benefits for offspring (Clutton-Brock 1991; Royle et al. 2012). Based on available data it is currently difficult to judge whether the limited support is due to the limited cases where parental care actually evolved under such conditions (providing evidence against evolution of parental care under harsh conditions), or if it is due to the limited amount of systematic research conducted in this field. Even if identified, a phylogenetic association between parental care and harsh environments does not prove that parental care evolved in response to selection imposed by such environments. Instead, such an association may reflect that species that have evolved parental care for some reason unrelated to the harshness of the environment, may be able to colonize habitats that otherwise would be inhospitable to ancestral species without parental care. There is clearly a need for further research on the question if and how harsh environmental conditions favor the evolution of parental care, that should involve a combination of phylogenetic analyses and manipulative experiments to test directly how environmental harshness affects selection on parental care (i.e., using fitness assays under different environments with and without care) and its change over generations (i.e., using experimental evolution experiments).

Do ephemeral or distant food sources and specialized foraging drive the evolution of insect parental care?

Parental care is expected to allow the offspring to obtain food resources indirectly through the provisioning parent when food sources are ephemeral and occur clumped in space or time, or if they are difficult to access or process (as is often the case in specialized foraging). A critical problem when offspring need access to ephemeral and rare food sources is the extent to which a suitable and safe site for the offspring (e.g., a burrow or nest) is spatially disconnected from the food sources required for energy uptake. If juveniles are less mobile than adults, a provisioning parent may be able to provide both sufficient food and safe shelter at sustainable cost, selecting for parental provisioning of the ephemeral food source. The co-evolution of parental food provisioning and egg-/offspring attendance for protection against natural enemies was recently modeled by Gardner & Smiseth (2011). In this model, parental food provisioning evolved from offspring attendance only if parental food provisioning was more efficient than offspring self-feeding. We suggest that parental food provisioning is more likely when food resources are ephemeral or difficult to access or process. Therefore, the model is in line with the general argument that these environmental factors are important for the evolution of food provisioning.

There are well-studied examples of food provisioning among insects where the species feed on ephemeral food sources and/or where the offspring are spatially disconnected from it. For example, females of the shield bug *P. japonensis* provision nymph-containing nests progressively with drupes of a single host tree: *Schoepfia jasminodora* (Olacaceae) distant from the nest (Filippi et al. 2000). Similarly in the burrower bug *Sehirus cinctus*, nymphs only eat seeds of a few plant species, in particular *Prunella vulgaris* (Labiaceae) and *Lamium purpureum* (Labiaceae), which are available for only a few weeks each spring and mothers might be better in competing for this limited resource (Kight 1997).

The cockroach *Cryptocercus punctulatus* is a clear example of a species where specialization for a food source may lead to the evolution of parental care. In this wood-feeding species nymphs are not able to directly process wood. First and second instar nymphs feed on hindgut fluids of both parents. Such behavior allows them to acquire endosymbionts (intestinal flagellata), which are necessary for cellulose digestion and, hence, for the maintenance of this specialized foraging behavior (Seelinger & Seelinger 1983; Nalepa & Bell 1997). In wood feeding passalid beetles, all stages must feed on the feces of mature adults. Feces are comprised of shredded, digested wood, inoculated with bacteria and fungi from the adult digestive tract (Schuster & Schuster 1997). Both *Cryptocercus* cockroaches and Passalid beetles feed on specialized food sources, but it should be noted that they also inhabit rather stable and structured environments (inside deadwood), another ecological factor that was hypothesized to promote the evolution of parental care (see below). It seems likely that a combination of these two factors was ultimately responsible for the evolution of parental care in these species.

In some species, females produce trophic eggs, i.e., unfertilized eggs that are used by hatched offspring as food sources; as e.g., in the Hemipteran *Adomerus triguttulus* (Kudô & Nakahira 2004). We refer the interested reader to Trumbo (2012) for a detailed discussion of this form of care.

Food provisioning is also present in species with non-specialized foragers feeding on non-ephemeral food sources. For instance, the European earwig, *F. auricularia,* is omnivorous and, offspring are only partly disconnected from the food source, since nymphs are able to self-forage independently from an early age (Lamb 1976a; b; Kölliker & Vancassel 2007). Still, female food provisioning is a phenomenon widespread across the whole order Dermaptera (Costa 2006).

Given the inconclusive qualitative evidence, the hypothesis that ephemeral food sources and specialized foraging enhance the evolution of parental care would need a full quantitative test. Such tests should take into account other ecological conditions experienced by the species, its life history, the nesting-habit and the feeding-habit of the species, because selection for parental care is most likely under the combined influences of multiple factors (i.e., survival costs; Bonsall & Klug 2011; Trumbo 2012), for example when safe nests cannot be built close to the food source (Smiseth & Gardner 2011), and/or when the offspring survival without parental assistance (mainly pre-digestion) is low.

Do natural enemies (predators, parasitoids, parasites, microbes) drive the evolution of insect parental care?

Predation was suggested early to play an important role in the evolution of parental care (Wilson 1975; Tallamy & Denno 1981). Whereas this hypothesis was originally put forward with regard to predators, it also applies in principal to any other natural enemy that can specifically impose harm upon offspring, such as parasitoids (Field & Brace 2004) or microbes competing with offspring for food resources (Rozen et al. 2008; see Trumbo 2012 for a detailed discussion). Exposure to natural enemies, especially of eggs and juveniles, may select for parental care only if the parents suffer substantially less from their exposure than the offspring. Protection can occur through egg/offspring attendance but other protective adaptations, like the ovipositor or the resistant egg shell can provide alternatives to enhance offspring fitness under pressure from natural enemies (Zeh et al. 1989).

The benefits of maternal egg/offspring attendance on offspring survival have been broadly studied and received consistent empirical support across insect species. For example, in the shield bug genus *Elasmucha,* females shelter the eggs and nymphs by covering them with their body and fanning their wings when attacked. Egg survival was reported to be very low without care (Melber & Schmidt 1975; Kaitala & Mappes 1997), mostly due to predation. Females of the lace bug *Gargaphia solani* also show maternal antipredator behavior and remain with their progeny throughout all five nymphal instars (Tallamy & Denno 1981). In the absence of predators, nymphs suffer no ill effects if raised without their mother, but when nymphs were experimentally orphaned under normal field conditions only very low numbers survive to maturity due to predation (Tallamy & Denno 1981). Such effects have also been described in a sister species, *G. tiliae* (Hardin & Tallamy 1992). In the staphylinid beetle *B. spectabilis,* maternal egg and offspring attendance protects eggs and larvae from predatory beetles or parasitoid wasps (Wyatt & Foster 1989a; b). In the treehopper *Publilia concava,* maternal egg attendance effectively keeps away predators and the eggs are substantially more susceptible to these predators than adults. Females exhibit two alternative tactics: immediate abandonment after oviposition or egg-attendance until and beyond hatching. Zink (2003a) showed that a female attending her eggs until hatching doubled her hatching success relative to a female that abandoned her eggs immediately after laying. However, in terms of lifetime reproductive success, the enhanced fitness of the tending females through higher offspring survival, was balanced by the reduced lifetime number and size of their clutches. Thus, tending and non-tending females had roughly similar fitness, which explains why the two alternative reproductive tactics are maintained in treehopper populations (Zink 2003a).

These are examples for inter-specific predation. But intra-specific predation (i.e., cannibalism) can also be an important agent of selection in predatory insect species. As an example, a recent study in the earwig *Anisolabis maritima* demonstrated experimentally that egg-attendance by females protects the eggs from being cannibalized by conspecifics (Miller et al. 2011).

Field & Brace (2004) experimentally showed in *Ammophila* wasps how progressive provisioning females can significantly reduce the impact of parasitism by cuckoo flies (Diptera: Miltogramminae), a major natural enemy of wasps. The cuckoo flies deposit live maggots that kill the immature wasp and then eat the provisions. Only wasp mothers of the progressively provisioning species could intervene and remove the fly maggots, which was not possible for mothers of mass provisioning species. Thus, there was an added benefit of progressive provisioning beyond the provided food in terms of protection against a parasite.

Empirical support for the benefits of parental care against competing microbes has been found in several species. Infestation by microorganisms is known to decrease offspring fitness either by killing the larvae or by decreasing progeny size and reproductive success. In the European beewolf (*Philanthus triangulum*), females provision brood cells with paralyzed honeybees as larval food. Because the brood is located in warm and humid cells, there is a high risk of microbial decomposition of the provisioned food. Preservation of prey is achieved by the maternal application of chemical secretions that reduce fungal growth (Strohm & Linsenmair 2001; Herzner & Strohm 2007). An analogous mechanism was recently described in the burying beetle *Nicrophorus vespilloides*, where parents obligatorily breed on carcasses of small vertebrates and larvae face intense competition with microbes over the carcass. The study by Rozen et al. (2008) showed that parents apply substances (e.g., lysozyme) that inhibit microbial growth and, hence, protect offspring by limiting the development of microbes that would otherwise quickly degrade the quality of the food source. The study further showed that the parental antimicrobial care resulted in higher larval body mass and survival. In the European earwig (*F. auricularia*), females have been shown to groom their clutch of eggs, a behavior that has been hypothesized to prevent fungal infections and the molding of eggs in their underground nests (Weyrauch 1927; Lamb 1976a).

Overall, the evidence seems robust for benefits of parental care in species where offspring face high risks of suffering fitness losses due to natural enemies that specifically target offspring or the resources they need for development and survival. However, whether the pressure exerted by natural enemies can favor the emergence of parental care remains to be confirmed experimentally, for instance by following changes in the level of parental investment in families reared under high and low predation pressures (i.e., experimental evolution). Furthermore, studies could also compare the effect of egg- or juvenile-specific predators versus general predators, which differentially affects the cost/benefit ratio of protection to the parents and therefore the strength of selection on pre- and post-natal care.

Do predictable environments & life history variation drive the evolution of insect parental care?

The reason why stable predictable environments may favor the evolution of parental care is linked with life history evolution. Wilson (1975) and Tallamy & Brown (1999) brought up two contrasting hypotheses regarding the evolution of parental care and the mode of parity. Wilson (1975) suggested, that when a species adapts to stable, predictable environments, K-selection for an iteroparous life history (i.e., multiple reproductive attempts) tends to prevail over r-selection for a semelparous life history i.e., single reproductive attempt). Under K-selection, individuals are predicted to live longer and grow larger, but also to produce only a small number of offspring over multiple reproductive attempts, each with high reproductive value and correspondingly high levels of parental investment. Following this line of argumentation, parental care is expected to predominately evolve among iteroparous species due to the high expected fitness returns on parental investment when each offspring represents a substantial fraction of lifetime reproductive success (here referred to as ‘iteroparity hypothesis’). Tallamy & Brown’s (1999) alternative hypothesis makes the opposite prediction that parental care should evolve more readily in semelparous species, because of the low evolutionary cost of care to parents in terms of residual fitness. Under this hypothesis iteroparous insects should provide either no parental care or less care than related semelparous species. The ‘iteroparity’ and ‘semelparity’ hypotheses suggest that the emergence of parental care does not primarily result from ecological selection pressures, but instead from life history pre-adaptations shaping the investment trade-off between current and future reproduction.

Qualitative comparisons have been carried out to test these two hypotheses with mixed results. Some studies provide support for the ‘semelparity hypothesis’. For example, Stegmann & Linsenmair (2002) tested this hypothesis in the membracid *Pyrgauchenia tristaniopsis*. Here, females exhibit relatively basic forms of care (i.e., egg attendance only) associated with a moderate degree of iteroparity (37 % females produced a second clutch), whereas other membracid species generally express more elaborate forms of maternal care (i.e., egg and offspring attendance) and are typically semelparous. The authors interpreted this result as consistent with the 'semelparity hypothesis' in that iteroparity was associated with low levels of maternal care. In another study, Nagano & Suzuki (2008) compared maternal investment in future reproduction between two species of Nicrophorine beetles: *Nicrophorus quadripunctatus*, which displays elaborate parental care (carcass preparation, offspring attendance and provisioning) and *Ptomascopus morio*, which displays simpler parental care (offspring attendance only). In contrast to predictions of the 'semelparity hypothesis', the authors found that *N. quadripunctatus* can oviposit several times in one breeding season and that they regulate their clutch size more strictly than *P. morio*. For more conclusive comparative tests, studies are now needed that relate parental care to parity across more than two species. Any two compared species are likely to differ in many ways that may also affect parental care (e.g., also ecology), which may confound the relationship and mask present patterns. Provided adaptive associations between life history and parental care exist, a different approach to test these hypotheses can be the comparison within species or within populations between individuals with different parity. Meunier et al. (2012) tested the association between the levels of maternal care and second clutch production in a population of the European earwig, *F. auricularia,* where semelparous and iteroparous females coexist. Contrary to the 'semelparity hypothesis', their results showed that iteroparous females provided significantly higher levels of maternal care in terms of food provisioning. They also produced larger first clutches and a larger total number of eggs (first and second clutch combined) than semelparous ones. The study suggests that the intrinsic condition of earwig females plays a key role in the level of maternal care and investment in future reproduction, in that high condition females can afford both being iteroparous and providing more care despite an underlying trade-off between current and future reproduction.

One potential reason for the mixed evidence for an association between mode of parity and parental care is that the distinction between evolutionary cause and effect of parental care in terms of life history remains ambiguous. Is maternal care the consequence of a semelparous life history (as suggested by the 'semelparity hypothesis')? Or is semelparity the consequence of the high costs of parental care (referred to as the 'cost-of-care hypothesis')? Both directions of effects are likely to occur at differing relative strength between species. The question of whether a particular parity is a life history pre-adaptation favoring the evolution of parental care, or whether it rather is the consequence of evolved parental care and the associated costs in terms of parental residual reproductive value has to our knowledge not yet been tackled theoretically or empirically. This distinction could be resolved through comparative phylogenetic studies by reconstructing the ancestral state and follow the gain and loss of parental care in association with changes in parity. In Fig. 2 we provide the three phylogenetic hypotheses for the evolutionary association of parental care and mode of parity in insects and explain the different possible scenarios.

To conclude, despite a wealth of descriptions of diverse forms of parental care across insect species that vary in life history and inhabit different ecological niches, only little research actually directly tested how the environmental factors originally proposed by Wilson (1975) affect the benefits and costs of care (see also Trumbo 2012). As previously mentioned, more experimental studies, but also phylogenetic analyses that combine potential effects of a species ecology and life history on the evolution of parental care are needed. Such an approach would provide a clearer picture on the importance of each ecological factor on the evolution of parental care, while correcting for phylogeny and taxon-biases resulting from differences in research effort across taxa, (e.g., the broadly studied cockroaches; see Table 1). Furthermore, some of the ecological parameters require standardized definitions (e.g., ephemeral food sources or harsh environments) and ways of measurement in particular if we aim at comparative tests between insect taxa.

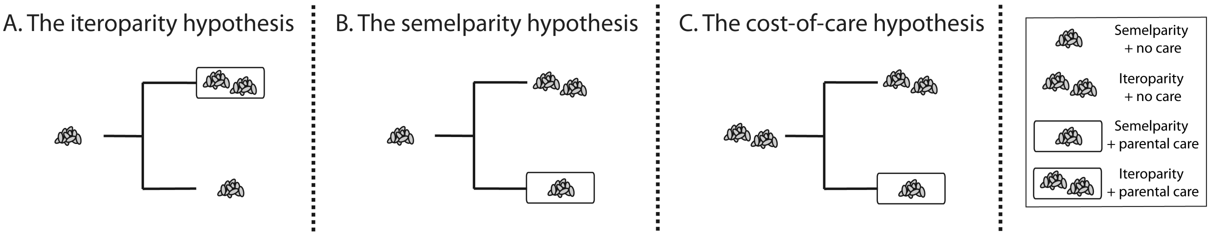

Figure 2. Phylogenetic hypotheses for the evolutionary association of parental care and mode of parity in insects. In each panel (a)–(c), the ancestral state is depicted to the left of the tree, and the predicted derived states under each hypothesis to the right of the tree. (a) Wilson's 'iteroparity hypothesis' (1975): Wilson's hypothesis would be supported if care evolves in an iteroparous species as novelty from a semelparous ancestor, and no care remains associated with semelparity. (b) Tallamy and Brown's 'semelparity hypothesis' (1999): The 'semelparity hypothesis' would be supported if care evolved in a semelparous ancestor without care and iteroparous species derived from the same ancestor show no care. (c) The 'cost of care hypothesis': The hypothesis that semelparity is the consequence of a cost of care would be supported if care evolved in an iteroparous ancestor without care resulting in lineages where maternal care and semelparity co-occur as evolutionarily derived states.

Social environment and the evolution of insect parental care

Parental care is typically associated with important social interactions, such as the ones between parents and offspring or among siblings. The transition from solitary to group (family) living entails the emergence of a novel – social – environment that is characterized by the aggregation of parents and offspring, the resources provided by parents and the ensuing intensified social interactions among family members. This social environment forms part of an individual's ecology and generates new selection pressures, for example through selection on the effective transfer and usage of parentally provided resources, or through conflicts of interest within and between families (Trivers 1974). Caring parents are a social environment to which offspring should adapt, and offspring are a social environment to which parents should adapt, and these novel selection pressures should expectedly lead to parent and offspring adaptations to family life and the co-adaptation of their traits (Kölliker et al. 2012).

When studying how the social environment influences costs and benefits of parental care we have to consistently partition fitness components between parents and offspring (Wolf & Wade 2001; see Smiseth et al. 2012 for a review). Parental care is beneficial to the offspring because it increases their direct fitness. From the perspective of the parent (or a parental care gene), the offspring fitness benefit is an indirect fitness benefit because the fitness of the genetically related recipient of care (i.e., the offspring) is enhanced, not of the donor of care (i.e., the parent). Similarly, parents may pay a direct fitness cost of care in terms of their fecundity. From the perspective of the offspring, the parental fitness costs are indirect costs, because they are paid by genetically related individuals (i.e., the parents). These benefits to offspring and costs to parents lead to genetic conflicts over parental care (Trivers 1974). It is expected that this selection favors mechanisms that contribute to a resolution of family conflicts, for example through the evolution of parent-offspring communication (reviewed in Kilner & Hinde 2012). In insects, offspring influences on parental care have been shown to include vibrational signals for example in treehoppers (Cocroft 1996; 2001), tactile and visual begging for example in burying beetles (*N. vespilloides*; (Smiseth et al. 2003) and chemical signaling, that is solicitation pheromones, for example in burrower bugs (*S. cinctus*) and earwigs (*F. auricularia*) (Kölliker et al. 2006; Mas et al. 2009; Mas & Kölliker 2011).

The reciprocal nature of parents and offspring influencing each other's fitness leads to selection on particular combinations of parental care and offspring traits, favoring co-adapted parent and offspring strategies. Co-adaptation models make two main predictions: first, that there is a genetic or epigenetic correlation between the levels of offspring demand and parental supply and, second, that a mismatch between parental and offspring strategies comes at a cost to family members (reviewed in Kölliker et al. 2012). Whereas these predictions were tested across numerous bird and mammalian species, parent-offspring coadaptation has been explored in only three insect species so far. The first prediction of co-adaptation models was tested in the burrower bug *S. cinctus* and the burying beetle *N. vespilloides* using cross-fostering experiments, both providing evidence for a genetic correlation between maternal food provisioning and offspring begging (Agrawal et al. 2001; Lock et al. 2004). The second prediction of co-adaptation models was tested in *N. vespilloides* and in the European earwig *F. auricularia*. In *N. vespilloides*, offspring reared by foster females, i.e., in families with mismatched parental and offspring strategies, survived significantly less than offspring reared by their own mother (Lock et al. 2004), and a recent study in *F. auricularia* demonstrated that earwig mothers caring for offspring with experimentally mismatched strategies suffered from fitness costs in terms of future reproduction (Meunier & Kölliker 2012a).

Social environment and the costs of parental care

When multiple parents are breeding in close proximity, the potential network of social interactions is greatly expanded beyond the core family (parents and offspring). Parents might interact with their own offspring, but also with other parents and their offspring. Such between-family interactions can be beneficial (in case of cooperative behaviors) or costly (in case of local competition for resources or brood parasitism). If the fitness or productivity of all individuals involved is increased simultaneously, we find a cooperative outcome due to direct benefits of communal breeding or brood mixing (Lin & Michener 1972). Brood mixing can occur in species where offspring are mobile and can join other families. However, if an individual's expected reproductive output is even slightly decreased by the invading individual, the invader is more appropriately termed a parasite (Eberhard 1986). In brood parasitism, one individual exploits the parental care invested by another individual. This could be through the female in case of egg dumping or through the offspring in case of brood mixing. Brood parasitic strategies are predicted to evolve for example when breeding sites are in close proximity and there is an opportunity for parental care to be misdirected. As a result, selection should favor kin recognition and guarding strategies in order for caring parents to avoid investment in foreign offspring, and offspring strategies to overcome such defense mechanisms in parents (reviewed in Keller 1997).

Intraspecific brood parasitism was described in a number of insect species, for example in the dung beetle *Onthophagus taurus* (Moczek & Cochrane 2006), females use cow or horse dung to form brood balls that also serve as food source for the larvae. Each brood ball contains a hollow chamber holding one egg. Females only oviposit one egg per brood ball, which constitutes the sole amount of food available for larvae to complete larval development (Moczek 1998). Egg dumping occurs as brood parasitic females were reported to replace conspecific eggs inside brood balls produced by another female with their own egg (Moczek & Cochrane 2006). The authors suggested that the re-filling of tunnels with previously excavated soil or sand by the caring parents is an adaptation to limit parasitism by conspecific females that makes it more difficult for other females to locate brood balls underground.

Parasitic strategies can also include social parasitism through the dispersal of mobile offspring invading foreign family groups. In the burrower bug *S. cinctus,* oviposition sites are aggregated in the field (Agrawal et al. 2004). The authors could not find evidence that neighboring females were closely related, so joining events could not have contributed to the females' inclusive fitness. Agrawal et al. reported that the joining of family groups happened frequently in experimental studies, mainly initiated by nymphs under limited food supply. This could suggest that under restricted food conditions nymphs change their strategy from remaining with their own mother to dispersing and exploiting care from unrelated females, which could reflect brood parasitism. A field study by Kölliker & Vancassel (2007) showed that offspring of the European earwig *F. auricularia* dispersed from their own burrow and joined foreign family groups and that this dispersal occurred more readily when the mother was removed (see also Kölliker 2007).

In the case of intraspecific brood parasitism, conspecifics provide the only hosts for brood parasites and obligate parasitism cannot become fixed in a population. De Valpine & Eadie (2008) suggested that the advantages of egg dumping are likely to be greatest when the frequency of parasitism is low and many host nests are available containing few parasitic eggs. Thus, the parasitic strategy is expected to evolve under negative-frequency dependent selection, and the advantages will decrease as the frequency of parasitism increases and more host nests contain many parasitic eggs. As already pointed out by Müller et al. (1990), so far, we are not aware of cases of intraspecific brood parasitism in which individuals are restricted to either exclusive parasitic or non-parasitic behavior. Intraspecific brood parasitism seems rather affected by environmental conditions such as population density (see section 'Ecological influences on social interactions') or the low likelihood for independent breeding by the parasitic individual.

Social environment and the benefits of parental care

Social interactions between families do not always result in parasitism. Sometimes both interacting sides can profit. For example, in cooperative breeders, some individuals postpone their personal reproduction in order to favor the reproduction of others, which was suggested to offer some of the strongest evidence of kin selection (Hamilton 1964; Wilson 1975). However, direct benefits such as communal territory defense, enhanced microclimate or enhanced foraging efficiency also favor the evolution of interactions between unrelated parents and between parents and offspring, including communal and cooperative breeding (e.g., Clutton-Brock 2002; Bergmüller et al. 2007).

The direct benefits of communal breeding received evidence from a study in the parent bug *Elasmucha grisea*, a species where two females sometimes attend and defend their clutches jointly (Mappes et al. 1995). In a field experiment, Mappes et al. showed that communally guarding females had more eggs in their clutches than singly guarding females. The authors then confirmed this result in the lab by showing that joint unrelated females lost fewer eggs to ant predation than single females, possibly because egg attendance is more than twice as effective with two females. The benefits of communal breeding are less clear in other species. In the burying beetle *Nicrophorus defodiens,* Eggert & Sakaluk (2000) showed that the presence of two females on a carcass did not reduce the risk of losing the carcass to other burying beetles. Scott (1994) suggested that communal breeding in the closely related *N. tomentosus*

reduces competition for carrion by fly maggots, a hypothesis that was later rejected by Eggert & Sakaluk (2000), who argued that large carrion flies cannot access carcasses once they are buried.
With regard to egg-dumping (see above; Tallamy 2005), what appears to be a parasitic behavior that is costly for the apparently parasitized individual might in some cases be beneficial for the dumper and the carer. Loeb et al. (2000) showed that females of the lace bug *G. solani* preferentially dump their eggs with kin, and that recipients gain indirect fitness by accepting these eggs. In their first bout of reproduction, significantly more of their own offspring survived to maturity in their first clutch than did controls without egg-dumping (Loeb 2003), most likely due to the predator dilution effect. In this case, egg-dumping does not appear to be a parasitic strategy, but rather provides direct and indirect benefits of alternative reproductive tactics among closely related individuals.

The potential for intraspecific cooperation between females of the membracid *Polyglypta dispar* was suggested by Eberhard (1986). Multiple females were reported to oviposit in the same egg mass and females sometimes adopted abandoned egg masses. Some guarding females attempted to prevent the visitor from ovipositing, whereas other guarding females just stepped aside. Guarding is a reproductively costly behavior, since it delays the time to the next oviposition. Eberhard suggested that the probability of high genetic relatedness, due to philopatry, contributes to the tendency for females to adopt abandoned egg masses. Furthermore, guarding females might benefit from the additional eggs, which are oviposited at the periphery of the egg mass, and Eberhard proposed that the eggs in the center might become less subject to parasitism by parasitic wasps.

Parental care and family interactions need to be on average beneficial for the offspring and the parent in order to evolve. Selection through direct or indirect benefits could have contributed to the evolution of parental care even if it is not purely directed to own genetic offspring, as previously shown by some exemplary studies. Such benefits could also partly explain why non-eusocial insect parents only rarely show sophisticated kin recognition and nepotism.

Ecological influences on social interactions

Ecological factors are expected to continue to shape selection on parental care once parental care originated. The evolutionary costs of certain amounts of parental care depend on the ecological context in which care is expressed. For instance, resource limitation in the environment is expected to modify the optimal investment in offspring, affecting the amount of resources transferred by parents to their offspring.

For example, in the European earwig (*F. auricularia*) females adjust the amount and duration of parental care to their own nutritional condition (Wong & Kölliker 2012), which at least partly reflects food availability in the environment. Females provided food to fewer nymphs and for a shorter period of time, if their access to food was limited. Furthermore, in a study with the same earwig species, (Meunier & Kölliker 2012b) showed that attendance by mothers can also be costly for offspring. Under food restriction, the usual fitness benefits of maternal presence for offspring (Kölliker 2007) turn into a net reduction of offspring survival. The study could rule out that this effect was due to brood size adjustment by the female through filial cannibalism, as reported in the burying beetle *N. vespilloides* (Bartlett 1987). Instead, it suggests direct competition for food between the female and her offspring under these conditions where the offspring pay the costs.

If offspring are not fully dependent on their parents (i.e., species with facultative care), they might take an active role in determining their own social environment. For example, work on the burrower bug *S. cinctus* showed that clutch joining initiated by the nymphs was especially high under insufficient food conditions (Agrawal et al. 2004). However, the consequences of joining between unrelated individuals, for example the increase in competition, were not investigated further. It would be interesting to test, if females or nymphs exhibit discrimination against foreign offspring. The potential direct benefits of an increase in group size could explain why mothers accept foreign nymphs.

An increase in group size can also lead to local resource depletion and thus modify population dynamics. For example, Evans (1988) suggested that an increase in population density could result in an increase in intraspecific brood parasitism. This higher density can lead to scarcity of resources, such as breeding sites. For example, vertebrate carcasses of suitable quality are probably a scarce, unpredictable resource for burying beetles. Females of *N. vespilloides* fight for the ownership of carcasses and larger females usually manage to monopolize the carcass (Müller et al. 1990). However, the smaller female might stay near the carcass to lay her eggs for which the winning female will provide care. The lower the chances of finding another carcass on which no larger female is present, the more it pays a small female to stay and try to parasitize the winner's brood rather than leave. This results in costs for the larger female. Since larvae hatching from the parasite's eggs consume part of the available carrion mass, the number of offspring from the caring female was reduced.

Overall, these studies provide examples for how ecological conditions like population density or food availability influence variation in condition or parent-offspring relatedness. Other ecological factors like natural enemies, climatic change and the abiotic and biotic properties of the environment can also influence selection through the social environment, for example by facilitating (or hindering) social interactions within and between families. This can affect social and family interactions and might modify or even reverse the usual benefits of parental care and turn them into costs paid by the parents and/or offspring.

Outlook

In this review, we have reviewed former hypotheses for how abiotic and biotic ecological factors can affect benefits and costs of different forms of parental care, and how their effect on the evolution of parental care is expected to depend on the pre-existing life history of the species. When considering the likely complex relationships between ecology, life history and parental care in insects (see also Costa 2006; Bonsall & Klug 2011; Trumbo 2012), we pointed out that the distinction between cause and effect is a critical one. Do some life history traits facilitate the evolution of parental care or do the costs of parental care that evolve under particular ecological conditions lead to certain life histories? The wide variety of literature available, some of which we presented here, is still lacking systematic experimental studies that disentangle cause and effect between life history and ecology and that directly test factors that contribute to the evolution of parental care. The empirical evidence presented in Table 1 shows an over-representation of certain orders, for example the Blattodea. The large amount of work already available in these orders, together with the increasingly detailed molecular phylogeny of taxa, should lead to further investigations on the association between ecology, life history and phylogeny in the evolution of parental care.

To date, few studies have used comparative approaches to study the evolutionary history of parental care (Trumbo 2012) but the following two are exemplary in demonstrating the scope that this approach has in answering evolutionary questions about the roles of ecology, life history and the social environment in the evolution of parental care. Lin et al. (2004) used the molecular phylogeny of the treehopper subfamily Membracinae. Their results indicate that the ancestral state of the Membracinae is estimated to have no maternal care and that there were three independent origins of egg attendance. The authors suggested that associated behaviors, life histories, and ecology may explain these origins, but the corresponding measurements were unfortunately not taken. Gilbert & Manica (2010) went a step further and used a phylogenetic approach using quantitative data on body size, life history and forms of care to test predictions about evolutionary associations between egg size, egg number (i.e., fecundity), and body size under different forms of parental care across 287 insect species from 16 orders. Their results showed that the association between body size and life history is reversed by particular forms of parental care, especially food provisioning (Gilbert & Manica 2010). Such phylogenetic studies hold great promise to further our understanding of the evolutionary origin of parental care when they are combined with close investigations and comparisons of the ecology, life history and the social environment (Trumbo 2012). This would comprise a large enough number of species, which show diversity in the forms of care within and among lineages as well as reliable measures of ecological factors and life history.

Besides phylogenetic work, more empirical studies investigating the evolution of parental care are required, and insects are probably uniquely suitable study systems to this end. Compared to mammals and birds that exhibit obligate forms of care, insects display a wide variability regarding the presence or absence of different forms of care, and regarding the degree of offspring dependence on parental care. Nevertheless, detailed experimental research on parental care and social interactions within and between families has been limited to comparably few species. Besides the investigation of causes and consequences of parental care and social environments, there is still also need for basic natural history work because our knowledge on the diversity in the forms and extent of care are still limited in many taxa (see also Trumbo 2012). Finally, the typically shorter generation time of insects compared to other model systems, and their easier maintenance under laboratory conditions enable us to investigate life history traits associated with divergent patterns of care between closely related species, as well as the effect of specific environmental factors (for example variation in predation pressure or food resources) on long-term changes in the form and strength of parental care. Thus, insects are a highly interesting and suitable system to address open key questions on the evolution of parental care.

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Contribution of authors

JWYW did the literature research, composed the table and drafted the manuscript. JWYW, JM and MK wrote the paper.

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CHAPTER 2

The effect of female condition on maternal care in the European earwig

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Abstract

Parental care typically enhances offspring fitness at costs for tending parents. Asymmetries in genetic relatedness entail potential conflicts between parents and offspring over the duration and the amount of care. To understand how these conflicts are resolved evolutionarily, it is important to understand how individual condition affects offspring and parental behaviour and whether parents or offspring make active choices in their interactions. Condition effects on offspring have been broadly studied, but the effect of parental condition on parent–offspring interactions is less well understood, in particular in species where care is facultative and offspring have the option to beg for food from the parents or to self-forage. In this study, we carried out two experiments in the European earwig *Forficula auricularia*, a system where females pro- vide facultative care, in which we manipulated female condition (through a high-food and low-food treatment) and the degree by which mothers and offspring could make active choices. In a first experiment, where female mobility was limited, female condition had no significant effect on the rate of offspring self-foraging, which increased with nymph age. In a second experiment, nymph access to food was limited and females in poor nutritional condition provided food to significantly fewer nymphs than high condition females. In both experiments, off- spring attendance remained at a constantly high level and was independent of female condition even after experimental separation of females and offspring. Our results show that earwig nymphs do not use cues of female condition to adjust rates of self-foraging, that females control food provisioning depending on their own condition, and that females and nymphs share control over offspring attendance, a form of care not influenced by female condition.

Introduction

Parental care evolved due to its fitness benefit to offspring and there is broad evidence for many forms of care to enhance offspring survival across taxa (Tallamy & Wood 1986; Clutton-Brock 1991; Kvarnemo 2010). Parent-offspring conflict is a direct consequence of sexual reproduction in species where parental care evolved, resulting from diverging interests of caring parents and their offspring over the amount of parental investment (Trivers 1974). The conflict over the amount and duration of care originates from asymmetries in genetic relatedness among family members. A focal offspring is 100 % related to itself, but only 50 % related to a full-sibling and even less to a half-sibling. Thus, personal fitness (direct fitness) weighs more than the fitness of its present and future siblings (indirect fitness) (Hamilton 1964; Trivers 1974). Caring parents, on the other hand, are 50 % related to all of their offspring, and the focal offspring has equal weight compared to all other offspring in terms of inclusive fitness (Trivers 1974).

This conflict is thought to partly drive the co-evolution of behavioural strategies parents use for providing resources and strategies offspring use for demanding resources. Various game theoretical models have been developed to understand how this conflict would be resolved evolutionarily assuming either parental control ('honest signalling models', Godfray 1991; Godfray 1995; Godfray & Johnstone 2000) or offspring control ('scramble competition models', Parker 1979; Parker 1985; Parker et al. 2002). These models focused on predicting effects of offspring condition on offspring begging as well as parental food allocation decisions and made different assumptions on whether parents or offspring make active choices in the interaction (Godfray and Johnstone, 2000; Royle et al. 2002). To fully understand the evolution of parent-offspring interactions, it is important to also test how female condition affects parental care (Bateson 1994) due to expected effects of condition on costs and benefits of care, and to investigate if parents or offspring make active choices and thereby exert behavioural control in the interaction (Royle et al. 2002).

The theoretical models and much of the experimental research typically focus on the co-evolution of a single particular form of care (usually food provisioning) and the corresponding offspring demand behaviour (usually begging for food). However, benefits of parental care are often mediated not only through food provisioning, but also through other forms of care (Wilson 1975; Tallamy & Wood 1986; Clutton-Brock 1991; Royle et al. in press), especially offspring attendance and defence against predators or pathogens (e.g. Cocroft 1996; Zink 2003; Cotter & Kilner 2010). This raises the questions as to whether parental condition has similar effects on different forms of care, whether the duration of different forms of care coincide or are distinct and whether active choices for each form of care are made by the same party or not. For example, parents may decide how much food is provisioned and for how long, but offspring decide when to disperse and, hence, determine the dynamics of offspring attendance. Conversely, parents may control both food provisioning and the termination of offspring attendance by dispersing themselves or making offspring disperse (for example by aggression). Finally, it may often be expected that both parties make active choices and behavioural control over each form of care is shared between parents and offspring (the 'power continuum', Royle et al. 2002).

Effects of female nutritional condition on fecundity or parental care duration have been tested in a few species (e.g. Laurien-Kehnen & Trillmich 2004; Steiger et al. 2007). Females in good condition were reported to lactate longer compared to low condition females of Weddell seals *Leptonychotes weddellii* (Wheatley et al. 2006) or vervet monkeys *Cercopithecus aethiops* (Lee 1984) whereas other studies reported a delay in weaning for poor condition females (baboons *Papio cynocephalus ursinus* (Lycett et al. 1998); domestic guinea pig *Cavia porcellus* (Laurien-Kehnen & Trillmich 2004)). These results illustrate that the expected directionality of the effect of female condition on parental care cannot easily be generalized because it depends on a species' life history and how variation in current condition affects future reproductive success (Lee et al. 1991).

In facultative care species, offspring benefit from parental care, but they are not fully dependent on it after hatching or birth (Krafft et al. 1986; Künkele & Trillmich 1997; Smiseth & Moore 2002; Kölliker & Vancassel 2007). Here, offspring food intake is partly determined by parental food provisioning and partly by self-foraging. In contrast to obligate care species, offspring of facultative care species are able to self-forage from an early age and long periods of offspring attendance are not vital for them, at least from the perspective of nutritional dependence (not necessarily from the perspective of other needs, e.g. the protection against natural enemies). If such offspring make active choices, they may be expected to use cues of parental condition to decide if they should remain with the mother or if they should start self-foraging to avoid ineffective and possibly costly begging from a female that is unable to provide the demanded food. Furthermore, because both parents and offspring are mobile, behavioural control over the termination of food provisioning and offspring attendance can be fully investigated in such systems.

The European earwig (*Forficula auricularia* L.; Dermaptera: Forficulidae) is a nocturnal insect species where females provide maternal care for their offspring (nymphs) in terms of food provisioning (regurgitation) and protection against predation, but care is only facultative and nymphs can survive without the mother after hatching (Lamb 1975; Lamb 1976a; Lamb 1976b; Kölliker & Vancassel 2007). Female earwigs produce one or two clutches in their lifetime indicating scope for mother-offspring conflict over the timing of termination of care. Caring for offspring is costly because it delays second clutch production (Kölliker 2007), and under continued offspring attendance, nymphs pose a direct threat to the second clutch due to oophagous sibling cannibalism (Lamb 1976a). In this study, we manipulated female condition in *F. auricularia* by manipulating female access to food and tested the effect of female condition on nymph self-foraging, maternal food provisioning and maternal attendance in two experiments that differed in the extent by which caring females were able to make active choices. Comparison between the two experiments allowed us to test if females or offspring make active choices and behaviourally control food provisioning and offspring attendance.

Methods

Study species & rearing conditions

Individuals of *F. auricularia* were collected in an olive grove in Dolcedo, Italy (7°56'55'E, 43°54'14'N, altitude 443 m) in May 2009 and were setup in the laboratory under standard conditions in plastic containers (37 x 22 x 25 cm), consisting of 30 males and 30 females each. All containers were lined with fluon (Whitford GmbH, Diez, Germany) to prevent escape by the insects and contained humid sand as a substrate as well as egg cardboard and plastic tubes as shelters. After random mating, females were set up individually in petri-dishes (100 x 20 mm) containing humid sand as substrate, plastic shelters as burrows and ad libitum artificial food (Meunier et al. in press). All females were subsequently transferred to complete darkness at 10 °C for one week to induce egg-production and then maintained in complete darkness at 60 % humidity and 15 °C until egg-laying and hatching. In this population, females lay on average between 46 - 52 eggs in their first clutches and approximately 85% of females lay second clutches (Meunier et al. in press).

Experimental design

We checked clutches daily for hatching and assigned the date of hatching as day 0. On day 1, the number of hatched nymphs was counted and families were transferred from 15 °C and complete darkness into the experimental room with 20 °C and a 14 h/10 h light/dark cycle and were provided with ad libitum food. Family groups were setup for the food manipulation (see below) on day 2. Each female and 20 of her nymphs (standardized clutch size) were transferred into an artificial burrow, that is, a small petri-dish (35 x 10 mm) divided by a grid with the female on one side and the nymphs on the other side. This allowed antennal contact and communication via chemical cues between mother and offspring, but prevented food provisioning by the female.

On day 4, family groups were transferred to the experimental containers for behavioural observations. The experimental containers consisted of a transparent plastic box (321 x 229 x 51 mm) with humid sand as substrate and two artificial burrows to provide shelter (Fig. 1). As before, the burrows consisted of a small petri-dish (35 x 10 mm) covered with a lid painted in black. Each burrow had a hole in the side of the dish serving as an entrance/exit. Females and nymphs were transferred to one of the burrows (in the following referred to as 'primal burrow'). The second burrow (in the following referred to as ‘substitute burrow’) was located on the opposite side of the container, where females or nymphs could disperse to after termination of offspring attendance.

The experimental design consisted of two experiments (N = 40 each), each consisting of two female condition treatments. In Exp. I, female mobility was experimentally restricted and in Exp. Π nymph access to food was limited. Both experiments were conducted simultaneously. Females were randomly assigned to the experiment and female condition treatment. Because female condition was manipulated in both experiments, we first describe this treatment before explaining in detail the design of Exp. I and Exp. Π.

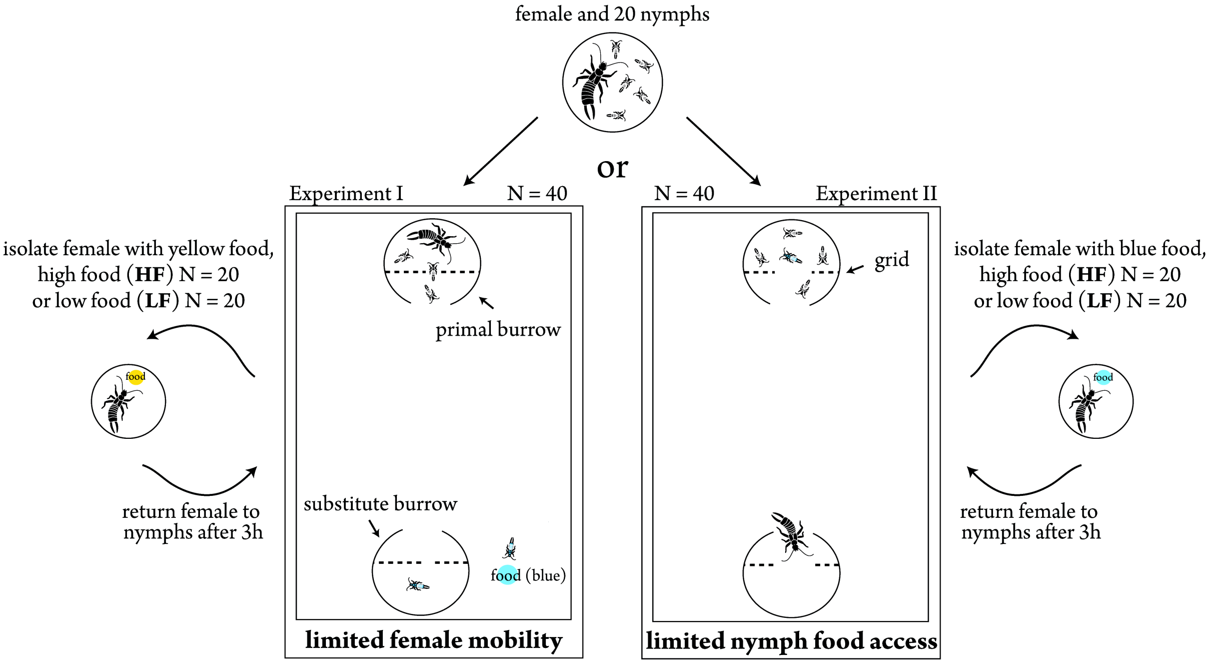

Figure 1. Experimental design: left side: set-up for Exp. I (limited female mobility), right side: set-up for Exp. II (limited nymph food access). Circles represent artificial burrows. Top circle: primal burrow, bottom circle: substitute burrow. The dashed lines illustrate the grids. The dot next to the substitute burrow indicates the food source (left side only). Large earwig: *Forficula auricularia* female, small earwigs: *F. auricularia* nymphs.

Female food manipulation

To experimentally manipulate female nutritional condition while keeping offspring nutritional condition as constant as possible, we daily manipulated the food access for each female independently of her nymphs. The first food treatment took place on day 2 after hatching. Feeding took place daily from 12:00 – 15:00 h in the dark period. Females were isolated from their nymphs during this time, and after 3 h of feeding, they were returned back to their nymphs, still separated by the grid. Nymphs had the possibility to feed ad libitum on pollen during the same 3 h period. To later confirm the effect of this experimental manipulation on female condition in terms of weight change, we measured female weight on day 1 and again on day 15 to the nearest 0.001 mg using a Mettler-Toledo MT5 Micro-balance (Mettler, Roche, Basel). The amount of food offered to females was based on previous measures of female food intake. Females eat on average between 3 – 4 mg of food every day when fed with ad libitum food (Kölliker 2007). In our experiments females in the low food treatment (LF) were allowed to feed on 0.5 – 0.9 mg pollen pellets every second day whereas females in the high food treatment (HF) were allowed to daily feed on 10 – 15 mg of pollen pellets (pollen: Swiss Extract Todon, Bonaduz, Switzerland; pellets prepared according to Staerkle & Kölliker (2008)). The food in the LF treatment was always completely eaten by these females indicating that food must have been limited. The quantities of food for HF females corresponded to ad libitum conditions during the 3 h of food manipulation. In both, Exp. I and Exp. II, the families were set up and observations initiated on day 4 after hatching.

Experiment I: Limited female mobility

Exp. I corresponded to a setup with limited female mobility (Fig. 1). Here, nymphs could receive food provisioned by the female or self-forage from a food source in the experimental container. The female could not leave the primal burrow. A grid, which had a small hole, allowed only the nymphs to pass, but prevented the female from leaving the primal burrow and from reaching the food source. We measured the initiation and dynamics of self-foraging as well as the number of days the nymphs continued to aggregate with the female in their primal burrow. In order to quantify the patterns of self-foraging by nymphs, a pollen pellet dyed with blue food dye was provided as ad libitum food source for the nymphs on the far side of the primal burrow next to the substitute burrow. Since first instar nymphs are transparent, self-foraging activity could be scored as the number of individuals with blue gut content (Staerkle & Kölliker 2008). The dyed pollen pellet was composed of 50 mg of flower pollen (Swiss Extract Todon, Bonaduz, Switzerland) and 10 ml blue food dye (werma Werner Schweizer AG: Blau Spezial E-131) and was changed daily. Nymphs had continuous access to the blue food source.

During the daily feeding (see above), the females in this experiment (HF and LF) received yellow pollen pellets, because blue guts of nymphs were used as marker to score nymph self-foraging activity only. After 3 h of feeding, females were returned to the primal burrow. Every morning the nymphs with blue gut content were counted and the locations of the nymphs and the female noted (in the primal or substitute burrow). The dynamics of self-foraging was measured as the pattern of change in the number of nymphs with blue guts over time.

In this experiment, only nymphs could make active choices to maintain or terminate offspring attendance. Offspring attendance was considered terminated when >75 % of the nymphs were found in the substitute burrow for 3 days in a row. The location of nymphs and the number of blue nymphs were determined daily from day 5 to day 15 during daytime, when individuals were inactive and usually found in the burrows. From day 16 to 34 we simplified the measure of offspring attendance and determined whether or not the majority of nymphs still aggregated with the female in the primal burrow.

Experiment Π: Limited nymph access to food

Exp. Π corresponded to a setup with limited nymph access to food (Fig. 1). Here, nymphs received food provisioned by the female, but self-foraging was restricted to the 3 h period of female food manipulation (there was no food source in the experimental arena). Contrary to Exp. I, the grid had a large hole and both the female and her nymphs were able to leave the burrow. Since it was not possible to restrict nymph mobility and allow free mobility for the female this experiment reflects a situation where female and nymphs have the potential to influence offspring attendance. Female food manipulation was continued daily as described above. In contrast to Exp. I, isolated females were provided with blue food during the daily 3 h feeding time according to their condition treatment (see above) and offspring were fed with yellow pollen during this time. To prevent premature nymph dispersal induced by handling disturbance, the food was provided to nymphs during this time directly in the burrow. By daily scoring the number of nymphs with blue guts, we could quantify in this experiment the daily amount of maternal food provisioning and, hence, its dynamics over nymph age and duration. In Exp. Π, we quantified when females would terminate offspring attendance and considered termination of offspring attendance to have occurred when only the females were found in the substitute burrow for 3 days in a row. The location of the nymphs, the number of blue nymphs and the location of the female were determined daily from day 5 to day 15. Female locations were scored until day 34.

Offspring attendance with and without limited female mobility

During the course of the experiments we observed a continued high level of offspring attendance (see results for details). To test whether this continued attendance was due to active aggregation tendencies of females, nymphs or both, we randomly transferred either the female (Exp. I: N = 13, Exp. Π: N = 14) or the nymphs (Exp. I: N = 13, Exp. Π: N = 15) to the substitute burrow on day 34. In Exp. I, the substitute burrow restricted female mobility in the same way as the primal burrow to ensure that in this experiment only nymphs could make the choice to re-aggregate. The location of the female and the nymphs was determined daily from day 35 to the end of the experiment on day 40. We also kept control replicates (N = 5 in Exp. I and Π, respectively) where we did not transfer the female or nymphs to be able to detect potential termination of offspring attendance after day 35. If the nymphs initiate attendance, we predicted an equal amount of re-aggregations in Exp. I and Π, since only nymphs could move freely in both set-ups. Conversely, if the females control attendance, we expected re-aggregations in Exp. Π, but not in Exp. I where female mobility was restricted.

Statistical analyses

Cases in which all nymphs died (Exp. I: N = 2, Exp. Π: N = 1) or the female died (Exp. Π: N = 1), were excluded from the analyses and final sample size was N = 19 for each treatment. To confirm the effect of the food manipulation treatment on female condition, we compared the relative weight change of females from day 1 until day 15 (absolute weight gain divided by the initial weight at day 1), using ANOVAs for each experiment. Here, the sample size was reduced to N = 36 in Exp. Π, due to one case of early female dispersal and one case of missing values. As expected, HF females gained significantly more relative weight than LF females (Exp. I: ANOVA, $R^2 = 0.666$, $F_{1,37} = 74.864$, $p < 0.0001$; Exp. Π: ANOVA, $R^2 = 0.217$, $F_{1,35} = 10.716$, $p = 0.002$). Although nymphs in Exp. Π were probably more food limited compared to nymphs in Exp. I, survival on day 40 was not significantly different between the two groups ($t_{63} = -1.262$, $p = 0.212$).

To analyse nymph food intake (the proportion of blue nymphs) across time in the two experiments, we used repeated-measures MANOVA with female condition as between-subjects factors and time as within-subjects factor (i.e. repeated measure) including the corresponding interaction. We also used repeated-measures MANOVA with the same factors as before and experiment as additional between-subjects factor to analyse the proportion of nymphs that aggregated with the female (i.e. in the same burrow) from day 35 until day 40 across the two experiments. Proportions were arcsine square-root transformed for statistical analysis. In the figures, means and standard errors for proportions were obtained by back-transforming the parameter estimates to the proportional scale. All *p*-values are two-tailed. Statistical analyses were conducted with JMP, Version 9.0.2. SAS Institute Inc., Cary, NC, 1989-2010.

Results

Experiment I: In this experiment, food intake by nymphs reflected self-foraging. The proportion of nymphs that self-foraged on the blue food was not significantly different between HF and LF females (main effect female condition: $F_{1,35} = 1.581$, $p = 0.217$) (Fig. 2). The proportion of blue nymphs gradually increased with time (main effect time: $F_{11,25} = 9289.498$, $p < 0.0001$) and reached 100 % within approx. 5 - 6 days, by the age of 9 days. The dynamics of self-foraging over time was not significantly different between HF and LF treatments (interaction female condition * time: $F_{11,25} = 1.529$, $p = 0.183$).

During the course of Exp. I (until day 34) nymphs terminated offspring attendance by dispersing to the substitute burrow in only 7 out of 38 replicates. Among these 7 cases 4 occurred in the HF and 3 in the LF treatment. In each of the remaining 31 replicates, the majority of nymphs stayed with the female until termination of the experiment (day 40).

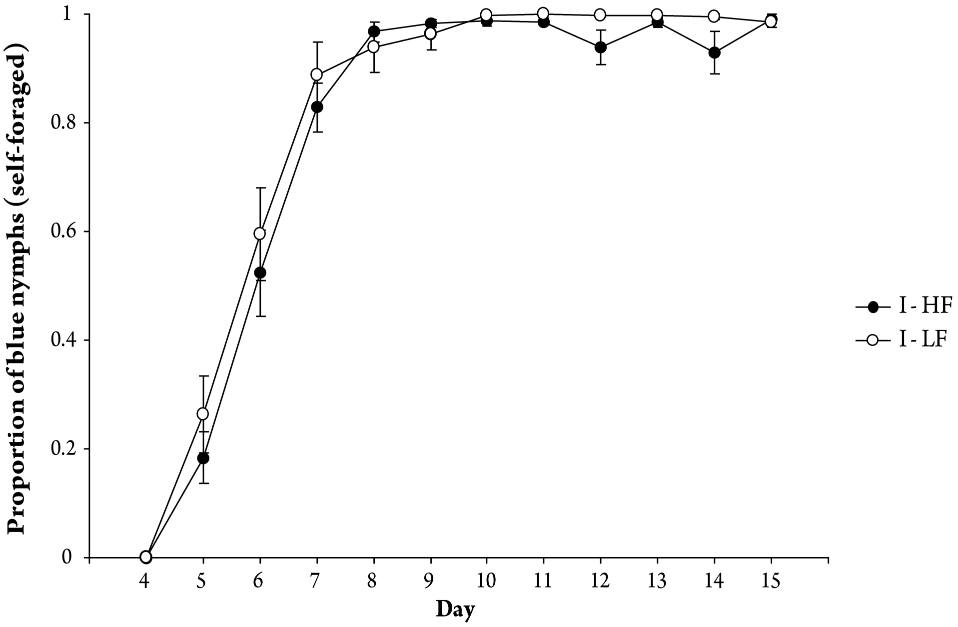

Figure 2. The proportion of nymphs, which self-foraged across time. In Exp. I, nymphs became blue because of self-foraging on a blue food source, when being with either a high-food female (black circles) or low-food female (white circles). Shown are means and standard errors that were estimated based on the arcsine square-root-transformed data and then back-transformed to the proportional scale.

Experiment II: In this experiment, blue food intake by nymphs reflected provisioning by the mother. HF females provided food to significantly more nymphs than LF females (main effect female condition: $F_{1,35} = 6.528$, $p = 0.015$) (Fig. 3). Not surprisingly, the dynamics of food provisioning changed with time increasing initially to a plateau and decreasing afterwards (main effect time: $F_{11,25} = 3.683$, $p = 0.003$). This pattern of provisioning over time proceeded significantly differently between HF and LF females (interaction female condition * time: $F_{11,25} = 2.663$, $p = 0.021$). HF females showed a faster initial increase in provisioning and a slower decrease until day 15.

Similar to Exp. I, offspring attendance was terminated in only very few cases, as reflected by the low incidence of dispersal to the substitute burrow. In Exp. II termination of offspring attendance was observed in 4 out of 38 replicates, twice in the HF and twice in the LF treatment. In all four cases it was the female that dispersed to the substitute burrow.

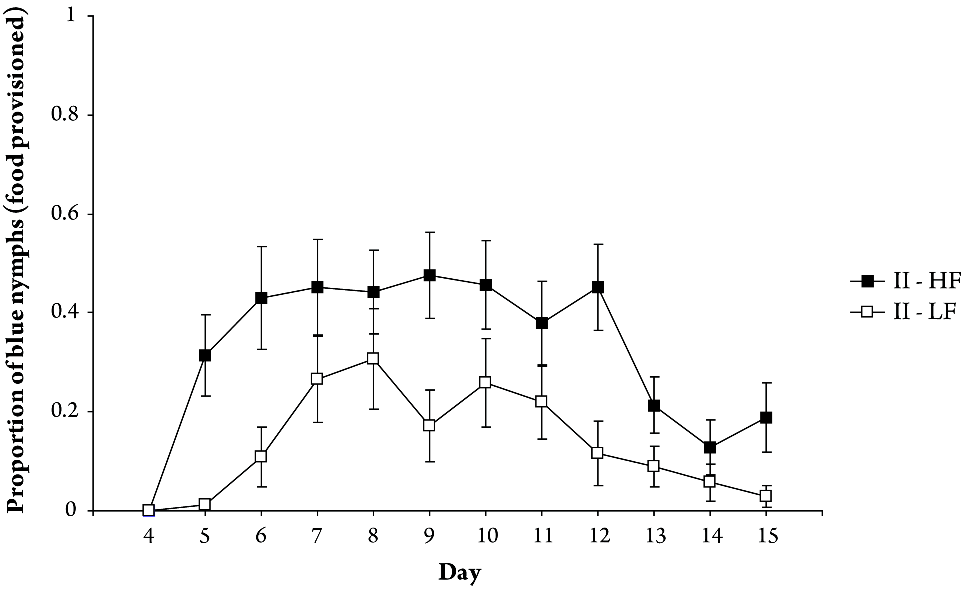

Figure 3. The proportion of nymphs, which received maternal food pro- visioning across time. In Exp. II, blue nymphs received food provisioning from either a high-food female (black squares) or a low-food female (white squares). Shown are means and standard errors that were estimated based on the arcsine square-root-transformed data and then back-transformed to the proportional scale.

Experiment I & Π: When comparing re-aggregation after the experimental separation of females and nymphs across the two different experiments and across time, we found a significantly higher level of offspring attendance, measured as the proportion of nymphs re-aggregating with the female, in Exp. Π where both females and nymphs could choose to re-aggregate compared to Exp. I where only nymphs were mobile (main effect experiment: $F_{1,51}$ = 17.109, $p < 0.0001$; Fig. 4). Re-aggregation did not significantly depend on female condition ($F_{1,51}$ = 0.005, p = 0.946) or on the interaction between experiment and female condition ($F_{1,51}$ = 0.556, p = 0.459). The proportion of nymphs that re-aggregated with the female significantly increased with time ($F_{5,47}$ = 3.319, p = 0.012) and showed a significant interaction between time and experiment ($F_{5,47}$ = 2.534, p = 0.041; Fig. 4). This interaction was due to the stronger increase over time in attendance in Exp. I compared to Exp. Π. The interaction between time and female condition as well as the three-way interaction between time, experiment and female condition were not significant ($p > 0.314$).

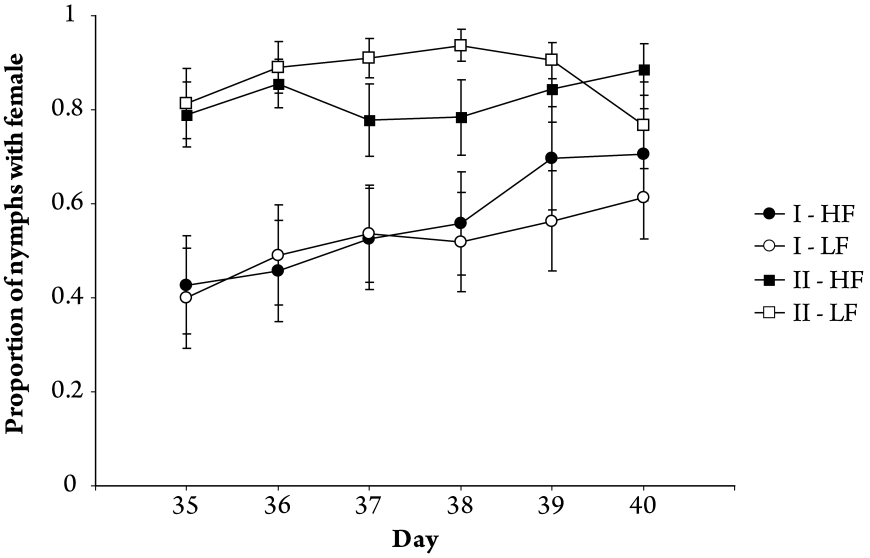

Figure 4. The proportion of nymphs that were in the same burrow as the female is shown for each day after the experimental separation of nymphs and females on day 34. In Exp. I, black circles represent nymphs from a high-food (HF) female, white circles indicate nymphs from a low-food (LF) female. In Exp. II, black squares represent nymphs from a HF female, white squares indicate nymphs from a LF female. Shown are means and standard errors that were estimated based on the arcsine square-root-transformed data and then back- transformed to the proportional scale

Discussion

This study investigated the effect of female nutritional condition on maternal food provisioning, nymph self-foraging and offspring attendance, and tested whether females or offspring make active choices to maintain or terminate maternal care in the European earwig *F. auricularia.* In Exp. I, where female mobility was limited and the amount of nymph self-foraging quantified, the rate of nymph self-foraging increased gradually to 100 % within few days irrespective of female condition. This result confirms earlier observations that earwig nymphs are mobile and quite effective self-foragers already early after hatching, and indicates that nymphs do not use cues of female condition to change their self-foraging activity as we hypothesized. In Exp. II, where nymph food-intake through self-foraging was limited and the amount of maternal food provisioning quantified, female food provisioning was significantly lower in LF females and decreased with time in both female condition treatments even though nymph access to food remained constant. Finally, the degree of offspring attendance by females from both conditions remained high throughout the whole period and was actively re-established by both the nymphs and the female after experimental separation. Thus, offspring attendance was maintained by active choices by females and nymphs for substantially longer time than maternal food provisioning, suggesting further social benefits of maintaining the family group besides food provisioning.

Bateson (1994) hypothesized that offspring should adjust their demand for parental care to cues of parental condition. Such adjustment would be particularly expected in facultative care species where offspring can choose between begging from parents and self-foraging (Smiseth et al. 2003). In earwigs it was shown before that nymphs leave their burrow for self-foraging earlier when their mother is absent (Kölliker 2007) and, hence, adjust their behaviour according to the presence/absence of their mother. In our experiment they did not significantly adjust their self-foraging activity according to their mother's nutritional condition (Exp. I). Thus, available maternal cues of condition (e.g. a correlate of body weight) were not used by the nymphs to adjust self-foraging activity, although LF-females provided less food.

Exp. Π was designed to quantify maternal food provisioning. Contrary to the dynamics of nymph self-foraging, there were marked effects of female condition on both overall amount and time dynamics. LF females provided food to significantly fewer nymphs, and their rate of provisioning increased more slowly and decreased earlier than the rate of provisioning of HF females. The difference in overall quantity could be explained by higher expected differential costs of care in low condition parents, or food limitations of LF females generally constraining the amount of food provided. However, the different time-dynamics especially the flatter increase in provisioning early and sooner decrease in the LF treatment (Fig. 3) rather suggest that females in poor condition may withhold more of their resources for themselves. Similar results were obtained in other studies where it was shown before that low quality parents provide smaller amounts of care for shorter durations across different species (Öst et al. 2003; Vergara et al. 2010).

Comparison of the results on nymph food intake in Exp. I versus Exp. Π offers some insight into female control over maternal food provisioning and further provides evidence that females make active choices to reduce provisioning when the nymphs reach an age of 10 days. When nymphs were not food limited (Exp. I), self-foraging reached a maximum and remained constantly high, indicating that nothing intrinsic to nymph development (e.g. moult to 2^{nd} instar) reduced their demand for food during the period when food provisioning declined. In Exp. Π female food provisioning decreased with time although nymph access to food remained limited. These results jointly suggest that females start to limit food provisioning with time. However, one could argue that a similar pattern of maternal food provisioning could arise under offspring control due to an increased efficiency in self-foraging with time and an associated reduction in nymph food intake through maternal provisioning. But since direct access to food for nymphs was continuously restricted in Exp. Π, it appears implausible that self-foraging became more efficient than begging. Yet mothers still reduced their feeding activity, which suggests female control over the duration of food provisioning in respect to her own condition.

Despite the decrease in maternal food provisioning, offspring attendance constantly remained at a high level throughout the course of the experiment (40 days), as confirmed by our control groups, where we did not separate females and nymphs on day 34. Overall, only a very low number of nymphs and females terminated offspring attendance and moved from the primal to the substitute burrow. There was no significant effect of female condition on the frequency of terminated offspring attendance, neither in nymphs nor in females. After the experimental separation of family groups on day 34, we found that the proportion of nymphs, which was in the same burrow as the female was higher in Exp. II than in Exp. I, i.e. higher in the set-up where the female could move freely. This suggests that the female actively contributed to a faster re-aggregation with nymphs and at least partially behaviourally controls offspring attendance. However, in Exp. I, we also observed re-aggregation that was necessarily initiated by the nymphs since female mobility was restricted, but re-aggregation levels were significantly lower, and continued to increase over time. Thus, both females and nymphs enhanced aggregation through their spatial activities thereby sharing behavioural control over offspring attendance. Females and nymphs continued to aggregate despite the drop in maternal food provisioning, suggesting further benefits of staying in family groups (e.g. predator defence, foraging efficiency or mutual grooming (Krause & Ruxton 2002)).

Bateson (1994) argued that female mammals in moderate nutritional condition should lactate their young longer, if offspring develop more slowly than under optimal conditions due to the females' reduced nutritional state. This hypothesis might explain the long period of offspring attendance by females in our experiment, because females from both condition treatments had access to food for a restricted amount of time. The only other study, which investigated the duration of maternal care in *F. auricularia* (Lamb 1976a) was carried out under ad libitum food conditions and reported termination of offspring attendance 23.2 ± 0.59 (mean ± SE) days after hatching, which is substantially earlier than in our study. Alternatively, the longer duration of offspring attendance in our study may partially reflect evolved population differences in maternal care due to local adaptation to different climatic and environmental conditions between Canada (Lamb 1976a) and Italy (this study). Females have to abandon their nymphs at some point in order to successfully produce a second clutch (Lamb 1976a; see introduction). This is why we predicted that females and nymphs should separate during the course of the experiment. The substitute burrow offered an alternative shelter for the females to disperse to and start a second clutch. However, the majority of females did not terminate nymph attendance. Further experiments are needed to test the roles of access to food and local adaptation on the maintenance and termination of parental care.

The effect of offspring condition is considered an important factor in current conflict resolution models and was also tested in empirical studies across a broad range of species (Kilner & Hinde 2008; Mock et al. 2011), including the European earwig (Mas et al. 2009; Mas & Kölliker 2011). Although female condition is known to influence the period of maternal care (Laurien-Kehnen & Trillmich 2004), current theoretical models do not incorporate female condition although female condition might be an important factor in shaping active choices by both sides (Bateson 1004). Furthermore, the dissociation of family groups via termination of offspring attendance and parental food provisioning have been frequently studied in obligate care systems like mammals (Zhao et al. 2008; but see Rehling & Trillmich 2008) or birds (Vergara et al. 2010; Tarwater & Brawn 2010; Naef-Daenzer et al. 2011). In such systems, the termination of offspring attendance and food provisioning usually coincides. Much less research has been devoted to species with facultative care (Smiseth et al. 2003), where offspring attendance and food provisioning can be loosely linked and, hence, behavioural control for different aspects of family life vary. Our results indicate that in the European earwig offspring attendance lasted substantially longer than maternal food provisioning (see Smiseth et al. 2003 for a similar result in burying beetles), that the females controlled the pattern of food provisioning over time, but that behavioural control over offspring attendance is shared between females and nymphs. These results jointly show that in species with facultative care like *F. auricularia*, the duration of and behavioural control over different aspects of parental care may not be identical and differentially affected by female condition. This potential divergence in termination of and behavioural control over different forms of parental care should be further investigated, both theoretically and experimentally, to better understand the diversity and evolution of parental care and family interactions.

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CHAPTER 3

The more the merrier? Condition-dependent brood mixing in earwigs

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The more the merrier? Condition-dependent brood mixing in earwigs, *Animal Behaviour*, 86, 845–850

Abstract

Parental care is beneficial for offspring, but costly for parents. Thus, it seems maladaptive to care for unrelated offspring unless the additional offspring provide direct benefits to the carer's own offspring or discrimination costs are high. Females of the European earwig, *Forficula auricularia*, provide maternal care for their own offspring (nymphs), but also for foreign nymphs that join their brood. Nymphs preferentially kill and cannibalize nonsiblings and individuals that are smaller or in poor condition. Here, we tested the effects of nymph condition and food availability on brood mixing in F. auricularia. We experimentally manipulated nymph condition by feeding or food depriving groups of unrelated nymphs. The resident groups remained with their mothers whereas the mothers were removed from the foreign groups. Since nymph condition determines the direction of cannibalism, we hypothesized that brood mixing is dependent on nymph condition and food availability in the environment. Our results show that brood mixing was initially driven by condition-dependent effects on nymph activity, and later by the fed foreigners invading resident broods, suggesting that foreigners control brood mixing, independent of food availability. These results are consistent with our hypothesis that the dynamics of brood mixing are condition dependent, possibly mediated by corresponding cannibalism threats

Introduction

Parental care is widespread across taxa and evolved because it increases offspring fitness (Clutton-Brock 1991; Royle et al. 2012; Wong et al. 2013). However, parents often pay a cost of care in terms of current and/or future reproduction, which is why it seems maladaptive for the parents to tolerate foreign offspring in their brood/litter and exhibit parental care for these unrelated offspring (alloparental care) (Roldan & Soler 2011). Nevertheless, intraspecific alloparental care has been reported from a variety of species in birds (Brown et al. 1995; Berggren 2006), mammals (reviewed by König 1997 and Roulin 2002, Gero et al. 2009), fish (McKaye & McKaye 1977; Wisenden 1999) or insects (Müller et al. 1990; Agrawal et al. 2004; Kölliker & Vancassel 2007).

Several factors may contribute to the occurrence of brood mixing and intraspecific alloparental care. For example, aspects of population structure such as high density and patchy food sources may affect the encounter rate between parents and unrelated juvenile conspecifics. These factors may facilitate alloparental care and the resulting brood mixing in particular if the juveniles are mobile (i.e., precocial) because they can actively join other broods. Furthermore, spatial proximity can enhance scope for different forms of interactions between breeding adults that result in brood mixing and alloparental care, e.g. communal breeding (house mouse *Mus musculus domesticus*; König 1993), egg-dumping (lace bug *Gargaphia solani*; Loeb 2003), takeover of nests/breeding sites including the offspring of the former breeders (burying beetle *Nicrophorus vespilloides*; Müller et al. 1990).

Food availability in the environment may also affect alloparental care and brood mixing, which was shown in a study conducted in the burrower bug *Sehirus cinctus* (Agrawal et al. 2004). In this species, females provision their young with food in the form of mint nutlets. Agrawal et al. found higher levels of brood mixing when food availability was experimentally limited. They also found that brood mixing occurred independently of the presence or absence of the mothers, suggesting that it was initiated by the offspring. This is in line with the findings in other species with mobile offspring, where insufficient care from the biological parents or their death may lead to offspring abandoning their own nest or burrow. Evidence for an effect of insufficient care on brood mixing comes from gulls (*Larus delawarensis, Larus occidentalis, Larus argentatus*), where chicks, which abandoned their natal nest and mixed with other broods, were fed at a significantly lower rate before (disadvantaged chicks) than similarly aged chicks that remained in their home broods until fledging (Pierotti & Murphy 1987; Brown 1998). Thus, offspring may abandon their own nest to sequester enhanced (allo)parental care elsewhere and thereby at least partly drive the brood mixing dynamics leading to alloparental care. Finally, in the Alpine swift (*Apus melba*), ectoparasites were shown to significantly increase the rate of brood mixing. The chicks of infested nests switched broods earlier and more often, apparently to evade ectoparasite exposure (Bize et al. 2003; Bize & Roulin 2006).

The reasons why parents should tolerate and care for foreign offspring seem less straightforward. Foreign offspring may reduce growth and/or survival of own offspring (Brown 1998), e.g. through enhanced competition or cannibalism (Mock & Parker 1997) or the introduction of ectoparasites (Bize et al. 2003). Evolutionary explanations why parents may tolerate invading foreign offspring revolve around costs of kin discrimination (reviewed in Keller 1997), various types of limitations (physical, cognitive, sensory, etc.) combined with a competition between different discrimination mechanisms that constrain parents to accept foreign offspring (reviewed in Grim 2006), and potential direct fitness benefits to the parent or the parents' own offspring (reviewed in Alonzo & Klug 2012). For instance, foreign offspring can contribute to a dilution of predation risk on own offspring (Hamilton 1971; Foster & Treherne 1981), as shown, for example, in the cichlid species *Cichlasoma nigrofasciatum* (Wisenden & Keenleyside 1992). Parents tending mixed broods containing foreign fry smaller than own fry enhanced survival of own offspring because of the predator dilution effect. Only fry of smaller or similar size than own fry were accepted and the smaller fry acted as primary predation targets. This differential predation effect added to the benefit of brood mixing in this species. Further evidence for direct benefits of alloparental care and brood mixing was shown in Canada geese (*Branta canadensis*; Nastase & Sherry 1997) and eider ducks (*Somateria mollissima*; Öst & Bäck 2003), where survival of own offspring was enhanced in mixed broods. In these examples, this effect may have been due to own offspring being physically closer to the tending parent than the foreign offspring, and a higher predation risk for chicks further away from the parent. The added cost of caring for foreign offspring to parents can thus be outweighed by direct gains of fitness to own offspring.

Brood mixing occurs also in the European earwig (*Forficula auricularia* L.; Dermaptera: Forficulidae), where females provide care for their offspring. They attend their eggs and, after hatching, provide food to their nymphs via regurgitation and protect them against predation (Lamb 1975; 1976b; 1976a; Kölliker & Vancassel 2007). Earwig nymphs are mobile soon after hatching and can join foreign clutches, as shown under field conditions (Kölliker & Vancassel 2007), and females tolerate and provide care for foreign offspring in cross-fostering experiments (Meunier et al. 2012). This indicates that females of *F. auricularia* do not discriminate against foreign nymphs (see also Radl & Linsenmair 1991 for indiscriminate maternal care in *Labidura riparia*, another earwig species). While females appear to care indiscriminately, Dobler & Kölliker (2010) showed that cannibalism occurred sooner and at higher rates between non-siblings than between siblings under restricted food conditions indicating recognition and defence mechanisms among nymphs. Furthermore, small/light nymphs are more likely to be the victims of cannibalism than large/heavy ones (Dobler & Kölliker 2011). Thus, the cost:benefit ratio of brood mixing to nymphs and of accepting foreign nymphs to caring females should depend on the relative condition of the resident nymphs compared to the foreign intruders.

In this study we investigated the effects of nymph condition and environmental food availability on alloparental offspring attendance and brood mixing in *F. auricularia.* It was shown before that the threat of cannibalism depends on nymph body condition (see above) and that environmental food availability influences the benefits of maternal care and earwig family life (Kölliker 2007; Meunier & Kölliker 2012). Here, we experimentally tested the hypothesis that patterns of brood mixing depend on nymph condition and food availability in the environment. To this end, we analysed the level of female-nymph aggregation with own versus foreign nymphs as a measure of brood mixing. We predicted that, if foreign nymphs generally drive brood mixing, foreigners in good condition should show more brood mixing than foreigners in poor condition, because the residents in poor condition would pose a smaller cannibalism threat. Conversely, if the female and the resident nymphs control brood mixing, they should restrict mixing with well-fed foreigners, but allow foreigners in poor condition to join the brood. We further expected higher levels of brood mixing in the environment with high food availability compared to the environment with low food availability. This prediction is opposite to the findings of the studies mentioned above because in earwigs the potential benefits of brood mixing are enhanced under high food availability, since the overall cannibalism threat is reduced and the benefits of maternal care are enhanced (Kölliker 2007; Meunier & Kölliker 2012).

Material & Methods

Rearing conditions

Individuals of *F. auricularia* were collected in an olive grove in Dolcedo, Italy in May 2010. Three groups of approx. 100 fourth instar nymphs were setup in the laboratory until they emerged as adults, and were then allowed to mate freely in plastic containers (37 x 22 x 25 cm) of approx. 50 males and females each over the duration of 7 months until the first female laid her clutch of eggs. All containers were lined with fluon (Whitford GmbH, Diez, Germany) to prevent escaping of the insects and the lids had slits for ventilation. Humid sand was used as a substrate. Egg cardboard as well as plastic tubes were provided as shelters. The food consisted of an artificial diet (see Meunier et al. 2012 for details) with the addition of 1 g sorbic acid to reduce mould growth (Carl Roth GmbH + Co, Karlsruhe, Germany) and 1 g ascorbic acid (Drogerie zum Chrüterhüsli, Basel, Switzerland), and the food was changed twice per week. The containers were kept in a climatic chamber at 60 % humidity, 14 h/10 h and 20 °C/20 °C light/dark cycle simulating summer conditions. In November 2010, all containers were transferred to fall conditions (60 % humidity, 10 h/14 h and 20 °C/15 °C light/dark cycle) to stimulate egg production. After the first oviposition, all females were set up individually in petri-dishes (10 x 2 cm) containing humid sand as substrate, plastic shelters and *ad libitum* food. They were transferred to complete darkness at 10 °C for one week and then maintained in complete darkness at 60 % humidity and 15 °C until hatching (= day 0). No food was provided after oviposition (Kölliker 2007).

Experimental design

From a total of 125 clutches from this stock of animals, 52 were used in the experiment. The other clutches were not part of the experimental samples because we needed an even number of clutches on each day to be able to form brood dyads of residents and foreigners of identical age. On day 1 after hatching, the broods were provided with ad libitum food. The hatched nymphs were counted and the clutch size standardized to 20 nymphs. Among these, the weight of five randomly chosen nymphs was measured to the nearest 0.001 mg using a Mettler-Toledo MT5 Micro-balance (Mettler, Roche, Basel). Females and nymphs were transferred to the experimental room for light cycle and climate adaptation (14:10 h light:dark cycle and approximately 20 °C during light and dark periods). On day 3, clutches were randomly assigned to the fed or the food-deprived treatment. Fed family groups were provided with ad libitum food on days 3 and 4. Food-deprived family groups received no food during this period. On day 5, each fed clutch was paired with one food-deprived clutch of the same hatching date. We used 52 families to form 31 replicates. Owing to low hatching success of some clutches 10 families were used twice; these families were assigned equally to the different treatment groups. After assigning fed and food-deprived clutches to clutch dyads on day 5 (see above), we randomly determined one clutch to be the residents (R = set-up with the nymphs' own mother in the experiment) and the other clutch as foreigners (F = set-up without the mother). The status of residents and foreigners was alternated between fed and food-deprived nymphs, resulting in 16 replicates in which the residents were fed and the foreigners were food deprived (R fed & F deprived) and in 15 replicates in which the residents were food deprived and the foreigners were fed (R deprived & F fed). To evaluate proper randomization, we used nymph weight on day 1 (see above) to confirm that there was no difference in body weight between nymphs of the different experimental groups before food manipulation (fed - deprived: $F_{1,59} = 0.388$, $P = 0.536$; resident-foreigner: $F_{1,59} = 0.202$, $P = 0.655$). We used 20 resident and 20 foreigner nymphs in 25 replicates. In the six remaining replicates, we set up groups with fewer nymphs because of some early nymph mortality (18 nymphs per group ($N = 1$), 17 nymphs ($N = 2$) and 15 nymphs ($N = 3$)), which were equally distributed among the treatments. Excluding these replicates from the statistical analysis did not change the results qualitatively. All nymphs were individually marked, by clipping the tip of either the left or the right forceps with a fine pair of dissection scissors. We clipped the forceps instead of colouring individuals with marker pens, as colours might influence the chemical profile of nymphs, which could disturb chemical communication and kin recognition. Any potentially harmful effects of this marking technique were ruled out in a pilot study. Individuals did not seem to be perturbed by the marking and completely regenerated the forceps after one or two moults (J. W. Y.

Wong, personal observation). The side of clipping (left or right) was alternated between residents and foreigners.

On day 5 after hatching, after food manipulation and marking, five residents and five foreigners were weighed to confirm successful manipulation of nymph condition. Afterwards, all individuals were transferred to the experimental containers (transparent plastic box 32.1 x 22.9 x 5.1 cm), holding humid sand as substrate. An artificial burrow, consisting of a small petri dish (3.5 x 1 cm) covered with a black lid, was placed on each far side of the container. The burrow had a small hole, which served as an entrance/exit, and all the individuals had the opportunity to seek shelter during daytime (nocturnal species; analogous to Wong & Kölliker 2012). The 20 residents and the female were put in one burrow (referred to as a 'resident burrow') whereas the foreigners were put in the opposite burrow (referred to as a 'foreign burrow'), alternating the left and the right burrow between the two groups of nymphs, respectively. The burrows were closed for 2 h to avoid immediate exploration and to allow adaptation to the new environment. After 2 h the burrows were opened and all individuals were free to move. The containers resembled either a set-up with high food availability with multiple food sources changed daily (R fed & F deprived ($N = 8$); R deprived & F fed ($N = 8$)) or one with low food availability without any food sources present (R fed & F deprived ($N = 8$); R deprived & F fed ($N = 7$)).

Brood mixing was quantified in the mornings of days 6 and 8 after hatching by recording the number of residents and foreigners alive as well as the proportion of residents and foreigners that aggregated with the female, that is, the proportion of nymphs that were in the same burrow as the female. The brood-mixing score from day 6 provided an early measure of aggregation (approximately 1 day after set-up of residents and foreigners in separate burrows) and the score from day 8 a later measure for which the study subjects had more time to make aggregation decisions. The observations were terminated on day 8 for two reasons. First, the condition differences between nymphs were expected to diminish over the course of the experiment under both high and low food availability. Second, we intended to keep actual cannibalism at low rates because it could have confounded our brood-mixing measurements (e.g. food-deprived foreigners could appear to aggregate less not because they actually did aggregate less, but because the aggregating individuals were cannibalized by the fed residents). It is important to emphasize that our goal was to manipulate (indirectly) the relative cannibalism threat that resident and foreigner broods pose to each other (Dobler & Kölliker 2011), and that actual cannibalism was not required or expected to occur in our experiment.

We did a side experiment to confirm that cannibalism was directed against food-deprived individuals (see also Dobler & Kölliker 2011). We took one fed and one food-deprived nymph (condition manipulation was equivalent to main experiment), marked them as mentioned before and transferred both individuals to a shared petri dish (10 x 2 cm) with no food available (*N* = 21). As soon as an individual died, we identified the survivor and recorded the date. We also noted whether the victim was cannibalized by the survivor (no corpse present) or not (corpse present). As a control, we also determined mortality of fed and food- deprived nymphs held singly under high and low food availability, ruling out cannibalism as the cause of mortality. After marking by clipping the forceps, fed (*N* = 20) and food-deprived (*N* = 20) individuals were singly transferred into a petri dish with no food provided (*N* = 20) or with food provided (*N* = 20), respectively. As 39 of 40 individuals survived until day 8, we did not expect any effects of nymph condition and/or food availability per se on nymph survival in the main experiment.

Statistical analysis

We used a Wilcoxon signed-ranks test to test if the two days of food manipulation had a significant effect on nymph weight and, hence, body condition. To test which group of nymphs drove the early phase of brood mixing (e.g. because they are more active), we used repeated-measures MANOVA with the proportion of residents and foreigners that left their own burrow during the first night as the dependent variable. Since residents and foreigners within one container could interact and influence each other they were not independent and were thus entered as repeated measures (factor 'status'). We entered the condition treatment (R fed & F deprived or R deprived & F fed) and the food availability as between-subjects factors including all possible interactions.

For our main analysis of brood mixing (female-nymph aggregation) on day 6 and day 8, we used again repeated-measures MANOVA. The models were the same as above except that we entered the proportion of residents and foreigners aggregating with the female as the dependent variable. Non-significant interaction terms were removed from the model (*P* > 0.370) and only the simplest models are given in the text (see also Table 1). Proportional values were arcsine square-root transformed. In the figures, means and standard errors for proportions were obtained by back-transforming the parameter estimates to the proportional scale. All *P*-values are two-tailed. Statistical analyses were conducted with JMP, Version 9.0.2. (SAS Institute Inc., Cary, NC, U.S.A.).

Results

Condition-dependent brood mixing and female-nymph aggregation

After two days of food manipulation, fed nymphs were significantly heavier than deprived nymphs ($Z = -4.308$, $N = 62$ $P < 0.0001$; $\text{mean}_{\text{fed}} \pm SE = 2.053 \pm 0.052$ mg; $\text{mean}_{\text{food-deprived}} = 1.615 \pm 0.069$ mg) confirming a successful manipulation of nymph condition.

As expected, significantly more foreigners compared to residents left their own burrow during the first night (main effect status: $F_{1,29} = 41.655$, $P < 0.0001$). Furthermore, the proportion of nymphs leaving their own burrow was influenced by nymph condition. Deprived foreigners left their own burrow in larger numbers than fed foreigners and deprived residents left the resident burrow in larger numbers than fed residents (interaction between status and condition: $F_{1,29} = 6.297$, $P = 0.018$). These effects were independent of food availability (interactions with food availability: all $P > 0.198$).

Our analysis of brood mixing showed that, on day 6, residents aggregated at a significantly higher rate with the female than foreigners (main effect status: $F_{1,29} = 10.868$, $P = 0.003$) (Fig. 1a). All individuals, residents and foreigners, aggregated with the female at a higher rate in the R fed & F deprived treatment compared to the R deprived & F fed treatment (main effect condition: $F_{1,29} = 13.184$, $P = 0.001$). Aggregation was not significantly dependent on food availability in the environment or any interaction with food availability (see Table 1).

On day 8, brood mixing was significantly dependent on the interaction between status and condition ($F_{1,29} = 5.040$, $P = 0.033$) (Fig. 1b). The residents aggregated with the female independent of their own condition. Conversely, deprived foreigners mixed relatively less with residents than fed foreigners. Brood mixing was not significantly influenced by food availability in the environment or any interaction with food availability (see Table 1).

To further investigate the time dynamics of brood mixing, we conducted paired t-tests to analyse who mainly influenced the change in female-nymph aggregation between day 6 and day 8. We found that only the fed foreigners significantly changed their rate of female-nymph aggregation. Here, the proportion of fed foreigners that aggregated with the female significantly increased from 0.45 to 0.83 ($t_{14} = 3.938$, $P = 0.002$) (see change from Fig. 1a to Fig. 1b). The changes from day 6 to day 8 of the other nymphs were not significant (fed residents decreased from 0.90 to 0.77, $t_{15} = -1.815$, $P = 0.090$; deprived residents increased from 0.66 to 0.78, $t_{14} = 1.067$, $P = 0.304$; deprived foreigners decreased from 0.78 to 0.70, $t_{15} = -1.194$, $P = 0.251$).

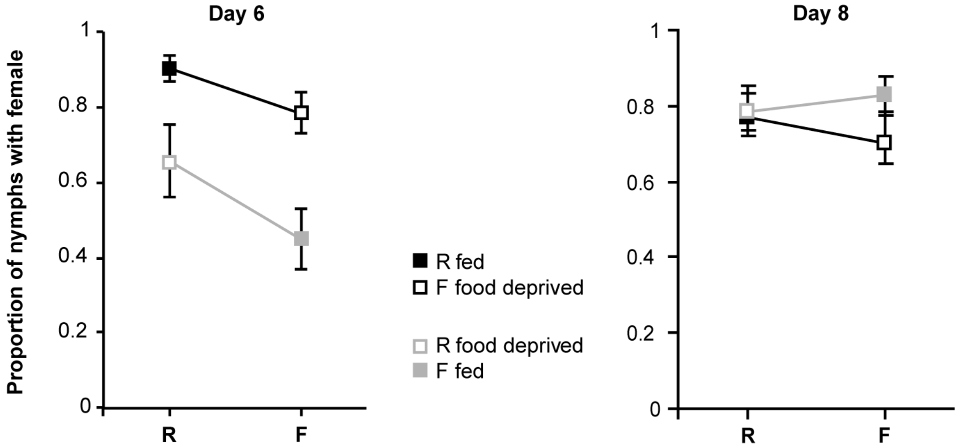

Figure 1. The proportion of nymphs that aggregated with the female, as a measure of brood mixing, on day 6 (left) and day 8 (right). R: resident nymphs; F: foreigner nymphs. In one treatment R were fed and F food deprived (black) and in the other R were food deprived and F fed (grey). Fed individuals are indicated with filled squares and food-deprived individuals with empty squares. Means and SEs are shown.

Table 1. Repeated-measures MANOVA on female-nymph aggregation on day 6 and on day 8.

		F-value	*P-value*
Day 6	**Final model**		
	condition	13.184	**0.001**
	status	10.868	**0.003**
	Rejected interactions and main effects		
	food availability	0.831	0.370
	status*food availability	0.631	0.434
	condition*food availability	0.017	0.899
	status*condition*food availability	0.286	0.597
Day 8	**Final model**		
	condition	0.431	0.517
	status	0.020	0.888
	status*condition	5.040	**0.033**
	Rejected interactions and main effects		
	food availability	0.074	0.788
	status*food availability	0.527	0.474
	condition*food availability	0.182	0.674
	status*condition*food availability	0.420	0.522

Nonsignificant ($P > 0.05$) effects and interactions were removed from the final model given in the text. The nonsignificant main effects remained in the model if they were included in a significant interaction term (see day 8: status*condition). Significant P values are shown in bold.

Condition-dependent nymph mortality

In the main experiment, nymph survival was, as expected high: 0.98 ± 0.005 (mean ± SE) on day 6 and 0.94 ± 0.010 on day 8. In the side-experiment, when one food-deprived nymph was paired with one fed nymph under low food availability, deprived nymphs died significantly earlier than fed nymphs (15 out of 21 cases: binomial test, $P = 0.039$). When cannibalism occurred, deprived nymphs were cannibalized by fed nymphs in 11 out of 16 cases and fed nymphs by deprived nymphs in only 5 out of 16 cases (binomial test, $P = 0.105$).

Discussion

Our study investigated the effect of nymph condition and environmental food availability on brood mixing dynamics and female-nymph aggregation in the European earwig *F. auricularia*. We predicted condition-dependent brood mixing and aggregation dynamics, since it was shown before that the cannibalism threat is higher among unrelated nymphs (Dobler & Kölliker 2010) and for individuals in poor condition (Dobler & Kölliker 2011). The period tested in our experiment is biologically relevant for female-nymph aggregation, because food provisioning is highest in the first week after hatching (Wong & Kölliker 2012) and female-nymph aggregation is positively correlated with nymph survival during this period (Gomez & Kölliker in press). Our results show that brood mixing was highly dynamic and indeed dependent on nymph condition. Early in the brood mixing process (i.e. on day 6), residents aggregated at significantly higher levels with the female than the respective foreigners, but both, residents and foreigners, from the R fed & F deprived treatment showed generally higher levels of brood mixing and aggregation with the female compared to the R deprived & F fed treatment. This pattern is consistent with our results that the early brood mixing dynamics were mainly driven by the foreign nymphs, since a higher number of foreigners left their own burrow compared to the residents. Furthermore, nymphs in poor condition were more likely to leave their own burrow during the first night, likely due to hunger and the correspondingly increased food searching activity. This activity difference can explain aggregation patterns early in the process, but not later on when there was more time for aggregation decisions to be made.

Later in the brood mixing process, the patterns were different. On day 8, resident nymphs aggregated with the female independently of their own condition. However, foreigners mixed relatively less with residents when they were deprived rather than fed. Our additional comparisons revealed that this change from day 6 to day 8 was mainly due to the fed foreigners who significantly increased and almost doubled their aggregation rate with the female. Therefore, mainly the fed foreigners drove brood mixing after an initial phase where aggregation merely reflected activity patterns. They successfully aggregated with the female and resident brood, whereas the deprived foreigners, who were the more likely victims of cannibalism, mixed at lower levels with the female and the resident brood. Since cannibalism among earwig nymphs is predominately directed against smaller (food deprived) individuals (Dobler & Kölliker 2011; this study), these findings are consistent with our hypothesis that the condition-dependent dynamics of brood mixing may at least partly reflect corresponding differential cannibalism threats. The major changes in the level and pattern of brood mixing from day 6 to day 8 further highlights that brood mixing is not a simple and static pattern, but shows substantial behavioural dynamics of group formation over time. The dynamic nature of aggregation in earwigs is not restricted to brood mixing, but also reflects their mother-offspring interactions more generally. Earwig maternal care is facultative, nymphs are already mobile shortly after hatching and female attendance of nymphs is characterised by substantial re-aggregation dynamics over time (Wong & Kölliker 2012; Gomez & Kölliker in press).

Contrary to our expectation, brood mixing and nymph survival were both independent of environmental food availability. Food availability has previously been associated with alloparental care and brood mixing. Specifically, Agrawal et al. (2004) showed that insufficient food availability leads to high levels of brood mixing in the burrower bug *Sehirus cinctus*, possibly because offspring that did not receive sufficient care would choose to disperse and mix with another brood. Contrary to this study, we predicted in the European earwig lower levels of brood mixing under low food availability because the benefits of care and, hence, of aggregation with a female are smaller under restricted than plentiful food conditions (Kölliker 2007; Meunier & Kölliker 2012). We showed that two days of food access or food restriction lead to a significant difference in nymph condition and body weight, which influences brood mixing dynamics, but food availability in the environment did not significantly affect the average extent of brood mixing. This suggests that brood mixing is not driven by the perceived food availability in the environment, but only by the condition of the nymphs.

Female earwigs do not discriminate against foreign nymphs (Radl & Linsenmair 1991; Meunier et al. 2012), and our results suggest an apparent lack of female control over the mixing of own brood with foreigners. One reason could be that the costs of making a discrimination error might outweigh the costs of caring for foreign offspring (Pierotti & Murphy 1987; Choudhury et al. 1993; Brown 1998). However, since maternal care in earwigs is facultative and nymphs are able to survive without their mother after hatching, the costs of making a discrimination error might be low in females of *F. auricularia* and therefore not the main reason for indiscriminate maternal care. The fact that resident and foreign nymphs mixed at high rates in our experiments suggests benefits of brood mixing despite the potential threat of cannibalism. We know from previous work that mother-offspring aggregation in *F. auricularia* continues even after the females refrain from food provisioning (Wong & Kölliker 2012; Gomez & Kölliker in press) indicating further benefits of group living besides maternal food provisioning. Group augmentation can be one direct benefit of brood mixing (Kokko et al. 2001) because individuals survive or reproduce better in large groups. Such benefits were shown in a study on greylag geese (*Anser anser*) where lone goslings preferred joining a dominant over a subordinate family (Kalmbach et al. 2005). Larger families are more dominant in this species, which leads to better access to high quality feeding areas and can result in better condition for each brood member including the parents. This could lead to benefits for the family and the foster offspring and thus favour parents that tolerate brood mixing and nymphs to aggregate and mix despite latent threats of cannibalism (also reviewed in Kalmbach 2006). Enhanced defence against predation, foraging efficiency or social immunity are candidate factors contributing to direct benefits to earwig nymphs when living in groups (Krause & Ruxton 2002). Another factor, which could explain brood mixing and alloparental care, is kin selection. For example, colonies of thick-billed murres (*Uria lomvia*) represent extended kin groups and alloparental care could be explained by altruistic behaviour through kin selection (Friesen et al. 1996). Benefits of living in kin groups were for example shown in the social spider *Stegodyphus lineatus* (Schneider & Bilde 2008). Communal feeding among kin spiderlings resulted in elevated feeding efficiency and growth rates compared to unrelated spiderling groups, independent of familiarity. In our experiment, since we sampled the earwig females in the field and randomly paired resident and foreigner broods only according to their age, it is conceivable that we might have paired related nymphs in a few cases. However, the likelihood for such cases must have been very small and, furthermore, females also provide care for foreign offspring in cross-fostering experiments (Meunier et al. 2012). We therefore do not consider kin selection as a likely explanation for brood mixing in *F. auricularia.*

The result that well-fed foreigners seem to drive brood mixing suggests that they may follow a brood parasitic strategy. Brood parasitism is predicted to evolve, for example, when breeding sites are in close proximity and there is an opportunity for parental care to be misdirected, e.g. by mobile offspring that invade foreign families (discussed in Wong et al. 2013). And because nymphs in good condition pose a greater threat of cannibalizing the resident nymphs, our results are consistent with the interpretation that broods without caring mother can use a brood parasitic strategy. However, an experimental proof of this interpretation would require a full test of the fitness consequences to resident females and their own nymphs of accepting foreign nymphs.

Our study demonstrated that brood mixing in *F. auricularia* is dependent on nymph condition and largely driven by foreign offspring in good condition. Females seem to passively tolerate this foreign offspring, despite the threat of cannibalism they might pose. As a consequence, females show offspring attendance for mixed broods and earwig families are characterised by dynamic aggregation not only within, but also between broods. Further research is needed to identify the fitness consequences of brood mixing to better understand if it reflects a form of communal living driven by direct benefits to the parents' own offspring, or a form of brood parasitism driven by offspring exploitation of critical resources from parents and their own offspring.

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CHAPTER 4

Maternal origin, mates and shared environment shape chemical signatures or earwigs

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Abstract

The ability to recognize and gain information about an encountered individual can be crucial in various contexts, for example to avoid inbreeding or express appropriate social behaviors. In insects, recognition is generally mediated by the cuticular hydrocarbons (CHC) displayed on each individual. CHC profiles can reflect information about the family origin of an individual (i.e., they can be heritable), but may also change during an individual's lifetime, for example due to age and exposure to social or abiotic environments. Such changes may affect the recognition process and outcome. Here we experimentally tested in the European earwig, *Forficula auricularia*, whether (1) chemical signatures of juveniles and adults reflect their family of origin, (2) these signatures are less informative for discrimination among families with higher genetic diversity, and (3) change depending on the environment shared by social groups of adults. Our results show that the CHC profiles of juveniles and adults could be used to statistically discriminate their family of origin. This prediction success was significantly lower among adults (but not juveniles) when their mother had been mated to multiple males, and it was significantly higher among adult males than females. Finally, we showed that groups of unrelated adults within a shared environment, exhibit group-specific CHC profiles. Hence, CHC profiles of *F. auricularia* display information about the genetic origin and (social) environment, and they can be used by conspecifics for example to direct social behaviors towards family members and to avoid costs of inbreeding.

Introduction

The evolution of group living selects for mechanisms that allow group members to recognize and gain information about encountered individuals, for example concerning their genetic, social or environmental origin. Such recognition mechanisms are crucial to ensure that cooperative or aggressive behaviors are directed towards appropriate individuals, as well as to limit the risks of sib-mating and inbreeding depression. Across taxa, a broad diversity of cues involving several sensory modes have been shown to display individual variation potentially useful for recognition, such as vocal cues (Rendall et al. 1996, Insley 2001), visual traits (Parr & de Waal 1999, Tibbetts 2002) and olfactory cues (Mehlis et al. 2008, Lihoreau & Rivault 2009).

In insects, the recognition system is typically based on chemical cues present on the waxy layer covering their cuticle (Singer 1998, Howard & Blomquist 2005, Blomquist & Bagnères 2010). This waxy layer is mainly composed of cuticular hydrocarbons (CHC), which form a chemical signature that can vary qualitatively and/or quantitatively among species, families and even individuals (Howard & Blomquist 2005). Whereas the primary function of CHC is to maintain the insect's homeostasis of water content (i.e., prevention of desiccation) (Lockey 1988, Gibbs 1998) and to act as a barrier against microorganisms (Howard & Blomquist 1982), their role in insect communication as a carrier of information about the individual likely evolved as a secondary function. For instance, CHC profiles can reflect very broad information about the species (Martin et al. 2008a), the sex (Ferveur 2005) and the breeding status (Steiger et al. 2007); as well as more fine-scaled information, such as in eusocial insects, where CHC profiles are known to reflect the colony of origin (Martin et al. 2008b), the social environment (Meunier et al. 2011) and even task specialization within a group (Wagner et al. 1998, Greene & Gordon 2003) (also all reviewed in Howard & Blomquist 2005).

Using CHC profiles to determine the origin of an encountered individual requires that such profiles vary between individuals and, to allow effective recognition after a period of separation, that at least a part of the profile remains stable over time. Such individual variation and long-term stability of CHC profiles can be due to a heritable component resulting in significant similarities of CHC profiles among individuals originating from the same family or colony. Such similarities were for example shown in cockroaches (*Blattella germanica,* Lihoreau & Rivault 2009), paper wasps and hornets (*Polistes dominulus* and *Vespa crabro,* Dani et al. 2004), as well as in termites (*Reticulitermes santonensis,* Dronnet et al. 2006). Interestingly, the paternally inherited genetic component in heritable CHC profiles may decrease the similarity of CHC profiles displayed among siblings sired by different males. Such patriline-specific inheritance of CHC profiles has been reported in the leaf-cutting ant *Acromyrmex octospinosus*, where workers from colonies headed by a multiply-mated queen exhibit patriline-specific variation in CHC profiles (Nehring et al. 2011).

In addition to possible heritable basis, CHC profiles may also be partly acquired from the environment, and may change over the course of an individual's life-cycle. More specifically, CHC have been shown to reflect diet composition (Liang & Silverman 2000, Florane et al. 2004), nesting substrates (Heinze et al. 1996, Bos et al. 2011), but also the age (Panek et al. 2001), and ovarian activity (de Biseau et al. 2004) of individuals. Interestingly, interactions with other individuals may also contribute to shape CHC profiles through active or passive transfer of chemical compounds among group members (Soroker et al. 1995b, Vienne et al. 1995, Vauchot et al. 1998, Boulay et al. 2000, Lucas et al. 2004, Soroker et al. 2003). Such exchange of CHC may ultimately generate a 'gestalt colony odor' when individuals live in stable groups, such as colonies of eusocial insects (Crozier & Dix 1979, Vander Meer et al 1998). However, the use of CHC profiles to recognize the genetic origin of an encountered individual (as required in kin recognition) needs that the information reflecting the genetic background of this individual is not fully blurred by the information reflecting its newly experienced environments and/or its life-cycle.

In this study, we investigated whether the CHC profiles exhibited by young and adult individuals of the European earwig *Forficula auricularia* L. (Dermaptera: Forficulidae) can be used to recognize their genetic origin, i.e., the family they originate from. Former research in this species showed that CHC profiles influence the nature of mother-offspring interactions (Mas & Kölliker 2011) and that they display information about the nutritional quality of young offspring (nymphs) (Mas et al. 2009). Furthermore, the potential additional role of CHC in the recognition of related and unrelated individuals can be particularly important in this species, because (1) discriminating between related and unrelated offspring could allow mothers to ensure that the care they provide to offspring (Lamb 1976, Costa 2006, Staerkle & Kölliker 2008) is directed to their own nymphs and not to unrelated ones that have joined their clutch (which could happen on the field as shown in field experiments by Kölliker & Vancassel 2007). (2) From the offspring point-of-view, kin recognition does also allow nymphs to direct aggressive behaviors and cannibalism towards unrelated young (Dobler & Kölliker 2010). Finally, (3) it would allow adults to avoid sib-mating and thus limit the costs of inbreeding depression (Meunier & Kölliker in press).

Using a series of mating experiments in *F. auricularia*, we investigated (1) whether CHC profiles of nymphs, adult males and adult females are informative to discriminate between families of origin, (2) whether the CHC profiles have an heritable basis and thus that discrimination is less effective in families with higher genetic diversity and (3) whether the shared environment contributes to variation in chemical signatures of a social group. If earwig chemical signatures can be used to discriminate their family of origin, we predicted that young and adults exhibit more similar CHC profiles when originating from the same family rather than different ones. If there is heritable variation, we predicted that statistically discriminating the family of origin of an individual based on its CHC profile is less accurate among the offspring of multiply mated as compared to singly mated females. Notice that a recent study in *F. auricularia* showed that paternal inheritance specifically influences offspring behavior and the outcome of earwig family interactions (Meunier & Kölliker 2012a). Finally, if earwig CHC profiles reflect their (abiotic and/or social) environment, we predicted that the CHC profiles of unrelated earwigs are more similar to each other when they live in a social group and share the same environment compared to unrelated individuals kept in separate groups.

Material & Methods

Experimental Design

The experimental design is graphically illustrated in figure 1. The chemical signatures of 112 nymphs and 329 adults of F. auricularia were extracted from 16 experimental clutches. These clutches were from the second clutches of either nine females mated to a single male ('singly mated females') or seven females previously mated with four successive unrelated males (each male was used only once across all the mating trials; 'multiply mated females'). The offspring from clutches of singly mated females were full siblings with low genetic diversity (treatment called 1M), while the offspring from the multiply mated females were assumed to be sired by more than one male, hence, displaying higher genetic diversity (treatment called 4M). The 16 mothers (and their mates) were from a second laboratory-born generation of individuals sampled in May 2009 in Dolcedo, Italy. They were reared under standardized laboratory conditions until each female produced her first clutch (see details in Meunier et al. 2012). Sixteen days after their first clutch hatched, each of the 16 females was isolated in a small Petri-dish (diameter 10 cm) to allow second clutch production. The Petri-dishes were kept in a climate chamber at 15°C, 60% humidity and complete darkness until egg laying and hatching.

One day after hatching of their second clutch (day 1), each mother and 37.6 ± 1.28 (mean ± SE) of its second clutch nymphs were transferred in new Petri-dishes and subsequently reared at 20°C, 60% humidity and 14:10h light:dark cycle. Six days later (day 7), 8 nymphs per clutch (from seven 1M and seven 4M clutches) were randomly sampled, singly isolated in glass-vials (300 µl) and immediately frozen at -30°C for chemical extractions. The remaining nymphs were kept with their mothers until day 14, on which mothers were removed to mimic the natural decrease in maternal care (Meunier et al. 2012). On day 21, all nymphs were transferred to large Petri-dishes (diameter 14 cm) in which they were kept until reaching adulthood. As soon as these nymphs molted into adults, males and females of each family were separated in two new large Petri-dishes to prevent mating and inbreeding. Once all individuals became adults (i.e., approximately eleven weeks after hatching), eight males and eight females were randomly sampled in each family and setup in new large Petri-dishes (called family groups) with seven 1M groups and seven 4M groups per sex. Due to small second clutch sizes, two 1M families were used for nymph chemical extractions and two different 1M families were used for adult chemical extractions (i.e., five 1M families have been used to extract both nymphs and adults). Simultaneously, to test that the chemical signature of each family does not only reflect its common environment (i.e., the Petri-dish) but also its genetic origin, we mixed adults from the 12 experimental clutches to form seven groups of eight unrelated females and seven groups of eight unrelated males (called unrelated groups). One month later, all adults were frozen at -30°C for 2 hours, individually transferred to a glass-vial (2 mL) and then put back at -30°C for later chemical extractions. Except when mentioned, all Petri-dishes were covered with humid sand, contained one plastic tube as shelter and received a*d libitum* food changed twice a week (see Meunier et al. 2012) for food composition).

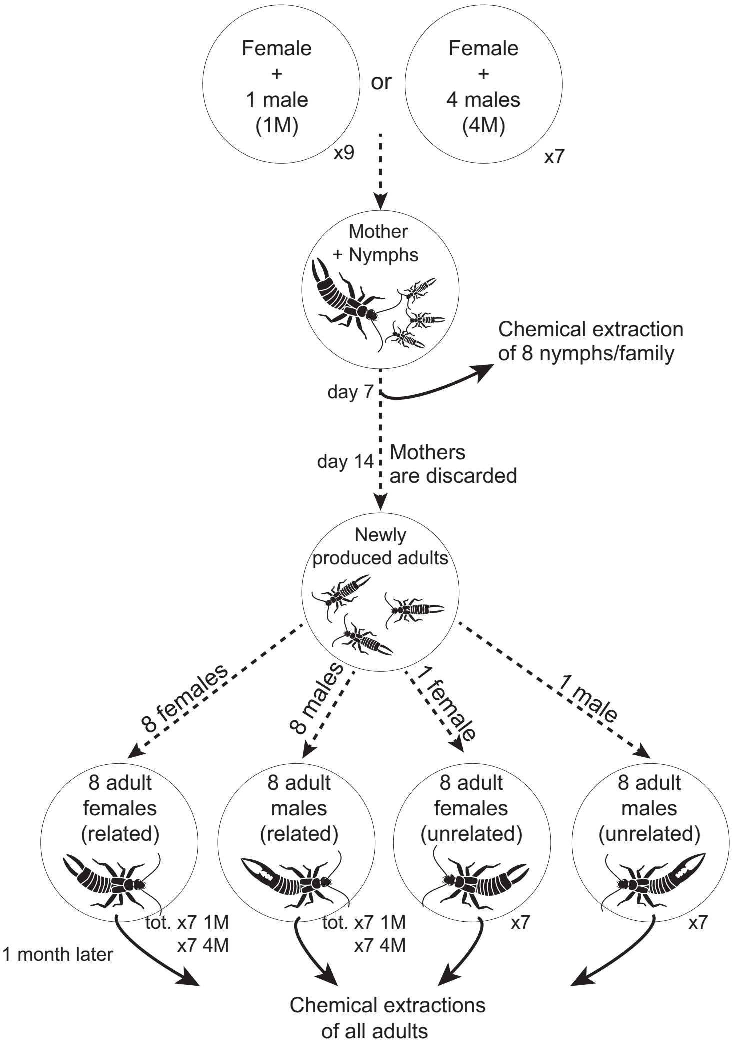

Figure 1. Experimental design used to extract the CHC profiles from nymphs and adult earwigs.

Chemical Extraction

Chemical extractions were performed on each frozen nymph, female and male sampled in the experimental clutches (see above). Each frozen vial was open for 15 minutes to thaw at room temperature before extraction. Individuals were extracted using 60 µl (nymphs) or 800 µl (adults) of a mix of n-Heptane (Carl-Roth AG, Arlesheim, Switzerland) and n-Octadecane at a concentration rate of 2.5 ng/µl (Fluka Analytical, Sigma-Aldrich, Buchs, Switzerland) during 10 min. Individuals were then carefully removed before storing samples at -30°C until their analyses by Gas Chromatography coupled with a Mass Spectrometry (GC-MS). A 2 µl sample of each extract was injected on an Agilent 7890A gas chromatograph fitted with a HP-5MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness; Agilent Technologies, USA) coupled to a mass analyzer (Agilent 5975C mass spectrometer). The injector was used in splitless mode with a splitless time of 2 min. Injector temperature was held constant at 250 °C. Nymph samples were injected using an oven program that began at 70 °C (hold 2 min) and was ramped at 15 °C/min to 232 °C, then 5 °C/min to 263 °C and 15 °C/min to 300 °C (hold 7 min). For adult samples, the oven program began at 70 °C (hold 2 min), then was ramped at 15 °C/min to 232 °C and then 5 °C/min to 300 °C (hold 7 min). Carrier gas was Helium at a flow rate of 1 ml/min. Electron impact positive ions at 70 eV were recorded in the scanning mode (mass range scanned 40–550 amu). The mass spectra were interpreted by fragmentation analysis and comparison to previous publications (McCarthy et al. 1968, Nelson 1978, Pomonis et al. 1980, Liu 1991). Retention indices based on a series of n-alkane standards (C8-C40, Fluka Analytical, Sigma-Aldrich, Buchs, Switzerland) were compared to published data. MSD ChemStation E02.00.493 Agilent Technologies software was used to calculate the retention time and total area of each peak for subsequent analysis.

Statistical Analyses

A series of linear discriminant analyses (DAs) was conducted to investigate whether the chemical signature of nymphs and adults reflected their family of origin, the genetic diversity of their clutch and/or their sex (Table 1). The significance of each DA was evaluated both using Wilks' Lamba tests and prediction success (by estimating the percentage of correct assignment of individuals to their family of origin) through both the statistical model fit to the data and cross-validation (Leave-one-out method). To avoid limitations inherent to analyses of compositional data, the area of each peak was transformed according to Aitchison formula (Aitchison 1986) prior to DAs. In this formula, $Z_{i,j} = \ln\left(\frac{Y_{i,j}}{g(Y_j)}\right)$, $Z_{i,j}$ is the transformed area of peak i for sample j, Y is the area of the peak i for sample j; and $g(Y_j)$ is the geometric mean of the areas of all peaks of sample j. Each DA was performed using all the extracted chemical compounds (Table 2). Notice that DA on the absolute quantity of each peak provided comparable results.

Table 1. Prediction success of earwig individuals according to their life-stage, their sex (only for adults), the type of rearing group, and the mating treatment (1M or 4M)

Indiv	Group rearing	Mating treatment	N fam	N ind	Lambda	Approx. F-value	P-value	Prediction success by model (%)	Prediction success by cross-validation (%)
Nymphs	Family	1M	7	56	< 0.0001	9.63	< 0.0001	100.0	87.5
	Family	4M	7	56	< 0.0001	12.79	< 0.0001	100.0	85.7
Males	Family	1M	7	55	< 0.0001	5.39	< 0.0001	100.0	83.6
	Family	4M	7	56	0.0001	4.53	< 0.0001	100.0	66.1
	Unrelated	-	7	55	0.0028	2.01	< 0.0001	90.9	29.1
Females	Family	1M	7	54	0.0001	4.25	< 0.0001	100.0	68.5
	Family	4M	7	55	0.0006	2.92	< 0.0001	100.0	47.3
	Unrelated	-	7	54	0.0026	1.98	< 0.0001	96.3	33.3

The table indicates the number of families used (N fam) and the total number of individuals (N ind) per type of experimental group.

Whether the chemical signature of each individual reflected its family of origin was first tested by conducting one global DA using the chemical signatures of nymphs across all treatments, and conducting another global DA using the chemical signatures of all the adults reared in family groups. In a second step, we tested whether prediction successes depended on the mating treatment of the clutch and the sex of the adults. To this end, we performed a total of six DAs using the chemical signature of nymphs sampled in 1M and 4M clutches, as well as using the chemical signature of either females or males sampled in 1M and 4M clutches (Table 1). To test whether the chemical signature of group members reflects the shared environment (i.e., the Petri-dish and social group), we conducted two DAs using the chemical signature of either females or males from the unrelated groups. Finally, we investigated whether males and females can be discriminated using their chemical profiles by conducting a DA using the chemical signature of all adults from the unrelated and family groups. Correlations between discriminating variables and the discriminant function (i.e., structure coefficients) were then used to assess the importance of each chemical compound in discriminating the sexes. Coefficients of correlations were obtained from Spearman rank correlations. According to Mardia's criterion (Mardia et al. 1979), coefficients of correlations above 0.7 times the largest coefficient in a discriminant function were considered to have contributed significantly.

The prediction successes obtained from the different data sets were compared using a series of General Linear Models (GLMs) with binomial error distribution. To this end, the prediction success obtained from each DA was converted into a binomial vector (1 or 0 values) of a length equal to the number of individuals involved in the DA and wherein the proportion of 1 was equal to the prediction success obtained from cross-validation method. The comparison between prediction successes of nymphs from

1M and 4M families was conducted using a GLM wherein 1M/4M was entered as fixed factor, and the two binomial vectors reflecting the respective prediction success concatenated to form the response variable. We then tested whether the female mating treatment (likely reflecting genetic diversity of the clutch) and the sex of the adult influenced the prediction success of adults living in family groups, using a GLM wherein these two factors and their interaction were entered as fixed factors, and the four corresponding binomial vectors concatenated to form the response variable. Finally, we tested whether the type of group (family group vs unrelated group) and the sex of the adults influenced the prediction success of adults using a GLM wherein these two factors and their interaction were entered as fixed factors, and the four corresponding binomial vectors concatenated to form the response variable. Because the genetic diversity of family groups influenced the prediction success (see results), this later analysis was conducted separately on the family groups with low and high genetic diversity. All statistical analyses were conducted using the software R.2.15.2.

Results

The cuticular extracts of individual earwigs provided a total of 36 peaks, among which 23 were found in nymphs, and 24 in adults (males and females, Table 2). Chemical signatures exhibited not only quantitative, but also qualitative differences between life-stages, with 12 chemical compounds specific to nymphs and 13 in adults. Between adults, no qualitative differences in the chemical signature of males and females were observed, but there were substantial quantitative differences between individual signatures (Wilk's lambda = 0.165, Approx. F= 66.9, $P < 0.0001$). 98.2% and 97.0% adult males and females, respectively, were correctly assigned to their sex by statistical models and cross-validation method. According to Mardia's criterion, 12 out of the 24 extracted peaks significantly contributed in discriminating males from females (Table 2).

Overall, the chemical signature of nymphs and adults clearly reflected their family of origin. The DAs performed on the individuals reared in family groups significantly separated nymphs (Figure 2; Wilk's lambda < 0.0001, Approx. F = 9.87, $P < 0.0001$) or adults (Wilk's lambda = 0.004, Approx. F= 4.40, $P < 0.0001$) according to their family of origin. The prediction success was 97.3% and 81.8% using the statistical model fit, and 92.0 % and 58.6% using cross-validation method for nymphs and adults, respectively. The DAs remained significant when taking into account the sex of the tested adults and/or the mating treatment (Table 1, all $P < 0.0001$), with prediction successes ranging from 90.9% to 100% using the statistical model fit, and from 29.1% to 87.5% using cross-validation method (Table 1).

Table 2. Mean relative peak area (%) oft he chemical compounds (CC) extracted from 112 nymphs, 163 females, and 166 males oft he European earwig

	Chemical Compounds	RT	Males	Females	Nymphs	Rs	P	
(1)	non HC	5.12	0.22	0.21	-	0.082	0.138	
(2)	non HC	5.23	0.14	0.13	-	0.082	0.138	
(3)	non HC	5.86	0.90	2.02	2.77	-0.089	0.107	
(4)	non HC	6.28	0.15	0.18	-	-0.178	0.001	
(5)	nC13	7.73	-	-	7.24	-	-	
(6)	nC15	9.51	-	-	1.18	-	-	
(7)	Acid benzoique 4 ethoxy, ethyl ester	9.76	-	-	0.28	-	-	
(8)	Phtalate	10.36	-	-	0.63	-	-	
IT	nC18	11.82	-	-	-	-	-	
(9)	nC21	13.96	-	-	4.77	-	-	
(10)	X-nC23:2 + X'-nC23:1	15.77	-	-	10.68	-	-	
(11)	X-nC23:1	15.83	-	-	2.86	-	-	
(12)	nC23	16.18	0.14	0.15	9.33	0.034	0.542	
(13)	11-, 9-MeC23	16.59	-	-	1.15	-	-	
(14)	5-MeC23	16.79	-	-	0.68	-	-	
(15)	3-MeC23 + (X-nC24:1)	17.14	-	-	1.45	-	-	
(16)	X-nC25:1	19.19	0.08	0.22	38.51	-0.303	< 0.0001	
(17)	nC25	19.74	1.37	2.20	3.84	-0.435	< 0.0001	
(18)	13-, 11-, 9-MeC25	20.58	1.03	2.60	2.77	-0.695	< 0.0001	*
(19)	3-MeC25 + (X-nC24:1)	21.61	0.20	0.88	1.19	-0.848	< 0.0001	*
(20)	13-, 11-, 9-MeC26	23.48	0.10	0.29	-	-0.786	< 0.0001	*
(21)	X, X'-nC27:2 + X-nC27:1	24.76	0.44	0.63	3.36	-0.304	< 0.0001	
(22)	nC27	25.61	1.62	1.58	0.37	0.012	0.822	
(23)	13-, 11-MeC27	26.34	-	-	2.88			
(24)	13-, 11-, 9-, 7-MeC27	26.59	8.58	13.68	-	-0.823	< 0.0001	
(25)	7-MeC27	26.77	17.33	22.26	2.34	-0.646	< 0.0001	*
(26)	7,15-; 7-19-; 11,15-; 11,19-diMeC27	27.10	-	-	0.58	-	-	
(27)	2,19-; 2,21-; 2,23-diMeC27 + (3-MeC27)	27.34	4.25	8.30	0.70	-0.868	< 0.0001	*
(28)	7,15-; 9,15-; 9,17-; 11,15-diMeC27	27.54	6.87	14.33	-	-0.89	< 0.0001	*
(29)	13-, 11-, 9-, 7-MeC28	28.96	0.94	1.15	-	-0.572	< 0.0001	
(30)	X,X'-diMeC28	29.63	1.30	1.76	-	-0.761	< 0.0001	*
(31)	13-, 11-, 9-, 7-MeC29	31.07	24.46	11.38	0.47	0.877	< 0.0001	*
(32)	7,19-; 9,19-; 11,17-; 11,19-diMeC29	31.57	22.94	13.22	-	0.87	< 0.0001	*
(33)	15-, 13-, 11-, 9-MeC30	32.86	3.69	2.00	-	0.804	< 0.0001	*
(34)	15-, 13-, 11-, 9-MeC31	34.47	1.58	0.39	-	0.935	< 0.0001	*
(35)	7,19-; 9,19-; 9,21-diMeC31	34.92	1.49	0.36	-	0.929	< 0.0001	*
(36)	13-, 11-MeC33	37.30	0.17	0.09	-	0.594	< 0.0001	

RT: mean retention time given in minutes. Rs: Spearman correlation test between the relative amount of each peak and the discriminant function (discriminating males from females). IT: Internal standard. * Compound that significantly contributes to the prediction between males and females (see Methods and Materials). Chemicals compounds in traces amount are reported between brackets.

In line with the hypothesis that variation in the chemical signature of family members reflects its genetic diversity, we found that the prediction success of adults was significantly higher in the 1M as compared to the 4M treatment (Table 1, GLM, LRχ^2= 9.69, P = 0.002). By contrast, this effect was not significant for nymph chemical signatures (Table 1, GLM, LR χ^2= 0.08, P = 0.781). The prediction success of adults was also significantly larger for males than females (Table 1, GLM, LR χ^2= 7.47, P = 0.006), but not significantly influenced by an interaction between the mating treatment and the sex of the adult individual (GLM, LR χ^2= 0.02, P = 0.90).

Finally, we found that the chemical signature of adults partly reflected the environment in which they had been reared. The DAs performed on the groups of unrelated adults significantly separated males and females according to their groups (unrelated, Table 1), with 90.9% of males and 96.3% of females correctly assigned to their group of origin by the statistical model fit, and 29.1% and 33.3% by cross-validation method. However, our results demonstrated that adult chemical signatures reflected significantly more their clutch of origin than their environment, as prediction successes were significantly lower among groups of unrelated individuals than family groups from the 1M treatment (GLM, effect of group type: LR χ^2= 23.60, P < 0.0001; effect of sex: LR χ^2= 2.67, P = 0.103, interaction: LR χ^2= 2.37, P = 0.123) or family groups from the 4M treatment (GLM, effect of group type: LR χ^2= 26.00, P < 0.0001; effect of sex: LR χ^2= 1.82, P = 0.177, interaction: LR χ^2= 2.40, P = 0.122).

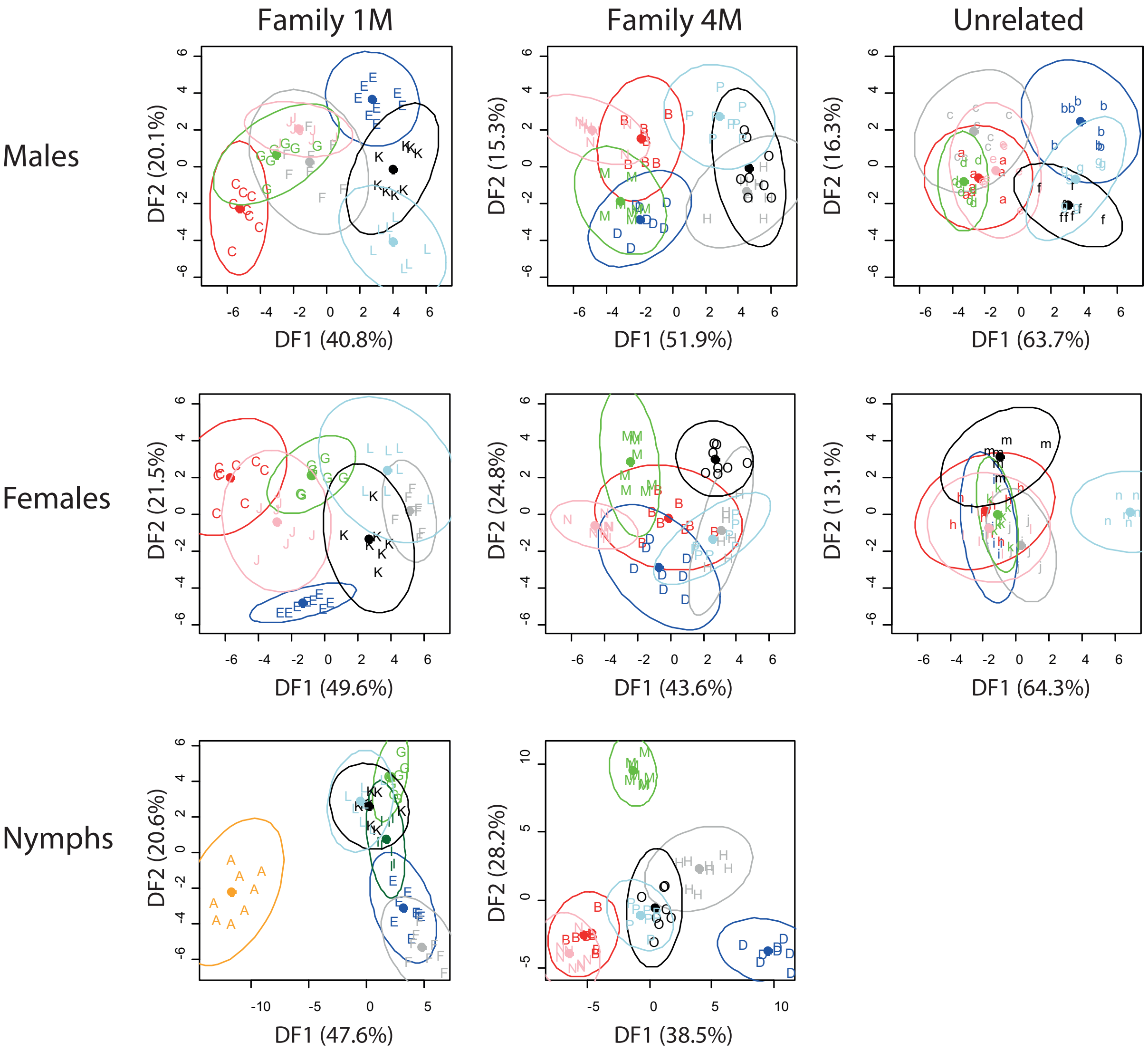

Figure 2. Discriminant analyses (DAs) on the basis of cuticular extracts of males, females and nymphs produced by singly (1M) or multiply (4M) mated females or reared in unrelated groups. The total data set includes 166 males, 163 females and 112 nymphs. Envelopes represent the 95% confidence ellipses and each letter refers to a different group of origin. The percentage of variance explained by each discriminant function (DF) is given between brackets.

Discussion

Using CHC profiles as cue to recognize the family origin of an encountered individual requires that such profiles contain this information and that it remains sufficiently stable over time (Page et al. 1991). Our study supports this prediction in the European earwig *F. auricularia* where we found that variation in CHC profiles allow discriminating accurately the family of origin of juveniles (nymphs), adult males and adult females. Our results also revealed that part of this variation is probably heritable and due to the genetic origin of the fathers, since the prediction success of family origin based on CHC profiles was significantly higher in broods of singly-mated 1M females (where all nymphs were full siblings) as compared to broods of multiply mated 4M females (where nymphs were likely sired multiply). Interestingly, this effect was only present in the adults, but not in the nymphs, in which the paternal contribution to heritable variation in CHC profiles was apparently not expressed. Finally, we showed that CHC profiles also partly reflect the environment and social group experienced by each individual, even though this information was not as strong as the one reflecting the family of origin of each individual.

Our result revealed that earwig young and adults have the possibility to use the CHC profile displayed by encountered conspecifics to discriminate kin from non-kin. In particular, we showed that the CHC profiles of individuals from the same family where more similar than the CHC profiles of individuals from different families, a result in line with other studies conducted, e.g., in wasps (Dani et al. 2004), cockroaches (Lihoreau & Rivault 2009), or subsocial spiders (Grinsted et al. 2011). In adults, kin recognition is generally needed to prevent inbreeding depression when choosing mates. Estimating similarities between CHC profiles displayed by potential mating partners could thus help adults to avoid sib-mating. In line with this hypothesis, mate choice has been shown to be preferentially directed towards partners sharing less similar CHC profiles in the European beewolf *Philanthus triangulum* (Herzner et al. 2006), the cockroach *Blattella germanica* (Lihoreau & Rivault 2009) and the cricket *Teleogryllus oceanicus* (Thomas & Simmons 2011). Moreover, in species exhibiting family interactions and group-living, kin recognition may also allow individuals to direct cooperative behavior towards related conspecifics, e.g., maternal care towards related offspring (Mathews 2011), or to limit costly contacts with unrelated individuals, e.g., maternal cannibalism directed towards unrelated offspring (Mathews 2011) or offspring-offspring competition for limited food source (Dobler & Kölliker 2010). In *F. auricularia*, sib-mating is known to entail inbreeding depression (Meunier & Kölliker in press) and kin recognition between nymphs has been demonstrated in the context of nymph cannibalism (Dobler & Kölliker 2010) and sibling food sharing (Falk J, Wong JWY, Kölliker M & Meunier J, submitted). Hence, our study shows that the CHC profiles of young and adults most likely mediate such recognition patterns in earwigs, and claim for further studies exploring the direct link between similarities in CHC profiles and sib-mating avoidance, as well as between similarities in CHC profiles and the nature of sibling interactions.

Predicting the family of origin of an earwig adult using its CHC profile was more successful when all offspring were sired by one male (1M families) as compared to multiple males (4M families). Because earwig males never encounter the eggs or the offspring they have sired (i.e., they cannot affect offspring CHC through a socially mediated paternal influence), our finding reveals that adult CHC profiles at least partly reflect their paternal genetic origin. Hence, the degree of variation in the CHC profiles of adults originating from the same family provides information about the number of sires in a brood.

Such influence of patrilines on individual CHC profiles were previously reported in several eusocial insects such as the leaf-cutting ant *Acromyrmex octospinosus* (Nehring et al. 2011), the paper wasp *Polistes dominulus* (Dani et al. 2004), the honey bee *Apis mellifera* (Page et al. 1991, Arnold et al. 2000) and the ant *Formica exsecta* (Martin et al. 2012). Whereas the presence of a patriline signal in the CHC profile of eusocial colonies is a contentious issue as it would allow workers to behave nepotistically by discriminating and favoring full-sisters over half-sisters (Keller 1997), it remains to be tested whether the occurrence of patriline signals triggers nepotistic behaviors among *F. auricularia* adults.

Although we found a significant difference in prediction success between adults from single and multiple sired families, we surprisingly did not find such difference in the nymphs. One possible explanation for the lack of cues of paternal origin in the nymph CHC profiles is that nymphs only exhibit immature profiles and that the paternal genetic contribution can only be expressed after a certain maturation time. It was shown in the ant *Cataglyphis niger* that the amounts of hydrocarbons in the postpharyngeal gland increased with maturation, especially in the first seven days after emergence (Soroker et al. 1995a). Another explanation is that maternal presence inhibits or prevents the CHC expression of patriline in the nymphs, e.g., through the maternal transfer of CHC to the nymph cuticle, a mechanism that was reported in early nymph instars of the German cockroach *Blatella germanica* (Fan et al. 2008). Maternal provisioning of lipids to eggs was also shown in several species of echinoderms (Villinski et al. 2002). The apparent lack of cues of paternal origin in the CHC profiles of nymphs could ultimately reflect either a by-product of maternal care to protect nymphs from desiccation, or a maternal strategy to actively reduce the risks of nepotistic conflicts and cannibalism in their clutch. In line with this last hypothesis, previous work in *F. auricularia* showed that variation in the number of male mates did not influence nymph sibling cannibalism (Meunier & Kölliker 2012b), whereas cannibalism was higher between unrelated nymphs from different families (Dobler & Kölliker 2010). The latter was also reported in other species, for example the spider *Stegodyphus lineatus* (Bilde & Lubin 2001). Further experiments will be required to disentangle these hypotheses and thus explain the lack of a signal of paternal origin in the earwig nymph CHC profile.

A somewhat surprising result was that the family-specificity of CHC profiles differed between sexes in the adults, suggesting a sex-difference in the heritability of the CHC. Differences in the chemical profiles of males and females are common for a variety of different species, for example in ants (*Diacamma ceylonense,* Cuvillier-Hot et al. 2001), flies (*Drosophila serrata* and *Drosophila melanogaster,* Chenoweth & Blows 2003, Everaerts et al. 2010) and fireflies (*Ellychnia corrusca*, Ming & Lewis 2010). We are aware of only one recent study that showed a sex-difference in the heritability of CHC in *Drosophila simulans* (Sharma et al. 2012), where CHC play a key role in female mate choice (Ingleby et al. 2013). The sex difference in the inherited information exhibited by earwig CHC profiles could be ultimately driven by sib-mating avoidance in *F. auricularia.* In this species, sib-mating is known to come at fitness costs that particularly affect male fertilization success (Meunier & Kölliker in press), so that females might select for males exhibiting reliable information on their family of origin. More proximately, other hypotheses are that genes underlying CHC metabolism are differently expressed in males and females (Dallerac et al 2000, Chertemps et al 2005), or that males and females exhibit behavioral differences that directly or indirectly shape the social transfer of CHC among adults. Because in our study we kept adults in separate sex groups to prevent sib-mating, the lower prediction success in female groups might reflect more CHC exchange between females compared to males, e.g., due to higher levels of allogrooming and close physical contacts (Soroker et al. 1995b, Vienne et al. 1995, Vauchot et al. 1998, Boulay et al. 2000).

Finally, our results showed that CHC profiles in *F. auricularia* adults partly reflect their shared environment and social group. In particular, experimental groups of unrelated males and females were successfully attributed to their new group, even if such success was significantly lower compared to the group of origin of related individuals. It was shown before that environmental information from multiple abiotic factors can influence the chemical profiles of individuals, such as the temperature (Savarit & Ferveur 2002), the nesting substrate (Singer & Espelie 1992, Heinze et al. 1996) or the diet (Liang & Silverman 2000, Florane et al. 2004). But since we did not vary the abiotic factors between our groups, we consider them an unlikely influence on group specific profiles. Environmental information may also become specific to each unrelated group due to social interactions among individuals, as reported in eusocial insects where the transfer of chemical compounds between colony members is mediated through trophallaxis, allogrooming or other forms of physical contacts (e.g., Soroker et al. 1995b, Vienne et al. 1995, Vauchot et al. 1998, Boulay et al. 2000, Soroker et al. 2003). In *F. auricularia,* older instar stages and adults are known to aggregate at high levels for foraging, resting and mating (Sauphanor & Sureau 1993, Hehar et al. 2008), and that there is scope for social transfer of chemical compounds (Bradbury 2011). Whether information of the environment reflected by the CHC profile of adult earwigs is driven by abiotic factors and/or social interactions should be tested in further experiments.

In conclusion, our results showed that the chemical signature of young, adult males and adult females of *F. auricularia* can be used to discriminate their family of origin. The CHC profiles display a component of heritable variation and the adult profiles are influenced by the sex and the genetic diversity of a family. Interestingly, mate number had no effect on the chemical profiles of the nymphs, suggesting that the maternal care provided in this species may cover heritable paternal contribution to variation in the chemical signatures of juvenile offspring. Additionally, the adult profiles partly reflected the shared environment of individuals, but this environmental effect was significantly weaker than the heritable component. Overall, these results demonstrate that CHC profiles can be used to avoid costs of sib-mating, as well as to direct cooperative behavior towards related instead of unrelated individuals, which are both particularly important in gregarious species exhibiting family interactions.

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CHAPTER 5

Cues of maternal condition influence offspring selfishness

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Abstract

The evolution of parent-offspring communication was mostly studied from the perspective of parents responding to begging signals conveying information about offspring condition. Parents should respond to begging because of the differential fitness returns obtained from their investment in offspring that differ in condition. For analogous reasons, offspring should adjust their behavior to cues/signals of parental condition: parents that differ in condition pay differential costs of care and, hence, should provide different amounts of food. In this study, we experimentally tested in the European earwig (*Forficula auricularia*) if cues of maternal condition affect offspring survival, mediated by sibling cannibalism. We experimentally manipulated female condition by providing them with different amounts of food, kept nymph condition constant, allowed for nymph exposure to chemical maternal cues over extended time, quantified nymph survival (deaths being due to cannibalism) and extracted and analyzed the females' cuticular hydrocarbons (CHC). Nymph survival was significantly affected by chemical cues of maternal condition, and this effect depended on the timing of breeding. Cues of poor maternal condition enhanced nymph survival in early broods, but reduced nymph survival in late broods, and vice versa for cues of good condition. Furthermore, female condition affected the quantitative composition of their CHC profile and predicted nymph survival patterns. Thus, earwig offspring are sensitive to chemical cues of maternal condition and nymphs from early and late broods have opposite reactions to the same chemical cues. Together with former evidence on maternal sensitivities to condition-dependent nymph chemical cues, our study shows context-dependent reciprocal information exchange about condition between earwig mothers and their offspring, mediated by cuticular hydrocarbons.

Introduction

Parental care evolved due to its fitness benefits to offspring, and it often comes at a cost for parents (Clutton-Brock 1991; Royle, Smiseth & Kölliker 2012a). Offspring that vary in condition are expected to differ in the gain in fitness per unit of provisioning obtained from their parents (Godfray 1991; Johnstone 1996; Kilner & Hinde 2012; Mock, Dugas & Strickler 2011; Royle, Hartley & Parker 2002), and parents that differ in condition should experience differential costs of provisioning (Bateson 1994; Hinde, Johnstone & Kilner 2010). Because parents and offspring are closely related, there is an evolutionary (kin selected) incentive for parents to adjust their provisioning to offspring condition (i.e., need or quality (Godfray 1995; Godfray & Johnstone 2000; Kilner & Hinde 2012; Mock et al. 2011; Mock & Parker 1997)) in order to maximize their returns on investment. However, it should also pay off to offspring to adjust their demand to parental condition to moderate the cost of investment that offspring impose on their parents (Bateson 1994). As a consequence, it is in the overall interest of both parents and offspring to be sensitive to variation in each other's condition, and selection may favor the exchange of information about condition between parents and offspring through cues or signals (Maynard Smith & Harper 2003). The evolutionary conflict between parents and offspring over parental investment (Godfray 1995; Kilner & Hinde 2012; Mock & Parker 1997; Trivers 1974) may have a modulating effect in the evolution of the signals, leading to exaggeration of signals, and to "information warfare" (Kilner & Hinde 2008) between parents and offspring and the evolution of costly signals.

Previous research has focused on offspring begging signals conveying information about offspring condition as signals of need or quality (Godfray 1991, 1995; Grafen 1990; Johnstone & Godfray 2002; Mock et al. 2011). The reverse expectation that offspring should be sensitive to cues of parental condition (Bateson 1994), or that parents even may have evolved signals to convey honest information about their condition to their offspring, has received substantially less theoretical or empirical scrutiny. We may ultimately often expect a reciprocal form of parent-offspring communication where parents and offspring exchange information about their respective condition (and maybe even beyond, an information exchange among all family members in a communication network; (Morales & Velando 2013); see also (Parker, Royle & Hartley 2002; Royle, Smiseth & Kölliker 2012b)). Based on these arguments, one may expect offspring to adjust their demand or selfishness to cues or signals of parental condition. The question how selfish offspring should be, how many resources they should demand from their parents, and how competitive they should be against their siblings is at the heart of parent-offspring conflict theory (Godfray 1995; Kilner & Hinde 2012; Mock & Parker 1997; Royle et al. 2002; Trivers 1974). In its most extreme form, offspring selfishness leads to siblicide, that is, the killing and possible consumption of a sibling offspring (Mock 1984; Mock & Parker 1997; O'Connor 1978; Parker, Mock & Lamey 1989). So, if parents provide cues or signals about their condition to their offspring (either as inadvertently released information or as evolved signal of parental condition), and offspring are sensitive to these cues, how should offspring respond in terms of their selfishness? The prediction partly depends on the consequences of the parent's condition on the amount of obtained care, and on whether sibling interactions are purely competitive or if there is scope for cooperation among siblings (see Roulin & Dreiss 2012 for review of evidence of sibling cooperation). Under pure competition over limited resources, offspring perceiving that their parents are in poor condition, which therefore will provide low levels of care, should attempt to kill their siblings (or alternatively disperse) earlier because the poor condition of the parents would indicate insufficient resources for all offspring, enhanced sibling competition and threat of brood reduction. In contrast, when cooperation between offspring can compensate partly for reduced care provided by parents in poor condition, offspring perceiving cues of poor parental condition may reduce their siblicidal drive due to the advantage of maintaining a larger number of siblings to cooperate with. Sibling cooperation may occur for example if larger groups/broods of young are better in predator defense, have enhanced foraging efficiency or directly cooperate for example by sharing food (Biedermann & Taborsky 2011; Yip & Rayor 2013; Falk et al. submitted).

Parental condition is often related to the timing of breeding, for example because individuals in good condition are able to breed early (Clutton-Brock 1988). Furthermore, early breeders may face quite different ecological conditions compared to late breeders in terms of population density, food availability, predation pressure, temperature etc., which are all factors that may also contribute to variation in their condition, in the benefits/costs of parental care (e.g., Klug, Alonzo & Bonsall 2012; Wong, Meunier & Kölliker 2013) and in the pay-off of sibling competition versus sibling cooperation. Correspondingly, parental cues/signals of condition and/or offspring sensitivities to these cues/signals may be expected to vary with the timing of breeding. Few studies investigated such context-dependent parent-offspring communication, but there is some evidence for different responses of parents to variation in offspring signals of quality by early and later breeders (Bize et al. 2006; Mas & Kölliker 2011).

The European earwig (*Forficula auricularia*) is an insect species with uniparental maternal care including egg- and offspring attendance and food provisioning (Kölliker 2007; Kölliker & Vancassel 2007; Lamb 1976; Staerkle & Kölliker 2008; Vancassel 1984; Weyrauch 1927).The offspring (nymphs) signal their condition by solicitation pheromones in the form of cuticular hydrocarbons (CHC) to which the females show two distinct responses: When exposed to CHC extracts from well-fed nymphs (as compared to poorly fed nymphs, or controls) females increase their food provisioning (Mas, Haynes & Kölliker 2009) and modify the timing of second clutch production (Mas & Kölliker 2011). This latter response depends on the timing of breeding, with early females advancing and late females delaying second clutch production. Furthermore, females in poor condition provide food to fewer nymphs

(Wong & Kolliker 2012) and they negatively affect their nymphs' survival under conditions of limited food availability, probably because of mother-offspring competition over the scarce food (Meunier & Kölliker 2012). This is in contrast to the beneficial effects of maternal presence under conditions of plentiful food where female food provisioning enhances nymph survival (Kölliker 2007). Finally, siblicide and cannibalism are a primary cause of mortality throughout nymph development (Dobler & Kölliker 2010, 2011), which makes *F. auricularia* an ideal model system to test the influence of maternal condition cues on offspring selfishness.

Material & Methods

The animals used in this experiment originated from a laboratory population held according to our standard laboratory rearing protocol and based on a large founder population (Meunier & Kolliker 2012; Meunier et al. 2012). In brief, groups of approximately 80 males and 80 females (randomly selected from the breeding stocks) were setup for mating in two plastic containers (37 x 22 x 25 cm) lined with fluon to prevent the insects from escaping, humid sand as a substrate, and egg-cardboard and plastic tubes as shelters. The food consisted of an artificial diet (Meunier et al. 2012; Wong & Kölliker 2013) and was changed twice a week. The containers were kept in a climatic chamber at 60 % humidity and 14 h/10 h 20 °C/20 °C light/dark photoperiod cycle ("summer conditions"). Upon observation of the first oviposition on 21 January 2011, all females were set up individually in petri-dishes (10 x 2 cm) with humid sand as substrate and plastic shelters as nests and ad libitum food. All females were then transferred to "winter conditions", which consisted of one week in darkness and at 10 °C for to trigger egg-production, and 80 % humidity and 15 °C afterwards. The females were held under these conditions until hatching (= day 0). Food was changed twice a week from isolation to oviposition. No food was provided from oviposition to hatching (Kölliker 2007). One day after hatching the number of hatched nymphs was counted, and the clutches were standardized to a maximum of 25 nymphs (give mean and SE) in preparation for the experimental setup (see below). The female and five randomly selected nymphs were weighed to the nearest 0.001 mg using a Mettler-Toledo MT5 Micro-balance (Mettler, Roche, Basel), provided with ad libitum food and transferred to summer conditions (see above).

Experimental design

The aim of the experimental design was to allow the earwig mother to release chemical cues in the substrate and to expose the nymphs to these cues over an extended time period, but preventing physical contact between mother and nymphs. We achieved this by keeping mothers and nymphs in separate petri dishes and swapping them daily between the two petri dishes. This treatment ensured that nymphs were exposed continuously to any chemical cues females released and left in the substrate, and that the cues were renewed every other day.

The experiment was initiated on day 2 after hatching. The female and 20 nymphs (between 15 and 19 nymphs when brood size was smaller; 7 out of 37 cases) were separated and transferred to a pair of petri-dishes (10 x 2 cm) containing humid sand as substrate and plastic shelters, respectively. The females were randomly assigned either to the high food (HF) or to the low food (LF) treatment. To obtain females in HF or in LF condition, while keeping nymph condition constant, we manipulated the degree of female food access (pollen pellets (Staerkle & Kölliker 2008)). HF females had daily access to large amounts of food (approx. 10 mg) for 3 hours. LF females had access only every second day to a smaller amount of food (< 1 mg) for a period of 3 h (see also Wong & Kolliker 2012). The nymphs had access to ad libitum food (pollen pellets) during these 3 h of female treatment. In all samples, the remaining food was removed after the 3h feeding period.

Because HF females had access to larger amounts of food for a longer total amount of time, we expected them to produce more frass, which would have biased nymph food intake through allo-coprophagy and, hence, potentially nymph condition. To prevent such an effect, female frass was removed daily before swapping females and nymphs between petri-dishes. The number of nymphs alive was counted daily. In this species, most deaths due to siblicide and cannibalism cannot easily be observed because the attacked nymphs are consumed quickly and completely. The number of nymphs alive is therefore mostly a consequence of nymph cannibalism (only 33 dead bodies were observed over the course of the experiments; out of 721 nymphs setup in total). On day 40 after hatching, we counted the number of surviving nymphs, and we took again the weight of the female and of five randomly chosen nymphs (or fewer, depending on the number of survivors).

The sample size consisted of 37 replicates (petri dish pairs), 18 females and their broods in the HF treatment and 19 females and their broods in the LF treatment. There were no significant differences between treatments in female egg-laying date (means ± s.e.; HF: 17.500 ± 3.607, LF: 19.684 ± 3.511; $t_{35} = 0.434$, $p = 0.667$), clutch size (HF: 67.556 ± 2.501, LF: 63.526 ± 2.434; $t_{35} = -1.155$, $p = 0.256$), hatching success (HF: 0.826 ± 0.042, LF: 0.810 ± 0.041; $t_{35} = -0.279$, $p = 0.782$), female body weight at hatching (HF: 52.178 ± 1.805, LF: 49.826 ± 1.757; $t_{35} = -0.933$, $p = 0.357$), or nymph body weight at hatching (HF: 1.585 ± 0.076, LF: 1.580 ± 0.074; $t_{35} = -0.045$, $p = 0.965$), confirming proper randomization of the experimental treatments.

Extraction and quantification of cuticular hydrocarbons (CHC)

After termination of the experiment on day 40, all females were individually frozen at -30 ºC for later CHC extraction. For extraction, each female was immersed for 10 minutes in 800 µl of the extraction solution which consisted of n-Heptane (Rotisolv 99% pure, Carl Roth AG, Arlesheim, Switzerland) and 2.5ng/µl n-Octadecane as an internal standard ($C_{18}H_{38}$; Fluka Analytical, Sigma-Aldrich, Buchs, Switzerland). The female was then removed from the vial and the extract stored at -30 ºC. Chemical analysis was carried out using Gas-Chromatography / Mass-Spectrometry (Agilent GC 7890A / 5975C MSD; electron impact: 70eV). For analysis, 2 µl extract were injected in the GC (containing 2x2.5ng=5ng of the internal standard) in splitless mode (splitless time = 2 min.) and a constant inlet temperature of 250 ºC. The GC-MS system was equipped with a HP-5MS fused silica capillary column (length: 30 m, inner diameter: 0.250 mm, film thickness: 0.25 µm; Agilent J & W GC columns, Agilent Technologies, USA). The GC temperature program started with a temperature of 70 ºC held for 2 min., then increased at 15 ºC/min to 232 ºC (11 min.), and then at 5 ºC/min to to 300 ºC (7 min.). This final temperature was held for 10 min. The column helium flow rate was 1 ml / min, ion detection started after a five minute solvent delay, and the MSD was set to a scan range of 40 – 550 m/z. For quantification of the CHC profiles, we integrated 31 peaks (of which one was the internal standard octadecane; nC18) from the chemical chromatogram using Chemstation software (Agilent Technologies, Inc.). For quantification, we divided the area of each peak by the area of the internal standard in the same chromatrogram and multiplied this ratio by 5 ng to obtain the absolute quantity for each peak in ng. We provide peak identifications based on comparison of retention times and mass-spectra with the ones from Wong et al. (submitted) where a detailed identification of earwig CHC profiles was carried out. Please refer to this study for detailed information on the compound identities of the peaks.

Statistical analysis

We analyzed the effect of the female condition treatment on the proportion of nymphs alive using a generalized linear model with a logit link, a binomial error distribution (correcting for overdispersion), the number of nymphs alive as the dependent variable, the number of nymphs originally present at experimental setup as denominator, and the female condition treatment, hatching date and their interaction as fixed effects.

The chemical peak quantities had distributions with positive skew. The power transformation $y=x^{0.2}$ applied to each of the compound quantities yielded approximately normal distributions. The values y of each peak were then standardized to a mean = 0 and standard deviation = 1 $(z_i = (y_i - \bar{y}))/\sigma_y$ [as recommended in 46]. The large number of peaks (n=30) relative to total sample size (N = 37), and the often tight correlations among them (50 / 465 pairwise correlations with $|r| > 0.8$) made it impossible to directly use the individual peak quantities in a multivariate analysis of variance (MANOVA). A reduction in the dimensionality of the CHC data was achieved by a combination of variable clustering (which is based on principle components) and variable selection (see Varmuza & Filzmoser 2009). The variable clustering resulted in sub-sets (i.e., clusters) of peaks with similar factor loadings on the principle components. For each cluster the peak showing the best fit to its own cluster compared to the next best other cluster (in terms of r^2) was used as the representative peak of this cluster for further statistical analysis. The selection of a peak as a cluster representative was for statistical reasons. Biological interpretation of an effect/relationship involving a cluster representative was done with regard to the cluster as a whole due to the general difficulty of statistical separation of effects among tightly correlated variables.

To analyze the effects of the female condition treatment and hatching date on the female's CHC profile we used a MANOVA with the cluster representatives as dependent variables (repeated measurements), and the treatment, hatching date and their interaction as fixed factors. To directly test for a quantitative relationship between the proportion of nymphs alive and maternal CHC we used a step-wise linear regression approach with hatching date dependent survival (see results for details on how this variable was calculated) as dependent variable and the cluster representatives as candidate explanatory variables. The model with the lowest value for the Baysian Information Criterion (BIC) was chosen as the final model and confirmed using both forward and backward variable selection procedures. All statistical analyses were carried out using JMP® 10.0.1 statistical software (SAS Institute Inc.) and all reported p-values are two-tailed.

Results

Females from the HF treatment gained significantly more weight over the course of the experiments (mean ± s.e.; 12.879 mg ± 1.254) than females from the LF treatment (3.945 mg ± 1.225; $t_{35} = -5.088$, $p < 0.0001$). As intended, the female food treatment did not affect nymph weight. The weight gain (from day 1 to day 40) of the nymphs from the HF treatments (mean ± s.e.; 3.993 mg ± 0.331) did not differ significantly from the weight gain of the nymphs from the LF treatment (4.258 mg ± 0.322; $t_{35} = 0.575$, $p = 0.569$). Thus, our food manipulation successfully generated variation in female condition while keeping nymph condition unaffected.

The proportion of nymphs alive on day 40 was affected by the female condition treatment through an interaction with hatching date (GLM; LR-$\chi^2_1 = 6.177$, $p = 0.013$; Figure 1), while the main effects of the female condition treatment (LR-$\chi^2_1 = 0.899$, $p = 0.343$) and hatching date (LR-$\chi^2_1 = 0.014$, $p = 0.907$) were not significant. The interaction was due to a significantly higher proportion of nymphs alive in the

LF treatment among early hatching broods (contrast; LR-χ^2_1 = 7.016, p = 0.008) and the opposite, marginally non-significant, trend among late hatching broods (contrast; LR-χ^2_1 = 3.456, p = 0.063) (see Figure 1).

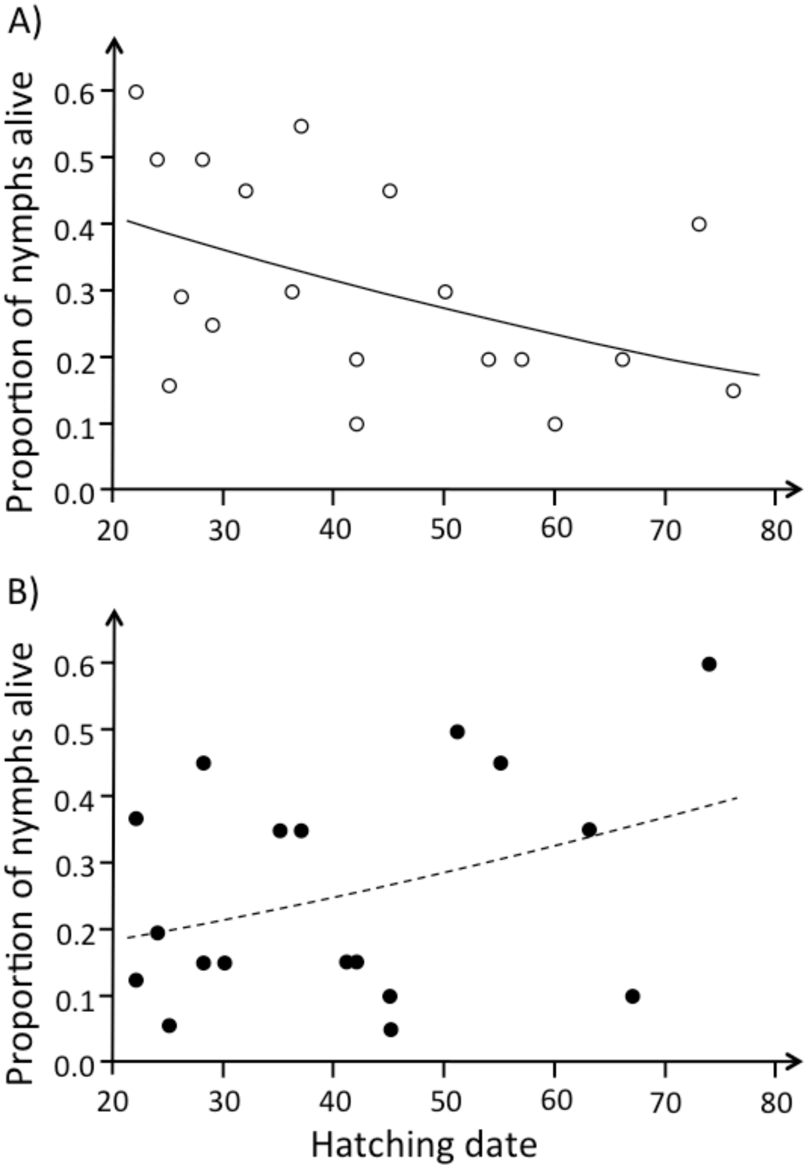

Figure 1. Relationship between the proportion of nymphs alive and brood hatching date for the two female condition treatments. A) low-condition treatment, B) high-condition treatment. Female condition was manipulated by varying experimentally the quantity of food to which the females had access (see Material & Methods). On the x-axis, a julian date is provided with 6.2.2011 corresponding to day 1.

The statistical clustering of the 30 peaks resulted in six clusters (summarized in Table 1) jointly explaining 80.2% of the total variance in compound quantities. Entering the representative chemical compounds for each cluster (Table 1) as repeated measures in a MANOVA with female condition treatment, hatching date and their interaction as fixed terms revealed a significant effect of female condition (but not hatching date or their interaction) on the relative CHC quantities and, hence, the composition of the CHC profile (within-subjects effects; compound x treatment: $F_{5,29} = 5.222$, $p = 0.002$; compound x hatching date: $F_{5,29} = 1.643$, $p = 0.180$; compound x treatment x hatching date: $F_{5,29} = 0.411$, $p = 0.837$). Conversely, the absolute CHC quantity was not significantly affected by female treatment, but significantly dependent on hatching date (between-subjects effects; treatment: $F_{1,33} = 0.254$, $p = 0.617$; hatching date: $F_{1,33} = 6.568$, $p = 0.015$; treatment x hatching date: $F_{1,33} = 0.131$, $p = 0.720$).

In order to correlate nymph survival patterns (Figure 1) to maternal CHC, a new variable for hatching date dependent survival was computed as the product of the standardized deviations of the proportion of nymphs alive and the standardized deviations of hatching date from their respective means. Positive values for this variable contribute to a positive covariance, negative values to a negative covariance between survival and hatching date. As predicted given the significant interaction between hatching date and female treatment (Figure 1), the average value for hatching date dependent survival was positive in the HF treatment and negative in the LF treatment ($t_{35} = -2.151$, $p = 0.038$; see Figure 2). To directly relate survival patterns to maternal CHC variation, we used hatching date dependent survival as the dependent variable in a step-wise linear regression, where we entered the female condition treatment and the six compound cluster representatives as predictor variables. The final model (BIC = 112.05, $r^2 = 0.229$) included cluster 3 (estimate ± s.e.; 0.479 ± 0.165, $F_{1,34} = 8.362$, $p = 0.007$) and cluster 6 (estimate ± s.e.; -0.355 ± 0.165, $F_{1,34} = 4.593$, $p = 0.039$) as the sole significant linear predictor variables (see Table I for cluster composition in terms of peak identity). The female condition treatment dropped from the model ($F_{1,34} = 0.332$, $p = 0.568$) as its formerly significant effect was explained by these two predictors. Their ratio was affected by the maternal condition treatment ($F_{1,33} = 11.618$, $p = 0.002$; Fig. 2), but it was not significantly related to hatching date ($F_{1,33} = 0.371$, $p = 0.546$) or to an interaction between hatching date and treatment ($F_{1,33} = 0.026$, $p = 0.874$). Thus, the relative quantity of cluster 3 CHC compared to cluster 6 CHC was a cue for female condition and significantly predicted the covariance between the proportion of nymphs alive and hatching date, but it was not in itself significantly related to hatching date.

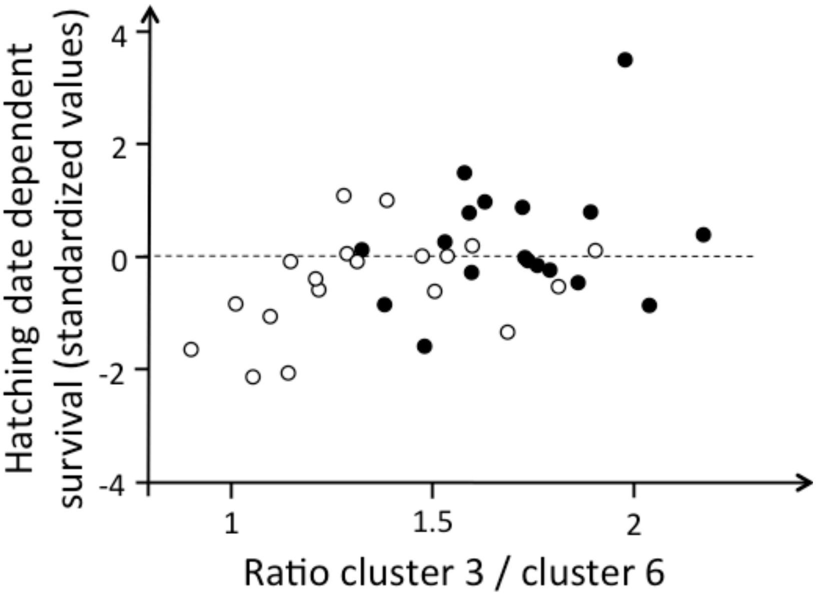

Figure 2. Hatching date dependent nymph survival in relation to the ratio of cluster 3 / cluster 6 cuticular hydrocarbons. The hatching date dependent survival rate was computed based on the deviations of nymph survival and hatching date from the means of the two female condition treatment. It is a measure for the covariance between the two original variables (see Results). Positive values imply lower than average survival in early hatching broods OR higher than average survival in late hatching broods. Negative values imply higher than average survival in early hatching broods OR lower than average survival in late hatching broods. The CHC clusters 3 and 6 were selected based on variable clustering and a step-wise linear regression (see Table 1 for their chemical composition).

Table 1. Summary of chemical compound clustering. The representative peak for each cluster is highlighted in bold[1]. Peaks and the contained chemical compounds are numbered according to the order of their retention times. Identifications of most peaks are according to Wong et al. (submitted), except for CC2, CC3, CC10, CC12 and CC16. Clusters 3 and 6 (bold) were significant predictors of nymph survival patterns.

Cluster	Nr. members	Ret. Time	Peak: Chemical Compounds	r^2own cluster / r^2next closest cluster / 1- r^2 ratio	Prop. variance explained
1	6	13.95	CC1: nC21	0.865 / 0.434 / 0.238	0.805
		14.40	CC2: X-MeC21	0.824 / 0.757 / 0.724	
		14.61	**CC3: X'-MeC21**	0.914 / 0.606 / 0.219	
		14.88	CC4: unknown HC	0.903 / 0.663 / 0.289	
		15.79	CC5: nC23:1/2[2]	0.502 / 0.195 / 0.619	
		16.05	CC6: nC23	0.824 / 0.594 / 0.433	
2	5	16.59	CC7: X-MeC23[2]	0.939 / 0.698 / 0.201	0.884
		16.77	CC8: X'-MeC23	0.734 / 0.515 / 0.549	
		17.11	CC9: X''-MeC23	0.941 / 0.757 / 0.241	
		17.29	CC10: X'''-MeC23	0.865 / 0.451 / 0.246	
		17.73	**CC12: X-MeC24**	0.939 / 0.698 / 0.201	
3	7	17.56	CC11: unknown HC	0.833 / 0.557 / 0.377	0.845
		19.52	CC14: nC25	0.704 / 0.338 / 0.447	
		20.34	**CC15: X-MeC25[2]**	0.932 / 0.574 / 0.160	
		20.56	CC16: X'-MeC25[2]	0.860 / 0.573 / 0.329	
		21.34	CC17: X''-MeC25[2]	0.914 / 0.672 / 0.261	
		22.26	CC18: unknown HC	0.779 / 0.667 / 0.663	
		23.33	CC19: unknown HC	0.892 / 0.625 / 0.289	
4	4	26.30	CC22: X-MeC27[2]	0.815 / 0.382 / 0.299	0.837
		26.51	CC23: Y-MeC27	0.789 / 0.569 / 0.491	
		27.10	CC24: X,X'-diMeC27[2]	0.874 / 0.308 / 0.183	
		27.29	**CC25: Y,Y'-diMeC27[2]**	0.871 / 0.269 / 0.176	
5	4	25.35	CC21: nC27	0.199 / 0.011 / 0.809	0.704
		28.86	CC26: X-MeC28[2]	0.791 / 0.433 / 0.369	
		30.94	**CC28: X-MeC29[2]**	0.946 / 0.140 / 0.062	
		31.50	CC29: X,X'-diMeC29[2]	0.878 / 0.144 / 0.142	
6	4	19.04	CC13: nC25:1	0.572 / 0.324 / 0.633	0.681
		24.60	CC20: nC27:1/2[2]	0.775 / 0.508 / 0.458	
		29.67	**CC27: X,X'-diMeC28**	0.785 / 0.272 / 0.295	
		33.38	CC30: X-MeC31	0.590 / 0.077 / 0.444	

[1] The compound with strongest correlation with its own cluster compared to the next closest cluster (i.e., compounds with lowest 1-r^2 ratio) were chosen as cluster representatives.

[2] Complex peaks containing more than one compound, usually isomers with double-bonds or methyl-groups at different positions. Different positions are indicated by single or multiple primes.

Discussion

Parents may transmit information about their condition or environmental conditions through pre-birth maternal effects, through their behavioral interactions with offspring, the provisioning of resources (Mousseau & Fox 1998; Royle et al. 2012a; Wendt Müller et al. 2007), but also through specific signals as part of a reciprocal exchange of information between parents and offspring. For example, treehopper (*Umbilia crassicornis*) nymphs signal predator threat to their tending mother through vibrational signals (Cocroft 1999), and the mothers produce vibrational signals to reduce the likelihood of falls alarms among her nymphs (Hamel & Cocroft 2012). In this study, we showed in the European earwig *Forficula auricularia* that condition-dependent chemical cues/signals from the mother, as encoded in her CHC profile, predict offspring survival, and that the direction of this effect depended on the timing of breeding. The nymphs had access to the same amount of food throughout and did not differ in their weight, and cannibalism occurred in almost all cases of nymph death. Thus, the difference in survival between treatments was most likely due to variation in nymph siblicidal and cannibalistic drive, induced by cues of maternal condition.

The information transfer about female condition was not direct through a behavioral interaction from mothers to their offspring. We experimentally prevented any physical (visual, tactile, or other) contact between mothers and nymphs by keeping the mother and her nymphs in separate petri dishes (and swapping them daily) to ensure that only chemical information about maternal condition, and not her behavior or the amount of maternal food provisioning, could mediate the observed effects on nymph siblicide and cannibalism. Thus, females must have released chemical cues in the form of non-volatile contact pheromones in the substrate, and the nymphs were exposed to these cues when subsequently placed in the same environment. This indirect substrate-born signaling would occur under natural conditions in the breeding burrows during the period of maternal care. Female earwigs “mark” their breeding burrow with pheromone secretions (shown for the sand earwig *Labidura riparia*; (Radl & Linsenmair 1991); pers. obs. for *F. auricularia*), to which the nymphs are then exposed while in the burrow.

The effect of maternal chemical cues of condition on nymph siblicide and cannibalism depended on the timing of breeding. Among early broods, nymphs exposed to maternal cues of poor condition showed a significantly and about two-fold higher survival rate than nymphs exposed to maternal cues of high condition. Interestingly, the effect was in the opposite direction among late broods. This effect could be either due to a quantitative or qualitative difference in the condition-dependent chemical cues among early and late breeding females or a temporal change in nymph response to the same condition-dependent cues. Although our data does not allow us to fully disentangle the two possibilities, our further analyses indicate that the latter is the more likely explanation. Variation between females in

CHC profiles was quantitative in nature. Early and late breeding females, and females in poor and good condition, had qualitatively the same CHC profiles. The composition of the female CHC profile (in particular the quantity ratio between cluster 3 and cluster 6 CHC) varied quantitatively with female condition but not with timing of breeding. We cannot fully rule out that chemical cues not measured by CHC extraction and GC-MS analysis (e.g., peptides or proteins), may also play a role, but given that the two identified CHC clusters explained well the treatment effect of female condition this seems rather unlikely. Thus, our results indicate that nymphs born early and nymphs born late have opposite responses to the chemical maternal cues of condition, expressing behavioral reaction norms (Smiseth, Wright & Kölliker 2008) of opposite sign. The ratio of cluster 3 compound quantities (composed mostly of nC25 – pentacosane, and several isomers of methylated C25; Table 1) to cluster 6 compound quantities (composed of a mix of alkene C25 – pentacosene, and C27 – heptacosene, and methylated C28 and C31) was lower in females of poor condition, and was associated with lower cannibalism rates among early broods AND higher cannibalism rates among late broods (and vice versa for higher ratios). This is evidence for context-dependence of offspring responses to maternal cues/signals. If variation in hatching date has a genetic component, these results would show genotype x family environment interaction (Kölliker, Royle & Smiseth 2012) with the maternal chemical cues of condition being a component of the family environment to which the nymphs are sensitive. G x E is an important factor in the maintenance of heritable variation of phenotypic traits (Lynch & Walsh 1998; Rutter 2006) and in the present case would contribute to maintained variation in cannibalistic tendencies.

We previously showed that the same manipulation of female food access affected the food provisioning rate of earwig mothers, with females in poor condition providing food to fewer nymphs than females in high condition (Wong & Kolliker 2012). Furthermore, the presence of a mother can reduce nymph survival when the mother is in poor condition and food is scarce, because mothers in poor condition compete with offspring for access to the limited available resources (Meunier & Kölliker 2012). As a consequence, nymphs can associate poor maternal condition with low expected food provisioning by their mother, and more costly interactions with her, and they should respond to the corresponding cues of maternal condition accordingly. Based on the predictions we formulated in the introduction, the higher cannibalism rate among late broods when exposed to chemical cues/signals of poor maternal condition fits a scenario of such enhanced competition when the mother is in poor condition. Conversely, the lower cannibalism rate among early broods when exposed to chemical cues of poor maternal condition would then suggest a differential benefit of living in larger sibships and/or of sibling cooperation when the mother is in poor condition. Recent experiments demonstrated that earwig nymphs not only compete (including siblicide) (Dobler & Kölliker 2010), but that they are also very gregarious over large parts of their juvenile development (Gómez & Kölliker in press; Wong & Kolliker 2012), and that they cooperate by exchanging food, a behavior particularly pronounced in the absence of physical interactions with their mother (Falk et al. submitted). Thus, there is scope for both sibling competition and cooperation in *F. auricularia*. But why should the benefits of cooperative versus competitive strategies vary with the timing of breeding? In earwigs, early broods are the first to emerge from their winter burrows and experience low densities, less cannibalism threat by other earwigs and more time for development before the next winter starts. The low density could imply that the costs of dispersing and self-foraging (to escape from a mother in poor condition with which nymphs would otherwise have to locally compete for food; (Meunier & Kölliker 2012)) may be lower for early brood nymphs. Concurrently, maintaining larger sib groups by keeping the level of siblicide low, may be beneficial for self-foraging for example because larger groups of nymphs are more efficient at foraging or provide a better protection against predators (Krause & Ruxton 2002 for a review). However, further studies are required to test this hypothesis.

Our results showed that the maternal CHC profile contained reliable information about condition and was associated with time-dependent responses in offspring that have immediate fitness consequences in terms of survival. Thus, there is selection on this cue, and it seems likely that variation in maternal CHC profiles may have evolved to some extent due to its signaling function. We do not know if the observed variation in CHC profiles carries strategic costs (i.e., is a signal of condition) or if it rather reflects a constraint of limited food intake (i.e., is an index of condition; (Holman 2012; Maynard Smith & Harper 2003)). Given that CHC derive from the fat-metabolism (which necessarily partly depends on the quality and quantity of ingested food (Blomqvist & Vogt 2003)), it is possible that limitation in food intake directly constrains the quantitative production of CHC influencing CHC profiles in turn. However, the female condition treatment did not affect the overall quantity of CHC, only its composition, implying that some CHC decreased (cluster 3 CHC; Table 1) and others increased (cluster 6 CHC; Table 1) under food restriction.

CHC are well known for their multitudes of functions in insect communication, especially their role as cues in insect (kin) recognition (Blomquist & Bagnières 2010; d'Ettorre & Hughes 2008; Guerrieri et al. 2009); Wong et al. submitted). A comparably well studied example in the context of parental care are burying beetles (*Nicrophorus vespilloides*), where adult CHC profiles display information about breeding status (breeding versus non-breeding), and to a lesser extent also about their sex and nutritional condition (Steiger et al. 2007). Male and female parents in this biparental beetle recognize each other based on these CHC (Müller, Eggert & Elsner 2003; Steiger et al. 2007), and CHC of adults in breeding status act as a trigger of begging behavior in the larvae (Smiseth et al. 2010). However, it is not known in burying beetles if larvae modulate their begging in response to condition-dependent variation in parental CHC. CHC have been invoked as signals of quality in other social contexts. For example, in black garden ants (*Lasius niger*) it was shown that ant queen CHC convey information about queen reproductive potential, and inhibits worker ovarian development and aggression (Holman 2012). While these studies previously showed that CHC can display information about various aspects of individual condition/quality, our study suggests that CHC act as maternal condition cues mediating offspring siblicide and cannibalism and, hence, their selfishness.

Conclusions: Taken together, our results on the effect of maternal condition-dependent cues on nymph siblicide and cannibalism reported here, and the former findings in *F. auricularia* showing that earwig nymphs express condition-dependent CHC profiles that affect maternal behavior (Mas et al. 2009) and reproductive physiology (Mas & Kölliker 2011), we provided to our knowledge the first evidence for CHC variation to be involved in a reciprocal information exchange about nutritional condition between parents and offspring in insects. The CHC exposure effects on nymph selfishness and maternal reproductive physiology both depend on the timing of breeding. Although the ultimate causes of this variation remain to be illuminated, our findings that behavioral consequences of information exchange depend on the timing of breeding suggest that adaptive responses in communication can be strongly context-dependent and include responses that are in opposite direction.

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Author contributions

Planned the experiment: JWYW, MK. Performed the experiment: JWYW. Analyzed the data: MK. Wrote the paper: MK, JWYW.

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CHAPTER 6

Food availability shapes juvenile development and adult life history in the European earwig

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Abstract

Variation in environmental conditions experienced during ontogeny can lead to differences in behavioral strategies, morphology and reproductive success. The necessity for differential allocation of limited resources in multiple life history traits can result in physiological trade-offs between these traits. In this experiment, we investigated the effects of food restriction on different stages during the juvenile and early adult development on survival, maternal care, and reproductive success in the European earwig *Forficula auricularia.* Individuals either received limited or unlimited access to food during their early and late juvenile, and their early adult development, and these treatments were applied in a fully crossed experimental design allowing me to also test for the combined effects of food limitation across these stages. Our results show that food restriction during the juvenile development has negative effects on survival and adult body weight. Moreover, restriction in the late juvenile development lead to smaller clutch size, lower probability of second clutch production and reduced hatching success in the second clutch. There were no effects of food restriction on maternal care behavior measured as egg clustering. Our findings show that food restriction during the juvenile development poses a constraint to reproductive success in general rather than reflecting a condition-dependent shift in the allocation trade-off between current and future reproduction. Female egg clustering was independent of food restriction. Thus, food limitation negatively affects female reproductive output, probably through an effect on nutritional condition, but not maternal care behavior in the form of egg clustering. Environmental conditions experienced early in an individual's lifetime can have detrimental effects once individuals become adult and need to be considered to understand individual variation in reproductive success and life-history trade-offs.

Introduction

Life history theory helps to explain variation in life history strategies that we find in natural populations. Within one species, life-history variation may be the results of developmental or physiological responses to certain environmental conditions (e.g., Stearns & Sage 1980). Abiotic environmental factors like temperature, precipitation and food availability often show high variability, exposing organisms to unpredictable environments over the course of their lifespan. This might lead to elevated levels of physiological stress, resulting in physiological trade-offs, when two or more functions compete for the same pool of limited resources (Stearns 1992; Flatt & Heyland 2011). Commonly studied trade-offs include, for example, growth vs. reproduction (Warner 1984; Werner & Anholt 1993; Sterck et al. 2006), reproduction vs. survival (Snell & King 1977; Tallamy & Denno 1982; Marshall & Sinclair 2010) or current vs. future reproduction (Candolin 1998; Richner & Tripet 1999; Desouhant et al. 2005).

Temporal variation in the acquisition and allocation of resources affects the individual resource pool and thus the optimal outcome of such trade-offs in terms of the association between different life-history traits ('house-car principle' Van Noordwijk & De Jong 1986). The 'house-car principle' states that when individuals are in good condition, they can invest more resources into several life history traits simultaneously compared to individuals in poor condition which are limited, and that this difference in condition can result in positive correlations between life-history traits despite underlying trade-offs. For example, a study of an island population of the song sparrow *Melospiza melodia* showed that the total number of fledglings produced per year was positively correlated with the subsequent survival of the females to the next breeding season (Smith 1981).

Phenotypic variation in condition can be influenced by current environmental effects, but also by the experience of the early life. Already in 1980, Stearns & Sage showed that the early environment experienced by the mosquito fish *Gambusia affini* had a significant effect on its body weight. And there is further evidence that the environment experienced early in life can strongly influence adult life histories. For example, larval nutrition affects female reproduction by mediating various aspects of adult body size in dipterans (Bergland 2011). These effects are often mediated by the size of the reproductive organs. Distinct phases of the juvenile development can be critical for adult life history traits. Studies in *Drosophila melanogaster* showed that females, which were reared on food with less or no yeast in their third (final) instar stage, are smaller and have fewer ovarioles than those reared with more yeast (Hodin & Riddiford 2000; Tu & Tatar 2003). Ovariole number correlates with potential reproductive output and, thus, with fitness (Cohet & David 1978).A recent study in the burying beetle *Nicrophorus vespilloides* revealed that when individuals were restricted in a subadult period of sexual maturation, they were less successful in competition for carcasses, which are compulsory for reproduction (Hopwood et al. 2013).

The European earwig *Forficula auricularia* has a juvenile development of approx. 60 days (JW & MK unpublished data), where individuals pass four instars before reaching the adult stage. Females from this species produce either a single clutch (i.e., they are semelparous) or two clutches (i.e., they are iteroparous) over the duration of their lifetime (Meunier et al. 2012), indicating scope for a trade-off between current and future reproduction. Results from Meunier et al. (2012) showed that semelparity and iteroparity coexist as alternative life history tactics in *F. auricularia*, that parity is partly the outcome of trans-generational effects and interactions with offspring (Meunier & Kölliker 2012a), and that females producing two clutches also produced larger first clutches (Meunier et al. 2012), a pattern consistent with the 'house-car principle' mentioned above. Furthermore, since females provide extensive care in the forms of egg and offspring attendance as well as predator defense and food provisioning (Lamb 1976; Costa 2006; Staerkle & Kölliker 2008), which increases offspring quality and survival (Kölliker 2007), there is further scope for a trade-off between number of offspring and maternal care.

Previous work in *F. auricularia* already showed that females in poor nutritional condition provide food to fewer nymphs and for a shorter period of time than females in good condition (Wong & Kölliker 2012). Hence, my final experiment investigated the effect of food restriction during several phases of the juvenile and early adult development on survival, maternal care, and reproductive success. Since fecundity varies with female body size in most insects (Honěk 1993), and since other studies have shown before that reduction in larval food quality or quantity generally results in reduced adult body size and fat content with consequent negative effects on fecundity (Awmack & Leather 2002; Bauerfeind & Fischer 2005; Boggs & Freeman 2005), I predicted a lower reproductive success in individuals that were restricted during their juvenile development, specifically with regard to the probability of second clutch production, that these females would be less able to provide efficient care (assuming care is costly). While previous studies have investigated the effect of nutritional restriction during development on reproduction in insects, I paid particular attention to the question if the effects of food restriction over several developmental periods are additive, or if their effects are interactive. For example, food restriction during the late juvenile phase may be particularly detrimental if the individual already experienced restricted food during early development.

Material & Methods

Study Species and Rearing Conditions

Individuals of *F. auricularia* were collected in July 2011 in an olive grove in Dolcedo, Italy (7°56'55'E, 43°54'14'N, altitude 443 m) and were set up in the laboratory under standard conditions in plastic containers (37 x 22 x 25 cm), consisting of 30 males and 30 females, respectively. To prevent escape by the earwigs, all containers were lined with fluon (Whitford GmbH, Diez, Germany). We used humid sand as a substrate as well as egg cardboard and plastic tubes as shelters. After random mating, females were set up individually in petri dishes (10 x 2 cm) containing humid sand as substrate, plastic shelters as burrows and ad libitum artificial food (Wong & Kölliker 2013). All females were subsequently transferred to complete darkness at 10°C for 16 days to induce egg laying and then maintained in complete darkness at 70% humidity and 15°C until egg laying and hatching. In this population, females kept under *ad libitum* conditions lay on average between 46 and 52 eggs in their first clutches and approx. 85% lay a second clutch (Meunier et al. 2012).

Experimental design

We checked clutches daily for hatching and assigned the date of hatching as day 0. One day after hatching (day 1), we counted the number of nymphs. We took the weight of the female, which was subsequently isolated again for second clutch oviposition. We also took the weight of 10 nymphs. Clutches, which contained fewer than 28 nymphs on day 2 were excluded. All clutches were subsequently transferred from 15°C and complete darkness to 20°C and a 14 h/10 h light/dark cycle and assigned to three consecutive treatments.

Manipulation of food availability – restriction treatments

We had three restriction treatments in a fully crossed experimental design: Restriction in the early juvenile development (EAR), restriction in the late juvenile development (LAT) and restriction in the early adulthood (AD). Restriction treatments always had two levels, restricted (yes; 'y') or not restricted (no: 'n'). All individuals were exposed to the three restriction treatments consecutively according to their assignment to the specific treatment combination (EAR-LAT-AD). For example, an individual was restricted in the early juvenile development, not restricted in the late juvenile development, and again restricted in the early adulthood (EAR: yes, LAT: no, AD: yes; abbreviated: yny). The fully crossed design resulted in eight possible combinations between the treatments: nnn, nny, nyn, nyy, ynn, yny, yyn and yyy. As the average developmental time until adulthood is approx. 60 days (JW & MK unpublished data), we defined the time period from day 1 until day 31 as the early juvenile development, and time period from day 31 until day 62 (or until the first adult molt in a clutch) as the late juvenile development. The period of early adulthood was from the day of the first adults until 16 days later. This period had to be kept shorter than the two juvenile restriction periods, because females start to develop unfertilized eggs, if kept without mating partners for too long after emergence as adult (personal observation).

Under unrestricted food conditions, food was provided in large quantities and renewed every three days. Under restricted conditions, food was provided only every 6 days, whereas the leftovers of the food (if any) were removed three days after supply. The quantity of food was adjusted according to the age of the nymphs, with 60 mg from day 1 to day 16, 120 mg from day 19 to day 28 and 240 mg from day 31 until molting into adults (Meunier & Kölliker 2012b).

Each brood started with 20 nymphs originating from the same family. Broods containing fewer than 20 nymphs (N = 20) were distributed equally between the different treatment combinations. Broods of nymphs were first reared in small petri-dishes as described above. On day 16, the number of nymphs was counted to monitor survival and all nymphs were transferred to large petri-dishes (14 x 2 cm). The food restrictions were sustained until day 31, which we had determined as the end of the first half of development. On this day, the number of surviving nymphs and the instar stages present were determined. We noted the date of emergence of the first adults and separated males and females in each group from this day onwards to prevent brother-sister matings. As soon as three males and three females had emerged here, three individuals of each sex were weighed individually. In individuals that were restricted during their juvenile development, either in early or late, we waited for the emergence of two males and two females as the average number of individuals was lower than in the groups that were never restricted. We took the weight of these individuals and of one additional male and female as soon as available. In groups where the nymphs were restricted throughout the whole juvenile development, early and late, we waited only for the emergence of one male and one female according to the small number of individuals and took their weight. We weighed additionally 2 males and 2 females after emergence, if possible. After 16 days in the adult treatment, 3 males and 3 females (if possible) were again weighed to compare the differences in weight change between group individuals that were restricted and not restricted as adults. We noted the date of emergence of the last adult and counted the total number of males and females in all groups. Then one male and one female from each group were set up for mating. As the individuals that were never restricted had developed faster compared to the other groups, we kept them under unrestricted food conditions for an additional 16 days. These individuals were thus set up for mating after 32 days of first adult emergence whereas individuals from the other groups were setup for mating already after 16 days of the first adults.

As mating partners, we used nymphs that were not setup on day 1, i.e. all clutches containing more than 40 nymphs (N = 62). These individuals were kept in family groups and raised to adults under unrestricted food conditions. Males and females were separated after emergence to prevent mating. Since these mating partners were all raised under the same (unrestricted) conditions, the expected effects on reproductive success and maternal care should be derived from the food restricted individuals in our treatments. We paired males and females randomly, but excluded sib-matings between individuals from the same family. All pairs were allowed to mate for a period of two months where food was unrestricted and changed twice per week. After that, males were removed and all females isolated in new petri dishes (10 x 2 cm) and transferred into fall conditions (20°C: 15°C and a 14 h:10 h light □ dark cycle) to stimulate egg production. Food was provided twice per week. After the first oviposition, all females were transferred to complete darkness at 10°C for 16 days to induce egg laying in the other females and then maintained in complete darkness at 70% humidity and 15°C until hatching of the first clutch.

Egg clustering

Approx. 15 days (+2 days) after oviposition of the first clutch, we conducted the egg clustering experiment. Egg clustering, i.e., the gathering of eggs into a pile that can be guarded by covering the eggs with the body, is an important maternal care behavior to prevent egg predation (Lamb 1976) and expected to be costly for the female. We took each egg and spread the whole clutch throughout the petri dish. By calculating the proportion of eggs the female clustered into a pile within one hour, I received a measure of the rate at which females retrieve their eggs and, hence, of maternal egg clustering. This measurement was taken for both first and second clutch clutches.

Clutches were daily checked for hatching and the date of hatching was assigned as day 0. One day after hatching (day 1), we counted the number of nymphs. We took the weight of the female, which was subsequently isolated again for second clutch oviposition. We also took the weight of 10 nymphs.

Second clutch

Females, whose first clutch hatched successfully, were isolated for second clutch oviposition on day 1 after hatching of the first clutch. Egg development from oviposition to hatching took on average 30 ± 0.629 days (mean ± SE). Females were then given a period of max. 60 days after hatching for second clutch oviposition (see Mas & Kölliker 2011). If they did not oviposit their second clutch within this period, they were considered as 1C females.

Females, whose first clutch did not hatch, were set-up for second clutch oviposition after a maximum period of 40 days after first clutch oviposition or on the day they had eaten their undeveloped first clutch. We chose a period of 40 days, as the observed maximum duration of egg development is 30 days and we added an additional buffer of 10 days. These females were given a period of 45 days for second clutch oviposition, equally resulting in a total period of approx. 85 days for oviposition of two clutches. If they did not oviposit within this period, they were considered as 1C females.

Statistical analysis

The effects of food restriction were tested on various measurements regarding (a) the development into adults (the proportion of nymphs that survived to adulthood, the body weight of newly emerged adult females), (b) clutch data (clutch size of the first and the second clutch, the probability of second clutch production), (c) maternal care behavior (the proportion of eggs clustered for the first and the second clutch), and (d) hatching data (the probability of hatching failure, hatching success, and nymph weight at day 1, all measures taken for both the first and the second clutch). Hatching success and weight of nymphs at day 1 was only calculated for those females whose eggs hatched successfully. The probability of first clutch production was not analyzed statistically, due to the very small number of females that did not lay a first clutch (3 out of 148).

In all statistical models, we entered the three restriction treatments (EAR, LAT and AD) and their interactions as fixed effects in all models. To analyze treatment effects on female body weight and nymph weight at day 1, we used linear models (three-way ANOVAs) (a log transformation was applied to nymph weight at day 1 to comply with normality assumptions of the models). For the remaining measurements listed above, we used generalized linear models (GLMs). In the experiment, we had to usually use two males and two females from the same family (N = 77 families), which implies that these replicates are not fully independent statistically. To check that our results are robust, we added the ID of the family as random effect in all model, hence checking the results of the GLMs using generalized linear mixed models (GLMMs) in R. All results were qualitatively similar, and we therefore present all results from the GLMs for simplicity. To allow for a direct comparison of each tested factor and all interactions across the analyses, only the three-way interactions that were non-significant across all the statistical models were removed from the statistical models. All models were corrected for overdispersion when necessary. All statistical analyses were conducted using the JMP® Pro 10.0.1(SAS Institute Inc., Cary, NC, U.S.A.) and *p*-values are two-tailed.

Results

(a) Development into adults

The proportion of individuals that successfully survived to adulthood was significantly reduced by food restriction in both, the early and the late juvenile development (binomial GLM, $n = 152$; EAR: $\chi^2 = 103.599$, $p < 0.0001$; LAT: $\chi^2 = 95.587$, $p < 0.0001$) (see Table 1). The proportion of adults was also influenced by an interaction between these two restriction periods. Individuals that were never restricted in their juvenile development (nn-) had the highest proportion of adults and individuals that were restricted during their whole juvenile development (yy-) had the lowest proportion of adults. Food restriction in only the early juvenile development (yn-) or only the late juvenile development (ny-) resulted in an approx. equal proportion of adults (binomial GLM, $n = 152$; interaction EAR*LAT: $\chi^2 = 7.507$, $p = 0.006$).

Individual weight of females molted into adults was significantly lower in individuals that were restricted in their late juvenile development (LAT: ANOVA, $F = 126.975$, $df = 1,144$, $p < 0.0001$; mean ± SE, no: 41.968 mg ± 0.795; yes: 31.201 mg ± 0.506), but was not significantly affected by restriction in the early juvenile development (EAR: ANOVA, $F = 2.702$, $df = 1,144$, $p = 0.102$) or the early adulthood (AD, ANOVA, $F = 0.685$, $df = 1,144$, $p = 0.409$) (see Table 1).

Table 1. Effects of food restriction during the early and late juvenile, and early adult development on the survival to adulthood and adult female weight. Significant *p*-values are in bold.

	(a)			
	Survival to adulthood (prop.)		**Weight females day 1**	
	χ^2	p	F	p
Restricted early (EAR)	**103.599**	**<.0001**	2.702	0.102
Restricted late (LAT)	**95.587**	**<.0001**	**126.975**	**<.0001**
Restricted adults (AD)	0.342	0.559	0.685	0.409
EAR:LAT	**7.507**	**0.006**	0.132	0.717
EAR:AD	0.250	0.617	0.125	0.725
LAT:AD	0.138	0.710	0.003	0.960
Type of statistical model; d.f. or n	Binomial GLM n = 152		ANOVA d.f. = 6,150	

(b) Clutch size and probability of second clutch production

Females that were restricted in their late juvenile development laid significantly fewer eggs in their first clutch (LAT: poisson GLM, $\chi^2 = 26.187$, $n = 145$, $p < 0.0001$; mean ± SE no: 78.039 ± 1.431; yes: 65.377 ± 2.041), but the size of the first clutch was unaffected by the EAR ($p = 0.217$) or AD ($p = 0.635$) treatments (or their interactions, all $p > 0.062$) (see Figure 1(top) and Table 2).

The probability of second clutch production was significantly reduced when females were restricted in their late juvenile development (LAT: binomial GLM, $\chi^2 = 6.373$, $n = 144$, $p = 0.012$) (Figure 2 and Table 2), whereas restriction during the period of early adulthood resulted in a higher probability of second clutch production (AD: binomial GLM, $\chi^2 = 4.661$, $n = 144$, $p = 0.031$). These two restriction treatments did also significantly interact with each other. Individuals that were restricted during both periods (-yy) had the lowest and individuals that were restricted only during their early adulthood (-ny) had the highest probability of producing a second clutch. Individuals that were never restricted in their late juvenile and their early adulthood (-nn) had medium probability of second clutch production such as the individuals that were restricted in their late juvenile development, but not as adults (-yn) (interaction LAT*AD: binomial GLM, $\chi^2 = 5.724$, $n = 144$, $p = 0.017$) (Figure 2 and Table 2).

Females producing a second clutch showed a significantly smaller number of eggs laid when they were restricted during their late juvenile development (LAT: poisson GLM, $\chi^2 = 10.699$, $n = 128$, $p = 0.001$; mean ± SE no: 52.986 ± 1.044; yes: 47.328± 1.419), while the early juvenile and early adult restriction treatment had not significant effects (EAR, $p = 0.246$) or AD, $p = 0.081$) treatments (or their interactions, all $p > 0.268$) (Figure 1(bottom) and Table 2).

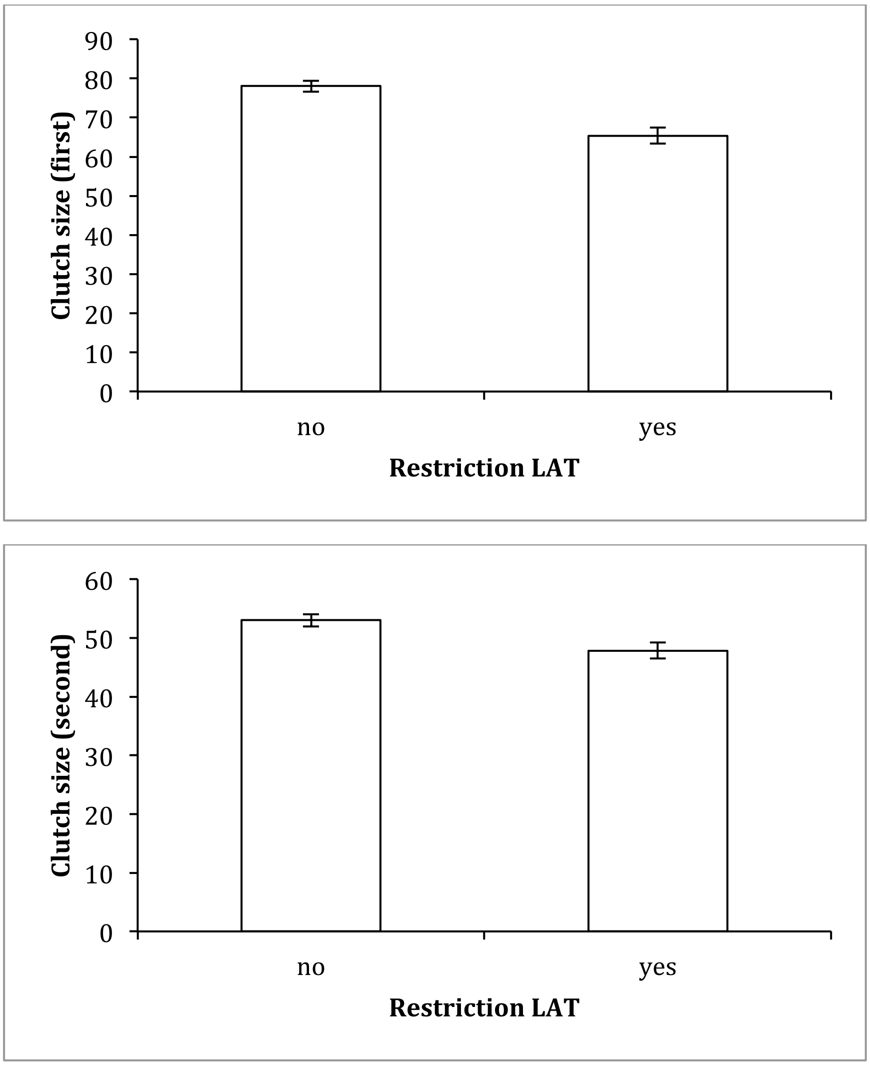

Figure 1. The effects of food restriction in the late juvenile development on first clutch size (top) and second clutch size (bottom). Data from non-significant effects were pooled for illustration purposes. The effects of all factors and interactions are listed in Table 2.

Figure 2. The effects of food restriction in the late juvenile development and early adulthood on the probability of second clutch production. Data from non-significant effects were pooled for illustration purposes. The effects of all factors and interactions are listed in Table 2.

Table 2. Effects of food restriction during the early and late juvenile, and early adult development on the clutch size and probability of second clutch production. Significant *p*-values are in bold.

(b)

	Clutch size				Prob. second clutch	
	First clutch		Second clutch			
	χ^2	*p*	χ^2	*p*	χ^2	*p*
Restricted early (EAR)	1.527	0.217	0.001	0.968	1.347	0.246
Restricted late (LAT)	**26.187**	**<.0001**	**10.699**	**0.001**	**6.373**	**0.012**
Restricted adults (AD)	0.225	0.635	0.0009	0.976	**4.661**	**0.031**
EAR:LAT	2.227	0.135	0.624	0.430	0.536	0.464
EAR:AD	3.475	0.062	1.570	0.210	1.225	0.268
LAT:AD	1.184	0.277	0.840	0.360	**5.724**	**0.017**
Type of statistical model; d.f. or n	Poisson GLM n = 145		Poisson GLM n = 128		Binomial GLM n = 144	

(c) Egg clustering

Female egg clustering of the first and the second clutch was not significantly influenced by any of the food restriction treatments or their interactions although in the first clutch there was a marginally non-significant trend for females that experienced food restriction during the early juvenile period to show a lower proportion of eggs clustered (Table 3; binomial GLM; first clutch: $n = 132$, $p > 0.067$; second clutch: $n = 123$, $p > 0.121$).

Table 3. Effects of food restriction during the early and late juvenile, and early adult development on the proportion of eggs clustered in the first and second clutch. Significant *p*-values are in bold.

	(c) **Egg clustering (prop.)**			
	First clutch		Second clutch	
	χ^2	p	χ^2	p
Restricted early (EAR)	3.360	0.067	1.118	0.277
Restricted late (LAT)	0.036	0.850	<0.0001	0.996
Restricted adults (AD)	0.847	0.357	0.009	0.923
EAR:LAT	0.528	0.468	1.060	0.303
EAR:AD	0.0002	0.988	0.634	0.426
LAT:AD	1.372	0.242	2.406	0.121
Type of statistical model; d.f. or n	Binomial GLM n = 132		Binomial GLM n = 123	

(d) Probability of hatching failure, hatching success, and nymph weight day 1

The probability of hatching failure of the first clutch was not significantly influenced by any of the restriction treatments or their interactions (binomial GLM, $n = 145$, $p > 0.123$; Table 4). The probability of hatching failure in the second clutch was also not significantly influenced by any of the restriction treatments or their interactions (binomial GLM, $n = 128$, $p > 0.050$; Table 4) However, there was a trend that hatching failure increased when individuals were restricted in both their early juvenile and early adulthood (y-y) or not restricted in their early juvenile and early adulthood (n-n). Hatching failure decreased when individuals were restricted in either the early juvenile (y-n) or their early adulthood (n-y) (interaction EAR*AD: binomial GLM, $\chi^2 = 3.856$, $n = 128$, $p = 0.050$).

Hatching success of the first clutch was not significantly influenced by any of the food restriction treatments or their interactions, but there was a non-significant trend that hatching success was influenced by the interaction between early juvenile and early adult development. Individuals that were restricted during either the early juvenile or the early adult development had a higher hatching success in their first clutch than individuals that were never or restricted during both periods (interaction

EAR*AD: binomial GLM, $n = 88$, $p > 0.050$; Table 4). Hatching success of the second clutch was significantly lower when females where restricted during their late juvenile development (LAT: binomial GLM, $\chi^2 = 7.035$, $n = 80$, $p = 0.008$) (Figure 3 and Table 4). The interaction between restriction during the early and the late juvenile development also influenced hatching success significantly. Individuals that were never restricted (nn-) had the highest, individuals that were restricted in the early juvenile development (yy- and yn-) had intermediate, and individuals that were restricted only in their late juvenile development (ny-) had the lowest second clutch hatching success (interaction EAR*LAT: binomial GLM, $\chi^2 = 6.074$, $n = 80$, $p = 0.014$) (Figure 3 and Table 4).

Nymph weights on day 1 (one day after hatching) of the first and the second clutch were not significantly influenced by any of the food restriction treatments or their interactions (Table 4; ANOVA; first clutch: $n = 87$, $p > 0.060$; second clutch: $n = 80$, $p > 0.106$).

Figure 3. The effects of food restriction in the late juvenile development and early juvenile development on the hatching success of the second clutch. Data from non-significant effects were pooled for illustration purposes. The effects of all factors and interactions are listed in Table 4.

Table 4. Effects of food restriction during the early and late juvenile, and early adult development on the probability of hatching failure, hatching success and nymph weight day 1. Significant *p*-values are in bold.

(d)

	Prob. hatching failure				**Hatching success**				**Nymph weight day 1**			
	First clutch		Second clutch		First clutch		Second clutch		First clutch		Second clutch	
	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	*F*	*p*	*F*	*p*
Restricted early (EAR)	0.154	0.695	0.057	0.812	0.117	0.733	0.002	0.965	0.001	0.978	2.686	0.106
Restricted late (LAT)	1.056	0.304	1.385	0.239	0.047	0.828	**7.035**	**0.008**	3.629	0.060	1.036	0.312
Restricted adults (AD)	0.273	0.601	0.091	0.762	0.864	0.353	2.779	0.096	0.175	0.677	0.961	0.330
EAR:LAT	1.446	0.229	1.271	0.260	2.272	0.132	**6.074**	**0.014**	0.980	0.325	1.271	0.263
EAR:AD	2.413	0.123	3.856	0.050	3.848	0.050	2.588	0.108	2.229	0.139	0.385	0.537
LAT:AD	1.149	0.238	2.313	0.128	0.171	0.679	0.541	0.462	1.986	0.163	1.559	0.216
Type of statistical model; d.f. or n	Binomial GLM n = 145		Binomial GLM n = 128		Binomial GLM n = 88		Binomial GLM n = 80		ANOVA d.f. = 6,86		ANOVA d.f. = 6,79	

Discussion

In this study, we investigated the effects of food restriction during different periods of the juvenile and early adult development on development, maternal care behavior, and reproductive performance in the European earwig *Forficula auricularia*. Our results revealed overall that food restriction experienced during the juvenile and/or early adult development influenced reproduction negatively, and could not be compensated for by unlimited food access during the period of mating and egg production. Specifically, we found that restriction during the early juvenile development decreased the survival to adulthood and hatching success of the second clutch in an interaction with restriction in the late juvenile development. Here, individuals that were never restricted (nn-) had the highest, individuals that were restricted in the early juvenile development (yy- and yn-) had intermediate, and individuals that were restricted only in their late juvenile development (ny-) had the lowest second clutch hatching success. Restriction during the late juvenile development showed several negative effects. It decreased the survival to adulthood, the weight of individuals that molted into adult females, clutch size of the first and the second clutch and the probability of second clutch production. Furthermore, it decreased hatching success of the second clutch, also in an interaction with restriction in the early juvenile development, as described already above. Interestingly, restriction in the early adulthood increased the probability of second clutch production, suggesting a benefit of food restriction in the early adulthood. However, this benefit was only present, when individuals where restricted only in their early adult development, but not in their late juvenile development, as shown by the interaction with restriction in the late juvenile development. Individuals that were restricted during both periods (-yy) had the lowest and individuals that were restricted only during their early adulthood (-ny) had the highest probability of producing a second clutch. Individuals that were never restricted in their late juvenile and their early adulthood (-nn) had medium probability of second clutch production, such as the individuals that were restricted in their late juvenile development, but not as adults (-yn).

The negative effects of restriction in the late juvenile development on various measurements taken, suggest that this is a key period in the development of *F. auricularia* and a lack of nutrition results in decreased fecundity. Individuals were in their fourth (last) instar for most of the time during the restriction in the late juvenile development and might require high amounts of energy to develop into adults. The amount of resources required for the development of the adult phenotype, including testes and ovaries, might be fixed and food restriction during this critical phase will lead to diminished fecundity. Also, since fecundity varies with female body size in most insects (Honěk 1993), this decline in fecundity might, at least partly, be the consequence of a reduced body size/weight of the restricted females.

Food restriction, especially in the late juvenile development, had stronger negative effects on the second clutch than on the first clutch, e.g., reduced probability of clutch production and reduced hatching success. However, I did not find an influence of food restriction on the trade-off between current and future reproduction, i.e. investment shifts between first and second clutch production. Second clutches were smaller than first clutches, but this effect is also visible when individuals develop under non-restricted conditions (Meunier et al. 2012). This suggests that in *F. auricularia* food restriction poses a general constraint to reproduction and does not permit females to flexibly reallocate their resources into the two clutches according to a nutrition-based shift in the trade-off between first and second clutches. The effects of food restriction might be stronger in the second clutch, as it is by definition always produced after the first clutch and thus later in an individual's lifetime. In order to produce a second clutch, females need to invest resources into maintenance and survival. Since this period of maintenance will be longer until the production of the second clutch, compared to the first clutch, the pool of resources will be much smaller at the time of second clutch production, which might lead to the stronger effects that I found.

Maternal egg clustering was not significantly influenced by food restriction during any period of the development. Individuals that were restricted during their late juvenile development produced fewer eggs in both their first and their second clutch and also had a lower probability of laying a second clutch at all. But female earwigs seem to rather reduce their clutch size according to their condition, but not the amount of care they provide when restricted in their juvenile or early adult development. My results do not reflect a trade-off between the number of eggs/offspring produced and maternal care. Other studies have demonstrated such a trade-off. One, for example, was conducted in gulls (*Larus fuscus*), where egg removal experiments revealed an intra-brood trade-off between the number of eggs produced and the capacity to rear a control clutch (Monaghan et al. 1998). Experimental females, which had laid one additional egg compared to control females, reared significantly fewer chicks, which also had lower body weight compared to control females, even though the reared clutch size was kept experimentally equal between two groups of females. However, females of *F. auricularia* provide many additional forms of care, which I did not measure. My previous work has shown that food restriction during the period of care reduces the rate of maternal food provisioning. Thus, females that were restricted in their juvenile development might also perform more poorly in food provisioning. Also, egg clustering might not be a very costly form of care for the female and any trade-off might be difficult to detect. Further experiments need to be conducted to better investigate the potential trade-off between clutch size and investment into maternal care behaviors.

Information about the environment experienced by the parents in their own juvenile development might be passed on to the offspring through a trans-generational effect. A study in cichlids (*Simochromis pleurospilus*), for example, showed that young of mothers, who were raised under low food availability themselves, were already larger at the end of brood care compared to offspring from mothers raised under high food availability (Taborsky 2006). This difference was not due to extended brood care, but females raised with little food laid eggs with a higher dry mass, resulting in larger larvae, which additionally showed a higher growth rate. These results suggest that mothers prepare their offspring for similar environmental conditions to those they encountered as juveniles. I did not find a difference in the body weight of hatched nymphs on day 1 between females that were restricted or unrestricted during their development. Possibly, environmental conditions and food availability might be highly unpredictable in the earwigs resulting in a high risk of wrong imprinting. However, to really exclude environmental-dependent maternal effects in *F. auricularia,* additional measurements regarding offspring development of the first and the second clutch need to be taken.

In my study, I could show that food restriction during the juvenile development has negative effects on the development and survival to adulthood as well as on female fecundity. Especially restriction in the late juvenile development lead to smaller clutch size and reduction in the probability of second clutch production, and hatching success. Late juvenile development seems to be a key period in determining adult fecundity. But interaction effects with the early juvenile and adult development weakened or enhanced the effects of restriction during the late juvenile development. Thus, the effect of environmental conditions experienced during the whole juvenile and early adult development needs to be considered when investigating fecundity and life history traits in future experiments. Food restriction during the juvenile development can hence explain the co-existence of semelparity and iteroparity, the two reproductive strategies we can find in *F. auricularia.*

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CONCLUDING REMARKS

Summary of results

The present work demonstrates how the environment and social interactions influence the condition and the behavior of individuals of the European earwig *Forficula auricularia.* I first investigated the roles of the ecology, life history and the social environment on the evolution of parental care in insects in a literature review (Chapter 1). I found that the strongest support for environmental drivers of parental care is given by natural enemies and that the social environment creates novel selection pressures from interactions within and between families. Thus, only the combined consideration of the abiotic, the biotic, and the social environment as well as the respective life history of a species will help us understand the evolution and maintenance of parental care.

In my first experiment, I showed that maternal nutritional condition influences the dynamics of food provisioning, where mothers in poor condition provided food to fewer nymphs for a shorter period of time than mothers in good condition. However, female condition had no significant effect on the rate of offspring self-foraging. Mother-offspring aggregation remained at a high level across treatments throughout the whole experiment, suggesting benefits of living in a (family) group (Chapter 2). My results jointly show that in species with facultative care like *F. auricularia*, the duration of and behavioral control over different aspects of parental care may not be identical and differentially affected by the condition of the caring parents.

I then demonstrated that brood mixing in *F. auricularia* is dependent on nymph condition and largely driven by foreign offspring in good condition. Females seem to tolerate these foreign offspring passively, despite the threat of cannibalism they might pose. Again, I found a high rate of female-nymph aggregation, suggesting benefits of group-living (Chapter 3). Female earwigs attend mixed broods and families are characterized by dynamic aggregations not only within but also between broods. My results show how the social environment in combination with the benefits and costs of parental care offer potential for brood parasitic strategies, which can drive the evolution of kin recognition.

In my third experiment, I identified kin recognition cues in nymphs and adults of *F. auricularia.* Information about the maternal origin, the mate number (adults only), and the social environment (adults only) was present in the form of cuticular hydrocarbons (CHC) (Chapter 4). Hence, CHC profiles of *F. auricularia* display information about the genetic origin and (social) environment, and they can be used by conspecifics for example to direct social behaviors towards family members. Such kin recognition cues can also be used in a mating context in order to avoid costs of inbreeding.

Subsequently, I showed that information about nutritional condition is also present in the CHC-profile of female earwigs and that nymph survival was significantly affected by cues of maternal condition, whereas this effect depended on the timing of breeding (Chapter 5). Thus, earwig offspring are sensitive to chemical cues of maternal condition and nymphs from early and late broods have opposite reactions to the same chemical cues. Together with former evidence on maternal sensitivities to condition-dependent nymph chemical cues, our study shows context-dependent reciprocal information exchange about condition between earwig mothers and their offspring, mediated by cuticular hydrocarbons. Not only information about the maternal origin and the social environment are reflected in the CHC-profiles of *F. auricularia,* but also the nutritional condition. The environment, including the social environment, influences the individual CHC profile and these chemical cues, in turn, are used by the social environment, for example, the parents, the offspring, or potential mating partners, to adjust their own behavioral strategies.

I finally demonstrated that food restriction during the juvenile development affects development, survival and reproduction negatively in female earwigs (Chapter 6). Consequently, not only the current environmental conditions experienced, but also the ones experienced early in an individual's lifetime influence its current condition. Understanding the occurrence of distinct behavioral strategies, which lead to differences in fitness between individuals, requires us to consider the conditions experienced throughout the whole ontogeny of an individual.

Further directions of research

The experiment investigating the effects of food restriction on different stages during the juvenile and early adult development on survival, maternal care, and reproductive success on conducted in Chapter 6 was done with females and males. I only presented data from restricted females in Chapter 6, but I collected the same measurements also for males. Analyzing the male data (which I will do after finishing my PhD) will allow the direct comparison of the effects of food restriction between the sexes. Ontogenetic effects due to food restriction during distinct phases of the juvenile development might have potential sex specific and trans-generational consequences. In Chapter 6, I already found significant effects of food restriction on body weight of adult females. Furthermore, additional samples from males of all the different treatment groups will allow me to measure and compare the effect of food restriction on male forceps size. Male forceps are suggested to be a sexually selected trait where females prefer to mate with large forceps males (Tomkins & Simmons 1998). Previous work suggested that male forceps size is a phenotypically plastic trait and depends on population density (Tomkins & Brown 2004) as well as the amount of protein available in the diet during the juvenile development (Tomkins 1999), but further support remains ambiguous. Analyzing the effect of food restriction on male forceps size will provide helpful insight on the potential phenotypic plasticity of this sexually selected trait.

While conducting my research and analyzing the results from my experiments, new ideas and questions arose. If I had the time for further experiments, I would, for example, investigate in a follow up study of my brood mixing experiment (Chapter 3), the level of maternal food provisioning received by residents and foreigners. My work has shown that the CHC-profile of nymphs allows predicting the maternal origin of individuals. Thus, cues of maternal origin exist, which could be used by the mother to discriminate her own offspring from unrelated, invading nymphs. The high level of brood mixing I found in my experiment suggests benefits of living in a (larger) group whereas the mother seems to not discriminate. When investigating maternal food provisioning, I could test whether the female does discriminate on a finer scale by provisioning preferentially her own offspring, when residents are foreigners are in the same condition. Since Mas et al (2009) showed that mothers exposed to chemical cues from well-fed nymphs foraged significantly more and allocated food to more nymphs compared with females exposed to chemical cues from poorly fed nymphs, I would measure maternal food provisioning also when residents and foreigners have opposing conditions, like in the brood mixing experiment I have already conducted. This would allow me to test, if females provision preferentially their own offspring or offspring in good condition, which might also be the foreigners. If females feed preferentially foreigners in good condition and not the own offspring in poor condition, females would behave maladaptive, which would open further room for investigations. Also, field experiments should be conducted to investigate dispersal, aggregation behavior, and brood mixing dynamics under natural conditions. All the environmental influences, which were missing in my laboratory experiments, like predators, pathogens, and food competitors could help to identify the benefits of brood mixing and mother-nymph aggregation and advance our understanding of the dynamics within and between families.

In Chapter 4, I showed a significant effect of mate number on the CHC-profiles of adult individuals of *F. auricularia*, but the effect was not present in the nymphs. To test whether the absence of the paternal influence on the CHC-profiles of nymphs is a maternal effect to homogenize the profiles between nymphs, I would first separate the eggs from singly mated and multiply mated mothers directly after oviposition and compare the CHC-profiles of the hatched nymphs. If there will be no difference between the profiles, I would test, if the mother transfers some CHC onto the nymphs already before oviposition. This would require using radiotracer approaches, to track the location and metabolic fate of ^{14}C- and ^{3}H-labeled hydrocarbons through vitellogenic females and their embryos and nymphs (see (Fan et al. 2008).

I showed in Chapter 5 that cues of maternal condition influence offspring selfishness dependent on the time of breeding. To confirm that these condition-dependent cues emitted by the female are CHC, one could expose the nymphs only to CHC extracts from females in either good or poor nutritional condition. Confirming that maternal CHC influence offspring selfishness would show a novel role of CHC in parent-offspring communication.

Final conclusion

The results of my research show that the environment influences the condition and the behavioral strategies that individuals will use according to their own condition. Maternal food provisioning, hatching date dependent offspring selfishness, and female survival and reproductive success were all dependent on the nutritional condition of the mother or the condition she experienced in her juvenile development. Thus, my work shows how individual condition affects not only parent-offspring interactions and family dynamics, but also major life history traits like reproductive success and the respective constraints and trade-offs associated with this trait. The importance of the social environment was demonstrated trough brood mixing dynamics and the transfer of CHC between adults of *F. auricularia.* Social interactions are not limited to within family interactions, but can occur, as my work showed, also between families and even between all the individuals of a population. Social interactions can result in cooperation or conflict, i.e., they can be beneficial or costly for both sides or only for one or the other side. Since the benefits and costs of social interactions can directly influence condition, e.g., through cooperative behaviors or parasitic strategies (Mappes et al. 1995; Moczek & Cochrane 2006), studying social interactions is important because fitness is not only the result of individual condition, but can be severely affected by interactions with conspecifics. Furthermore, my work demonstrated how condition and the social environment influence the CHC-profile of individuals and how these chemical cues can be used to communicate relatedness and nutritional condition to interacting conspecifics.. The role of chemical cues is studied comparatively little in parent-offspring interactions, although they constitute the most important means of communication in insects. Further investigations of how chemical cues affect social interactions will allow us to better comprehend not only variation in parental and offspring strategies, but also communication in general. Parental care is considered an important step in the evolution of sociality and conducting research with a facultative care species like *F. auricularia* promotes our understanding of other social systems.

To conclude, my thesis represents a major advancement demonstrating the significant influence of the environment and chemical communication on maternal care and within and between family interactions. My results offer many directions for further research on the evolution of parental care, social interactions, chemical cues and communication, as well as life history traits and trade-offs. A better understanding of all these fields is an important step in understanding the evolution of sociality and understanding the existence of variation in fitness driven by distinct behavioral strategies.

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APPENDIX

Further work to which I contributed during my PhD

APPENDIX I

One clutch or two clutches? Fitness correlates of coexisting alternative female life-histories in the European earwig

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Abstract

Whether to reproduce once or multiple times (semelparity versus iteroparity) is a major life-history decision that organisms have to take. Mode of parity is usually considered a species characteristic. However, recent models suggested that population properties or condition-dependent fitness payoffs could help to maintain both life-history tactics within populations. In arthropods, semelparity was also hypothesised to be a critical pre-adaptation for the evolution of maternal care, semelparous females being predicted to provide more care due to the absence of costs on future reproduction. The aim of this study was to characterize potential fitness payoffs and levels of maternal care in semel- and iteroparous females of the European earwig *Forficula auricularia*. Based on 15 traits measured in 494 females and their nymphs, our results revealed that iteroparous females laid their first clutch earlier, had more eggs in their first clutch, gained more weight during the two weeks following hatching of the first clutch, but produced eggs that developed more slowly than semelparous females. Among iteroparous females, the sizes of first and second clutches were significantly and positively correlated, indicating no investment trade-off between reproductive events. Iteroparous females also provided more food than semelparous ones, a result contrasting with predictions that iteroparity is incompatible with the evolution of maternal care. Finally, a controlled breeding experiment reported full mating compatibility among offspring from females of the two modes of parity, confirming that both types of females belong to one single species. Overall, these results indicate that alternative modes of parity represent coexisting life-history tactics that are likely to be condition-dependent and associated with offspring development and specific levels of maternal care in earwigs.

Introduction

Whether the resources available for reproduction are invested in a single reproductive attempt (i.e. semelparity), or partitioned among multiple attempts (i.e. iteroparity), is a critical question in life-history theory (Charlesworth 1994; Charnov and Schaffer 1973; Cole 1954; Ranta et al. 2002; Stearns 1992). Mode of parity is usually considered a species characteristic (Stearns 1992). In line with this view, the early deterministic models for the evolution of modes of parity predicted that either semelparous or iteroparous life-histories would be evolutionarily stable and spread to fixation (Charlesworth 1994; Charnov and Schaffer 1973; Cole 1954; Stearns 1976; Stearns 1992). The models predicted that reproductive lifespan of individuals (e.g. age at first reproduction, adult versus juvenile viability), adult fecundity, extrinsic rates of juvenile and adult mortality (Charlesworth 1994; Stearns 1992) or stochastic risk associated with a single reproductive event (bet-hedging effect, Stearns 1976) influence whether a semel- or iteroparous life-history should evolve in a population. Recently, new theoretical models identified ultimate reasons to explain why semelparous and iteroparous life-history tactics may coexist within populations. In particular, the maintenance of alternative modes of parity may be facilitated in species with structured populations (Ranta et al. 2000a), unstable population dynamics (Ranta et al. 2000b), stochasticity in offspring and adult viability (Ranta et al. 2002) or differential effects of population density on offspring versus parental mortalities (Bulmer 1985). Furthermore, when considered as alternative allocation phenotypes (defined as discontinuous inter-individual variation in a trait of interest, Brockmann and Taborsky 2008; Taborsky et al. 2008), semelparous and iteroparous life-histories are also expected to coexist whenever the fitness payoffs of expressing the two alternatives are condition-dependent (Taborsky et al. 2008; Tomkins and Brown 2004).

To date, most empirical work testing factors associated with the evolution of modes of parity (e.g. life-history traits, ecology and fitness payoffs) used comparisons across taxa, related species or populations within species (references listed in Crespi and Teo 2002; Moerkens et al. 2010). Only little empirical research was carried out on semelparous and iteroparous life-history tactics that coexist in a population (Grosberg 1988; Iguchi and Tsukamoto 2001; Leggett and Carscadden 1978; Seamons and Quinn 2010). However, populations with coexisting alternative life-histories are of particular interest because they offer a unique opportunity to understand mode of parity as alternative life-history tactics and to study the ultimate and proximate factors maintaining coexistence. More generally it is possible to investigate in such populations the evolution of parity independently from population idiosyncrasy and its coevolution with other specific life-history or behavioural traits with important fitness consequences, such as parental care (Gilbert and Manica 2010; Stegmann and Linsenmair 2002).

Maternal care is known to have evolved repeatedly in insects and spiders, where it generally provides important benefits to eggs and juveniles (Choe and Crespi 1997). Both across and within species, tending females can express variable duration and complexity of care, ranging from nest building to egg grooming, protection against predators or food provisioning (Choe and Crespi 1997; Mas and Kölliker 2008; Tallamy and Wood 1986). Maternal care may evolve when the associated fitness benefits exceed costs for tending mothers, such as a reduced future reproduction or an increased mortality rate (Clutton-Brock 1991). For arthropods, it has been hypothesized that semelparity is a critical life-history pre-adaptation for maternal care to evolve (Tallamy and Brown 1999). Because semelparous females pay no cost of care in terms of future reproduction, already small benefits of care to offspring are sufficient for the evolution of maternal care. Based on this hypothesis, semelparous females were predicted to provide higher levels of care to current offspring than iteroparous ones (Gilbert and Manica 2010; Tallamy and Brown 1999).

Females of the European earwig *Forficula auricularia* are known to provide maternal care to eggs and nymphs, and to show a life-history dimorphism in terms of the number of clutches they produce over their lifetime (reviewed in Costa 2006). Maternal care is provided in the form of tending the clutch of eggs and nymphs, supplying food to the nymphs and protecting them against predation (Costa 2006). Females from this species produce either a single clutch (in the following referred to as 1C) or two clutches (referred to as 2C) where the second clutch is produced without remating. 1C and 2C strategies were originally thought to characterize different *F. auricularia* populations (Vancassel 1984) and later as characteristic of potential cryptic sister species (Guillet et al. 2000; Wirth et al. 1998). However, recent work repeatedly showed that 1C and 2C females co-occur within populations (in Switzerland, Kölliker 2007; in Italy, Mas and Kölliker 2011). This indicates that both strategies can be maintained within natural populations and calls for further studies both on fitness-related traits associated with mode of parity and the question if 1C and 2C life-histories represent one single or two cryptic sister species.

The current study aimed at investigating associations between alternative life history tactics (semelparity and iteroparity) and other life-history, morphological and behavioural traits in the European earwig *F. auricularia*. To this end, we recorded reproductive, morphological and behavioural characteristics of 600 females (and their offspring) sampled in the field and reared under standardized laboratory conditions. If the 1C-2C polymorphism reflects coexisting alternative life-histories with equivalent fitness consequences (Charlesworth 1994; Charnov and Schaffer 1973; Cole 1954), we predicted 1C females to produce a larger clutch than the first clutch of 2C females, as well as a similar life-time number of offspring, and/or offspring of better quality e.g. by providing more care (measured in terms of nest building, egg guarding, clutch attendance and food provisioning) than 2C females. Conversely, the polymorphism could represent condition-dependent alternative life-history tactics where poor female condition (e.g. nutritional, physiological, genetic) could limit the production of a second clutch. In this case, we predicted 2C tactic to reflect better intrinsic conditions and, hence, 2C females to be able to produce a larger lifetime number of offspring and display higher levels of maternal care. Finally, to confirm that 1C and 2C strategies do not characterize sympatric sister species (Wirth et al. 1998) in the studied population, we tested reproductive isolation between offspring from 1C and 2C females using cross-breeding of progeny (males and females) from 1C and 2C females.

Material & Methods

Field sampling and laboratory rearing

A total of 600 females and 600 males of the European earwig *Forficula auricularia* were sampled in May 2009 in Dolcedo, Italy (7°56'55'E, 43°54'14'N, altitude 443 m). All individuals were setup in the laboratory under standard conditions for random mating for 5 months in 20 plastic containers (37 x 22 x 25 cm), consisting of 30 males and 30 females each. In this species, males and females have multiple mating partners (promiscuous mating system) and clutches are generally sired by multiple fathers (Guillet 2000). All containers were lined with fluon to prevent the escape of the insects, contained humid sand as a substrate, and egg carton and plastic tubes as shelters. The food consisted of an artificial diet (composition: 10g Agar, 60g carrots, 30g flower pollen, 30g bird food (Schweizer Bekfin, Eric Schweizer SAMEN AG, Thun, Switzerland), 30g cat food (Perfect fit 8+ senior, Wathan, Switzerland) and 900 ml water) and was changed three times a week. The containers were kept in a climatic chamber at 60% humidity, 10 : 14h light:dark cycle and 20 : 15°C during light and dark periods, respectively (= autumn condition).

After observation of the first oviposition on the 19th of October 2009, all 600 females were isolated in individual Petri-dishes. Each female was subsequently placed in complete darkness at 10°C for one week to further stimulate oviposition, and then maintained in complete darkness at 15°C and 60% humidity (= winter condition) until egg-laying and hatching. No food was provided from egg-laying to hatching (Kölliker 2007). One day after hatching, females and nymphs were set up in new Petri-dishes. From hatching (= day 0) to day 14, clutches were kept at 60% humidity, 14 : 10h light : dark photoperiod and a constant temperature of 20°C (= summer condition).

Fourteen days after hatching of the first clutch, females were separated from their nymphs to initiate the production of second clutches. Fourteen days were chosen because it has been reported that female care for nymphs may end early in their second instars, which occurs approximately 12 days after hatching (Lamb 1975). On this day, 20 nymphs per clutch were setup in new Petri-dishes (14 x 2 cm) and reared under summer conditions until they were used in mating experiments (see below). Mothers were set up in new Petri-dishes for oviposition of second clutches. To compare properties of first and second clutches while controlling for environmental effects, Petri-dishes for second clutch production were held under winter conditions for egg-laying and hatching, and then transferred to summer conditions from hatching to day 14. Except when specified, all Petri-dishes were of 10 x 2 cm dimension and contained humid sand, aluminium shelters and *ad libitum* artificial diet.

Measures of life-history traits, morphology and development

A total of 15 measurements on clutches and mothers were taken during laboratory rearing. Clutch size (eggs and nymphs), hatching success and nymph survival were obtained by counting the number of eggs per clutch one day after egg-laying and the number of nymphs per clutch one and 14 days after hatching. Fresh weights of mothers and nymphs on day one and 14 were measured to the nearest 0.001 mg using a Mettler-Toledo MT5 Micro-balance (Mettler, Roche, Basel). Each mother and a random subsample of usually 10 nymphs were weighed. If the number of nymphs in a clutch was smaller than 10, all the available nymphs were weighed. Embryonic developmental time was defined as the number of days between oviposition and hatching, and the developmental rate of nymphs as the number of days from hatching to the first observation of a second instars nymph in a clutch. Finally, structural body size of females was estimated by measuring their pronotum area, which is strongly correlated to the measure of pronotum width ($r^2 = 0.87$) used in other studies (Tomkins and Brown 2004; Tomkins and Simmons 1998). To obtain a measure of pronotum area, we measured pronotum width and length under a binocular scope (Leica, MZ 12.5) to the nearest 0.001 mm using ImageJ© software v1.43n (http://rsb.info.nih.gov/ij/), and calculated the area by multiplying the largest width by the largest length. All characteristics of eggs and nymphs from first and second clutches were measured in a standardised way.

Measures of maternal care

Four distinct maternal care behaviours were scored for first clutches. (i) Nest building: at egg-laying, the complexity of the nest built by each female was ranked from 1-4. 1: females laid their eggs without building a nest, 2: they laid the eggs into a small cavity in the sand, 3: females burrowed a small nest to 4: females built a complex nest (i.e., wider crater surrounded by wall-like extensions). (ii) Egg guarding: during the daily checks we scored if the female was within a 2.5 cm radius (= diameter of the experimental nests) of her eggs. Egg-guarding was defined as the proportion of days a female was observed within that radius over the total number of days between egg-laying and hatching. (iii) Family aggregation: after hatching we counted the proportion of nymphs within a 2.5 cm radius of the female's head on days 2, 5, 8, 11 and 14 after hatching. Family aggregation was defined as the average of these five values. Notice that high values of family aggregation were unlikely to be obtained by chance: nymphs form tightly aggregated groups typically located near the mother's head, and not near an arbitrary female body part (J.M., MK, personal observation) as would be expected in case of spatial proximity that occurs by chance. The measures involved in egg guarding and family aggregation were recorded once a day, between 9:00 and 11:00 am, while the individuals were inactive (i.e., during light conditions). (iv) Food provisioning: this scoring took place between days 6 and 7 after hatching, a period where mouth-to-mouth contacts between mothers and nymphs are the most frequent (Liu 1991). At day 5, the usual food was removed from each Petri to standardize nutritional condition of nymphs and females, and to increase female foraging behaviour on the subsequent day. On day 6, mothers were isolated for one hour in small Petri-dishes (5.5 x 1.2 cm) with humid sand as substrate and a pollen pellet dyed with blue food-dye (synthetic E-131, patent blue V, Werner Schweizer AG, Switzerland, see Staerkle and Kölliker 2008) as food source. After one hour, mothers were replaced in their original Petri-dish with a standardized group of 20 nymphs (sometimes slightly fewer due to small clutch size: on average 19.65 ± 2.81 (mean ± SD) nymphs). Groups of nymphs were standardized to avoid confounding effects of clutch size on the provisioning score, and food provisioning was not estimated in clutches containing fewer than 10 nymphs. The remainder of nymphs was temporarily set up in another small Petri-dish with humid sand. Fifteen hours later (overnight), the level of food provisioning was scored as the proportion of nymphs with blue/green gut among all nymphs in the experimental group (see also Mas et al. 2009; Staerkle and Kölliker 2008). After the provisioning experiment, the nymphs previously separated were returned to their original Petri-dish, together with their respective mothers and the remaining nymphs. The labour intensive scoring of food provisioning could only be carried out in a subset of 328 clutches that were chosen haphazardly from the base populations.

Clutches that were not chosen to be involved in the measurement of food provisioning were treated identically (i.e., food was also removed), except for the food provisioning. The developmental rate of nymphs was significantly different between clutches involved in the food provisioning experiments (mean ± SE = 23.76 ± 0.07 days) and clutches that were not involved (23.27 ± 0.06 days; Welch *t*-test, $t = -5.11$, $df = 419.147$, $P < 0.0001$). By contrast, none of the other measured traits were significantly associated with the inclusion of clutches in the provisioning experiment (all $P > 0.08$). Note that our results did not qualitatively change when excluding/including clutches involved in food provisioning measurements.

Finally, each female was retrospectively categorised as presenting 1C or 2C life-history tactic, depending on whether they had produced a single clutch or two clutches in the period between day 14 and day 60. We selected 60 days as temporal delimiter for egg laying because previous work indicated that females were very unlikely to produce a second clutch more than 60 days after egg hatching (Mas and Kölliker 2011). Females laying a second clutch that did not hatch (N = 32) were discarded from the analyses as they could not be assigned unambiguously to the 1C or 2C tactic. Results did not significantly change when these females were included, irrespectively of their assignment to the two categories.

Cross-breeding among offspring from 1C and 2C females

To confirm that individuals with 1C and 2C life-histories represent one single species, we tested for mating compatibility between offspring of the 1C and 2C females from the previous experiment. A total of 56 controlled single-matings were conducted between all possible combinations of sons and daughters of 1C and 2C females. For these matings, adult sons and daughters were obtained from 51 and 60 randomly chosen first clutches of 1C and 2C females, respectively (see above). To ensure virgin status of individuals, males and females of each clutch were separated in two new Petri-dishes of equal size within a maximum of 3-4 days after emergence (during which no fertilizations occur; MK unpubl. results). Two weeks after completed emergence the 56 mating pairs were setup in new Petri-dishes where they were allowed to mate during three months. Subsequently, the females were isolated and the standard breeding procedure was used for earwig breeding (see above). In addition, eggs were cross-fostered within and between females with different parity background in order to isolate potential mating incompatibility from other potential incompatibilities such as egg care. Clutch size, hatching success and the body mass of hatched nymphs were recorded as before (see above). If the 1C/2C polymorphism in *F. auricularia* populations represents coexisting alternative life-history tactics, we expected similar clutch sizes, number of nymphs and body weight of hatched nymphs) for breeding-crosses among parity background as compared to within background (i.e., lack of interaction between 1C and 2C backgrounds).

Statistical analysis

Correlates between female reproductive type (1C or 2C) and parameters of first clutches were tested on 494 out of the 600 females (82.3 %), of which 305 were involved in the scoring of food provisioning. A total of 106 females were excluded because they (i) did not produced a first clutch (N = 6 females; 1.0 % of the total number of females), (ii) escaped from their Petri-dishes or were mixed by mistake with other clutches during the experiment (N = 18; 3.0 %), (iii) died during their first clutch (N = 8; 1.3 %), (iv) had at least one morphological, physiological or behavioural measure that was accidentally unrecorded during the experiment (N = 42; 7.0 %), and finally (v) could not be attributed to either 1C or 2C reproductive strategies as they produced a second clutch that did not hatch (N = 32; 5.3 %). These latter 32 clutches were either eaten by their mother few days after egg laying, suffered from early infection (eggs did not develop and became reddish in coloration) or from late infection (eggs developed but became brownish in coloration).

We tested for differences between 1C and 2C females in their reproductive and developmental parameters using two Multivariate Analyses of Variance (MANOVA) where the measured traits (either from the clutches or the mothers, see below) were entered as dependent variables and the reproductive type of females was entered as a fixed factor. The first MANOVA was conducted on "clutch traits" using the entire data set (N = 494 females). This analysis included egg number, hatching success, time of embryonic development, survival rate of nymphs from day 1 to day 14, mean weight of nymphs at day 1, relative weight gained by nymphs between day 1 and day 14, and developmental rate of nymphs as dependent variables (Table 1). The second MANOVA was conducted on maternal traits using the subsample of females involved in food provisioning measurements (N = 305 females) since MANOVA omit objects with missing values (Quinn and Keough 2002). This analysis included laying date of the first clutch, maternal weight at hatching, proportion of weight gained by mothers between day 1 and day 14, pronotum area of mothers and the four measurements of maternal care – nest building, egg guarding, family aggregation and food provisioning - as dependent variables (Table 1). Interpreting which morphological, physiological or behavioural trait contributed significantly to each MANOVA was then tested using independent *t*-tests. To correct for inflated type-I errors due to multiple testing, the significance level $\alpha = 0.05$ was adjusted to $\alpha = 0.027$ using the False Discovery Rate correction (FDR, Benjamini and Hochberg 1995).

To explore potential trade-offs in female investment between first and second clutches, each of the seven traits measured in both clutches of the 2C females was compared using paired *t*-tests and Spearman's correlation tests, with $\alpha = 0.027$ (FDR correction). Trade-offs were tested partly on the whole set of 409 females with second clutches (for trade-offs involving egg-number, hatching success, embryonic developmental time) and partly in a randomly chosen sub-sample of 59 females with second clutches for trade-offs involving long-term rearing of family groups (i.e. survival rate of nymphs, nymph weight at day 1, their weight gain from day 1 to 14 and their developmental rate). None of the measures significantly differed between this subsample and the rest of the population (all $P > 0.46$).

Finally, reproductive isolation between offspring from 1C and 2C females in the cross-breeding experiment was tested using three independent analyses of variances (ANOVA), where the number of eggs produced in the first clutch, the subsequent number of nymphs and their weight at hatching were used as response variables, while parity background of sires and dams (their mother being 1C or 2C) and their interaction were entered as fixed factors. To fulfil normal distribution of residuals in all our statistical analyses, hatching success, nymph survival, weight gained by mothers, egg guarding, food provisioning and family aggregations were arcsine square-root transformed, nymph, weight at day1 was square-transformed and the date of egg laying was log-transformed. Values for parameter estimates were back transformed in tables and figures. Statistical analyses were done using the software R v2.11.1 (http://www.r-project.org/).

Table 1. Measures of first clutches of females adopting one-clutch (1C females) or double-clutches strategy (2C females) strategies. *P*-values in bold remained significant after False Discovery Rate corrections (reported *P*-values are uncorrected).

	1C females				2C females						
	Mean	±	SE	(N)	mean	±	SE	(N)	*t-values*	*df*	*P*
Clutch traits											
Egg number	46.92	±	1.31	(74)	52.47	±	0.51	(420)	-4.2	492	**<0.0001**
Embryonic developmental time (day)	23.16	±	0.14	(74)	23.51	±	0.05	(420)	-2.57	492	**0.011**
Hatching success (%)	82.32	±	2.38	(74)	83.83	±	0.91	(420)	-0.64	492	0.525
Nymphs survival from day 1 to day 14 (%)	88.53	±	1.28	(74)	88.40	±	0.48	(420)	0.61	492	0.540
Nymph's weight at day 1 (mg)	1.39	±	0.03	(74)	1.40	±	0.01	(420)	-0.72	492	0.473
Weight gained by nymphs until d14 (%)	122.41	±	4.27	(74)	122.13	±	1.45	(420)	0.07	492	0.944
Developmental speed of nymphs (day)	12.42	±	0.20	(74)	12.18	±	0.08	(420)	1.15	492	0.253
Mother traits											
Date of egg laying (day) *	36.12	±	1.65	(49)	28.98	±	0.47	(256)	4.89	303	**<0.0001**
Weight at day 1 (mg)	51.57	±	0.74	(49)	51.73	±	0.37	(256)	-0.18	303	0.857
Weight gained between day 1 and day 14 (%)	16.75	±	1.45	(49)	27.86	±	0.74	(256)	-6.35	303	**<0.0001**
Pronotum area (mm^2)	3.60	±	0.04	(49)	3.56	±	0.02	(256)	0.79	303	0.428
Nest building	2.71	±	0.15	(49)	2.50	±	0.06	(256)	1.33	303	0.184
Egg guarding (%)	92.64	±	1.52	(49)	90.20	±	0.63	(256)	2.34	303	**0.020**
Food provisioning (%)	26.43	±	4.62	(49)	48.85	±	2.01	(256)	-4.53	303	**<0.0001**
Family aggregation (%)	65.48	±	2.79	(49)	71.74	±	1.16	(256)	-2.04	303	0.042

* The date of egg laying was reported as the difference between the day of egg laying of each female and the first day where egg laying has been observed in the population (i.e. the 19th of October 2010).

Results

Overall, 74 out of the 494 females (15.0 %) were attributed to 1C females and 420 (85.0 %) to 2C females (Fig. 1). In the subsample of females involved in food provisioning measurements, 49 out of 305 (16.1%) were 1C females and 256 (83.9%) were 2C females (difference: $\chi^2 = 0.17$, $df = 1$, $P = 0.68$). The bimodal distribution of the size of second clutches relative to lifetime number of produced eggs (Fig. 1) illustrates that 1C and 2C females reflect clearly distinct modes in the distribution. 1C females constituted a distinct mode in the distribution (mode = 0) without variance, while 2C females a second mode with some continuous variation around it (mode = 0.39).

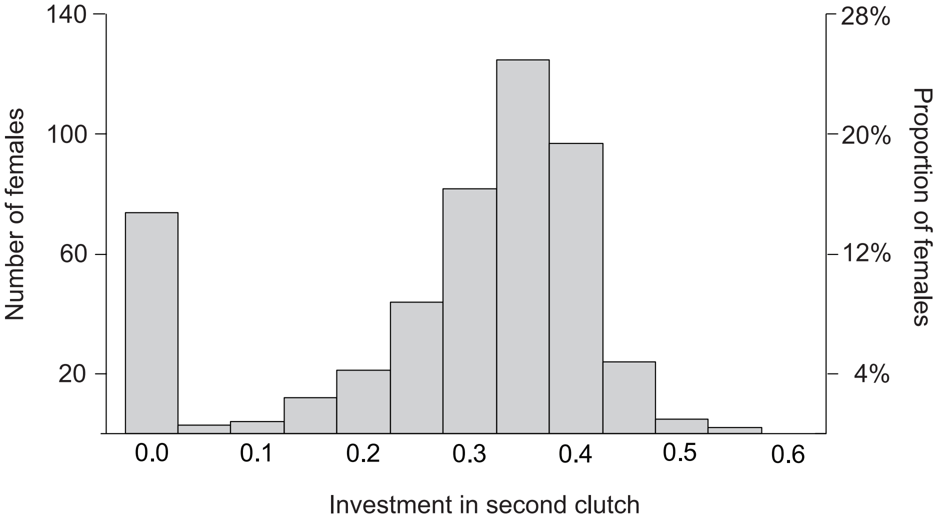

Figure 1. Distribution of the size of second clutches relative to lifetime number of eggs produced. "0" implies no eggs in second clutches and, hence, refers to the 1C females.

Parity mode of females (1C or 2C) was significantly associated with both clutch traits and maternal traits (Table 1; MANOVA on clutch traits: Wilk's lambda = 0.94, $F_{7,486} = 4.09$, $P = 0.0002$; MANOVA on maternal traits: Wilk's lambda = 0.79, $F_{8,296} = 9.74$, $P < 0.0001$). In particular, the first clutches of 2C females contained significantly more eggs and these eggs had a significantly slower development time than the single clutch of 1C females (Table 1). Concerning maternal traits, 2C females gained significantly more weight, laid eggs significantly earlier, provided more food to their nymphs but also spend less time in egg guarding than 1C females (Table 1). In addition, the lifetime number of eggs produced by 2C females was significantly larger (by a factor of almost two) than the one produced by 1C females (2C females: (mean ± SE) 83.10 ± 0.86 eggs; 1C females: 46.92 ± 1.31 eggs; Welch *t*-test, $t = -23.07$, $df = 144.5$, $P < 0.0001$).

Comparisons between first and second clutches of 2C females revealed major differences between the characteristics of first and second clutches. All taken measurements differed significantly (Table 2). Second clutches were significantly smaller (in terms of egg number), had lower survival rates (in terms of hatching success and nymph survival), but had faster development (embryonic developmental time, developmental rate of nymphs, heavier weight at hatching and larger weight gain from day 1 to 14, Table 2). Egg number, hatching success and nymph weight at day 1 were significantly and positively correlated between first and second clutches (Table 2). The other variables were not significantly correlated between first and second clutches (Table 2).

Table 2. Differences between first and second clutches of 2C females. Negative means (Mean diff.) indicate larger values in first than second clutch. *P*-values in bold remained significant after False Discovery Rate corrections (reported *P*-values are uncorrected).

	Pairwise difference						**Correlations**	
	Mean diff.	±	SE	*t*-value	*df*	*P*	r_s	*P*
Egg number	-21.89	±	0.53	-41.22	408	**<0.0001**	0.43	**<0.0001**
Embryonic developmental time (day)	-6.29	±	0.08	-82.47	408	**<0.0001**	0.06	0.212
Hatching success (%)	-9.94	±	1.27	-7.82	408	**<0.0001**	0.29	**<0.0001**
Nymph weight at day 1 (mg)	0.17	±	0.03	6.42	58	**<0.0001**	0.35	**0.007**
Nymph survival from day 1 to day 14 (%)	-14.41	±	2.78	-5.13	58	**<0.0001**	0.09	0.490
Weight gained by nymphs until d14 (%)	43.55	±	6.11	7.12	58	**<0.0001**	0.02	0.866
Developmental rate of nymphs (day)	-0.90	±	0.24	-3.80	58	**0.0004**	0.19	0.149

Our cross-breeding experiment confirmed the hypothesis that the observed dimorphism represented coexisting alternative life-history tactics within a single species. In the first clutches, neither the parity background of sires and dams (Fig.2; two-way ANOVAs, all $F_{1,52}< 1.7$, all $P > 0.19$), nor their interaction significantly influenced the number of eggs laid, the number of nymphs hatched or the body mass of nymphs at hatching (interaction in two way ANOVAs; number of eggs: $F_{1,52}=0.03$, $P = 0.87$; number of nymphs: $F_{1,52}= 1.12$, $P = 0.29$; weight of nymphs: $F_{1,52}=0.06$, $P = 0.81$).

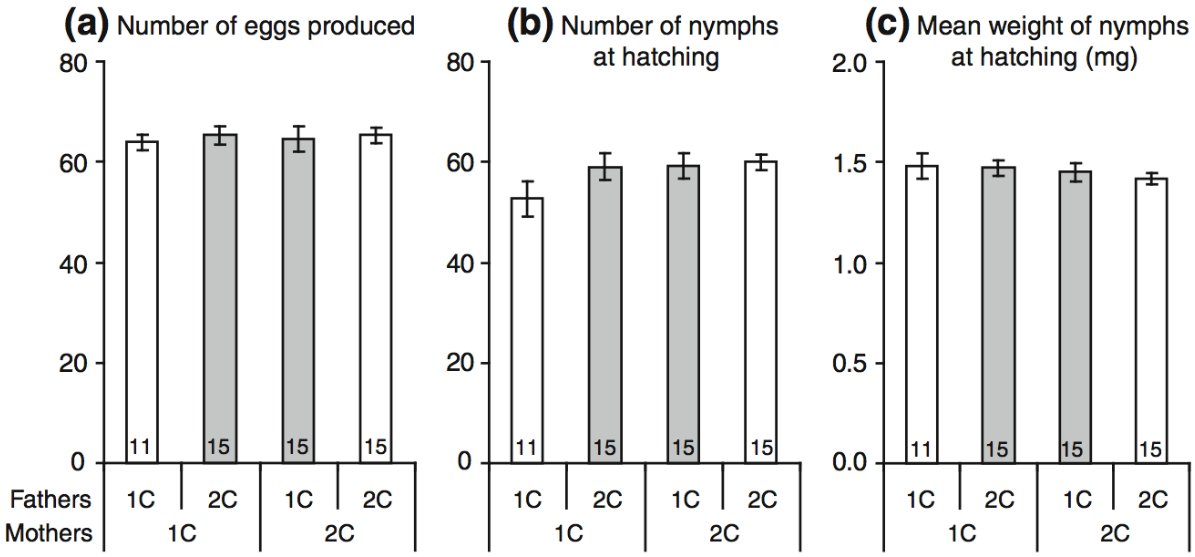

Figure 2. Number of eggs (a), number of nymphs (b) and mean weight of nymphs (c) produced in experimental mating between adults from 1C and 2C clutches. Adults from clutches with similar (white) or alternative (grey) parity background were mated. Values are for the first clutch only. The number of mated females is at the bottom of each bar.

Discussion

The aim of this study was to characterise the polymorphism in the production of second clutches observed in the European earwig as coexisting alternative life-history tactics. Our large scale sampling and multivariate analysis revealed that 1C and 2C females significantly differed in a number of clutch and maternal traits, but not in terms of female morphology or quality of offspring. As compared to 1C females, 2C females laid their first clutch earlier, produced a larger first clutch and lifetime number of offspring (with a slower embryonic developmental time), gained more weight during the first 14 days after hatching of first clutch, provisioned more food to their nymphs but were slightly less assiduous in egg guarding. Experimental cross-breeding also showed that mating among adult progeny of 1C and 2C females produced almost identical clutch size (eggs and nymphs) and nymphs of similar weight than 1C-1C and 2C-2C mating pairs. This finding clearly indicates that discontinuous distribution of investment in second clutches represent coexisting alternative life-histories tactics within a single species, rather than divergent life-histories of two sympatric sister species present in the studied population.

The two-fold lower level of maternal care (in term of food provisioning) reported in 1C as compared to 2C females contrasts with predictions that reduced costs of care in terms of future reproduction should favour enhanced maternal investment towards the current offspring (Gilbert and Manica 2010; Tallamy and Brown 1999). Hence, semelparity appears not to be a strict precondition for the evolution and maintenance of maternal care (Tallamy and Brown 1999). Our results also revealed that access to *ad* *libitum* food did not homogenise the level of maternal care provided to current offspring as would be expected if the level of care was determined by benefits and costs as mediated by concurrent conditions. This suggests that variation in maternal care at least partly depends on factors independent from food availability, such as intrinsic female or nymph quality (e.g. Mas et al. 2009), or variation maintained by coadaptation between maternal care and offspring traits (which would contribute to the maintenance of intrinsic variation in maternal care, Kölliker et al. 2005).

Our findings are not consistent with the hypothesis that 1C and 2C females reached equivalent fitness over lifetime through alternative investment trade-off between current and future reproduction. This interpretation is further supported by the result that egg number, hatching success and nymph weight at day 1 were positively correlated between first and second clutches. The lack of evidence for trade-offs between first and second clutches has to be interpreted with caution, however. Subtle trade-offs could indeed be covered and reversed when individuals vary in quality and potential for reproductive output (house-car principle, van Noordwijk and de Jong 1986). These trade-offs may also be harder to observe due to the low cost of maternal care when individuals are reared under standardised laboratory conditions such as with easy access to food and in the absence of predators and pathogens (Kölliker and Vancassel 2007; Little et al. 2003; Stearns 1992). For instance, we cannot rule out the possibility that under predation pressure, offspring of 1C females would have had higher survival because 1C females spent more time or effort in protecting their young against predators.

The production of single clutches by 1C females is unlikely to simply reflect limitations in critical resources for reproduction, such as female viability, age at reproduction, senescence after clutch production or sperm depletion. First, 62 out of the 74 (83.8%) 1C females survived until the end of the experiment (i.e. 60 days post-hatching), indicating that single clutch production was not a mere consequence of premature death of these females. Second, 1C and 2C females were likely to be of a similar age in the experiment, as all individuals moulted into adults within a period of approximately two weeks. Third, 1C females were unlikely to already be in a state of senescence after having produced their clutch, as it was produced only 8 days later on average than the first clutch of 2C females and the average latency between the successive clutches of 2C females was 37 days. Finally, single clutch production was unlikely to result from enhanced sperm limitation in 1C than 2C females (e.g. due to a lower number of mating partners), as previous paternity analysis using microsatellite markers reported that a similar number of males sired progeny from 1C and 2C females (Guillet 2000). Furthermore, mating is not a prerequisite for oviposition in *F. auricularia*, and females lay their clutches also if the eggs are not fertilized, for instance due to lack of sperm (M.K. unpubl. results).

Alternatively, the coexistence of 1C and 2C females could reflect alternative life-histories where females adopt the tactic that provides the highest fitness returns given her condition and according to an evolved threshold (Gross 1996; Tomkins and Brown 2004). Thereby, condition can be a property of the individual female itself (what is usually meant by "condition"; e.g., nutritional, genetic, ontogenetic or environmental influences on reproductive capacity), but might also reflect the outcome of interactions between the female and her social environment, such as specific properties of their male mates or of the tended offspring (Wolf et al. 1998). Consistent with a role of female condition in underlying the observed discrete variation in clutch production, we found that 2C females gained significantly more weight during nymph care than 1C females. This finding could reflect that 2C females chose their reproductive tactic before or during care for their first clutch, putting more effort in increasing their weight in order to produce a second clutch. Alternatively, 1C females may have been limited in their ability to gain weight for second-clutch production because of their intrinsic physical condition, or because of properties of their social environment (i.e., mates, offspring). However, further research is required to elucidate the determinism of the 1C-2C dimorphism in *F. auricularia*.

In 2C (iteroparous) females, all the measured characteristics of second clutches significantly differed from the ones of first clutches. In particular, second clutches showed faster development, larger nymph weight at hatching, higher weight gain, but lower survival. Ultimately, the development of nymphs is expected to be faster in second than in first clutches as it may help to (1) alleviate to some degree cannibalism risk caused by offspring from first clutches (second clutch nymphs have to survive in an environment populated by advanced instars nymphs from first clutches, Vancassel and Quris 1994), (2) catch up in development to be able to effectively compete with offspring from first clutches for mating when adult and (3) oviposit before winter starts. Proximately, this enhancement of growth rate could be mediated by different mechanisms: for instance, a maternal effect may induce different developmental programs in first as compared to second clutches, or the expectedly higher levels of maternal care for second clutches may have such an effect on developmental rate (i.e. terminal investment, Williams 1966). Alternatively, it is possible that a higher cannibalism rate in second than first clutches may explain this result. Because mortality in earwig clutches is almost always associated with cannibalism (Dobler and Kölliker 2010), our finding of significantly lower survival rate together with higher weight gain of nymphs in second than first clutches are consistent with the hypothesis that cannibalism is higher in second clutches.

It is characteristic for alternative allocation phenotypes (Brockmann and Taborsky 2008) that the frequency of the different tactics varies among populations due to ecological and population dynamic factors. For example, the relative frequency of two coexisting wing-morphs (reflecting alternative dispersal tactics) in the planthopper *Prokelisia marginata* depends on population density and environmental persistence (Denno et al. 1996), with an average of 92% of the dispersing morph in populations along the North American Atlantic coast and 17% in populations along the Gulf of Mexico. Although we lack a quantitative analysis of variation in the parity dimorphism across populations in earwigs, the example of the wing-dimorphism in *P. marginata* illustrates that the relative frequencies for the 1C and 2C life-histories in our study (i.e. 15% and 85%, respectively) is in a range that can be expected for an alternative allocation phenotype.

The frequency of the 2C tactic in the Mediterranean population studied here (85%) was substantially higher than in a more northern and higher altitude, central Swiss, population (approx. 33%, Kölliker 2007), and similar to a low-altitude (albeit northern) population near Rennes (Bretagne, France) (86%; Vancassel 1984). Previous studies suggested season length and population density as likely major agents of selection on mode of parity. Short seasons (i.e., at high altitude and/or latitude) were expected to provide less time to successfully raise two clutches and therefore to selectively favour the 1C tactic (Charlesworth 1994; Stearns 1992), whereas long seasons allow longer reproductive periods which may favour the 2C tactic (Vancassel 1984). High population densities are also predicted to favour the 1C tactic because of the higher risks of cannibalism for second clutch nymphs by the older first clutch nymphs, which differentially reduces the juvenile survival rate and, hence, reproductive value for second clutch nymphs (Moerkens et al. 2009). The known differences in seasons between Mediterranean and Swiss climates are consistent with the season length hypothesis and could explain the observed differences in the frequency of 2C tactic between populations. Density estimates for the two populations are however not available. In the future, transplant experiments within and between populations of different density and/or season length may shed light on the selective agents and evolutionary dynamics underlying the coexisting 1C or 2C life-history tactics in earwig populations.

To conclude, our results demonstrate that semelparous and iteroparous modes of reproduction coexist in *F. auricularia* populations, most likely as condition-dependent alternative female life-history tactics. Overall, 15% of females did not produce second clutches despite access to *ad libitum* food and absence of predators. This observation suggests that the alternative life-history tactics may not be completely phenotypically plastic, but may also contain a heritable component. To shed light on the mechanism expressing the 1C-2C dimorphism, further experiments will be required manipulating early (juvenile) and late (adult) nutritional condition, and/or breeding experiments to disentangle environmental from genetic sources and the role of social interactions with mates and offspring in generating variation in the expression of the alternative modes of parity.

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APPENDIX II

Sibling cooperation through food sharing in an insect with facultative parental care

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Sibling cooperation through food sharing in an insect with facultative parental care, *American Naturalist*

Abstract

In families where offspring exhibit capabilities of early dispersal and independent survival, the persistence of family life requires that young gain a net fitness benefit from remaining with parents and siblings. Parent-offspring interactions are well known to provide such benefits through parental care, while research on sibling interactions traditionally report fitness costs to offspring due to competitive behaviors. Here we show that sibling interactions also reflect cooperative behaviors in the form of food sharing in the European earwig, *Forficula auricularia*, an insect species with facultative maternal care. Using a series of laboratory experiments, we demonstrated that the food ingested by individual offspring was transferred to their sibling through proctodeal trophallaxis and active allo-coprophagy. These transfers occurred both in presence and in absence of the tending mothers, even though the direct contact with the mothers limited sibling food sharing. Neither food deprivation nor relatedness influenced the total amount of transferred food, but relatedness affected the behavioral mechanisms mediating food sharing. Specifically, related offspring obtained food predominately through allo-coprophagy, whereas unrelated offspring obtained food through proctodeal trophallaxis. Overall, this study emphasizes that sibling cooperation could be a key parameter in the early evolution of family life.

Introduction

Family life is a common phenomenon in nature that has been described in almost all animal taxa (Clutton-Brock 1991; Kokko & Jennions 2008; Davies et al. 2012; Wong et al. 2013). The frequent and intimate interactions between parents and offspring are generally associated with parental care, which are known to provide benefits to offspring in terms of development and survival (Gross 2005; Royle et al. 2012; Smiseth et al. 2012). However, family interactions and parental care also come at fitness costs to parents and/or offspring. Such costs are due to the conflicts between parents and offspring over the level of parental care (reviewed in Kilner and Hinde 2012) or over limited food resources (Meunier & Kölliker 2012a), between mothers and fathers over their respective investment in care (reviewed in Lessells 2012) and among offspring over their selfish access to the limited parental/food resources (reviewed in Roulin & Dreiss 2012). Hence, family life can only evolve if its associated benefits for offspring outweigh the costs to the different family members (Alonso-Alvarez & Velando 2012). Gaining a better understanding of the emergence and persistence of family life therefore requires identifying not only the competitive behaviors that mediate fitness costs, but also the cooperative behaviors that potentially provide fitness benefits to offspring.

To date, most studies investigating the nature of sibling interactions reported antagonistic behaviors associated with fitness costs for the offspring (reviewed in Mock & Parker 1997; Roulin & Dreiss 2012). However, few studies also showed forms of cooperation among siblings. For instance, nestlings preen each other in the Mississipi kite *Ictinia Mississippiensis* (Botelho et al. 1993), cups form coalitions with litter-mates against unrelated juveniles in the spotted hyena *Crocuta crocuta* (Smale et al. 1995) and old chicks delay their time of fledgling to provide more maturation time for their younger siblings in the house wrens *Troglodytes aedon* (Bowers et al. 2013). A few additional studies even found that siblings possibly express forms of cooperation regarding limited food/parental resources, a keystone parameter typically enhancing sibling rivalry not cooperation (Stearns 1992; Mock & Parker 1997). First, siblings may negotiate about instead of fight over limited food resources, a behavior considered as cooperative because it prevents starved offspring to suffer from the costs of intense physical fights, while it provides indirect genetic benefits to the related cooperative offspring (Johnstone & Roulin 2003). Such sibling negotiation has been reported in the barn owl *Tyto alba* and the spotless starling *Sturnus unicolor*, where starved chicks vocally negotiate with non-starved siblings for their later access to parentally-provided preys (Roulin et al. 2000; Roulin 2002; Bulmer et al. 2007; Dreiss et al. 2010, 2013). Second, sibling may coordinate their begging behavior when parents return from foraging trips, a cooperative interaction that provides benefits to offspring by increasing parental feeding rate, while limiting their personal investment in costly begging (Johnstone 2004). Such sibling coordination received support from studies conducted in the black-headed gull *Larus ridibundus* (Mathevon & Charrier 2004) and in the banded mongoose *Mungos mungo* (Bell 2007). Finally, siblings may restrain competition for resources, as found in the blue-footed booby *Sula nebouxii*, where dominant chicks permit subordinates to have access to the limited food source in spite of their capacity of siblicide, a behavior again possibly driven by the indirect fitness benefits gained by the cooperative sibling (Anderson & Ricklefs 1995).

The few above examples for sibling cooperation over food resources mostly derive from studies of altricial birds and mammals, in which the mobility of offspring is limited and their survival mainly relies on the resources provided by the tending parents. While these studies provide information about the possible occurrence of sibling cooperation when offspring do not have early dispersal capabilities, these studies are of limited relevance to understand the importance of sibling cooperation for the early evolution of family life (Smiseth et al. 2012). In particular, it remains unknown whether forms of sibling cooperation occur in species where offspring have the capabilities to both actively disperse from their family group and survive in absence of tending parents, the likely scenario that prevailed in the early evolution of family life. Interestingly, early dispersal and independent survival can be found in offspring from precocial species (also called partially begging species in insects), which are very common across taxa (e.g. Smiseth et al. 2003; Rehling & Trillmich 2007; Dial & Carrier 2012) and thus provide ideal biological models to better understand the evolutionary mechanisms that favored the emergence and persistence of family interactions (Smiseth et al. 2003).

The current study aimed at testing the occurrence of sibling cooperation in form of food sharing in families of the European earwig, *Forficula auricularia*. In this partially begging species, mothers tend their clutch of mobile offspring (called nymphs) for several weeks after the eggs have hatched (Costa 2006). During this period, mothers frequently interact with their nymphs to provide different forms of care, such as the protection against predators and the provisioning of food through regurgitation (Staerkle & Kölliker 2008; Meunier & Kölliker 2012a, 2012b, 2013). Although tending mothers are known to enhance nymph survival under natural and good laboratory conditions (Kölliker & Vancassel 2007; Kölliker 2007), maternal attendance is not required to ensure such survival (Lamb 1976; Kölliker 2007) and may even come at costs to nymph survival when food is limited (Meunier & Kölliker 2012a). Nevertheless, earwig nymphs have been shown to actively aggregate with their mothers both under good and harsh conditions (Wong & Kölliker 2012). Besides mother-offspring contacts, frequent and tight interactions among nymphs also shape earwig family life. These interactions are known to be partly competitive, as both large clutches and limited food availability have been shown to speed up nymph development and to reduce their survival (Kölliker 2007; Meunier & Kölliker 2012a), as well as because siblicide and cannibalism are relatively frequent (Dobler & Kölliker 2010). However, recent observations suggests that nymph-nymph interactions may also take the forms of allo-grooming as well as mouth-to-mouth and mouth-to-anus contacts (pers. Obs.), which possibly reflect cooperative behaviors.

In this study, we conducted a series of three laboratory experiments to address the four following questions. First, do nymphs exchange food during family life and what is the effect of maternal presence on this exchange? Second, can allo-coprophagy at least partly mediate food transfer among nymphs? Third, is food transfer between siblings an active or a passive process? Active processes were defined as stomodeal trophallaxis (mouth to mouth feeding), proctodeal trophallaxis (anus to mouth feeding) and/or active allo-coprohagy (i.e. resulting from the socially-induced release of frass), whereas passive processes are mainly defined as passive allo-coprophagy, which reflects an opportunistic feeding on waste produced by other nymphs. Finally, what are the influence of nymph-nymph relatedness and nymph starvation on the level of food transfer? These two factors were selected because relatedness is considered a key parameter in the evolution of cooperation (Kin selection, Hamilton 1964; West et al. 2002), relatedness between earwig nymphs naturally varies within clutches due to the promiscuous mating system of adults and the phenomenon of brood-mixing by nymphs (Kölliker & Vancassel 2007; Meunier & Kölliker 2012a) and nymph starvation was expected to enhance nymph incentive to obtain food from siblings.

Material & Methods

Origin of the tested individuals

All the clutches used in the following experiments were produced by a total of 125 *F. auricularia* females that had been field-sampled in Dolcedo, Italy, in May 2009 (experiments 1 and 2) and in September 2012 (experiment 3). Random groups of females and males collected in May 2009 were first maintained in plastic containers (37 x 22 x 25 cm) to allow mating (Meunier et al. 2012). These containers were kept at 60% humidity, 14:10 h light: dark, constant 20°C and received a*d libitum* artificial food (food composition detailed in Meunier et al. 2012). After five months, the resulting mated females were isolated in Petri dishes (diameter 10 cm) for the production of their first clutch of eggs. Because individuals sampled in autumn are generally mated, the females collected in September 2012 were directly isolated in Petri dishes for egg production. At isolation, all females were maintained under complete darkness at 15°C, 60% humidity and received artificial food twice a week. These conditions were maintained from egg laying to hatching, except that no food was provided during this period (Kölliker 2007). At hatching, females and their nymphs were transferred to new Petri dishes that had been covered with humid sand and contained a plastic shelter used as a nest. These families were then maintained in a climate chamber at 20°C, 60% humidity and 14:10 light:dark cycle, and they received artificial food every other day (except when specifically mentioned) until the end of the experiments.

Notice that females sampled in September 2012 were kept under such standardized laboratory conditions (Meunier et al. 2012) until they had produce their second clutches, which were used to conduct experiment 3. In *F. auricularia*, females produce up to two clutches in their lifetime (Meunier et al. 2012).

Experiment 1: Do nymphs exchange food and what is the effect of maternal presence on this exchange?

This first experiment involved the clutches produced by 45 females, which were divided among three experimental treatments, each of them containing 15 replicates (i.e. one clutch per replicate). For all treatments, the replicates consisted of one four-days-old focal nymph that had been starved for one day before the setup to slightly increase its nutritional needs and marked with a dot of red paint for identification (focal nymph), as well as three nymphs from the same family fed on green-colored food one day before the setup. The green-colored food (mix of blue dye and yellow-natural color of pollen) was used as a marker of food sharing, because ingested green food is visible through the cuticle of the partially transparent nymphs (fig. A1 in the appendix, available online; Staerkle and Kölliker 2008).

In the first treatment, the groups of nymphs were set up with their mother and could freely interact with her (maternal interaction treatment). In the second treatment, the groups of nymphs were set up with their mothers, but separated by a mesh to prevent physical contact between nymphs and mothers (maternal presence treatment). Finally, in the third treatment, the nymphs were set up without their mother (maternal absence treatment). One day after the setup of the experimental family groups, we scored whether the focal nymphs were green or not, i.e. received food from the nymphs previously fed on green-colored food. The mothers were fed with naturally yellow-colored pollen the days before the experimental set up to ensure that any green food ingested by the focal nymphs could exclusively originate from siblings. The nymph's changes into green were determined using a stereomicroscope to record even low amount of food-transfer, and following a blind-procedure, i.e. the experimenter did not know the experimental group of the respective focal nymph. Each group was set up in 50 mm diameter Petri dishes, containing humid sand and a metal mesh used to separate the mother from the nymphs or, when not required, simply separating an empty side of the Petri-dish.

Experiment 2: Can allo-coprophagy at least partly mediate food transfer among nymphs?

A split-clutch experiment was conducted using the clutches of 40 females different from the ones used in experiment 1. Five days after hatching, six nymphs per clutch were distributed to the three following treatments. In the first treatment, one focal nymph was set up in a new Petri dish without any food (control, *n* = 40). In the second treatment, one focal nymph was set up in a new Petri dish, to which we added three nymphs from the same family that had been fed on green-colored food during the two days before the setup (colored nymphs treatment, *n* = 40). Finally in the last treatment, one focal nymph was set up in a Petri dish, which contained the frass that had been released by the above three colored nymphs during the two days before the setup (colored frass treatment, *n* = 40). Each focal nymph was starved for two days before the setup to enhance their nutritional need, and marked with a dot of red paint. One day after the setup, we determined whether or not the focal nymphs were green using a stereomicroscope and following a blind-procedure (see above). All experimental groups were set up in 15x15x20 mm plastic boxes preliminary covered with humid sand.

Experiment 3: (1/2) Do allo-coprophagy, stomodeal trophallaxis and/or proctodeal trophallaxis mediate food transfer? (2/2) What is the influence of nymph-nymph relatedness and nymph starvation on the level of food transfer?

This third experiment aimed at disentangling (1) whether food transfer between nymphs was mediated by the active release of frass by the encountered fed nymphs (i.e. socially-induced allo-coprophagy), by head-to-head contacts (which typically mediates stomodeal trophallaxis) and/or by head-to-anus contacts (which mediate proctodeal trophallaxis), as well as to test (2) whether the transfer of food between nymphs was shaped by nymph-nymph relatedness and/or nymph starvation. To this end, a full-factorial experiment was conducted using 40 pairs of nymphs that were either related or unrelated and where the focal nymphs were either starved or fed *ad libitum* (each of the four combinations: *n* = 10). The clutches produced by 40 females were first maintained in their original Petri-dish for 16 days with *ad libitum* food, after which nymphs were transferred to a new petri-dish, while mothers were discarded to mimic natural family disruption (Meunier et al. 2012). The 40 groups were then maintained in these Petri dishes for 10 ± 0.8 (mean ± SE) more days. Three nymphs per group were separated between two new Petri-dishes. In the first one, two nymphs were set up for four days with green-colored food (donor nymphs) whereas in the second one, one focal nymph was isolated (recipient nymph) with either non-colored pollen (fed *ad libitum*) or without any food source (starved). Such experimental starvation reduced the fresh weight of nymphs by 35.7% at setup (fig. A2 in the appendix, available online; $t = -3.16$, df = 38, $P = 0.003$) and therefore ensured high nutritional needs in the starved nymphs. After these four days, one of the two donor nymphs was randomly paired with the focal recipient nymph to obtain the four possible combinations of related or unrelated pairs with starved or fed focal nymphs.

At setup, each experimental pair was videotaped for 1h (Sony Handycam® HDR-CX 200) under red light to mimic night conditions (this species is nocturnal). The resulting movies were then analyzed to quantify the number of head-to-head contacts, the total duration of head-to-head contacts, the number of head-to-anus contacts from the recipient to the donor nymph and the total duration of these head-to-anus contacts. Video analyses were done using the free software VLC (http://www.videolan.org/) and following a blind observation procedure (see above). All nymphs were 26-days-old at setup and emerged from second clutches. We used 26-days-old nymphs as compared to the younger ones used in the first two experiments, because the movies recorded under red-light conditions were of relatively poor quality, thus only allowing fine-scale observations of trophallactic behaviors in larger nymphs. Nymphs of that age are still very keen to aggregate with their mothers (Wong and Kölliker 2012). Because the 26-days-old nymphs are less transparent than the younger ones used in the two first experiments, the amount of food transferred from non-focal to focal nymphs was here estimated by calculating the weight gained by each focal nymph over the 1h of filming, and not through changes in body coloration. This weight gain was calculated by subtracting the fresh weight of each focal nymph measured to the nearest µg after the experiment (Mikrowaage MYA 5, Pescale Wägetechnik) from the one measured before the experiment. After 1h of filming, each Petri dish received a piece of wet paper towel to provide water and humidity. The number of colored frass (i.e. frass released by the donor nymphs) present in the Petri dishes was counted after 1h and 24h in six replicates per combination (i.e. in a total of 24 pairs). All Petri dishes were 50 mm in diameter, covered with humid sand.

Notice that in 20 out of the 40 families (equally distributed across the four treatments) the mothers were already removed one day after hatching. Because such early presence or absence of tending mothers did not significantly influence any of our measurements (all $P > 0.23$) and did not qualitatively change any of our conclusions, this factor was not included in the following statistical analyses.

Statistical analyses

The results of the first and second experiments were analyzed using a Generalized Linear Model (GLM) and a Generalized Linear Mixed Model (GLMM), respectively. In these two models, the coloration of the focal nymphs was entered as binary response variable (with binomial error distributions) and the experimental treatments as explanatory factor. Because each family contributed to the three experimental groups in experiment 2, the GLMM was conducted using family of origin as random effect. Pairwise comparisons between the treatments were conducted using Chi-squared tests in which the significance level was Bonferonni-adjusted to $\alpha = 0.025$.

The third experiment partly aimed at testing the influence of trophallactic behaviors on the weight gained by focal nymphs. Because we found significant correlations among the mean duration and the number of head-to-head and head-to-anus contacts (see Table A1 in the appendix, available online), we first conducted a Principal Component Analysis (PCA) to obtain four non-correlated principal components (PCs) reflecting single or combinations of trophallactic behaviors (see details in Table 1). This PCA was conducted using the log(x+1)-transformed mean duration and number of head-to-head and head-to-anus contacts between nymphs. The PCs were then used to test if the weight gained by focal nymphs was mediated by trophallaxis (i.e. associated with the PCs) and/or by coprophagy (i.e. associated with frass number). To this end, we conducted a GLM in which nymph weight gain was entered as response variable, while the number of frass counted after 1h, as well as the four PCs were entered as explanatory variables. We finally tested whether trophallactic behaviors triggered frass release using a GLM, in which the log(x+1)-transformed number of frass counted after 1h (and then 24h) was entered as response variable and the four PCs as explanatory variables. These two last models were started by including all possible interactions between the factors and then proceeded with stepwise simplification by removing the interaction terms that were not significant (all $P > 0.19$).

Table 1. Loadings of the PCA conducted on the trophallactic behaviors.

	PC1	PC2	PC3	PC4
Head-to-head contacts				
Mean duration	-0.560	-0.262	-0.566	-0.546
Number	-0.594	-0.418	0.131	0.674
Head-to-anus contacts				
Mean duration	-0.299	0.813	-0.386	0.316
Number	-0.494	0.308	0.717	-0.384
Variance explained (%)	47.0	25.9	19.3	7.8

The influences of relatedness and starvation on the level of food transferred between nymphs in the third experiment were tested using three GLMs, in which these factors (and their interaction) were entered as explanatory variables, while the log(x+1)-transformed number of frass counted after 1h or 24h and the weight gained by focal nymphs were separately entered as response variables. Furthermore, the effect of relatedness and starvation (and their interaction) on the trophallactic behaviors was tested using a Multivariate Analysis of Variance (MANOVA) in which the four PCs were entered as response variables. The influence of these fixed factors on each of the PC was then tested using separate GLMs. In all statistical analyses, the log(x+1)-transformation was used to fulfill residual normality of the models. All statistical analyses were conducted using the software R v2.15.2 (http://www.r-project.org/) loaded with the libraries "car" and "Hmisc".

Results

Experiment 1: Do nymphs exchange food and what is the effect of maternal presence on this exchange?

Focal nymphs received food from the non-focal ones in all three experimental treatments, but to variable degree. 33.3% of the focal nymphs became colored in the maternal interaction treatment, 87% in the maternal presence treatment and 66.7% in the maternal absence treatment (fig. 1A; Binomial GLM, *LR* χ^2_2 = 9.70, ***P*** = 0.008). The number of colored nymphs was significantly lower in the maternal interaction than the maternal presence treatments (χ^2_1 = 8.89, ***P*** = 0.003), but not significantly different between the maternal presence and the maternal absence treatments (χ^2_1 = 1.67, ***P*** = 0.195) or between the maternal interaction and the maternal absence treatments (χ^2_1 = 3.33, ***P*** = 0.068). Overall, Food transfer between nymphs occurred significantly more in the groups where nymphs did not have any possible physical contact to their mothers (76.7% versus 33.3%, respectively; Binomial GLM, n = 30, *LR* χ^2_1 = 9.51, ***P*** = 0.0021), but was not significantly influenced by the presence of mother (prevented from physically interacting with the nymphs, or not) in the petri-dish (60.0% versus 66.7%, respectively; Binomial GLM, n = 30, *LR* χ^2_1 = 0.19, ***P*** = 0.662).

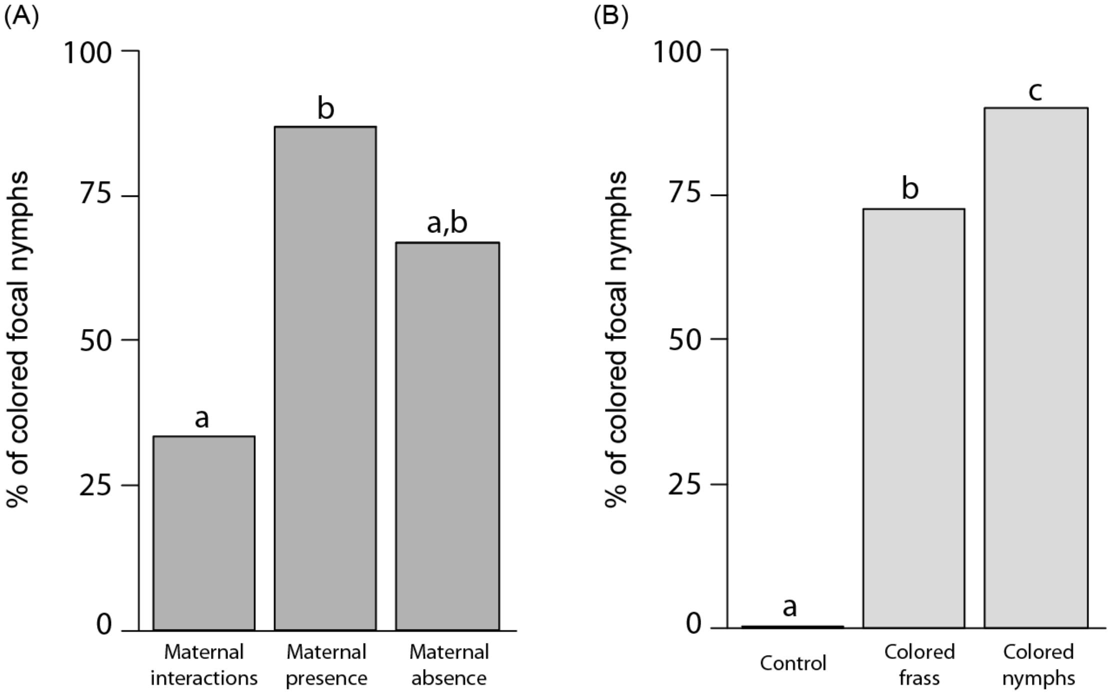

Figure 1. Percentage of experimental groups where the focal nymph became colored after being in contact to colored nymphs (A) and either plus their mothers (maternal interactions), plus their mothers but not in contact to them (maternal presence), or without any mother (maternal absence); and (B) either alone (control), in contact to colored frass or to colored nymphs. Different letters indicate significant differences between the treatments (see text).

Experiment 2: Does allo-coprophagy at least partly mediate food transfer among nymphs?

Colored focal nymphs were found both in the presence of colored frass and of colored non-focal nymphs. In particular, 72.5% of the focal nymphs became colored in the colored-frass treatment, 89.7% in the Petri dishes with colored nymphs and 0% in the Petri dishes without colored nymphs or frass (Fig. 1B, Binomial GLMM; *LR* $\chi^2_2 = 28.13$, $P < 0.0001$). The number of colored nymphs was significantly lower in the control than both the colored frass ($\chi^2_1 = 45.49$, $P < 0.0001$) and the colored nymph ($\chi^2_1 = 67.84$, $P < 0.0001$) treatments. Furthermore, a significantly larger proportion of focal nymphs became colored in the Petri dishes with colored nymphs than in the ones with colored-frass ($\chi^2_1 = 5.31$, $P = 0.021$), indicating that other factors than allo-coprophagy at least partly mediate food transfer among nymphs.

Experiment 3: (1/2) Do allo-coprophagy, stomodeal trophallaxis and/or proctodeal trophallaxis mediate food transfer?

The PCA conducted on the mean duration and number of head-to-head and head-to-anus contacts between nymphs provided four orthogonal PCs (1). All four traits exhibited consistent negative loadings on PC1, indicating a (low) general time investment in trophallactic behaviors. In other words, high values for PC1 reveal low frequencies and low durations for the trophallactic behaviors. Conversely, the mean duration of head-to-anus contacts primarily and positively loaded on PC2, which thus reflects the duration of proctodeal trophallactic behaviors. The number of head-to-anus contacts primarily and positively loaded on PC3, which indicates the number of proctodeal trophallactic behaviors. Finally, the number and duration of head-to-head contacts strongly but oppositely loaded on PC4, thus reflecting a trade-off between number and duration of stomodeal trophallactic behaviors.

The weight gained by the recipient nymphs in experiment 3 was significantly and positively associated with the number of frass released by the donor nymph counted after 1h (Fig. 2A; GLM; *LR* $\chi^2_1 = 7.77$, $P = 0.005$), as well as to PC2 (Fig. 2B; GLM; *LR* $\chi^2_1 = 5.37$, $P = 0.020$). In other words, recipient nymphs gained more weight when they had access to more frass, but also when they exhibited longer proctodeal trophallactic behaviors. By contrast, the weight gained by focal nymphs was not significantly influenced by PC1 (*LR* $\chi^2_1 = 2.35$, $P = 0.130$), by PC3 (*LR* $\chi^2_1 = 0.10$, $P = 0.756$), by PC4 (*LR* $\chi^2_1 = 2.37$, $P = 0.123$) or any interactions among them (all $P > 0.19$). Trophallactic behaviors did not triggered frass release, as revealed by the fact that the number of frass counted after 1h was non-significantly influenced by PC2 (*LR* $\chi^2_1 = 2.23$, $P = 0.135$) and any other PCs (PC1: *LR* $\chi^2_1 = 3.36$, $P = 0.07$; PC3: *LR* $\chi^2_1 = 1.03$, $P = 0.310$; PC4: *LR* $\chi^2_1 = 1.76$, $P = 0.184$).

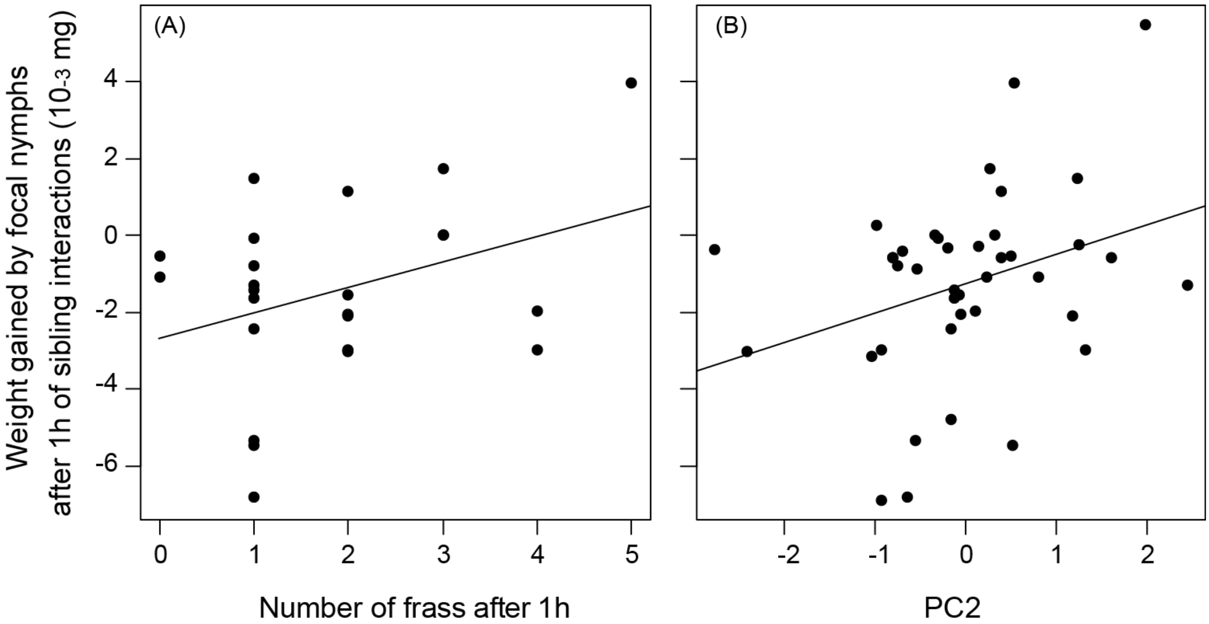

Figure 2. Positive association between the weight gained by focal nymphs and (A) the number of frass released by the non-focal nymphs over 1h and (B) PC2, which mainly reflects the mean duration of head-to-anus contacts.

Experiment 3: (2/2) What is the influence of nymph-nymph relatedness and nymph starvation on the level of food transfer?

As predicted if frass production is a (socially-induced) kin-directed process, we found that donor nymphs produced twice more frass after 24h when in contact to a related than an unrelated nymph (Fig. 3A; $LR\,\chi^2_1 = 7.46$, $P = 0.006$). Notice that relatedness did not significantly influence the number of frass counted in the short-term (i.e. 1h; $LR\,\chi^2_1 = 2.13$, $P = 0.145$), even if the trend followed the same direction as the significant one observed after 24h (Fig. 3B). The total number of frass counted after 24h and 1h, respectively, was not significantly influenced by starvation (24h: $LR\,\chi^2_1 = 0.001$, $P = 0.975$; 1h: $LR\,\chi^2_1 = 0.85$, $P = 0.357$) or by an interaction between food deprivation and relatedness (24h: $LR\,\chi^2_1 = 1.81$, $P = 0.179$; 1h: $LR\,\chi^2_1 = 0.57$, $P = 0.450$).

In addition to an effect of relatedness on frass release, we found an overall effect of relatedness on the trophallactic behaviors (MANOVA on the four PCS; approx. $F_{4,33} = 3.27$, $P = 0.023$). This overall effect was mainly driven by PC2, which was significantly larger in non-related than related pairs (Table 2; Fig. 3C) thus indicating that the durations of head-to-anus contacts were longer between unrelated than related individuals. None of the other PCs was significantly influenced by relatedness, starvation or an interaction between these two factors (Table 2). Finally, the trophallactic behaviors were overall not significantly influenced by starvation (approx. $F_{4,33} = 0.50$, P = 0.735) or by an interaction between starvation and relatedness (approx. $F_{4,33} = 0.50$, $P = 0.739$). Interestingly, the weight gained by the recipient nymphs over 1h was not significantly influenced by relatedness ($LR\,\chi^2_1 = 1.87$, $P = 0.172$), starvation (*LR* χ^2_1 = 0.06, *P* = 0.805) or by an interaction between relatedness and starvation (*LR* χ^2_1 = 0.29, *P* = 0.591).

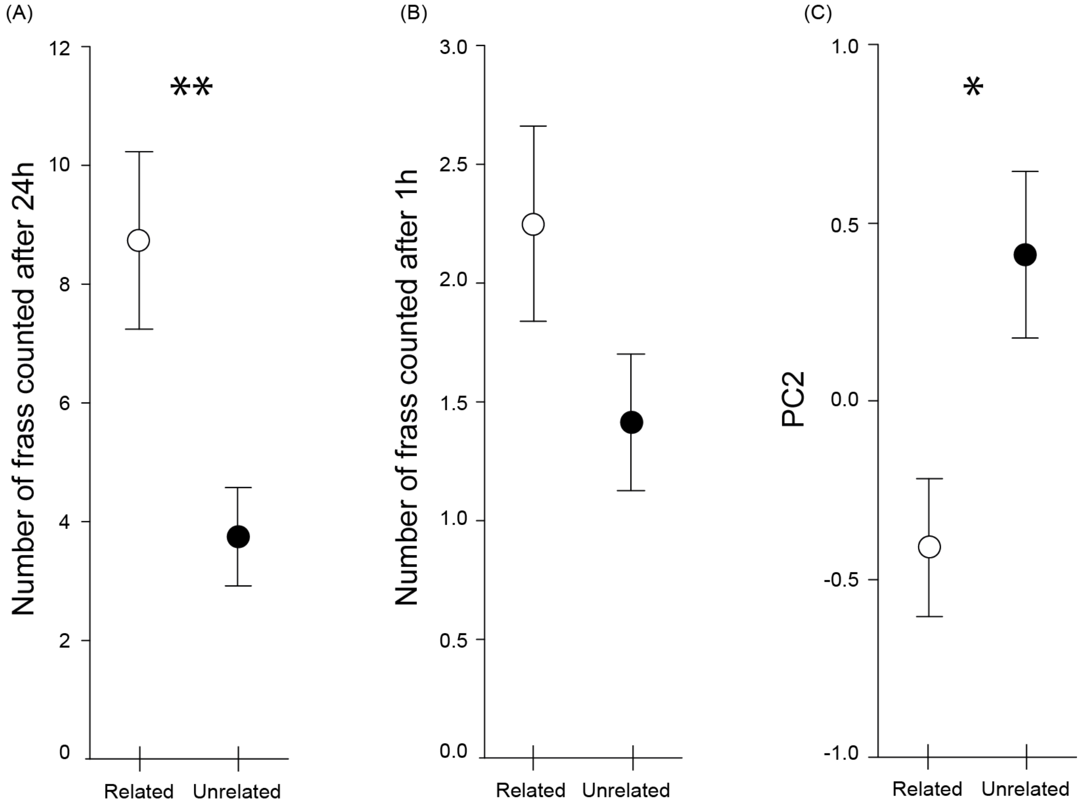

Figure 3. Influence of relatedness between focal and non-focal nymphs on (**A**) the number of frass produced by the non-focal nymphs after 24h, (**B**) the number of frass produced by the non-focal nymphs after 1h, and finally on (**C**) PC2, a principal component positively associated to the mean duration of head-to-anus contacts between nymphs. All values are Mean ± SE. * $P < 0.05$, ** $P < 0.01$.

Table 2. Influence of relatedness and starvation on the four principal components obtained from PCA on the trophallactic behaviors. The significant effect is in bold.

	PC1		PC2		PC3		PC4	
	LR χ^2_1	*P*	LR χ^2_1	*P*	LR χ^2_1	*P*	LR χ^2_1	*P*
Relatedness	3.23	0.072	7.09	**0.008**	0.35	0.552	1.13	0.287
Starvation	0.22	0.640	0.25	0.614	1.57	0.210	0.10	0.753
Relatedness:Starvation	0.27	0.603	0.02	0.891	0.43	0.513	1.41	0.235

Discussion

Our study overall demonstrates that sibling cooperation through food sharing occurs in families of the European earwig. Specifically, we showed that (1) the food ingested by individual offspring is frequently transferred to other offspring during family life and that (2) active allo-coprophagy and proctodeal trophallaxis mediated such food exchange. Interestingly, our results also reveal that (3) relatedness shaped allo-coprophagy and proctodeal trophallaxis in opposite directions: Offspring interacting with a related nymph produced significantly more frass (thus favoring food transfer through allo-coprophagy), whereas offspring interacting with an unrelated nymph exhibited longer head-to-anus contacts (thus favoring food transfer through proctodeal trophallaxis). This effect of relatedness on the two mediators of food transfer between nymphs (4) did not translated into an overall effect of relatedness on the total amount of food transferred to the recipient nymphs. Hence, these results reveal that relatedness influences the strategy used by nymphs to provide and/or obtain food from other nestmates, but that both strategies have equivalent outcomes in terms of amount of food transferred (at least over the course of the experiment). Finally, our data revealed that (5) physical interactions with the mother reduced, albeit did not prevent, the likelihood of food transfers between nymphs and that (6) starved nymphs did not receive more food from their siblings than non-starved ones.

The fact that nymphs exchanged food through active allo-coprophagy and proctodeal trophallaxis supports the hypothesis that food sharing reflects an active form of cooperation, rather than an opportunistic feeding on waste produced by other individuals. Sibling food sharing is a well-known and an obligate behavior in cooperative breeding vertebrates and insect societies (e.g. ants, bees, wasps and termites), where the development and survival of newly produced offspring rely on the care (including food provisioning) provided by siblings (e.g. Wilson 1971; Hatchwell and Komdeur 2000; Biedermann and Taborsky 2011). However, such behavior has been rarely studied and described in species with simple forms of family life consisting of caring parents and their offspring. Furthermore, the few other forms of sibling cooperation that have been described in such species (see introduction) were done in altricial species, where young offspring cannot disperse and may have secondarily evolved forms of cooperation to help them dealing with antagonistic sibling interactions enforced by the inability to disperse. By demonstrating that food sharing among contemporary siblings occurs in a partially begging species where offspring exhibit a mobile feeding system, our study emphasizes that sibling cooperation can evolve even in relatively simple family structures, where offspring cohorts do not overlap and where young are capable of early dispersal (Kölliker & Vancassel 2007). Based on these results, we suggest that cooperation between contemporary siblings may be a key parameter in the emergence and persistence of family life, by increasing the incentive of mobile offspring to stay with their siblings to benefit from their cooperative behaviors (and not only from parental care).

Although we showed that nymphs received nutritional benefits from sibling food sharing in terms of weight gain, the experimental reduction of weight in recipient nymphs did not trigger higher rate of food transfer. This finding reveals that starvation is not the main driver of sibling food sharing in earwigs and suggests that food sharing provides benefits additional to the ones reflected by weight gain. In line with this hypothesis, our experiment demonstrated that food transfer was mediated by allo-coprophagy and proctodeal trophallaxis, two behaviors typically associated with three benefits for the receiving individuals (reviewed in Nalepa et al. 2001; Weiss 2006). First, allo-coprophagy allows the digestion of microbes that quickly colonize frass pellets after gut transit, a mechanism known to provide an important and sometimes the unique source of protein, lipid, carbohydrates or micronutrients to the ingesting individuals (Martin & Reddy 1984). Second, allo-coprophagy and proctodeal trophallaxis also permit specific food sources to be pre-processed by microbes or individuals, respectively, thereby facilitating their otherwise difficult assimilation in the recipient organism. Such process (called external rumen; Nalepa et al. 2001) is known to enhance the digestion of cellulose, allow the detoxification of allelochemicals, and/or soften the food in several insect and mammal species (Nalepa et al. 2001). Finally, allo-coprophagy and proctodeal trophallaxis allow individuals to acquire the microbes present in the ingested frass to establish a mutualistic hindgut fauna. Such a process is broadly used in cockroaches and termites to exchange microbial fauna, such as flagellate, required in wood digestion or developmental processes (Nalepa & Bell 1997; Nalepa et al. 2001; Bignell 2011). In *F. auricularia*, the benefits of digesting microbes (i.e. the first benefits described above) are unlikely to drive sibling food sharing, as individuals are mobile and omnivorous, and thus likely to have direct access to all the types of nutrients required for their development and survival (Albouy & Caussanel 1990). Whether frass consumption is required to facilitate the assimilation of certain types of food and/or to establish a mutualistic hindgut fauna in the European earwigs will have to be investigated in further studies.

Relatedness is recognized as a keystone in the evolution of cooperation, because the indirect fitness benefits gained by helping relatives possibly outweigh the direct costs of cooperation (Kin selection, Hamilton 1964; West et al. 2002). Surprisingly, our study shows that relatedness did not influence the total amount of food exchanged between nymphs. Instead, it reveals that relatedness had more subtle effects on food sharing by determining the behavioral mechanism by which individuals provided and/or obtained food from their nestmates. In particular, high relatedness increased frass release (which in turn favored food transfer through allo-coprophagy), whereas low relatedness increased the duration of head-to-anus contacts (which favored food transfer through proctodeal trophallaxis). The increase in frass release among related individuals is likely to be an effect on donor generosity, and resembles the kin-directed production of a public good (e.g. Griffin et al. 2004; Diggle et al. 2007) that could benefit all nymphs in the same family. Conversely, it is unclear whether longer proctodeal trophallaxis between unrelated individuals reflects a cooperative behavior of the donor (which increased extra-frass supply), or a competitive behavior of the recipient nymph (which increased its demand and monopolized frass early). The use of proctodeal trophallaxis instead of allo-coprophagy to mediate food transfer could thus reveal a behavioral conflict between unrelated nymphs, a scenario in line with the higher rates of cannibalism reported between unrelated as compared to related earwig nymphs (Dobler & Kölliker 2011).

Physical contacts between mothers and offspring are known to mediate multiple behaviors in European earwigs, such has grooming, aggression, antennations and the transfer of food from mothers to offspring (Staerkle & Kölliker 2008; Mas & Kölliker 2010). Here we showed that direct physical contacts between mothers and offspring limited the frequency of sibling food sharing. This result suggests that offspring prefer the maternally-provided food to the sibling-provided food, possibly because the former comes in larger quantities or with a better quality. However, we found that sibling food sharing still occurred in 33% of the experimental groups tended by a mother. The level of maternal care and food provisioning are known to vary broadly among *F. auricularia* mothers, ranging from females exhibiting high provisioning rates to females provisioning none of their nymphs (Mas & Kölliker 2010; Meunier & Kölliker 2012b; Meunier et al. 2012). The occurrence of sibling food sharing observed in the maternal interaction treatments could thus reflect a context-dependent strategy of earwig nymphs to ensure the gain of food already ingested by another individual (and its possibly associated benefits): nymphs obtain food from siblings when the mother is absent or shows a low level of provisioning.

To conclude, our study demonstrates that sibling interactions during *F. auricularia* family life reflect cooperative behaviors in the form of food sharing. Together with previous studies showing the occurrence of siblicide and cannibalism between earwig nymphs (Dobler & Kölliker 2010, 2011), our results reveal the broad spectrum of behaviors from antagonistic to cooperative characterizing sibling interactions in this species. Because *F. auricularia* is a species with facultative forms of maternal care (Kölliker 2007), our findings also provide evidence that the evolutionary forces promoting the emergence and the persistence of family interactions do not (necessarily) only rely on the benefits of parental care for offspring, but may also involve the benefits of cooperative sibling interactions. These results claim for more considerations in further theoretical and empirical studies investigating the influence of sibling cooperation on the outcomes of offspring-offspring interactions and more generally, on the early evolution of parental care and family life.

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Online Appendix

Sibling cooperation through food sharing in an insect with facultative parental care

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Table A1 – Correlations between the four measures of trophallactic behaviors. Pearson correlations are given on the lower part of the diagonal, while the corresponding *P-values* are given on its higher part. Significant correlations are in bold.

		Head-to-anus		Head-to-head	
		Duration	Number	Duration	Number
Head-to-anus	Duration		*P* = 0.073	*P* = 0.196	*P* = 0.958
	Number	r = 0.29		*P* = 0.243	***P* = 0.008**
Head-to-head	Duration	r = 0.21	r = 0.19		***P* < 0.001**
	Number	r = 0.01	**r = 0.41**	**r = 0.57**	

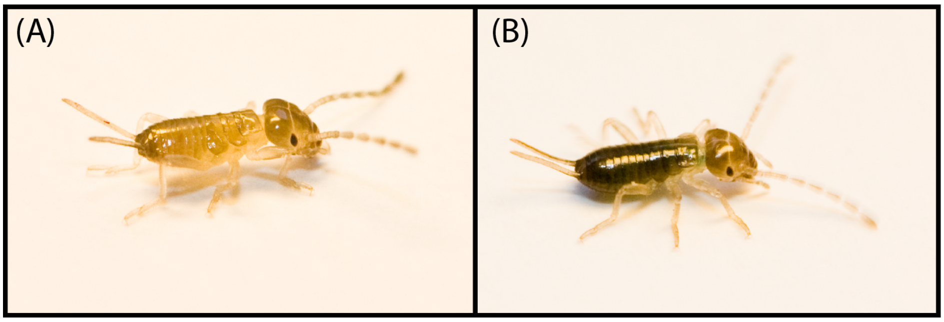

Figure A1 - First instars nymphs fed with (A) non-colored pollen or (B) green-colored pollen.

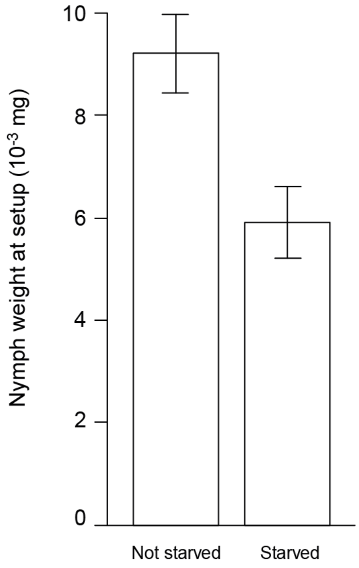

Figure A2 – Effect of starvation on the fresh weigh of recipient nymphs at set up.