# Influence of Ecology and Social Interactions on the Early Evolution of Family Life

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# SUMMARY

A core research area in evolutionary biology is devoted to the investigation of universal mechanisms that drive transitions to social life. The formation of animal societies out of formerly independent, solitary organisms has long taken center stage in the quest for these mechanisms. However, while transitions from family systems to the highly integrated societies of cooperatively breeding vertebrates and eusocial insects have been thoroughly explored, little attention has thus far been paid to the evolutionary origin of the allegedly simple family systems themselves. Hence, the mechanisms promoting the early evolutionary stages of family life remain poorly understood, despite the pivotal role they might play in explaining transitions from solitary to social life. Here, we investigated evolutionary mechanisms shaping the family life of the European earwig *Forficula auricularia,* a precocial insect with facultative post-hatching maternal care that recently emerged as a model system for the early evolution of family life. In particular, we assessed the impact of social interactions, basic life-history characteristics, and environmental conditions on the costs and benefits of earwig family life, and thus sought to determine how their interplay might affect the evolutionary origin and subsequent consolidation of family life.

The first part of this thesis investigates the relationship between different types of social interaction among European earwigs to elucidate potential mechanisms underlying the fitness effects of facultative social life. Chapter 1 shows that the cooperative food transfer among earwig nymphs increases if mothers do not provision their offspring at a sufficiently high level, indicating that sibling cooperation could complement the benefits of facultative parental care in driving the early evolution of family life. Chapter 2 demonstrates that the propensity of earwig nymphs to share food depends on the condition of their mother. Offspring thus seem to flexibly adjust their behavior toward siblings in response to changes in parental care. However, such complex behavioral patterns do not seem to promote gregariousness in adult earwigs; chapter 3 reveals that the intimate social interactions typical for family life only partly extend into the semisocial adult stage. Overall, these results affirm the pre-eminent role of the family (as compared to other types of) group in the evolution of advanced animal societies, and demonstrate that early family systems are not just simplistic imitations of their more advanced counterparts, but rather reflect surprisingly sophisticated social environments in their own right.

The second part of this thesis explores how the complex interdependencies of social interactions, basic life-history characteristics, and environmental conditions affect the costs and benefits of facultative family life. Specifically, chapter 4 shows that parent-offspring competition drastically reduces offspring survival under food limitation, suggesting that this mechanism might hamper the evolution of family life under harsh environmental conditions. By contrast, chapter 5 demonstrates that maternal loss unexpectedly increases the body size of offspring, but impairs

their expression of parental care in the adult stage – pointing at a crucial role of such long-term costs in the maintenance of early forms of family life. Finally, chapter 6 shows that life-history traits of mothers and their offspring vary between the first and last reproductive event in a partly population-specific manner, indicating that this variation reflects age-dependent changes in parental investment, and population idiosyncrasies that likely arose as the result of different environmental conditions. Altogether, these findings point at a crucial role of the joint effects of environmental conditions and life-history characteristics in the early evolution of family life.

Finally, the third part of this thesis takes a broad theoretical perspective, and explores how the study of family life might integrate into the current theory of social evolution. In particular, chapter 7 reviews the enduring controversy surrounding two major theories of sociobiology – kin selection theory and multilevel selection theory – and suggests that both approaches provide different, but complementary perspectives on social evolution that could jointly advance our understanding of evolutionary transitions from solitary individuals to complex animal societies.

In conclusion, this dissertation demonstrates that facultative family systems can give rise to surprisingly sophisticated social environments that are shaped by a unique set of behavioral mechanisms, and thus often differ profoundly from the social environments in obligatory family systems. Specifically, sophisticated forms of parental care are here paralleled by sophisticated forms of sibling cooperation, and parents not only confer long-term benefits to their offspring, but also directly compete with them for limited food resources. Importantly, the social behaviors expressed during family life resonate in the behaviors expressed in the adult stage, as well as affect life-history characteristics found across populations. The interplay between all these parameters is ultimately likely to shape the evolution of family life and thus more generally, the transition from simple to complex family systems.

# GENERAL INTRODUCTION

## The early evolution of family life

Jos Kramer

> "Furthermore, in many groups of organisms, from the social insects to the primates, the most advanced societies appear to have evolved directly from family units."
>
> Edward Osborne Wilson, 1975. *Sociobiology*

## TRANSITIONS TO SOCIAL LIFE

Throughout the history of life on earth, previously independent units have formed social collectives that allowed them to cope with the numerous challenges imposed by their ever-changing environment: genes grouped together in cells; cells grouped together in multicellular organisms; and multicellular organisms grouped together in societies. Transitions from solitary to social life were the incipient steps in such *major transitions in evolution*, and hence often had far-reaching repercussions on the diversity, complexity, and hierarchical organization of life itself (Szathmáry and Maynard Smith 1995; Bourke 2011a). On these grounds, the investigation of the mechanisms driving transitions to social life has emerged as a core research area in evolutionary biology (Alexander 1974; Krause and Ruxton 2002; Bourke 2011a).

The formation of animal societies out of formerly independent, solitary organisms has long taken center stage in the quest for general mechanisms that drive social evolution. Indeed, it has prepossessed scientists ever since Charles R. Darwin first speculated on the evolution of eusocial societies in his book *On the Origin of Species* (he suggested that selection at the family-level would resolve his 'special difficulty' of explaining the emergence of sterile worker castes; Darwin 1859). Since then, the mechanisms driving transitions from simpler social systems to the highly integrated and often permanent societies of cooperatively breeding vertebrates and eusocial insects have been thoroughly explored (e.g. Wilson 1971; Bourke and Franks 1995; Crozier and Pamilo 1996; Koenig and Dickinson 2004; Koenig and Dickinson 2016). This process was paralleled by the development of *inclusive fitness theory*, the central theoretical framework underpinning modern sociobiology (Hamilton 1964a; Hamilton 1964b; Frank 1998; Marshall 2015). However, less consideration has been given to the evolutionary origin of the simpler social systems themselves (Trumbo 2012; Falk et al. 2014), and the incipient steps in transitions to social life thus remain poorly understood.

### *Pathways of Group Formation*

In principle, transitions from solitary to social life in simple (temporary and often facultative) animal societies can roughly follow two different pathways: the semisocial and the subsocial (Michener 1969; Bourke 2011a). The semisocial pathway occurs when group formation results from the aggregation of individuals of the same generation, a process that, for instance, gave rise to the larval societies of sawflies and colonies of communally nesting halictid bees (Michener 1969; Costa 2006; Bourke 2011a). By contrast, the subsocial pathway occurs when group formation results from the continual association of parents with their offspring, an event that is tantamount to the emergence of social interactions among the closely related family members (Queller 2000; Bourke 2011a). It has long been recognized that this latter pathway gave rise to vast majority of advanced animal societies (Wheeler 1928; Michener 1969; Wilson 1975b; Bourke 2011a). Nevertheless, surprisingly little is known about the mechanisms shaping the evolutionary origin and the subsequent consolidation of such subsocial family systems (cf. Smiseth et al. 2012; Trumbo 2012), despite their crucial role in the early evolution of animal sociality.

## SOCIAL LIFE IN THE FAMILY

Social life in family groups is a rare, but taxonomically widespread phenomenon that occurs not only among widely known vertebrate groups such as mammals, birds and (non-avian) reptiles, but also among numerous invertebrate taxa including arthropods, molluscs, and annelids (Clutton-Brock 1991; Balshine 2012; Wong et al. 2013). Both within and across these groups, family life – the associations of caring parents with their offspring after birth or hatching (cf. Clutton-Brock 1991) – can vary tremendously in the nature, intimacy, and duration of family interactions (Balshine 2012; Klug et al. 2012; Trumbo 2012), ranging from temporary and often facultative aggregations over cooperatively breeding groups to permanent and highly integrated eusocial societies (Hölldobler and Wilson 1990; Costa 2006; Koenig and Dickinson 2016).

The evolution of family life entails the emergence of a novel – social – environment characterized by the ensuing social interactions among the family members (Badyaev and Uller 2009; Uller 2012). The interactions most profoundly shaping this social environment commonly reflect parental care (cf. Clutton-Brock 1991; Royle et al. 2012b), a phenomenon encompassing "any parental trait that enhances the fitness of a parent's offspring, and that is likely to have originated and/or to be currently maintained for this function" (Smiseth et al. 2012). Parental care can thus comprise non-behavioral traits such as gamete provisioning and gestation, as well as behavioral traits ranging from nest construction over brood attendance to food provisioning (reviewed in Clutton-Brock 1991; Smiseth et al. 2012). The expression of parental care often has far-reaching consequences for the fitness of all family members (see below) and can create long-lasting bonds between parents and offspring. Such bonds preceded the evolution of many derived social behaviors, and thus likely drove the transformation of simple family systems to advanced societies (Darwin 1871; Wilson 1975b; Royle et al. 2012a). The investigation of the evolution and fitness effects of parental care is hence crucial to shed light on the evolution of family-living and more generally on the mechanisms shaping transitions from solitary to social life.

### *Benefits and Costs*

The evolution of family life generally presumes that the fitness benefits of family-living outweigh the concomitant costs (Alexander 1974; Clutton-Brock 1991; Klug et al. 2012). Both the benefits and costs of family life are typically composed of two types of fitness effect; they may comprise effects that are inherent to all types of group-living (Krause and Ruxton 2002), as well as effects that are specific to family life and closely linked to the expression of parental care (Alonso-Alvarez and Velando 2012). The inherent fitness effects of group-living arise independent of the expression of parental care and can in principle accrue to both parents and offspring. They comprise benefits that predominantly arise from an improved foraging efficiency and anti-predator defense, as well as costs that typically arise from increased competition and pathogen transmission among the family members (reviewed in Alexander 1974; Krause and Ruxton 2002). Notably, these costs are often especially pronounced in family groups, because the high genetic similarity within the family facilitates the horizontal transmission of pathogens (Schmid-Hempel 1998; Godfrey et al. 2006), and exacerbates the competition among the family members (West et al. 2002; Svanbäck and Bolnick 2007).

The benefits and costs specific to family life predominantly derive from the expression of parental care, and are usually split across generations: parental care is beneficial to offspring, as it neutralizes environmental hazards and thus raises the lifetime reproductive success of the offspring (Alonso-Alvarez and Velando 2012; Klug and Bonsall 2014) by increasing their survival and/or their quality (e.g. Eggert et al. 1998; Zink 2003; Salomon et al. 2005; Kölliker 2007). However, parental care also often entails high costs for parents (such as an increased energy loss or an elevated risk of predation) and may hence reduce their condition or survival – that is their capacity or opportunity to invest into future offspring (Trivers 1972; Alonso-Alvarez and Velando 2012). In addition to these direct costs, the expression of parental care often prompts consequential costs that accrue from evolutionary conflicts of interest among the family members over the allocation of care (Parker et al. 2002; Royle et al. 2004). Such conflicts arise because the family members are not perfectly related to each other, and can occur between parents (*sexual conflict*; Trivers 1972; Lessells 2012), among offspring (*sibling rivalry*; Mock and Parker 1997; Roulin and Dreiss 2012), as well as between parents and their offspring (*parent-offspring conflict*; Trivers 1974; Kilner and Hinde 2012). Note that parental care can evolve despite these costs, because caring parents gain indirect benefits that might outweigh the costs of care and thus increase the parents' (aggregate) inclusive fitness (Hamilton 1964a; Smiseth et al. 2012).

### *Origin and Evolution*

A plethora of studies have investigated the evolution of family life by elucidating the mechanisms that shape cooperative and competitive family interactions (reviewed in Clutton-Brock 1991; Royle et al. 2012b). However, the strong historical bias towards studying these mechanisms in the advanced social systems of mammals and birds has led to a neglect of the (mechanisms promoting the) evolutionary emergence of family life itself (Smiseth et al. 2003b; Trumbo 2012; Falk et al. 2014). Recent theoretical approaches have begun to augment early conjectures about the role of various factors ranging from life-history characteristics over environmental conditions to coevolutionary dynamics (reviewed in Klug et al. 2012). Nevertheless, the evolutionary origin and the subsequent consolidation of family life remain poorly understood, as the interdependencies among these factors have rarely been scrutinized (Bonsall and Klug 2011a; Klug et al. 2012). Moreover, the paucity of empirical studies in liaison with the intricacies of exploring the evolutionary past make it likely that additional factors have been overlooked (Trumbo 2012).

### (1) The emergence of family life

The evolutionary emergence of family life requires the concurrence of a wide range of factors that jointly make sustained social interactions among family members possible and – should the occasion arise – able to spread in the population (Klug et al. 2012). The initial step in the emergence of family life is promoted by life-history characteristics which ensure that social behaviors are primarily directed toward family members (Tallamy and Wood 1986; Lion and van Baalen 2007). This propensity to mainly interact with closely related family members increases the scope for the evolution of cooperative behaviors (such as parental care), and reduces the likelihood that such behaviors are misdirected toward non-kin (Hamilton 1964a; Lion and van Baalen 2007). Family life is accordingly most likely to emerge if parents and offspring recognize (by means of kin or familiarity recognition; cf. Evans 1998; Fellowes 1998; Dobler and Kölliker 2011), or regularly encounter each other (typically due to spatial clumping resulting from limited dispersal; Hamilton 1964a; Lion and van Baalen 2007). Additionally, the emergence of family life can be promoted by the existence of behavioral precursors of parental care (Royle et al. 2012a). For instance, the evolution of offspring attendance and guarding has been suggested to derive from ancestral defensive or aggressive behaviors (Tallamy 1984).

Once the preconditions for the emergence of family life are met, effects of (additional) life-history characteristics and environmental conditions jointly determine whether it can spread in the population against the background of the prevalent solitary lifestyle (Tallamy 1984; Clutton-Brock 1991; Klug et al. 2012). In particular, environmental conditions – including the availability of limited resources and the presence of predators or parasites (reviewed in Wilson 1975b; Krause and Ruxton 2002) – typically modify the impact of basic life-history conditions (such as stage-specific mortality and maturation rates) on the benefits and costs of family interactions (Bonsall and Klug 2011a; Klug et al. 2012). For instance, harsh conditions and the concomitant intense competition for limited resources have been predicted to increase the mortality rate of solitary individuals (Wilson 1975b; Clutton-Brock 1991). This, in turn, should promote the evolution of parental care and thus family life, because the then uncertain prospects of future reproduction decrease the relative costs of care to adults (Klug and Bonsall 2010; Bonsall and Klug 2011a), and increase its potential benefits to offspring (Webb et al. 2002; Klug and Bonsall

2010). However, recent studies suggest that the joint effects of environmental conditions and life-history characteristics may often themselves interact with the (baseline) benefits and costs of family interactions (Bonsall and Klug 2011a; Bonsall and Klug 2011b; Meunier and Kölliker 2012a). Subtle disparities in such synergistic effects might offer an explanation as to why even closely related species exposed to ostensibly identical environmental conditions often differ in the occurrence and nature of social interactions among family members (Costa 2006; Trumbo 2012).

### (2) The consolidation of family life

Following the emergence of family interactions, coevolutionary feedback-loops between parental and offspring traits can promote the evolution and diversification of (additional forms of) parental care and thus lead to the rapid consolidation of family life (Kölliker et al. 2012; Uller 2012). For instance, the initial evolution of parental provisioning may trigger evolutionary changes in other components of care as well as in offspring traits, allowing parents to choose safer nest sites, but also increasing the competition among offspring for parentally provided food. This increased sibling rivalry may, in turn, further advance the evolution of parental provisioning, thereby closing the coevolutionary feedback-loop between parental provisioning, the choice of safer nest sites, and sibling rivalry (Smiseth et al. 2007; Gardner and Smiseth 2011). Such mutual reinforcement between parental and offspring traits can lead to a unidirectional trend from simple ancestral forms toward more complex forms of family life (cf. Wilson 1975b; Smiseth et al. 2012) by fostering an increasingly tight phenotypic integration of parental care and offspring development (Kölliker et al. 2012; Uller 2012). In the sophisticated and highly-derived family systems of altricial species, this phenotypic integration is advanced to such an extent that juveniles cannot survive without at least some care early in their life (Kölliker 2007; Uller 2012).

### *Emergence versus Consolidation*

A considerable number of studies have investigated the benefits and costs of parental care and family life in altricial birds and mammals (reviewed in Clutton-Brock 1991; Roff 1992; Stearns 1992; Balshine 2012). However, the advanced consolidation of family life characteristic for altricial species (see above) implies that these studies are only of limited relevance in elucidating the mechanisms shaping the emergence of social interactions among family members and their maintenance during the incipient steps of the evolution of family life (Smiseth et al. 2003b; Falk et al. 2014). This is because the current adaptive value of a suite of traits – such as parental care – typically does not explain how these traits increased fitness in an ancestral state (Williams 1966a). For example, the current benefits of food provisioning in altricial species accrue from the prevention of offspring starvation, and thus reflect the dependency of offspring on parentally provided food. However, this condition cannot explain the ancestral adaptive value of food provisioning, since this dependency would have only evolved after the origin of food provisioning (Smiseth et al. 2003b; Klug et al. 2012; Smiseth et al. 2012). The elucidation of the mechanisms driving the early evolution of family life thus requires their investigation in species with less derived family systems (Kölliker 2007; Falk et al. 2014).

Precocial species typically exhibit family systems that are far less consolidated than the systems of altricial species. Family life in precocial species is consequently often only facultative, and the limited phenotypic integration of parental and offspring traits enables juveniles to survive even in the absence of – nonetheless beneficial – parental care (Smiseth et al. 2003b; Kölliker 2007). Notably, these characteristics of family-living in contemporary precocial species suggest that it often (still) bears a close resemblance to the presumed ancestral state of family life (Smiseth et al. 2003b; Kölliker 2007; Falk et al. 2014). Despite these resemblances, the benefits and costs of facultative parental care in precocial species have received comparably little attention (but see, for instance, Eggert et al. 1998; Zink 2003; Smiseth et al. 2003b; Kölliker 2007; Meunier and Kölliker 2012a; Falk et al. 2014). However, investigating the interplay of evolutionary mechanisms that underlie the benefits of family-living in these species might be crucial to clarify the incipient steps in the evolution of family life, and more generally the mechanisms promoting the emergence and consolidation of social life from an ancestral, solitary state (Smiseth et al. 2003b; Kölliker 2007; Falk et al. 2014).

### *Subsocial insects as models of family evolution*

Subsocial insects long stood in the shadow of the more advanced eusocial societies they gave ultimately rise to (Wheeler 1928; Michener 1969; Wilson 1975b; Bourke 2011a). However, recent years have seen a growing interest in these allegedly ‘primitively social’ insects themselves, as they offer unprecedented opportunities to study the origin and maintenance of early forms of parental care and family life (Smiseth et al. 2003b; Kölliker 2007; Trumbo 2012). Subsociality in insects is conventionally thought to arise from the extension of parental care beyond offspring hatching or birth (Michener 1969; Costa 2006), and has independently evolved in various taxa ranging from cockroaches over burying and scarab beetles to shield bugs, crickets, and earwigs (reviewed in Tallamy and Wood 1986; Costa 2006; Trumbo 2012; Wong et al. 2013). The juveniles of many subsocial insects are precocial (also called partially begging), and family life is thus usually facultative (Smiseth et al. 2003b; Agrawal et al. 2004; Costa 2006; Kölliker 2007) – a scenario that likely also prevailed during early stages of the evolution of family life. This resemblance to ancestral family systems, and the great diversity of family interactions across species, render subsocial insects prime models of family evolution.

## STUDY SYSTEM

The European earwig *Forficula auricularia* L. (Dermaptera: Forficulidae) is a cosmopolitan insect species native to Europe, eastern Asia, and northern Africa. It commonly occurs in temperate habitats ranging from wastelands over meadows to anthropogenic fruit groves and gardens (Lamb and Wellington 1975), where it subsists on a wide range of dead and alive plant and animal materials (such as flower pollen, fruits and aphids) that are collected during nocturnal foraging excursions (Lamb and Wellington 1974; Costa 2006). European earwigs have a reddish-brown colored, elongated body of 12 to 15 mm length that features a shield-like pronotum and legs as well as forewings of a brighter, yellowish-brown colorization. They exhibit a conspicuous pair of sexually dimorphic forceps – elongated and, in males, curved and heavily sclerotized cerci – which are used in defense against predators (Costa 2006), during courtship (Tomkins and Simmons 1998), and as weapons in intraspecific competition for resources and mating partners (Lamb 1975; Radesäter and Halldórsdóttir 1993a; Forslund 2000; Forslund 2003). The name earwig (and the specific name *auricularia*) derives from the distinctly human ear-like shape of the membranous hindwings. These hindwings are only rarely used for flight, and usually remain folded under the tegmina; the short, leathery forewings that are referenced by the ordinal name Dermaptera (the Greek words *derma* and *pteron* mean skin and wing, respectively; Costa 2006).

**Figure I.1.** Impressions of earwig social life. (A) A female stands guard over her newly emerged offspring. (B) Stray nymphs are swiftly carried back to the nest by the mother. (C) A female cleans her tightly packed clutch. (D) Two newly emerged adult males (recognizable by their forceps) aggregate with siblings in their last developmental instar. [all pictures © J. Meunier]

### *Parental Care and Family Life*

European earwigs are precocial insects that form subsocial family groups during a part of their life cycle (see Box 1 for details). The formation of these family groups results from the extension of egg care beyond hatching, and entails the expression of elaborate forms of maternal care toward the newly emerged offspring (called nymphs; Lamb 1976a; Costa 2006). Pre-hatching maternal behaviors include the guarding of eggs and their relocation in response to changes in the microclimate within the nest. Moreover, females regularly clean their eggs (thereby mechanically removing microbial pathogens such as fungal spores; Figure I.1) and apply protective chemical substances on the egg surface (Lamb 1976a; Costa 2006; Boos et al. 2014). Conversely, post-hatching maternal care comprises the protection, relocation, and grooming of nymphs (Figure I.1), as well as their provisioning with food through regurgitation or the placement of pieces of food in the nest (Lamb 1976a; Staerkle and Kölliker 2008; Mas and Kölliker 2011; Meunier et al. 2012).

In contrast to the pre-hatching (egg) care, these forms of post-hatching care are – despite their sometimes substantial benefits – not obligatory for offspring survival (Kölliker and Vancassel 2007; Kölliker 2007). This is because the precocial nymphs can forage independently soon after hatching and are thus not fully reliant on resources supplied by their mother (Lamb 1976a; Wong and Kölliker 2012). Moreover, earwig nymphs have been shown to share food with their siblings by proctodeal trophallaxis and active allo-coprophagy, processes characterized by a socially induced increase in feces excretion by donor nymphs and the subsequent consumption of these feces by recipient nymphs (Falk et al. 2014); see also Körner et al. 2016). As a consequence of these alternative offspring survival strategies (cf. Smiseth et al. 2003b), the cost-benefit ratio of family life in *F. auricularia* strongly depends on environmental factors such as predation or resources availability (Kölliker and Vancassel 2007; Kölliker 2007; Meunier and Kölliker 2012a).

### THE LIFE-CYCLE OF THE EUROPEAN EARWIG **BOX 1**

**Adult mating and aggregation:** A new generation of *F. auricularia* adults emerges each year in early summer (Lamb and Wellington 1975; see Figure I.2). Adult European earwigs are polygamous (Costa 2006; Sandrin et al. 2015) and gregarious (Lamb 1975; Sauphanor and Sureau 1993). They typically congregate in mixed-sex aggregations of up to several hundred individuals in daytime-shelters throughout summer (Lamb 1975; Lamb and Wellington 1975).

**Egg-laying and pre-hatching care:** In late autumn, females dissociate themselves from the daytime-aggregations and dig a nest into the soil, often next to partially-buried stones or plant roots (Lamb 1976a). Although single males may initially linger in the nest, they are eventually driven away by the female over the course of winter, before she deposits her first clutch (comprising about 50 eggs on average; Lamb 1975; Lamb and Wellington 1975; Lamb 1976a). Upon egg deposition, females enter a period of starvation (Lamb 1976a; Vancassel 1984) and continuously tend their clutch until their offspring hatch in the spring of the following year (Lamb and Wellington 1975; Lamb 1976a).

**Post-hatching care and family life:** After egg hatching, females stay with their brood for several weeks, usually until after the juveniles reached the second of four developmental instars (Lamb 1976b; Wong and Kölliker 2012). During this period, females typically provide extensive forms of maternal care to their offspring, for instance in the form of guarding and food provisioning (Lamb 1976a; Costa 2006; Kölliker 2007; Meunier et al. 2012).

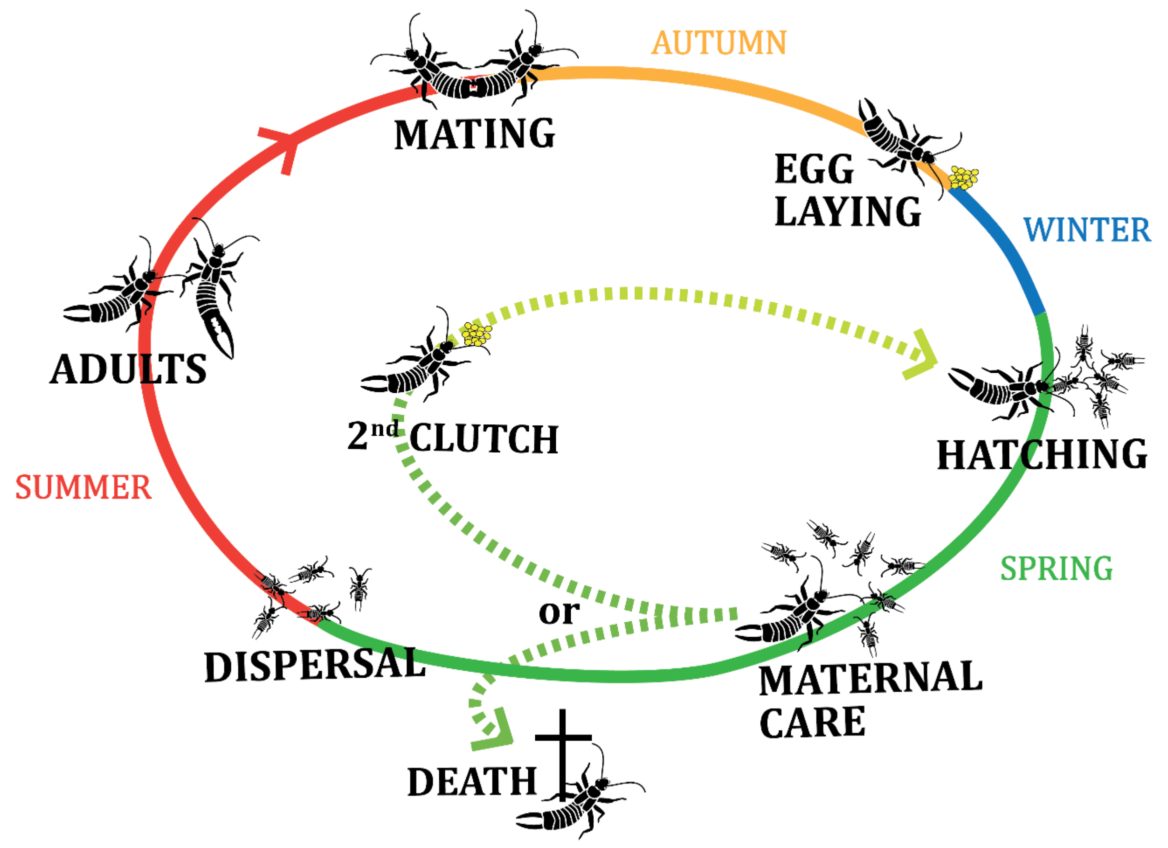

**Figure I.2.** The life-cycle of the European earwig [modified with permission; © J. Meunier].

**Dispersal, maturation, and 2nd clutch production:** After the period of family life, the offspring disperse from their natal nest and enter a free-foraging phase to complete their development into the adult stage (Lamb 1976b). Likewise, the females disperse from the nest and attempt to produce a second, usually much smaller, clutch in late spring (without re-mating; Lamb and Wellington 1974; Lamb 1976b; Vancassel 1984; Meunier et al. 2012).

## THIS THESIS

Despite our extensive knowledge of the mechanisms shaping social interactions in derived family systems, we still know only little about the selective forces that promoted the evolutionary origin and subsequent consolidation of family life. In particular, our understanding of the behavioral mechanisms shaping these early evolutionary stages is sketchy at best, because only few empirical studies so far investigated these mechanisms in non-derived family systems. Moreover, we remain largely unaware of how the interplay among such mechanisms interacts with environmental and ecological conditions to shape the life-history and fitness of family members. The research project presented in this thesis aimed at contributing to the closure of this gap in knowledge. In particular, we (1) investigated the nature and interplay of family interactions in the precocial European earwig *F. auricularia* to improve our knowledge of the behavioral mechanisms shaping the early evolution of family-living. Moreover, we (2) studied the joint effects of social interactions, life-history characteristics, and environmental conditions on the benefits and costs of earwig family life to determine how their interplay might shape the evolution of early family systems. Finally, we (3) reviewed an enduring controversy between two major theories of sociobiology – kin selection and multilevel selection theory – to clarify how they might be fruitfully combined to gain a better understanding of the evolutionary transition from solitary to family life.

### *Part 1 – The behavioral mechanism shaping family life*

In the first part of this thesis, we investigated the relationships between different types of social interaction among European earwigs. We thus aimed at elucidating the interplay between behavioral mechanisms underlying the fitness benefits of facultative social life.

Parental behaviors are the most prominent type of behavioral mechanisms affecting family life, since they typically confer substantial benefits to the offspring. However, offspring can also benefit from cooperating with each other (Roulin and Dreiss 2012), and earwig nymphs indeed regularly share food with siblings (Falk et al. 2014). Sibling cooperation could hence be an important mechanism complementing the benefits of facultative parental care in non-derived family systems. In chapter 1, we examined this intriguing possibility by investigating the potential codependency of sibling food transfer and maternal food provisioning. We showed that the levels of maternal provisioning and sibling food sharing are indeed negatively correlated, indicating that high levels of sibling cooperation might compensate for insufficient levels of maternal care.

The level of parental care is generally condition-dependent, because parents in poor condition incur higher relative costs when providing care (Hinde et al. 2010). The association between maternal care and sibling cooperation hence suggests that the propensity of earwig nymphs to share food with their siblings might depend on the condition of their mother – a possibility that we investigated in chapter 2. Indeed, maternal condition did not only affect the level of maternal care, but also the level of sibling food sharing, suggesting that juveniles can flexibly adjust their behavior towards siblings in response to changes in parental care.

The far-reaching consequences of (changes in) parental behaviors are triggered by their often substantial fitness effects, and thus render parental care the prime driver of group-living in family units. By contrast, the mechanisms mediating the benefits of other forms of group-living are often less clear (Krause and Ruxton 2002). In chapter 3, we investigated whether intimate behavioral interactions characteristic for family life (such as food sharing and allo-grooming) might extend into – and thus help to explain the benefits of – the gregarious adult stage. We showed that adult earwigs only infrequently engaged in social interactions, indicating that adult gregariousness is only partly motivated by benefits of behaviors characteristic for family life.

### *Part 2 – Social behavior, life-history, and environmental conditions*

In the second part of this thesis, we studied the interplay of family interactions, life-history characteristics, and environmental conditions. We thereby sought to determine how these factors might jointly shape early stages in the evolution of family life.

Environmental conditions are often granted a crucial role in the early evolutionary stages of family life. However, the effect of specific conditions is often hard to predict as long as the behavioral interactions affected by these conditions remain unknown. For instance, harsh environments have traditionally been predicted to promote the evolution of family life (Clutton-Brock 1991), but Meunier and Kölliker (2012a) recently showed that they can actually render facultative family life detrimental to offspring fitness – possibly because offspring have to compete with their parents for limited resources. In chapter 4, we explored this intriguing possibility by investigating the occurrence, as well as potential determinants and consequences of parent-offspring competition under food limitation in the European earwig. We showed that competitive weight gains of mothers reduced the survival of their offspring, suggesting that parent-offspring competition might generally hinder the evolution of family life in resource-poor environments.

The possible occurrence of parent-offspring competition notwithstanding, parental presence is usually beneficial in caring species. In these species, the loss of parents amounts to the discontinuance of care, and thus usually entails short-term – and sometimes even long-term and transgenerational (Klug and Bonsall 2014). However, these long-term costs have so far only been investigated in altricial vertebrates, and their occurrence in precocial species – and thus their potential impact on the early evolution of family life – remains unknown. In chapter 5, we thus investigated the consequences of maternal loss in the European earwig. Surprisingly, maternal loss increased offspring body size while impairing their expression of parental care in the adult stage, indicating that maternal absence was beneficial in the short run, but costly in the long run. This suggests a crucial role of such long-term costs in the maintenance of early family life.

The costs of parental loss impressionably illustrate the usual benefits of parental care to offspring. These benefits, however, typically come at costs for the parents, and can affect both their survival and their ability to invest into future reproduction. Parental effort should hence change over time, either because the deteriorating state of older parents reduces their reproductive investment (McNamara and Houston 1996; Javoiš 2013), or because older parents attempt to increase their investment into their terminal reproductive attempt (Williams 1966b; Javoiš 2013). In chapter 6, we examined these predictions by assessing the variation of maternal and offspring life-history traits between the initial and the terminal reproductive event of *F. auricularia* females from three distinct populations. We showed that females produced larger first clutches, but expressed higher levels of care toward second clutches, indicating that both parental senescence and terminal investment jointly shape the variation between reproductive events. Moreover, we found that part of this variation was population-specific, suggesting that population idiosyncrasies might have an important influence on parental reproductive investment.

## *Part 3 – The behavioral mechanism of early family life*

In the third and last part of this thesis, we took a broad theoretical perspective on the theoretical foundation of sociobiology. We thereby wanted to ascertain how the study of family life might inform – and be informed by – the further development of the current theory of social evolution.

When Charles R. Darwin first suggested that selection at the family-level might explain the evolution of sterile worker castes in eusocial insects, he unknowingly laid the foundation for the development of two major theoretical frameworks of modern sociobiology – kin selection theory and multilevel selection theory (Okasha 2006; Foster 2009; Marshall 2015). However, he thereby also inadvertently initiated an enduring controversy about the relevance of selection acting on the (family) group level. In the third and final part of this thesis (chapter 7), we reviewed this controversy and explored the applicability of the kin and multilevel selection approaches to the study of the ephemeral family groups of subsocial insects. We suggest that both approaches provide different, but complementary perspectives on social evolution, and thus could be fruitfully combined to gain a better understanding of the evolutionary transition from solitary to social life. Intriguingly, kin selection theory might provide a better causal representation of the underlying evolutionary process as long as groups are facultative and ephemeral, whereas multilevel selection theory might be more causally apt once the fate of the group as a whole fully determines the fate of the constituent individuals.

# CHAPTER 1

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

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*Based on*

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## ABSTRACT

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

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

## INTRODUCTION

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

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

Over the last decades, offspring benefits of family life were traditionally attributed to interactions between parents and offspring in the form of parental care (reviewed in Royle et al. 2012b). However, an often overlooked source of benefits is sibling cooperation (Forbes 2007; Roulin and Dreiss 2012), which is promoted by the additional indirect fitness benefits of assisting genetically related individuals (Hamilton 1964a; West et al. 2002). Cooperation among adult siblings is common in nature, such as in cooperatively breeding vertebrates (Clutton-Brock 2002; Koenig and Dickinson 2004) or in colonies of eusocial insects (Wilson 1971), but an increasing number of studies also reports cooperation among juvenile siblings. For instance, offspring express mutual cleaning in the Mississippi Kite *Ictinia mississippiensis* ("allo-preening; Botelho *et al.*, 1993) and the ambrosia beetle *Xyleborinus saxesenii* (Biedermann and Taborsky 2011), as well as share food in the Common Barn-owl *Tyto alba* (Roulin et al. 2012), the huntsman spider *Delena cancerides* (Yip and Rayor 2013), and the European earwig *Forficula auricularia* (Falk et al. 2014).

Although both sibling cooperation and parental care may provide substantial benefits to juveniles during family life, it remains surprisingly unexplored how these behaviors are related when they co-occur. Assessing the modality of their co-occurrence would allow to determine their independent or joined roles, as well as their respective importance in the evolutionary transition from solitary to group living (Falk et al. 2014). The association between sibling cooperation and parental care - if any - could either be complementary or compensatory. In the first case (here termed complementarity hypothesis), the level of sibling cooperation is predicted to be positively correlated with the level of parental care. This scenario could, for example, be based on a higher propensity of siblings to cooperate with each other when the level of parental care is high, which in turn should reduce offspring competition and conflict (Roulin and Dreiss 2012) that are otherwise predicted to hamper cooperation (Frank 1998). Such a positive correlation could be expected in altricial species, in which offspring exclusively rely on parental resources. In the second case (here termed compensation hypothesis), the level of sibling cooperation is expected to be negatively associated with the level of parental care. This scenario likely applies to precocial species, which exhibit a non-derived and non-obligatory form of family life. In these species, offspring do not exclusively rely on parental resources, but instead either have direct access to the resources used as nest material (e.g. carrion and dung) or are mobile and capable of independent resource acquisition in the vicinity of the nest site. Consequently, offspring competition over parental resources could be reduced and offspring could benefit from sharing independently acquired resources with siblings (Falk et al. 2014), particularly when parental investment is insufficient. Under such circumstances, resource transfer among siblings could even release parents (at least partly) from offspring provisioning.

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

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

## MATERIAL AND METHODS

### *Study animals and laboratory rearing*

The adult female earwigs used in our experiment descended from 160 individuals collected in a natural population in Dolcedo, Italy in September 2012. These field-caught earwigs had been maintained in the laboratory under standard rearing conditions (detailed in (detailed in Meunier et al. 2012; Koch and Meunier 2014) for one generation. After emergence, F1-adults were maintained in large plastic containers (37 x 22 x 25 cm) for three months to allow uncontrolled mating in sex-balanced groups of adults with different genetic origins. The following experiment involved a random sample of 54 of these F1 females and their subsequent first clutch of offspring.

### *General experimental setup*

We successively measured the levels of sibling food transfer and maternal food provisioning in 54 clutches (see experimental details in Figure 1.1). Four days after egg hatching, mothers were isolated from their clutches and the nymphs randomly attributed to either of two groups of equal size termed SFT- and MFP-groups. The SFT-groups were used to measure the level of sibling food transfer (details below), whereas the MFP-groups were used to measure the level of maternal food provisioning (details below). Because the mothers were involved in both types of measurements, 28 clutches were used to first measure sibling food transfer (days four to five) and then maternal food provisioning (days eight to nine), whereas the order of tests was reversed in the other 26 clutches (Figure 1.1). In between the two measurements (i.e. from day five to day eight), the two groups of nymphs were maintained separately and received an *ad libitum* amount of uncolored pollen pellets as food source (naturally yellow colored flower pollen formed into pellets; Hochland Bio-Blütenpollen, Hoyer GmbH, Polling, Germany). During the same period, mothers were isolated and likewise fed with uncolored pollen pellets.

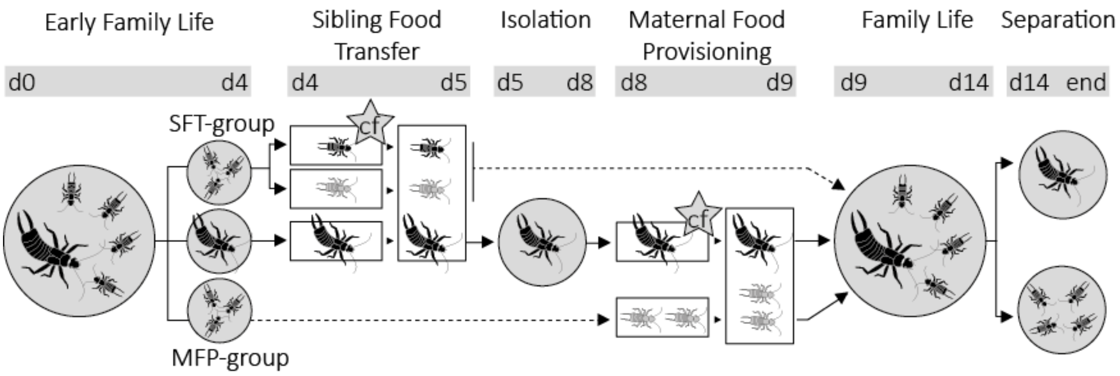

**Figure 1.1. Experimental setup.** White boxes indicate measurements of sibling food transfer and maternal food provisioning. Sibling food transfer was measured by providing the SFT-group of nymphs (called donor nymphs) with colored food (indicated with "cf"), then reassembling these newly colored nymphs with a group of food deprived siblings (called recipient nymphs; grey individuals) and their mother, allowing family interactions overnight and finally counting the number of recipient nymphs that ingested the colored food provided by the donor nymphs. Conversely, maternal food provisioning was measured by providing the mother with colored food, then reassembling the fed mother with the MFP-group of nymphs previously set aside, allowing them to interact overnight and finally counting the number of nymphs that ingested the colored food provided by the mother. Note that the order of food transfer test was reversed in half of the tested families.

Once sibling food transfer and maternal food provisioning had been measured, we investigated the association between these measurements and offspring fitness and/or female quality by measuring nymph development and survival, as well as maternal investment in their 2nd clutch. To this end, nymphs from SFT- and MFP-groups were reassembled with their mother on day nine. Five days later, the mothers were isolated to mimic natural family disruption and allow the production of a second clutch (Meunier et al. 2012), whereas the nymphs were maintained in groups until adult emergence. Nymph development time was recorded by checking daily for the emergence of the first second-instar nymph in each clutch, and the proportion of nymphs that survived until adulthood was assessed by counting the number of nymphs that molted into adults and then dividing this number by the number of nymphs that initially entered the experiment. Finally, maternal investment in their 2nd clutch - if any – was measured by counting (1) the number of days between their isolation for 2nd clutch production and oviposition, (2) the number of 2nd clutch eggs present three days after the first egg laying had been observed (egg laying takes up to three days), as well as (3) the number of 2nd clutch nymphs present one day after the first hatching had been observed (egg hatching generally occurs over a single day).

Groups of nymphs (and, when not isolated separately, their mother) were maintained in medium sized Petri dishes (9 x 2 cm) until the end of family life (on day 14) and subsequently in large Petri dishes (13.8 x 2 cm) until adult emergence. Each Petri dish contained humid sand as ground material and a plastic tube as shelter. During their isolation from day five to day eight, mothers were maintained in small Petri dishes (5.5 x 1.2 cm) inlaid with a moist paper towel. Mothers and nymphs received *ad libitum* uncolored pollen pellets as food source from hatching to day three. Conversely, nymph and mothers were provided with an *ad libitum* amount of artificial diet twice a week from day nine until the end of the experiment (food composition detailed in the supplementary material). Note that we used pollen instead of this artificial diet during sibling food transfer and maternal food provisioning tests because of its better dyeability (see below). Groups of nymphs and mothers were always food deprived one day prior to the sibling food transfer and maternal food provisioning tests to increase foraging and solicitation behaviors (of the nymphs) on the following day (Staerkle and Kölliker 2008; Falk et al. 2014).

### *Measuring sibling food transfer and maternal food provisioning*

The measurements of sibling food transfer and maternal food provisioning were implemented by taking advantage of an exceptional property of *F. auricularia* nymphs: ingested colored food is visible through the partially transparent cuticle of first-instar nymphs and can thus be used as a marker of food transfer between family members (Staerkle and Kölliker 2008; Falk et al. 2014). In brief, sibling food transfer was measured by (1) providing half of the nymphs of the SFT-group with green-colored food, then (2) reassembling these colored (donor-) nymphs with their uncolored remaining siblings (recipients) and their mother, (3) allowing family interactions overnight and finally (4) counting the number of newly colored recipient nymphs. To this end, we first divided each SFT-group into two sub-groups of equal size (Figure 1.1). All nymphs of one of the sub-groups were marked by clipping off the distal third of the right cercus (Wong and Kölliker 2013). This marking had no influence on the proportion of newly green-colored nymphs in the sibling food transfer tests (Wilcoxon rank sum test; W = 288.5, P = 0.967). After marking, we randomly selected either the marked or the unmarked subgroup and transferred it for one hour to a small Petri dish containing an *ad libitum* amount of green-colored food (donor sub-group; naturally yellow colored pollen mixed with a blue food dye; Dekoback, Online Ideen GmbH, Germany). Meanwhile, the other (recipient) sub-group was food-deprived whereas the mother was provided separately with uncolored pollen. The nymphs of the donor and recipient sub-group were then assembled overnight with their mother in a medium-sized Petri dish. Fifteen hours after the setup, we counted all nymphs in their respective sub-groups and determined the number of newly green-colored nymphs in the recipient sub-group under a stereo-microscope. We fed mothers, and thus allowed for maternal provisioning during sibling food transfer tests, because this ensured a more direct link between our measures of maternal food provisioning and sibling food transfer. Note that mothers were isolated and fed with uncolored pollen pellets between day five and eight to ensure that the feces of mothers first involved in the maternal food provisioning measurement (see below) had lost their coloration before sibling food transfer measurements.

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

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

### *Statistical analyses*

All statistical analyses were performed using the software R 3.0.1 (http://www.r-project.org/) complemented with the packages “car” and “MASS”. We first tested the overall importance of sibling food transfer (SFT) and maternal food provisioning (MFP) on the gain in coloration of recipient nymphs using a generalized linear mixed model (GLMM) with a binomial error distribution corrected for overdispersion. In this model, the proportion of colored recipient nymphs was entered as response variable (via the “cbind”-function in R), while the type of test (SFT or MFP; categorical), the order of the tests (SFT/MFP or MFP/SFT; categorical), clutch size (continuous) and all interactions among these three factors were entered as explanatory variables. Because each clutch was used to measure both sibling food transfer and maternal food provisioning, clutch-ID was entered as a random factor into the model. To control whether differences in the number of recipient nymphs involved in the sibling food transfer and maternal food provisioning tests drove the results of the above model, we conducted an additional linear mixed model (LMM), in which we used the same set of explanatory and random variables but entered the absolute number of colored recipient nymphs as response variable. The potential influence of clutch size on the distribution of food among multiple recipient nymphs during sibling food transfer tests was analyzed in a generalized linear model (GLM) with binomial error distribution corrected for overdispersion. In this model, we entered the proportion of weakly colored nymphs among all colored recipients as response and clutch size (continuous) as explanatory variable.

We then analyzed whether the level of sibling food transfer was positively (complementarity hypothesis) or negatively (compensation hypothesis) associated with the level of maternal food provisioning in each clutch. To this end, we tested the correlation between the deviations from the predicted levels of sibling food transfer and of maternal food provisioning using a Pearson product-moment correlation. These deviations were defined as the residuals of sibling food transfer and maternal food provisioning from the first model, i.e. the parts of the proportions of colored recipient nymphs in sibling food transfer and maternal food provisioning tests, respectively, that were not explained by clutch size and order of testing. Note that we back-transformed the residuals to their original (i.e. proportional) scale to facilitate their interpretation in the figures.

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

Statistical models were simplified stepwise by removing non-significant interaction terms ($P > 0.05$). The significance-level for the analyses of maternal 2nd clutch production and fitness traits in offspring was adjusted to correct for multiple testing using the MFDR (Mean False Discovery Rate) approach to $\alpha c = 0.029$ according to $\alpha c = \frac{n+1}{n \times 2} \times 0.05$ where n denotes the number of tests. Our analyses involved 48 of the 54 clutches initially set up. Among the 6 clutches not used in the analyses, (1) three were excluded because nymphs of the donor-group either escaped or were cannibalized by their siblings, which potentially could have biased our measure of food transfer, (2) two clutches showed an exceptionally high proportion of donor nymphs that failed to feed on the green colored food prior to the sibling food transfer test (50 and 70%, respectively vs. 4 ± 8 % (Mean ± SD) in the remaining clutches) and finally (3) one clutch was excluded because the mother still produced green-colored feces prior to the sibling food transfer test, which prevented reliable measurements of sibling food transfer. As a result, the analyzed data set comprised 24 of the 28 clutches that were subjected to sibling food transfer measurements on day four, and 24 of the 26 clutches used to measure sibling food transfer on day eight. A total of 41 out of 48 (85.4%) mothers produced a 2nd clutch and were used to analyze the time from isolation to 2nd clutch production, as well as the hatching success of the 2nd clutch.

## RESULTS

Overall, food transfer among nymphs occurred more frequently than maternal provisioning (94% vs. 67% of all clutches; $\chi_1^2 = 9.45$, $P = 0.002$). The proportion of newly-colored recipient nymphs varied substantially between families and ranged from 0 to 100% after both types of food transfer test, with a median value of 75% of recipient nymphs newly colored in sibling food transfer test and 22% in maternal food provisioning tests (Wilcoxon signed rank test, $V_{47} = 169$, $P < 0.001$; Figure S1.1). These values are comparable to the proportions presented in previous studies (Meunier et al. 2012; Meunier and Kölliker 2012b; Falk et al. 2014), indicating that they are unlikely to only reflect the different initial numbers of recipient nymphs during the two types of food transfer tests, as well as the limited contacts between mother and nymphs during the experiment.

**Table 1.1.** Effects of type of test (sibling food sharing or maternal food provisioning), clutch size and day of food-transfer test on (A) the proportion and (B) the number of nymphs that received food from family members. Significant P-values are in bold print.

| | (A) Proportion of nymphs colored | | (B) Number of nymphs colored | |
|---|---|---|---|---|
| | Wald $\chi_1^2$ | p | Wald $\chi_1^2$ | p |
| Type of food-transfer (TFT) | 44.44 | **< 0.0001** | 2.72 | 0.0992 |
| Clutch size (CS) | < 0.01 | 0.9533 | 19.46 | **< 0.0001** |
| Day of food-transfer test | 8.19 | **0.0042** | 5.95 | **0.0147** |
| TFT:CS | 6.85 | **0.0089** | 5.33 | **0.0209** |

An interaction between clutch size and the type of food transfer shaped the proportion of recipient nymphs that became colored during the food transfer tests (Table 1.1A, Figure 1.2A). Specifically, the proportion of colored nymphs was positively associated with clutch size in the sibling food transfer tests (Estimate ± SE: 0.035 ± 0.015, $t_{46} = 2.43$, $P = 0.019$), but not in the maternal food provisioning tests (Estimate ± SE: -0.016 ± 0.019, $t_{46} = -0.86$, $P = 0.386$). The contrasting influence of clutch size on maternal food provisioning and sibling food transfer was also present when analyzing the absolute number of newly colored recipient nymphs (Table 1.1B, Figure 1.2B). Accordingly, the number of nymphs that had received food increased with clutch size in the case of sibling food transfer (Estimate ± SE: 0.240 ± 0.028, $t_{46} = 8.635$, $p < 0.001$), but not in the case of maternal food provisioning (Estimate ± SE: 0.086 ± 0.073, $t_{46} = 1.18$, $p = 0.243$). Contrary to the expectation that competition for a constant per capita amount of feces increases with clutch size, the ratio of nymphs that were weakly colored after sibling food transfer tests did not depend on clutch size ($\chi_1^2 = 0.44$, $P = 0.501$). Independent of other effects, the proportion and absolute number of nymphs that received food from family members was higher on day eight (Mean ± SE; proportion = 0.55 ± 0.05; absolute = 7.75 ± 0.82) than on day four (Proportion = 0.42 ± 0.05; absolute = 5.67 ± 0.69) after hatching (Table 1.1A and 1.1B, Figure S1.2), presumably reflecting the increased nutritional needs of older nymphs (Wong and Kölliker 2012).

The level of sibling food transfer was negatively associated with the level of maternal food provisioning after taking the influences of clutch size and the day of the respective food transfer test into account ($\rho = -0.306$, $S_{46} = 24062$, $P = 0.035$, Figure 1.3). This result is in line with the hypothesis of a compensatory relationship between sibling cooperation and parental care.

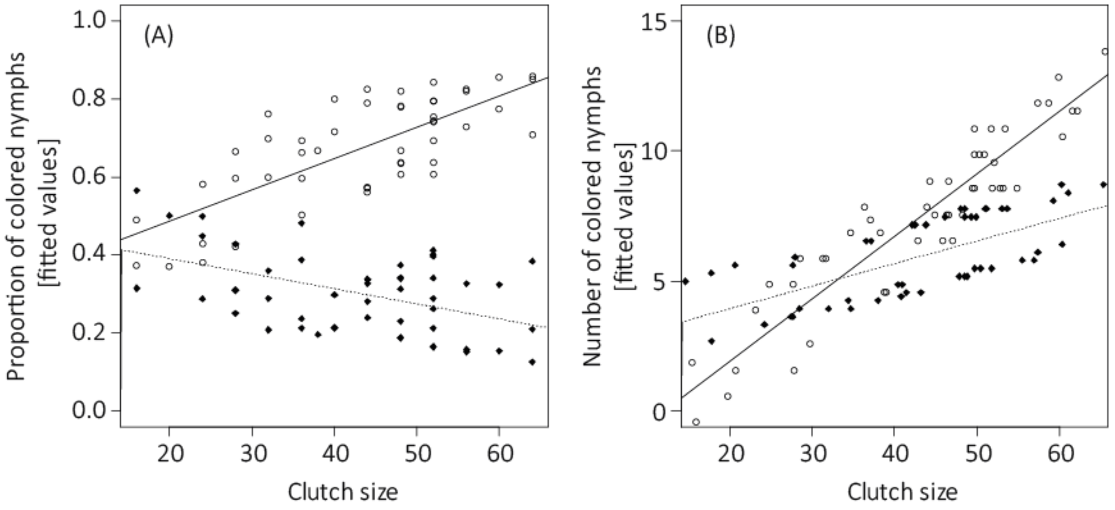

**Figure 1.2**. Influence of clutch size on the proportion (A) and number (B) of nymphs that received food during maternal food provisioning (filled squares) and sibling food transfer (open circles).

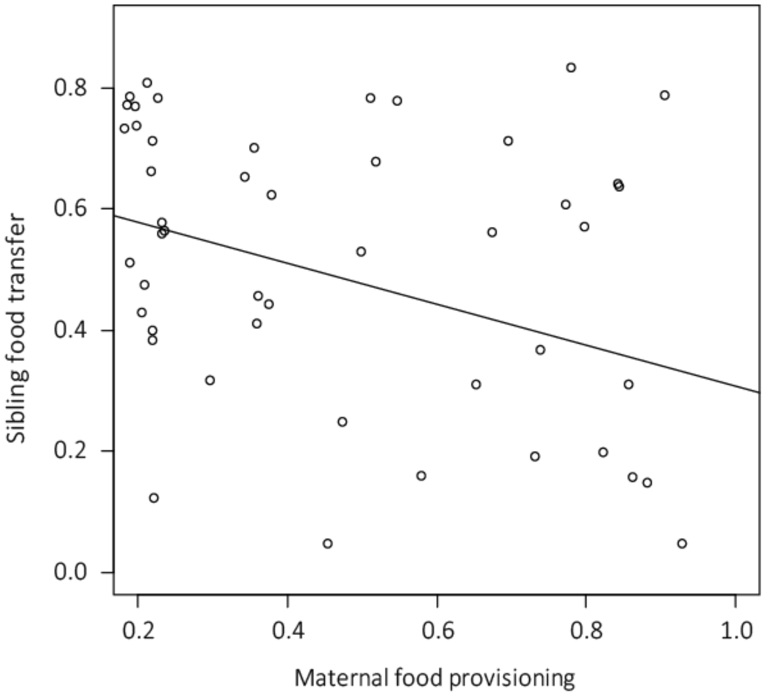

**Figure 1.3**. Correlation of the residuals of sibling food transfer and maternal food provisioning after taking the influences of clutch size and the day of the respective food transfer test into account.

Finally, the level of food transfer among siblings was correlated with the expression of fitness-relevant traits in mothers, but not in their offspring. Specifically, higher than predicted levels of sibling food transfer were associated with increased delays in the mother's production of a 2nd clutch (Table 1.2A, Figure 1.4A) and reduced numbers of 2nd clutch eggs (Table 1.2A, Figure 1.4B). They were however not linked to the occurrence of 2nd clutch production (Table 1.2A), the hatching success of the 2nd clutch eggs (Table 1.2A) or the number of resulting nymphs (Table 1.2A). Higher than predicted levels of sibling food transfer were neither associated with the development time of 1st clutch offspring (Table 1.2B), nor with the probability of their survival until adulthood (Table 1.2B).

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

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

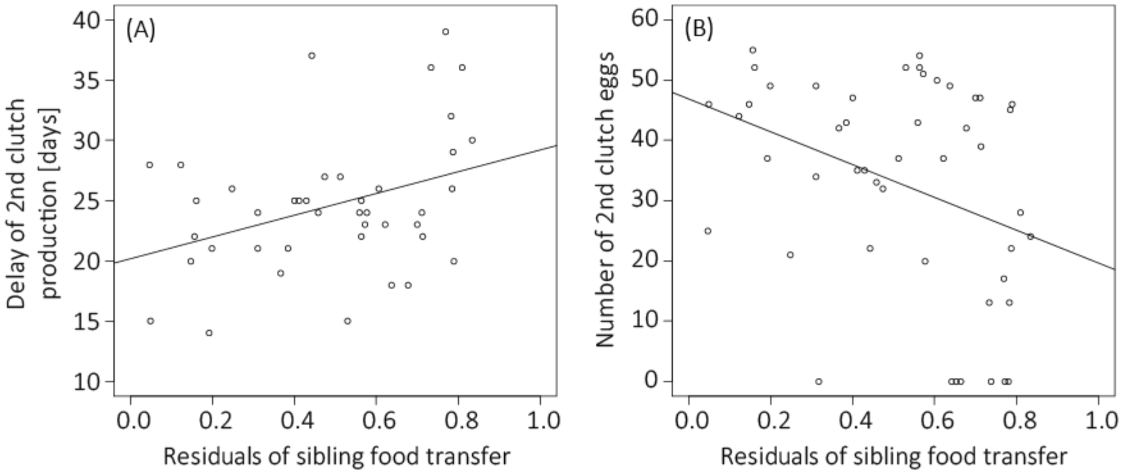

**Figure 1.4**. Correlation of the residuals of sibling food transfer with (A) the duration from maternal isolation until 2nd clutch production, and (B) the number of eggs in the 2nd clutch.

## DISCUSSION

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

In this study, we showed that, in the European earwig, the level of sibling food transfer (a form of sibling cooperation characterized by a socially induced, kin-directed production of feces that can be consumed by other group members) - but not of maternal food provisioning (a form of parental care) - increased with group size. Notably, this increase was not associated with a change in food distribution among offspring, a result expected under the assumption that recipient nymphs do not compete more intensively for donor feces in larger clutches. Furthermore, higher than predicted levels of sibling food transfer (with regards to clutch size and the day of measurement) were associated with lower than predicted levels of maternal food provisioning and *vice versa*. This finding is in line with the hypothesis of a compensatory relationship between sibling food transfer and maternal food provisioning. Finally, higher than predicted levels of sibling food transfer were associated with a delayed production and reduced size of the mothers' 2nd clutch, but not with the development time and the survival of 1st clutch nymphs until adulthood, the hatching success of the 2nd clutch, or the resultingnumber of nymphs.

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

2012a). The overall higher prevalence of sibling food transfer as compared to maternal food provisioning is in line with this scenario, as it suggests that sibling food transfer also occurred in families in which the mother did not provision (this study), or was experimentally prevented from provisioning her offspring (Falk et al. 2014). Conversely, sibling food transfer might have evolved secondarily to compensate for low levels of maternal food provisioning. This alternative scenario is however unlikely since the evolution of parental provisioning also drives the evolution of increased levels of sibling competition (Smiseth et al. 2007; Gardner and Smiseth 2011), which in turn should impede the evolution of sibling cooperation (Frank 1998; West et al. 2001). As a consequence, some forms of sibling cooperation, especially if they involve the exchange of resources acquired independently from parents, could be lost when the evolution of parental provisioning progresses.

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

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

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

Sibling cooperation by definition entails benefits for offspring, but cooperative interactions among their offspring could also benefit parents, for example by reducing offspring demand and hence allowing parents to reduce investment in parental care. However, earwig mothers tending clutches with higher than predicted levels of sibling food transfer did not produce larger 2nd clutches, despite the fact that they simultaneously showed low levels of maternal food provisioning. Interestingly, these mothers even produced fewer 2nd clutch eggs and delayed the production of their 2nd clutch longer than mothers tending nymphs that showed lower levels of sibling food transfer. Hence, mothers likely do not selectively retain resources for the production of their 2nd clutch when their 1st clutch offspring shows high levels of sibling food transfer. Instead, the combination of low levels of maternal food provisioning during 1st clutch family life and the small size and delayed production of the 2nd clutch suggests that variation in maternal investment into offspring care and future reproduction reflect differences in intrinsic female quality (Reznick et al. 2000; Koch and Meunier 2014). Whether and how such differences in female (and nymph) quality affect sibling food transfer will be investigated in further studies.

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

## ACKNOWLEDGEMENTS

[removed for privacy purposes]

## SUPPLEMENTARY MATERIAL

**Table S1.1**. Effects of the deviation from the predicted level of maternal food provisioning on either (A) traits of mothers and nymphs in the second clutch or (B) traits of nymphs in the first clutch. Statistical values were obtained from linear models (LM) or generalized linear models (GLM). P-values that remained significant after correction for multiple testing are in bold.

| | Deviation from predicted sibling food transfer | | | | |
|---|---|---|---|---|---|
| | Model | n | Estimate ± SE | t | P |
| **(A) Second clutch** | | | | | |
| Occurrence of 2$^{nd}$ clutch production | GLM | 48 | 0.49 ± 0.49 | 1.01 | 0.320 |
| Days between isolation and egg laying | LM | 41 | -2.51 ± 0.60 | -4.19 | **<0.001** |
| Egg number | LM | 48 | 5.72 ± 1.91 | 3.00 | **0.004** |
| Hatching success | GLM | 41 | 0.14 ± 0.20 | 0.67 | 0.510 |
| Nymph number | LM | 48 | 4.62 ± 2.05 | 2.25 | 0.030 |
| **(B) First clutch** | | | | | |
| Nymph development time | LM | 48 | 0.18 ± 0.11 | 1.72 | 0.092 |
| Nymph survival until adulthood | GLM | 48 | -0.01 ± 0.04 | -0.17 | 0.864 |

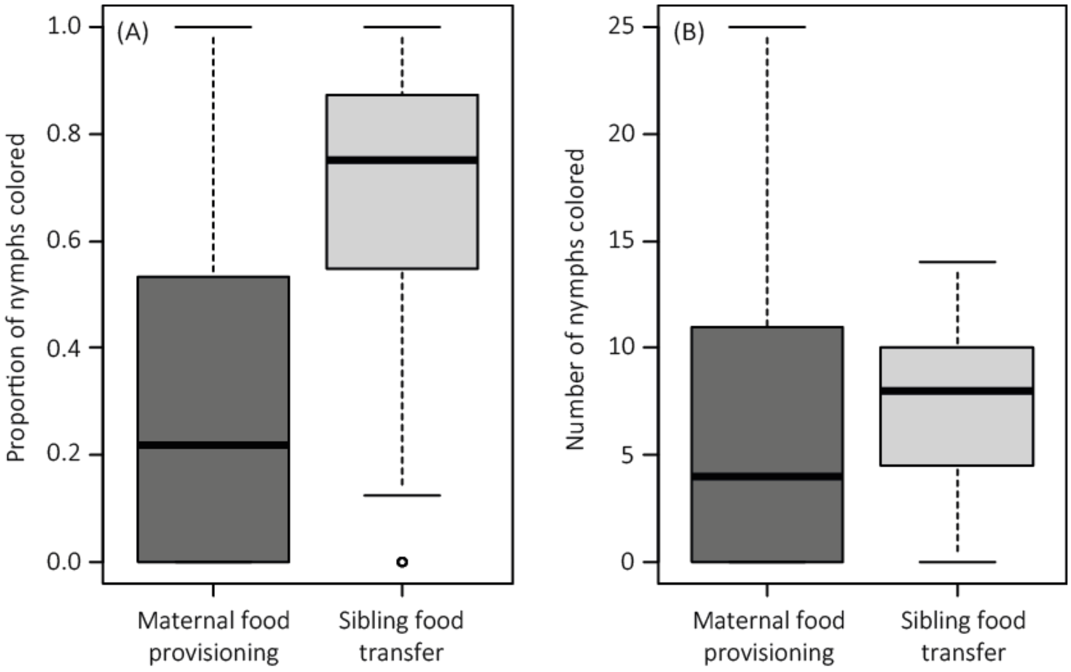

**Figure S1.1.** Proportion (A) and number (B) of colored recipient nymphs after the maternal food provisioning and sibling food transfer tests, respectively.

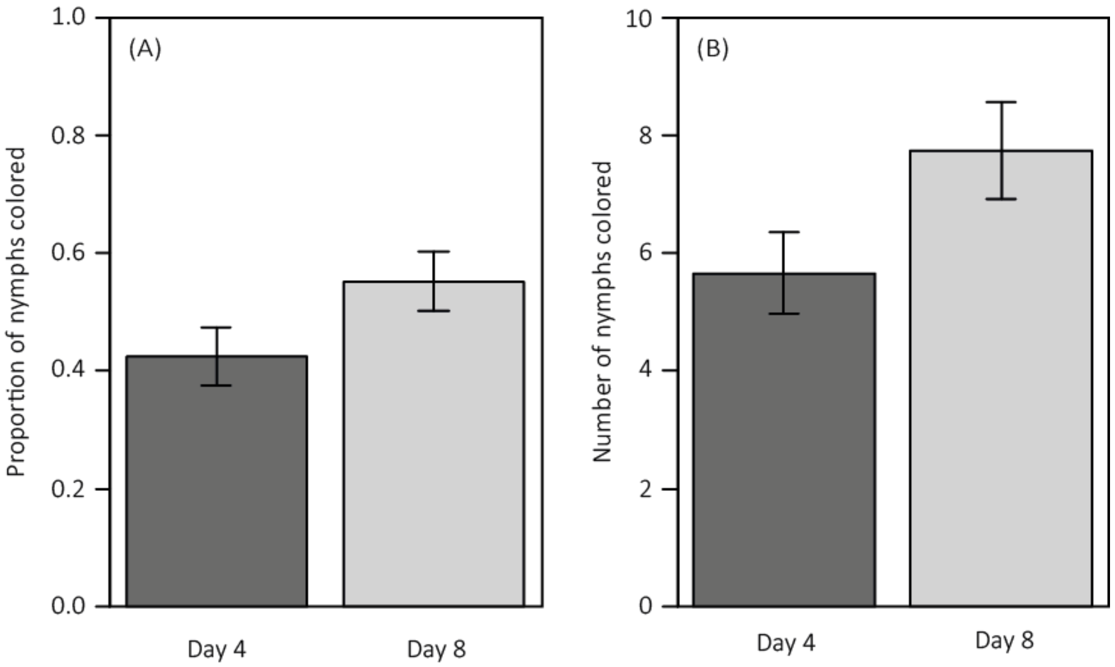

Figure S1.2. Proportion (A) and absolute number (B) of recipient nymphs colored after the food transfer tests on day 4 and day 8 after hatching, respectively.

**Composition of the artificial diet:** 60g weed germ, 120g carrots, 60g bird food (Beo Special, Vitakraft, Bremen, Germany), 60g cat food (Whiskas Senior, Mars GmbH, Viersen, Germany), 60g pollen (Hochland Bio-Blütenpollen, Hoyer GmbH, Polling, Germany), 1 egg (including a piece of its shell), 40g Agar, 2g Sorbic Acid, 2g Ascorbic Acid and 1600ml water

# CHAPTER 2

# Maternal condition determines offspring behavior toward family members in the European earwig

**Jos Kramer** & Joël Meunier

*Based on*

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## ABSTRACT

Parental care confers benefits to juveniles, but is usually associated with substantial costs for parents. These costs often depend on parental condition, which is thus considered as a key determinant of the level of parental care expressed during family life. However, how parental condition affects the behaviors that juveniles express towards their siblings and parents remains poorly explored. Here, we investigated these questions in the European earwig *Forficula auricularia*, an insect in which mothers provide extensive forms of care to their juveniles. We measured maternal body condition at egg hatching, subsequently manipulated maternal nutritional state and finally assessed both food transfer among siblings and the nature of mother-offspring interactions. We also considered variation in brood size, an important parameter in family interactions. We found that food transfer among siblings increased with brood size when the tending mothers were in a deteriorated nutritional state. This effect was masked when the nutritional state of mothers was enhanced. By contrast, the frequency of care-related behaviors that juveniles expressed towards their mother was independent of brood size, but overall higher when the mother was in a deteriorated rather than an enhanced nutritional state. Finally, increasing values of maternal body condition entailed a shift from a positive to a negative association between maternal care behaviors and brood size, but only when the mothers' nutritional state was deteriorated. Overall, our results demonstrate that parental condition and brood size do not only affect parental behaviors, but can also be important and entangled drivers of offspring behaviors during family life.

*Keywords:* social evolution; precocial species; parental care; sibling rivalry; *Forficula auricularia*

## INTRODUCTION

Parental care is a common and taxonomically widespread phenomenon in nature and usually confers substantial benefits to the tended juveniles (Royle et al. 2012b). However, providing care often entails high costs for parents, such as an increased loss of energy or an elevated risks of infection and predation (Alonso-Alvarez and Velando 2012), which can reduce their ability to invest in future reproduction (Trivers 1972). Since parents in a bad condition are expected to incur higher costs of care than parents in a good condition (Hinde et al. 2010), they are generally predicted to adjust the expression of parental care to their body condition as well as to short-term changes in their nutritional state in order to maximize their reproductive success (Bateson 1994). Several studies provided support for this prediction (e.g. Markman et al. 2002; Gorman and Nager 2003; Laurien-Kehnen and Trillmich 2004; Bleeker et al. 2005; Segers et al. 2011; Wong and Kölliker 2012), thus revealing the central importance of parental body condition and nutritional state in the expression of parental care. For instance, mothers in a poor nutritional state were shown to reduce parental care towards their current offspring in the mouthbrooding cichlid *Simochromis pleurospilus* (Segers et al. 2011), whereas food-deprived guinea pig (*Cavia porcellus*) mothers prolonged the expression of nursing behavior while maintaining a constant body condition, thus ultimately reducing the growth rate of their pups (Laurien-Kehnen and Trillmich 2004). Conversely, artificial food supplementation with a sucrose solution allowed parent palestine sunbirds (*Nectarinia osea*) to increase the rate at which they provisioned their nestlings with arthropods (Markman et al. 2002).

The optimal level of care from the point of view of the parents, however, does not necessarily coincide with the optimal level of care from the offspring's point of view. Because of relatedness asymmetries among family members, offspring often behave more selfishly than their parents desire (Trivers 1974; see also Mock and Forbes 1992), both by trying to monopolize resources at the expense of their siblings (sibling rivalry; Mock and Parker 1997; Roulin and Dreiss 2012), as well as by manipulating their parents into increasing their parental investment (Kilner and Hinde 2012). The resulting parent-offspring conflict over the amount of parental investment into care compels parents and offspring to monitor each other's state (Royle et al. 2002; Morales and Velando 2013) and to adjust their strategy of providing and demanding care accordingly (e.g. Godfray and Johnstone 2000; Parker et al. 2002; Smiseth et al. 2003a). By analogy, parental body condition and nutritional state should not only influence the behavior of tending parents, but might also affect how offspring interact with each other, as well as how they act towards their parents (Bateson 1994).

While a number of studies have tested the influence of parental body condition on the expression of parental care and the (begging) behavior of offspring towards their parents (see above), it remains poorly explored how parental condition and its changes during family life affect the behavior of juveniles towards their own siblings (but see White et al. 2010; Wong et al. 2014a for effects on aggressive behavior). Here, we investigated this effect in the European earwig *F. auricularia*. In this insect species, mothers provide non-obligatory forms of care to their mobile offspring (called nymphs) for several weeks after hatching (Lamb 1976b). Maternal care comprises multiple behaviors including the provisioning of nymphs (Lamb 1976a; Staerkle and Kölliker 2008), the amount of which is reduced when mothers had limited access to food resources during family life (Wong and Kölliker 2012). While such condition-dependent behavioral changes in maternal care might enable nymphs to indirectly monitor short-term changes in their mother's condition, nymphs can also assess maternal condition based on cues/signals that are encoded in the profile of the mother's cuticular hydrocarbons (Wong et al. 2014a). Interestingly, maternal presence and post-hatching care are not obligatory for offspring survival (Lamb 1976a; Kölliker and Vancassel 2007; Kölliker 2007). Earwig nymphs do not exclusively rely on maternal provisioning, but may also forage independently soon after hatching (Lamb 1976a; Wong and Kölliker 2012) and obtain food from their siblings (Falk et al. 2014; chapter 1). This food transfer among juveniles is predominantly mediated by active allo-coprophagy, a process defined as a socially-induced increase in feces production by donor nymphs and the subsequent consumption of these feces by recipient siblings (Falk et al. 2014).

To unravel whether offspring behaviors towards family members were associated with maternal condition at egg hatching (initial body condition; a proxy for the mother's long-term energetic state) and/or the post-hatching access of mothers to food resources (nutritional state; a proxy for short-term changes in the satiety level), we first determined the body condition of mothers at egg hatching, subsequently manipulated their nutritional state during four days and then assessed sibling food transfer and mother-offspring interactions. If the mother's nutritional state influenced the behavior of offspring towards their siblings and towards their mother, we would expect (1) a decrease in food transfer among the nymphs, as well as (2) a lower frequency of care-related offspring behaviors (such as begging) in families tended by mothers in an enhanced as compared to a deteriorated nutritional state. This is because mothers in an enhanced nutritional state typically show higher levels of maternal care (Wong and Kölliker 2012). We also expected that mothers with a high body condition at egg hatching could generally afford higher levels of care than mothers with a low initial body condition. Accordingly, we predicted that (3) a high maternal body condition at egg hatching would reinforce the positive effects of an enhanced nutritional state on the level of parental care and (4) allow, in comparison to a low initial body condition, higher levels of care when a mother's current state is deteriorated. Note that we also considered (differences in) family size in our analyses of the effects of maternal condition and nutritional state on family interactions, because group size is generally assumed to affect the competition among group members (Alexander 1974; Shen et al. 2014) and could thus modify reactions of family members to changes in maternal condition or state. Moreover, family size has been suggested to influence mortality and developmental rates of nymphs in European earwigs (Kölliker 2007; Meunier et al. 2012; Meunier and Kölliker 2012a) and is linked to the level of food transfer among their offspring (chapter 1).

## MATERIAL AND METHODS

### *Study animals and laboratory rearing*

The adult *F. auricularia* females used in the experiment descended from 80 female and 73 male earwigs collected in September 2012 in a natural population in Dolcedo, Italy. These individuals were maintained in the laboratory under standard rearing conditions (detailed in Koch and Meunier 2014) for two generations. The experiment comprised of two randomly chosen, independent subsets of adult females and their respective first brood of offspring derived from the first (n = 19 families used in 2013) and the second (n = 29 families used in 2014) filial generation, respectively. These subsets neither differed with respect to maternal body size, weight, or body condition at egg hatching (details on these measurements below) nor with regards to brood size (see Manova results below).

### *Experimental setup*

A total of 48 families were used to disentangle the effects of maternal body condition at egg hatching and subsequent changes in maternal nutritional state on the level of sibling food transfer and the frequency of mother-offspring interactions. The corresponding experimental design is detailed in Figure 2.1. Females had no access to food between egg laying and hatching (mean duration ± se: 33 ± 2 days), as *F. auricularia* females generally do not consume food during that period of time (Kölliker 2007). One day after egg hatching, families were randomly attributed to one of two treatments. In the first treatment (n = 24 families; 9 in 2013 and 15 in 2014), mothers did not receive any food before the behavioral observations (i.e. until day four, deteriorated state). In the second treatment, mothers had access to uncolored food (naturally yellow colored flower pollen formed into pellets; Hochland Bio-Blütenpollen, Hoyer GmbH, Polling, Germany) for 20 minutes on the first and the fourth day after hatching (enhanced state; n = 23 families; 9 in 2013 and 14 in 2014). As intended, the nutritional state of females in the first treatment deteriorated, whereas the state of females in second treatment improved (see details in the supplementary material). In both treatments, nymphs were provided with uncolored food for 1.5 hours on the first and second day after hatching. No food was provided on the third day after hatching to increase their foraging and solicitation behavior towards mothers and siblings on the following day (Staerkle and Kölliker 2008; Falk et al. 2014). On day four after hatching, the nature and frequency of mother-offspring interactions as well as the level of sibling food transfer were investigated in each family (see details below).

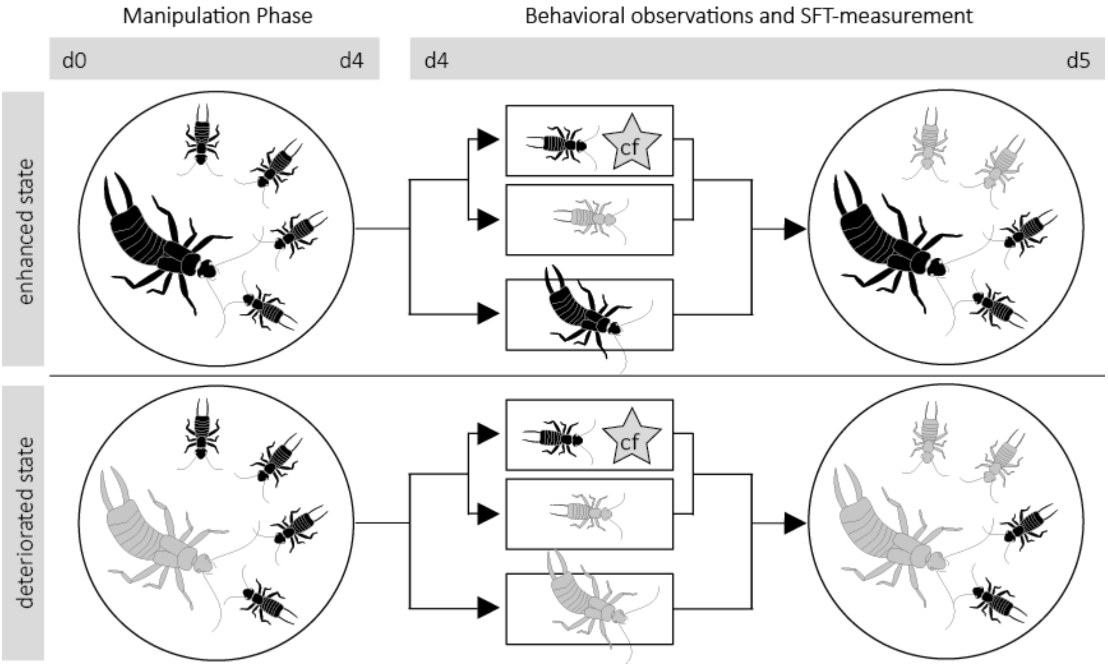

**Figure 2.1. Experimental setup.** Female nutritional state was manipulated by selectively feeding (enhanced) or starving (deteriorated) her prior to the behavioral tests. Sibling food transfer (SFT) was measured by providing half of the nymphs (called donor nymphs) with colored food (indicated with "cf"), then reassembling these newly colored nymphs with their remaining, food-deprived siblings (called recipient nymphs) and their mother, allowing family interactions overnight and finally counting the number of recipient nymphs that ingested the colored food provided by the donor nymphs. Grey individuals were food-deprived in the corresponding experimental phase.

Throughout the experiment, families were kept in their original, medium-sized Petri-dish (9 x 2 cm) with humid sand as ground material and a plastic tube as shelter. During offspring feeding and (where applicable) to provide them with food, mothers were isolated in a small Petri dish (5.5 x 1.2 cm) that only contained humid sand as ground material. Brood size was established by counting the number of nymphs one day after the first egg hatching had been observed. To control that our treatments induced changes in maternal nutritional state, females were weighed on the first and fourth day after hatching (before the onset of behavioral observations) to the nearest 0.01 mg using a micro-scale (model MYA5; PESCALE, Bisingen, Germany). After the behavioral observations, females were sedated with $C0_2$ and their eye distance was measured to the nearest 0.001 cm using a camera coupled to a binocular (Leica DFC425, Leica Microsystems Ltd., Heerbrugg, Switzerland) and the software *Leica Application Suite 4.5.0*. Using eye distance as a measure of body length together with female weight at egg hatching, we calculated the initial body condition for each female based on the 'scaled mass index' (Peig and Green 2009; Peig and Green 2010). In brief, this index standardizes body mass at a fixed value of a linear body measurement based on the scaling relationship between these measures (Peig and Green 2009). Accordingly, this index indicates which mass a particular female would have at the average eye distance. Note that initial body condition, brood size, as well as maternal weight and size at egg hatching did not differ between the 'enhanced state' and 'deteriorated state' treatments in both experimental seasons (Manova; interaction treatment:season: $\Lambda_{\text{Pillai},1} = 0.052$, P = 0.700; treatment: $\Lambda_{\text{Pillai},1} = 0.032$, P = 0.848; season: $\Lambda_{\text{Pillai},1} = 0.106$, P = 0.308).

### *Assessments of sibling food transfer and mother-offspring interactions*

The measurement of sibling food transfer relied on the fact that ingested colored food remains visible through the partially transparent cuticle of young *F. auricularia* nymphs (Staerkle and Kölliker 2008; Falk et al. 2014). Sibling food transfer was measured in three successive steps according to a previously established, standard protocol (chapter 1). First, we provided one half of the nymphs (called donor nymphs) with colored food (naturally yellow colored pollen mixed with a blue food dye; Dekoback, Online Ideen GmbH, Germany) for 1h in a small Petri dish, while the remaining nymphs were starved separately. Simultaneously, mothers were either starved or had access to uncolored pollen for 20 min (see treatments above). Note that temporary separations of nymphs from their mother commonly occur in nature during independent foraging trips (Lamb 1976a). In the second step, we reassembled the newly colored donor nymphs with their remaining siblings (called recipient nymphs) and their mother to allow family interactions. Fifteen hours later, we finally counted the number of recipient nymphs that ingested the colored food provided by the donor nymphs using a stereo-microscope. To be able to discriminate between donors and recipients nymphs, either all donor (n = 27 trials) or all recipient nymphs (n = 20 trials) of a given family were chosen at random and marked by clipping off the distal third of their right cercus (Wong and Kölliker 2013) prior to the experiment. Nymphal coloration was scored by one single observer and blindly with regard to the treatment of the mother throughout the experiment. The level of sibling food transfer (measured as the proportion of recipient nymphs that received colored food from siblings) was independent of marking (Wilcoxon rank sum test; W = 263, P = 0.889).

The behavioral interactions between mothers and their offspring were assessed in the course of the measurement of sibling food transfer and categorized into care-related behaviors expressed by offspring, but also care and aggressive behaviors expressed by mothers. The behaviors were recorded using a scan sampling approach (one observation every 5 min for 45 min, i.e. 10 scans in total per replicate) starting 15 min after the family members had been reassembled in their original Petri dish to allow food transfer (see above). Care-related offspring behaviors comprised (1) 'licking' behaviors, during which nymphs manipulate the inter-segmental skin between abdominal segments and/or the leg-joints of the mother with their mouthparts, (2) begging behaviors, during which nymphs try to establish mouth-to-mouth-contact with the female and (3) mouth-to-mouth contacts. The number of occurrences of each of these behaviors (i.e. the number of times a given behavior was performed by at least one nymph) was then summed up across all ten scans obtain the overall frequency of care-related offspring behaviors. Maternal care behavior was defined following Mas & Kölliker (2011) and was recorded as the sum of (1) antennal contacts with the nymphs, (2) allo-grooming during which the mother manipulated nymphs with her mouth parts and (3) mouth-to-mouth contacts. Mouth-to-mouth contact was considered as both female and nymph behavior because it required coaction of the interacting individuals. Finally, aggressive behaviors expressed by mothers were the sum of threat displays, during which the female raised her forceps in the direction of a nymph, and abdomen shaking, a behavior allowing females to cast off riding nymphs. As this study only focuses on mother-offspring interactions, we did not analyze self-directed female behaviors such as resting, self-grooming and exploring. All behavioral observations were conducted blindly with respect to the nutritional state of the female. Note that all of the above detailed measurements were based on half of the nymphs per family (half of the nymphs were haphazardly chosen and removed from their families before the onset of the observations reported in this study. These nymphs were used in a different experiment; data not shown).

### *Statistical analyses*

The effects of initial body condition, offspring number and nutritional state on sibling food transfer and mother-offspring interactions were tested in three Generalized Linear Models (GLMs). In these models, initial body condition (continuous), brood size (continuous), nutritional state (enhanced or deteriorated; bimodal) and all their interactions were entered as explanatory variables. As the experiment was conducted in two consecutive seasons, we additionally entered 'season' as a bimodal (2013 and 2014) explanatory variable in all models to account for potential differences caused by this confounding factor. We initially also included the interaction between 'season' and 'nutritional state' into our models, but subsequently removed it because it was never significant (all $P < 0.128$). The frequency of maternal care behaviors and the relative frequency of care-related nymphal behaviors were analyzed in two separate GLMs with a Poisson error distribution corrected for overdispersion. We used the relative instead of the absolute frequency of care-related nymphal behaviors (i.e. the absolute frequency divided by the brood size) to avoid a potentially confounding effect of differences in nymph number on this measure of offspring behavior. The proportion of recipient nymphs colored after the sibling food transfer test (entered as odds ratio using the 'cbind'-function in R) was analyzed in a GLM with binomial error distribution corrected for overdispersion. Note that we did not statistically analyze the frequency of female aggressions against her offspring, as such aggressions occurred infrequently, both within broods (none of the 47 families featured more than 1 aggression) and across broods (aggressive behavior was only observed in 5 of 47 families).

All statistical analyses were performed using the statistics software R 3.0.3 (http://www.r-project.org/). Mixed model analyses were implemented using the package *lme4*. Significance levels of effects in these models were assessed using the packages *car* (*Anova*-function) and *lmerTest* (*summary*-function). Note that we centered 'brood size' and 'initial body condition' on their mean to avoid any model bias due to collinearity between these explanatory variables (variance inflation factor (VIF) < 5.5 after centering in all models). All statistical models were simplified stepwise by removing non-significant interaction terms (all P > 0.187), as retaining these terms can bias estimates of other effects in the model (Engqvist 2005). A log-Likelihood Ratio test was used to test the explanatory power of each model after the removal of a variable. Where applicable, models were checked for normality of residuals and homogeneity of variance before and after the model selection procedure. Finally, interactions between continuous variables were plotted using the package *effects* to display the predicted relationship between the response variable and one explanatory variable for different, fixed values of the interacting variable(s) (details in Fox 2003).

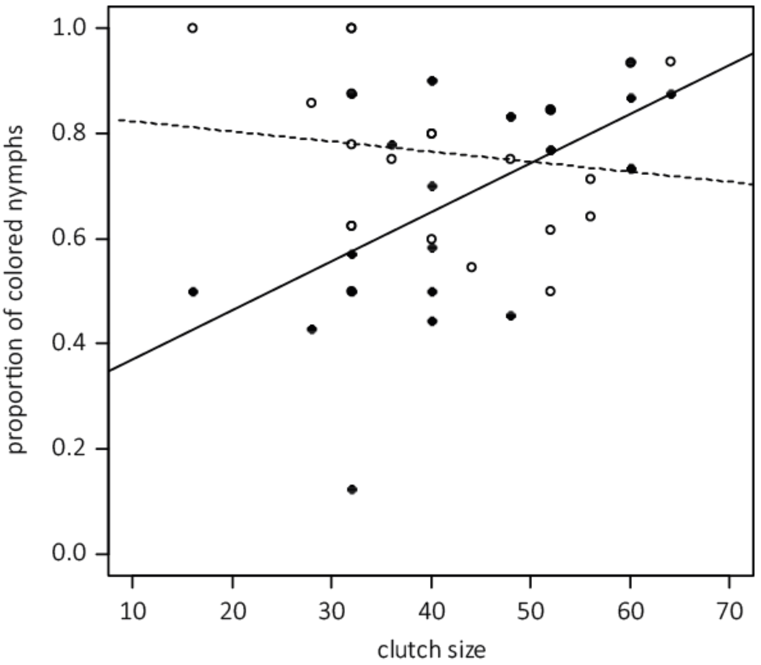

**Figure 2.2.** Influence of brood size on the proportion of recipient nymphs that received food during sibling food transfer tests conducted when maternal nutritional state was enhanced (open circles, dashed line) or deteriorated (filled circles, solid line). Note that the discrepancy between the number of visible points and the sample size is due to the overlap of some data points.

## RESULTS

Maternal nutritional state influenced the exchange of food among her offspring, but only through an interaction with brood size (Figure 2.2; Table 2.1A). Specifically, sibling food transfer increased with brood size when the mother's nutritional state was deteriorated (Estimate ± SE: 0.048 ± 0.016, $t_{46} = 3.073$, $P = 0.004$), whereas this association disappeared when maternal nutritional state was enhanced (Estimate ± SE: -0.0003 ± 0.0163, $t_{46} = -0.021$, $P = 0.983$). Overall, the level of food transfer did not differ among clutches that had been tended by females in a deteriorated or enhanced state, respectively (Table 2.1A). Notably, sibling food transfer was independent of the initial body condition of the mother (Table 2.1A).

**Table 2.1.** Effects of the initial body condition of mothers, their brood size and their nutritional state (enhanced or deteriorated) as well as the experimental season on (A) the level of sibling food transfer (measured as the proportion of recipient nymphs that received colored food from their siblings), (B) the frequency of care-related nymph behaviors and (C) the frequency of maternal care behaviors. Note that we report the results of the full models here to facilitate comparisons among them. The (qualitatively unchanged) results of the corresponding reduced models can be found in Table S2.1. Significant P-values are highlighted in bold print.

| | (A) Sibling food transfer | | (B) Nymph behavior | | (C) Maternal care | |
|---|---|---|---|---|---|---|
| | $\chi_1^2$ | P | $\chi_1^2$ | P | $\chi_1^2$ | P |
| Initial body condition (IBC) | 0.57 | 0.449 | 0.63 | 0.429 | 0.21 | 0.649 |
| Brood size (BS) | 4.49 | **0.034** | 0.10 | 0.755 | 1.35 | 0.245 |
| Nutritional state (NS) | 0.89 | 0.345 | 3.95 | **0.047** | > 0.01 | 0.937 |
| Season | 0.11 | 0.736 | 1.92 | 0.166 | 0.13 | 0.716 |
| IBC:BS | 0.68 | 0.409 | > 0.01 | 0.933 | > 0.01 | 0.982 |
| IBC:NS | 0.14 | 0.708 | 0.02 | 0.900 | 1.68 | 0.196 |
| BS:NS | 4.82 | **0.028** | 0.14 | 0.707 | 2.99 | 0.084 |
| IBC:BS:NS | 0.20 | 0.653 | 0.24 | 0.624 | 6.24 | **0.013** |
| Type of model / error family | GLM / binomial | | GLM / poisson | | GLM / poisson | |

The relative frequency of care-related behaviors expressed by nymphs was overall higher when mothers had a deteriorated compared to an enhanced nutritional state (Figure 2.3A; Table 2.1B), but was independent of brood size, the initial body condition of the mother and any interactions between the three tested variables (Table 2.1B). In contrast, a triple interaction between the initial body condition of the mother, brood size and her nutritional state influenced the frequency of care-related behaviors expressed by mothers (Table 2.1C). When the nutritional state of mothers was deteriorated, the frequency of care behaviors increased with brood size if they initially had been in bad condition, but surprisingly decreased with brood size if they had been in good condition (Figure 2.4; interaction between initial body condition and brood size: Likelihood ratio (LR) $\chi_1^2 = 5.35$, P = 0.021; initial body condition: LR $\chi_1^2 = 0.97$, P = 0.325; brood size: LR $\chi_1^2 = 0.30$, P = 0.583). By contrast, when the nutritional state of mothers was enhanced, their initial body condition did not influence the frequency of maternal care behaviors, irrespective of brood size (interaction between initial body condition and brood size: LR $\chi_1^2 = 1.74$, P = 0.187; initial body condition: LR $\chi_1^2 = 1.08$, P = 0.298; brood size: LR $\chi_1^2 = 3.26$, P = 0.071). Finally, aggressive behavior of mothers against their offspring overall occurred infrequently and were distributed across seasons and experimental treatments without any obvious pattern (occurrences per family; 'enhanced state': 2014 = 0, 2013 = 2; 'deteriorated state': 2014 = 2, 2013 = 1). Note that 'season' never had a significant effect in any of our models (Table 2.1).

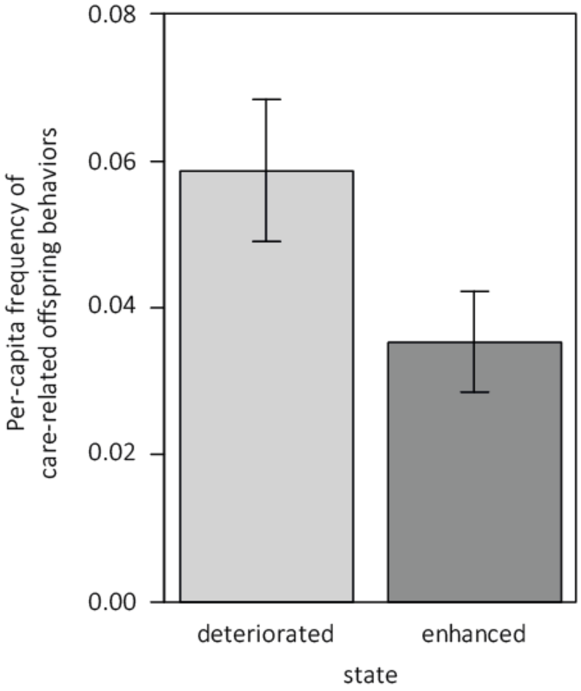

**Figure 2.3**. Effects of the mother's nutritional state on the frequency of care-related offspring behaviors.

## DISCUSSION

In this study, we showed that behavioral interactions among family members in the European earwig *F. auricularia* reflect an interplay of the mother's current nutritional state, her condition at offspring emergence and the number of her offspring. Our data revealed that sibling food transfer increased with brood size when the female was in a bad nutritional state, but was independent of brood size when she was in an enhanced nutritional state. The level of sibling food transfer was, however, not linked to the female's condition at egg hatching. We also found that the expression of care-related behaviors by offspring was overall higher if the mother was in a deteriorated rather than an enhanced nutritional state. Finally, regarding maternal care, our results demonstrated that the frequency of caring behaviors - but likely not the rare occurrence of aggressions - was shaped by an interaction between the current state of the mother, her body condition at egg hatching and brood size. If the mother was in a deteriorated state at the time of measurement, the frequency of maternal care behaviors increased with brood size if she had also been in a bad condition at egg hatching, but surprisingly decreased with brood size when she had been in a good initial condition. Notably, this interactive effect was masked when the female was in an enhanced nutritional state.

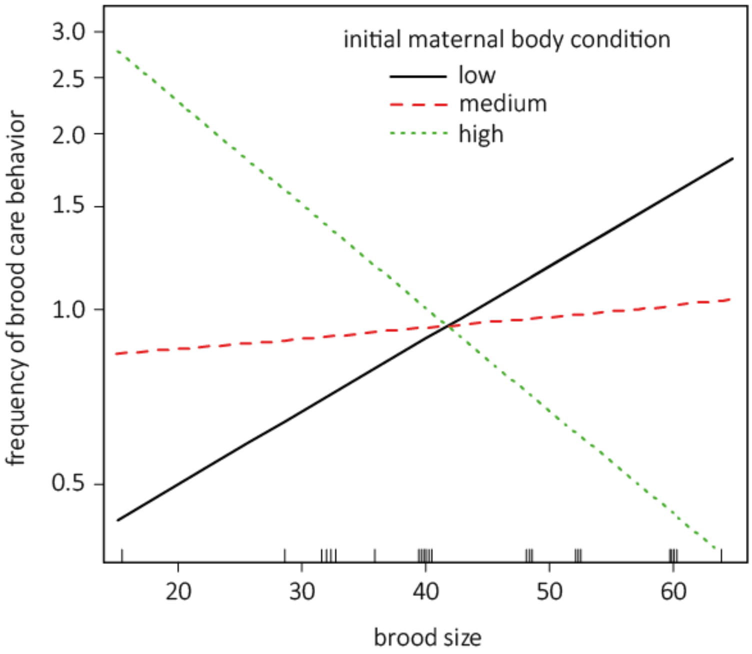

**Figure 2.4**. Interacting effect of the initial maternal body condition and brood size on the frequency of parental behaviors of mothers in a deteriorated nutritional state. To illustrate the interaction, regression lines are given for an average value of the initial maternal condition (Median = 0.0464; dashed line) as well as for a comparatively low (1st quartile = 0.0442; solid line) and high (3rd quartile = 0.0501; dotted line) value, respectively.

Our results are in line with the prediction that a mother's nutritional state shapes the behavioral interactions among her offspring. When mothers were in a deteriorated state (and thus unable to provision their offspring), the increase of sibling food transfer with brood size could reflect a density-dependent increase of competition among recipients for publicly available feces and/or brood-size dependent changes in the propensity of donors to produce these feces (chapter 1). Conversely, the absence of such an association when mothers were in an enhanced state and thus able to provision their offspring might reflect that females provided sufficient levels of care to limit selfishness among offspring that react to cues of female condition (Wong et al. 2014b; Wong et al. 2014a) and/or to mask the above described density-dependent effects. Interestingly, such a masking effect has recently been described in the burying beetle *Nicrophorus vespilloides*, where a density-dependent shift from cooperation to competition was only evident in the absence of parental care (Schrader et al. 2015). Further experiments are needed to differentiate among the above possibilities in earwigs. Nevertheless, the deteriorated state of mothers is unlikely to have specifically triggered a density-dependent increase of feces production by donor nymphs, as the overall level of sibling food transfer was found to be independent of female state. Instead, a deteriorated maternal state is likely to increase the competition among recipient nymphs, which might in turn reduce (rather than increase) the feces production of donor nymphs. The fact that the differences in the level of food transfer between treatments were most pronounced in small broods suggests that the reduction in feces production might be larger in these broods. Hence, while sibling food transfer can possibly mitigate detrimental effects of low maternal care by providing access to additional resources in the form of feces (chapter 1), its extent might be ultimately limited because donor nymphs neither seem to adjust the production of feces directly to their siblings' nutritional need (Falk et al. 2014), nor indirectly by monitoring and reacting to their mother's condition (this chapter).

The frequency of care-related behaviors that offspring directed towards their mother was higher when she was in a deteriorated rather than an enhanced nutritional state. This result is in line with the assumption of increased offspring begging in broods tended by mothers in poor condition. In this situation, offspring likely increased their begging efforts either cooperatively (e.g. Bell 2007; Madden et al. 2009) or competitively (e.g. Neuenschwander et al. 2003; Smiseth et al. 2003a) to elicit sufficient levels of maternal care. Irrespective of the mediating mechanisms, the effects of maternal state on care-related offspring behaviors, as well as on the food transfer among these offspring, illustrate that offspring behaviors during family life are flexible and change according to their mother's condition.

Maternal state did not only shape sibling interactions, but also affected the frequency of care-behaviors mothers expressed towards their offspring. When the nutritional state of mothers was deteriorated (but not when it was enhanced), our data showed that increasing values of maternal body condition at egg hatching entailed a shift from a positive to a negative association between maternal care and brood size. The increase of maternal care with brood size in families tended by females with both low initial body condition and deteriorated nutritional state suggests a trade-off between providing care and somatic maintenance that depends on the number - and thus on the value – of current offspring. Accordingly, females tending small broods might favor somatic maintenance over providing (some forms of) care, e.g. to retain the ability to provide other forms of care such as predator defense (Bateson 1994) or a prospect of future reproduction (Thorogood et al. 2011), whereas females tending large broods might do the opposite (and possibly forfeit chances of future reproduction). In line with this latter reasoning, stitchbird, or hihi (*Notiomystis cincta*) parents were shown to be largely insensitive to the experimentally enhanced begging displays of their brood when they attempted two breeding attempts in one reproductive season, but responded with an increased rate of nestling provisioning when they bred just once (Thorogood et al. 2011).

Surprisingly, the trade-off between providing post-hatching care, somatic maintenance and the prospects of future reproduction seemed to be differently solved by mothers in a deteriorated nutritional state that had featured a high body condition at offspring emergence. Under these conditions, maternal care decreased with brood size. This result could reflect that mothers with a high initial body condition generally favor future over (additional) current reproductive investment (McNamara et al. 2009), but are nevertheless capable of providing high levels of care to small broods without compromising their prospects of future reproduction. Future studies should investigate the nature and adaptive significance of this apparent trade-off between self-maintenance, care and future reproduction. However, our findings reveal that, independent of the underlying mechanisms, females are not forced into this trade-off if their nutritional state is enhanced after offspring emergence.

In summary, we demonstrated that maternal condition did not only influence parental care, but also affected interactions among juveniles and the behaviors they expressed towards their mother. Moreover, we showed that the influence of the mother's current nutritional state on offspring behaviors critically depended on both, her body condition at offspring emergence and the number of offspring she tended. These results thus call for a better integration of female nutritional state and quality in studies on behavioral interactions between parents and offspring as well as among offspring. More generally, our findings illustrate that life-history traits and environmental conditions interact to shape the complex interplay of behaviors characteristic for family life.

## ACKNOWLEDGEMENTS

[removed for privacy purposes]

## SUPPLEMENTARY MATERIAL

### Effect of food access on changes in female condition

The effect of the experimental manipulation on female condition was tested in a Linear Model (LM) in which the relative change in female body condition during the manipulation phase was entered as continuous response and nutritional state (enhanced or deteriorated) as bimodal explanatory variable. The relative change in female condition was calculated by first subtracting her initial condition (i.e. the standardized weight at egg hatching) from the corresponding condition prior the behavioral tests and then dividing this difference by the initial body condition. Because the experiment was conducted in two consecutive seasons, we also entered 'season' (bimodal factor) and its interaction with 'nutritional state' into the model.

As expected, the relative change of a mother's condition markedly differed between the treatments manipulating the nutritional state (Figure S2.1; $F_1 = 64.584$, $P < 0.001$). In particular, maternal body condition improved in the 'enhanced state' treatment (t-test vs. 0; $t_{22} = 7.357$, $P > 0.001$) and declined in the 'deteriorated state' treatment (t-test vs. 0; $t_{23} = - 3.348$, $P = 0.003$), thus confirming the efficiency of our experimental manipulation of the mothers' nutritional state. In contrast, the relative change of a mother's condition was independent of the season ($F_1 = 0.066$, $P = 0.799$) or its interaction with the mother's nutritional state ($F_1 = 0.275$, $P = 0.603$).

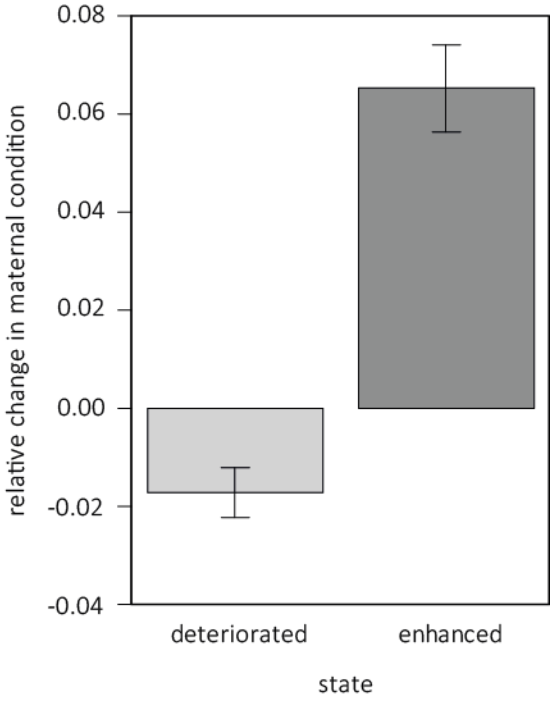

**Figure S1:** Relative change in maternal body condition in the 'deteriorated state' and 'enhanced state' treatment, respectively.

**Table S2.1.** Effects of the initial body condition of mothers, their brood size and their nutritional state (enhanced or deteriorated) as well as the experimental season on (A) the level of sibling food transfer (measured as the proportion of recipient nymphs that received colored food from their siblings), (B) the frequency of care-related nymph behaviors and (C) the frequency of maternal care behaviors. Significant P-values are highlighted in bold print.

| | (A) sibling food transfer | | (B) nymph behavior | | (C) maternal care | |
|---|---|---|---|---|---|---|
| | $\chi_1^2$ | P | $\chi_1^2$ | P | $\chi_1^2$ | P |
| Initial body condition (IBC) | 0.60 | 0.437 | 0.64 | 0.422 | 0.21 | 0.649 |
| Clutch size (CS) | 5.24 | **0.022** | 0.10 | 0.749 | 1.35 | 0.245 |
| Nutritional state (NS) | 0.99 | 0.321 | 4.25 | **0.039** | > 0.01 | 0.937 |
| Season | 0.31 | 0.575 | 1.85 | 0.174 | 0.13 | 0.716 |
| IBC:CS | - | - | - | - | > 0.01 | 0.982 |
| IBC:NS | - | - | - | - | 1.68 | 0.196 |
| CS:NS | 4.80 | **0.028** | - | - | 2.99 | 0.084 |
| IBC:CS:NS | - | - | - | - | 6.24 | **0.012** |
| Type of model | GLM | | GLM | | GLM | |

# CHAPTER 3

# Influences of relatedness, food deprivation and sex on adult behaviors in the group-living insect *Forficula auricularia*

Charlotte Weiß, **Jos Kramer**, Kai Holländer & Joël Meunier

*Based on*

Ethology 120 (2014), 923-932

## ABSTRACT

The evolution of group living is generally associated with the emergence of social behaviors that ensure fitness benefits to group members. However, the expression of these behaviors may depend on group composition, which can vary over time with respect to sex, starvation status and relatedness. Here, we investigated (1) whether adults of the group-living European earwig, *Forficula auricularia* show cooperative behaviors towards conspecifics, and (2) whether sex, food availability and relatedness shape the nature and frequency of these behaviors. We conducted a full-factorial experiment using 108 unisexual pairs of adults, in which we manipulated these three factors and video-recorded the earwig behaviors for 45 minutes. Our results revealed that adults mostly expressed self-directed and aggressive behaviors. Nevertheless, they also showed allogrooming, a social behavior that offers scope for cooperation. Pairs of males displayed longer bouts of aggression and allogrooming (when it occurred) than pairs of females. Food deprivation had no effect on male behaviors, but females spent less time self-grooming and walking when they were food deprived. Finally, low relatedness between adults did not influence any of the measured behaviors, but exacerbated frass production, possibly due to social stress. Overall, these results indicate the limited role of cooperation among *F. auricularia* adults during their group-living phase.

*Keywords:* social life; cooperation; evolution; insect; sex-specific behaviors

## INTRODUCTION

Group living can be found in almost all animal taxa and encompasses a broad diversity of social systems. Within these systems, forms of group living range from simple and temporary aggregation to complex and permanent societies with obligatory forms of cooperation between their members (reviewed in Wilson 1971; Costa 2006; Lihoreau et al. 2012; Wong et al. 2013). The emergence and persistence of these multiple forms of group living in nature generally relies on the fact that the benefits a social system provides to group members outweigh their its associated costs (reviewed in Krause and Ruxton 2002; Bourke 2011a). Gaining a better understanding of the conditions promoting the evolution of group living therefore requires to characterize the diversity of social systems across animal taxa and to identify the factors shaping their associated costs and benefits.

Testing the occurrence of social behaviors between conspecifics may provide key insights into social systems and the nature of the benefits possibly associated with group living (Costa 2006; Legendre et al. 2008). For instance, one of the most common social behaviors found across species is allogrooming (Schmid-Hempel 1998; Mooring et al. 2004; Clutton-Brock 2009), a process during which one individual grooms another individual (also called allo-preening in birds, e.g. Gill 2012). Allogrooming is known to provide key benefits to group members, by mediating the removal of pathogens and parasites from the recipient individuals (Rosengaus and Maxmen 1998; Zamma 2002; Hamilton et al. 2011), allowing self-immunization by low-dose infections in donors (Konrad et al. 2012) and enhancing social bonding (reviewed in Dunbar 2010; Semple et al. 2013). Allogrooming also improves the accuracy of nestmate recognition in eusocial insects, by mediating the transfer of chemical compounds between group members and thus contributing to the homogenization of colony odors (Boulay et al. 2004; Turgis and Ordon 2012).

Another behavior that can be shaped by contacts between group members is frass (= feces) production. In insects, frass production does not only fulfill the needs of waste disposal, but may also be selected to actively help group members (Weiss 2006). For instance in many group-living cockroaches and eusocial termites, frass production provides key benefits to group members by allowing, through allo-coprophagy, the transfer of nutrients and microbial fauna within the group (reviewed in Nalepa et al. 2001; Weiss 2006). Interestingly, a recent study in the European earwig, *Forficula auricularia*, also revealed that young offspring both express allo-coprophagy during family life and actively increase their frass production when encountering a related sibling (Falk et al. 2014).

The expression of social behaviors does not only depend on species traits, but may also depend on group composition, which can vary over time with respect to the relatedness between interacting individuals as well as under different environmental conditions affecting the level of starvation of the encountered conspecifics. The inclusive fitness theory predicts that relatedness favors the occurrence of cooperation between related individuals, because the indirect fitness benefits gained by helping relatives can outweigh their direct costs (Hamilton 1964a; West et al. 2002). This prediction received empirical support from a large number of studies across animal species, even if cooperation occurs between unrelated conspecifics and even between individuals from different species (reviewed in Clutton-Brock 2009; Davies et al. 2012). Independently from relatedness, food shortage is predicted to increase the frequency of aggression between individuals to selfishly monopolize the limited resource (Mock and Parker 1997; Meunier and Kölliker 2012a; Paul et al. 2014).

Although aggressive and cooperative behaviors are keystones of sibling interactions during family life in the European earwig, *F. auricularia* (Dobler and Kölliker 2011; Falk et al. 2014), the importance of these two behaviors during adult group living remains unknown so far. Earwig family life lasts several weeks (Costa 2006), during which mothers provide multiple, albeit facultative, forms of care to their few dozen offspring (Kölliker 2007; Staerkle and Kölliker 2008; Meunier et al. 2012; Meunier and Kölliker 2012b). During family life, offspring frequently interact with each other through both competitive (Dobler and Kölliker 2011) and cooperative behaviors. Forms of sibling cooperation include allo-grooming, kin-directed frass production and allo-coprophagy (JM personal observation; Falk et al. 2014). Low relatedness and food deprivation are known to increase the level of sibling competition and sibling cannibalism during family life (Kölliker 2007; Dobler and Kölliker 2010; Meunier and Kölliker 2012a), but have limited effects on sibling cooperation (Falk et al. 2014). Offspring disperse from their burrow several weeks after their emergence (late spring) and then live in groups until early winter (Costa 2006). These groups are generally mixed in terms of sex and their size ranges from pairs to several dozens of individuals (Suckling et al. 2006; JM personal observation). To date, nothing is known about the genetic composition and the stability of these groups. Furthermore, it is unclear whether the cooperative and competitive interactions that shape offspring interactions during early family life also occur during adult group-living. In cases like this – that is. when only limited information is available on the biology of a group-living species – exploring the occurrence and form of social behaviors even during simple dyadic interactions may provide key insights into social systems and the nature of the benefits associated with group living (Costa 2006; Legendre et al. 2008).

Our study was designed both to characterize the nature of the behaviors expressed by paired *F. auricularia* adults, and to determine the influence of relatedness, food deprivation and sex on their expression. We conducted a full-factorial experiment to test whether (1) paired adults express cooperative (allogrooming or active frass production), aggressive (forceps waving and backwards approach with spread forceps; Eisner 1960) and/or self-directed behaviors (e.g. self-grooming, walking, resting) and (2) whether the expression of these behaviors was shaped by the relatedness between and/or by food deprivation of the paired individuals. Because our primary goal was to describe the nature of behavioral interactions in a non-mating context (for the mating context, see Walker and Fell 2001), only pairs of males or females were set up. We thus also tested whether the expression of the above behaviors differed between pairs of males and females.

## MATERIAL AND METHODS

### *Origin of the tested individuals*

The tested *F. auricularia* adults were offspring of 45 field-mated females collected in September 2012 in Dolcedo (Italy) and maintained under standard laboratory conditions until their second clutch production (rearing details in Meunier et al. 2012). Fourteen days after the hatching of the second clutch eggs, the resulting offspring (called nymphs) were separated from their mothers and set up in large Petri dishes (diameter 14 cm). From then on, they were referred to as family groups. The Petri dishes contained humid sand as a substrate and a plastic shelter as a nest. The nymphs received food every other day until they reached adulthood (for food composition, see chapter 1). To avoid sib-mating, males and females were separated in two Petri dishes per family at adult emergence (Meunier and Kölliker 2013). The following experiments were conducted using (Mean ± SE) 5.1 ± 0.2 of these newly bred virgin adults per family, which were randomly distributed across the treatments.

### *Experimental design*

A total of 108 unisexual pairs of adults were set up to investigate (1) the nature of their behaviors, (2) whether relatedness, food availability and sex shape the frequency and duration of these behaviors, and (3) whether these three factors affect frass production and weight changes after 24h of group living. To this end, a full-factorial experiment was conducted to obtain the eight possible combinations of the three factors: relatedness, food deprivation and sex (Figure 3.1). In particular, relatedness was manipulated by pairing individuals, which had the same (related) or different (unrelated) mothers. Note that this treatment reflects differences in both familiarity and genetic relatedness. The level of food deprivation was manipulated in one of the two individuals (called "recipient"), which received either *ad libitum* food or no food for one week prior to the experiment. The other individual (donor) was fed *ad libitum* during this week. Finally, sex of the pairs was manipulated by setting up either two females or two males.

One week before the behavioral observations, all the tested individuals were isolated in Petri dishes (diameter 5 cm), because such isolation (1) possibly occurs after the nocturnal foraging activities of the adults and (2) is known to stimulate aggregation and interactive behaviors between *F. auricularia* adults (K. Holländer and J. Meunier, unpublished results). At isolation, half of the males and females were randomly assigned to a "donor" group and marked by tying a wire (Conrad, enameled copper wire, 0.05mm$^2$) between head and thorax, whereas the other half was assigned to a "recipient" group and was not marked. Note that one week after this manipulation, we did not observe any signs of stress or abnormal behaviors in the donor group (wired) as compared to the recipient group (non-wired). During the week of isolation, we also manipulated the feeding condition of the recipient individuals by providing half of them with *ad libitum* pollen (yellow color; Hochland Bio-Blütenpollen, Hoyer GmbH, Polling, Germany) and by food depriving the other half. All donor individuals were fed *ad libitum* with green pollen (mix of naturally yellow-colored pollen and blue food dye; Dekoback, Online Ideen GmbH, Germany). This coloration allowed us to later count the number of (green-colored) frass produced by the donor individuals and the number of (yellow-colored) frass released by the recipient individuals.

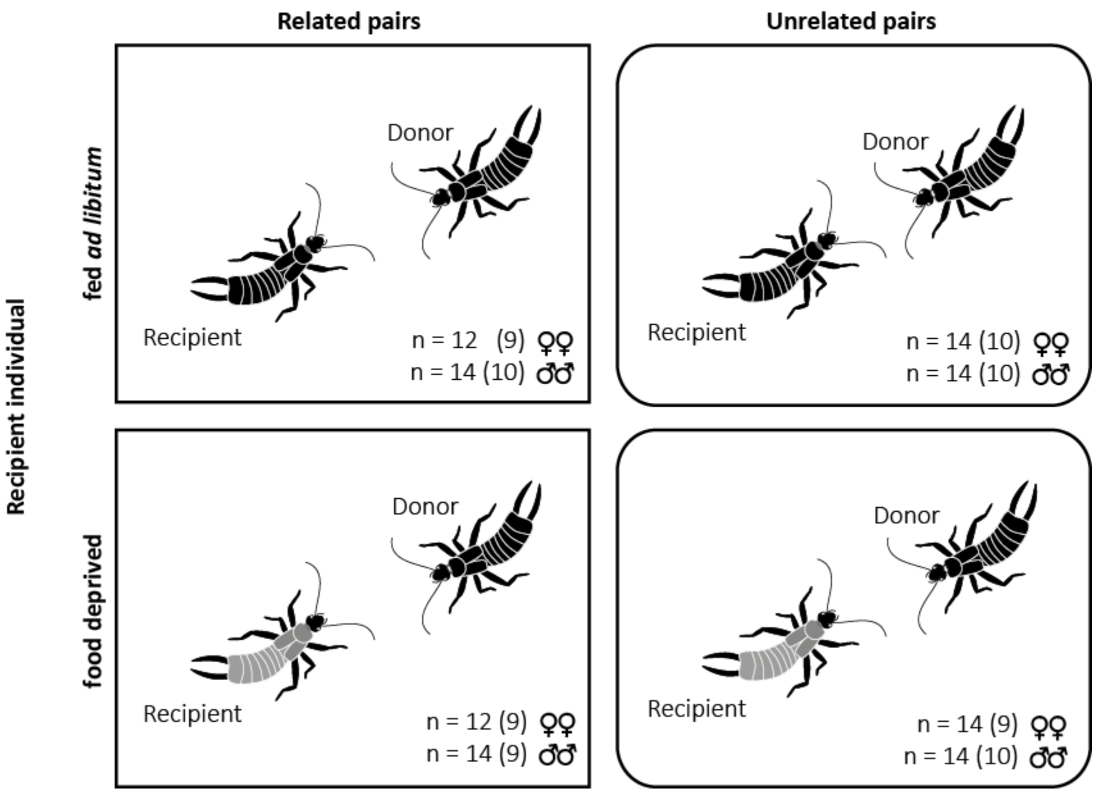

**Figure 3.1. Experimental design.** Pairs of same-sex adults where either related (square boxes) or unrelated (rounded boxes), and the recipient individuals either fed *ad libitum* (black) or food deprived (grey) prior the experiment. The number of videos recorded per combination is given between brackets. *n* refers to the number of replicates.

The behavioral observations were conducted in a rectangular arena (7.5 x 5.5 cm) formed by two glass sheets (15 x 10.5 cm) put on top of each other and separated by four transparent plastic spacers of 0.5 cm height. The donor individuals were always put in the center of the arena first and the recipients were added at the same place two minutes later. The behaviors of the two individuals were then video recorded for 45 minutes (JVD HD Everio GZ-GX1). Because earwigs are nocturnal, the recordings were conducted under red light (see, for example, Walker and Fell 2001; Mas and Kölliker 2011). The videos were then analyzed using the software OBSERVER XT 11 (NOLDUS Information Technology) to measure the frequency and total duration of six behaviors involving the recipient individuals. These behaviors were (1) allogrooming (i.e. mouth-to-mouth contact, mouth-to-back contact, grooming given and grooming received), (2) resting in group (head of the recipient within a body-length radius – i.e. 3 cm - from the head of the donor individual), (3) aggression (forceps waving and backwards approach with spread forceps; Eisner 1960), (4) walking, (5) resting alone (head of the recipient more than 3 cm away from the head of the donor individual) and (6) self-grooming. The limited resolution of the videos (due to red-light conditions) did not allow us to quantify allo-coprophagy. We recorded 9 or 10 videos per treatment (i.e. in 76 out of the 108 pairs; Figure 3.1). All video-analyses were conducted by a single observer, as well as randomly and blind with respect to the treatment.

Because frass release is known to be a key form of cooperation between earwig nymphs (Falk et al. 2014), we additionally counted the number of frass released by the donor (green-colored frass) and recipient (yellow-colored frass) individuals 24h after the start of the behavioral observations. To this end, each pair was transferred to a new Petri dish (diameter 5 cm) for 24h with moist paper towel as a source of humidity. To disentangle whether variation in frass number observed between the treatments (see results) reflects an increase or a decrease in frass production, we compared these numbers to the number of frass produced by individuals set up in a control treatment, in which either one male ($n$ = 12) or one female ($n$ = 11) was kept individually in a Petri dish (diameter 5 cm) with moist paper towel for 24h. These control individuals were previously isolated for one week in Petri dishes (diameter 5cm) and fed *ad libitum* with green-colored food (i.e. same rearing conditions than the donor individuals).

Finally, we investigated the influence of frass production, relatedness, sex, and food deprivation on the absolute weight change of both recipient and donor individuals over 24h. To this end, all donor and recipient individuals were weighed to the nearest 1.0 µg (Pescale Wägetechnik MYA Mikrowaage) just before the setup of the behavioral observations, as well as 24h later. The absolute weight change of each individual was calculated by subtracting the second measurement from the first one.

### *Statistical analyses*

Because we found significant correlations in the duration of the six measured behaviors (Table S3.1, supplementary material), we first conducted a Principal Component Analysis (PCA) to obtain non-correlated principal components (PCs) reflecting single or combinations of different behaviors. Such statistical approach allowed us to investigate the effects of relatedness, food deprivation and sex on potential trade-offs between the measured behaviors (e.g. Falk et al. 2014; Stahlschmidt and Adamo 2015). The PCA was conducted using the square root-transformed duration of each behavior and by scaling the variables. The resulting PCs (Table 3.1) were then analyzed using linear mixed models (LMMs), in which relatedness, food deprivation and sex, as well as all interactions were entered as independent factors. Because each family provided individuals in several treatments, the family of origin of each recipient individual was entered as a random factor in the models. The same LMM procedure was conducted to analyze the square-root transformed number of frass produced by the donor individuals and counted after 24h. Finally, whether allogrooming or aggression occurred at least once during the 45 minutes of observation (i.e. their occurrence) was analyzed using General LMMs (GLMMs) with binomial error distribution and a cloglog link-function to correct for unbalanced representation of 0 and 1 in the data set.

Whether relatedness, food deprivation, sex and the square-transformed number of frass released by the donor individuals influenced the weight change of these individuals was analyzed using a LMM. In this model, the four above factors and their interactions where entered as independent factors, the absolute weight change as the dependent factor and the family of origin as random factor. We also tested whether the number of frass released by the donor and by the recipient individuals (and their interaction) influenced the absolute weight change of the recipient individuals (e.g. due to ingestion through allo-coprophagy and/or simple frass production, respectively) using a LMM. All statistical models were simplified stepwise, starting by removing the interaction terms that were not significant (all $p > 0.14$). All statistical analyses were performed with the software R 3.0.2 loaded with the packages “car”, “lme4” and “MASS”.

Table 3.1. Occurrence of each behavior and the respective loading on the six principal components. The behaviors having absolute PC loadings higher than 0.5 appear in boldface type.

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| Allogrooming | 0.140 | **-0.533** | 0.476 | -0.337 | **-0.595** | -0.049 |
| Resting in group | **0.570** | -0.318 | 0.109 | 0.121 | 0.484 | **-0.560** |
| Self-grooming | **-0.568** | -0.278 | -0.327 | 0.224 | -0.222 | **-0.627** |
| Walking | **-0.523** | -0.104 | 0.341 | **-0.541** | **0.552** | -0.046 |
| Resting alone | 0.174 | **0.618** | -0.073 | **-0.526** | -0.232 | **-0.501** |
| Aggression | -0.170 | 0.380 | **0.730** | **0.502** | -0.065 | -0.194 |
| Variance explained | 29.6% | 27.2% | 16.1% | 12.1% | 9.4% | 5.6% |

## RESULTS

Earwigs in unisex pairs mainly expressed self-grooming behavior (Mean ± SE, 24 ± 1 min), followed by resting (in pairs: 12 ± 1 min; alone: 4 ± 1 min) and walking (4 ± 1 min, Figure 3.2A). Interactive behaviors were also present, albeit shorter, in the forms of aggressions (43 ± 18 sec) and allogrooming (12 ± 3 sec). The PCA conducted on the duration of these six behaviors provided six orthogonal PCs, out of which we extracted the first four components (total variance explained = 85.0%; Table 3.1). Loadings of the first component (PC1) showed that it measured a negative association between being active (self-grooming and walking) and resting alone. The second component (PC2) represented a negative association between allogrooming and resting alone, while the third component (PC3) was singly and positively loaded by aggression. Finally, the fourth component (PC4) reflected a negative association between aggressiveness on the one hand, and walking and resting alone on the other hand.

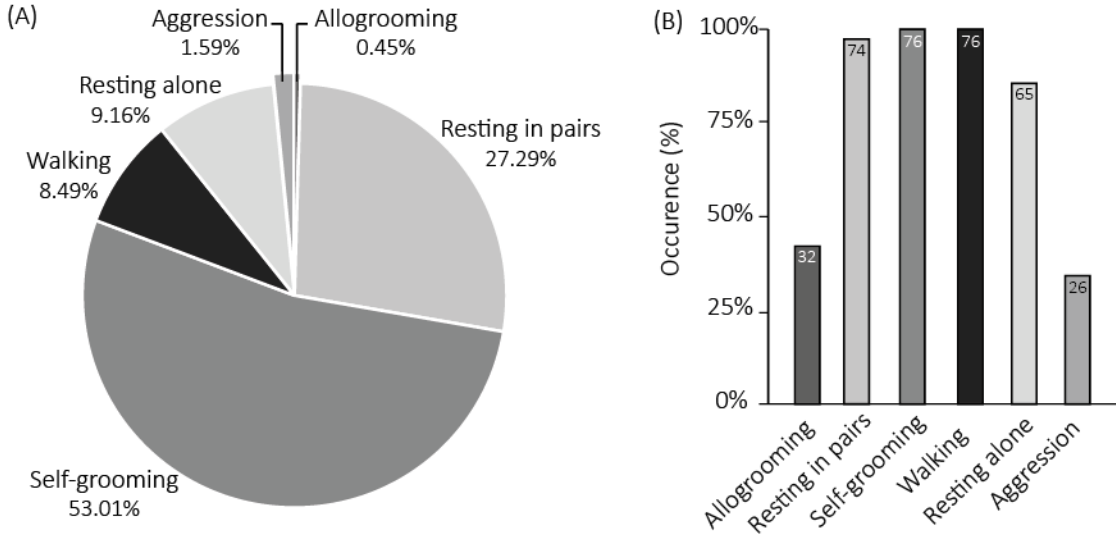

**Figure 3.2.** (A) Distribution of the time spent by recipient individuals on each of the six measured behaviors and (B) occurrence of these behaviors during the experiment, measured as the proportion of pairs in which the behavior has been observed at least once over the 45 minutes of observation (the corresponding number is written within each bar). The reported values are based on the 76 recipient individuals that were filmed.

An interaction between the sex of the group and food deprivation significantly shaped the negative association between activity and resting (PC1 scores; Table 3.2; Likelihood Ratio (LR) $\chi^2_1 = 4.65$, $P = 0.031$). Compared to females fed *ad libitum*, food deprived females were less active and rested more (i.e. higher PC1 scores; Figure 3.3A; LR $\chi^2_1 = 10.26$, $P = 0.001$), whereas food deprivation had no comparable effect in groups of males (Figure 3.3A; LR $\chi^2_1 = 0.06$, $P = 0.807$). Males behaved also more aggressively than females (higher PC3 scores; Figure 3.3B; LR $\chi^2_1 = 31.33$, $P < 0.0001$).

By contrast, relatedness had no effect on any of the four components (Table 3.2) and none of the three tested factors influenced the PC2 and PC4 scores (Table 3.2).

**Table 3.2.** Influence of food deprivation, relatedness and sex of the group on the four principal components (PC1 to PC4) and on the number of frass released by the donor individuals and counted after 24h (Donor frass). Values obtained from LMMs. Significant P-values are in bold.

| | PC1 | | PC2 | | PC3 | | PC4 | | Donor frass | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LR $\chi^2_1$ | p | LR $\chi^2_1$ | p | LR $\chi^2_1$ | p | LR $\chi^2_1$ | p | LR $\chi^2_1$ | p |
| Food dep. | 6.37 | **0.012** | 0.18 | 0.667 | 0.03 | 0.867 | 1.59 | 0.207 | 0.00 | 0.988 |
| Relatedness | 0.66 | 0.418 | 0.92 | 0.337 | 0.05 | 0.827 | 0.01 | 0.942 | 9.18 | **0.002** |
| Sex | 1.26 | 0.262 | 2.16 | 0.142 | 31.33 | **<0.0001** | 0.00 | 0.991 | 10.63 | **0.001** |
| Food dep:Sex | 4.65 | **0.031** | 0.24 | 0.624 | 0.01 | 0.903 | 1.79 | 0.180 | 2.15 | 0.142 |

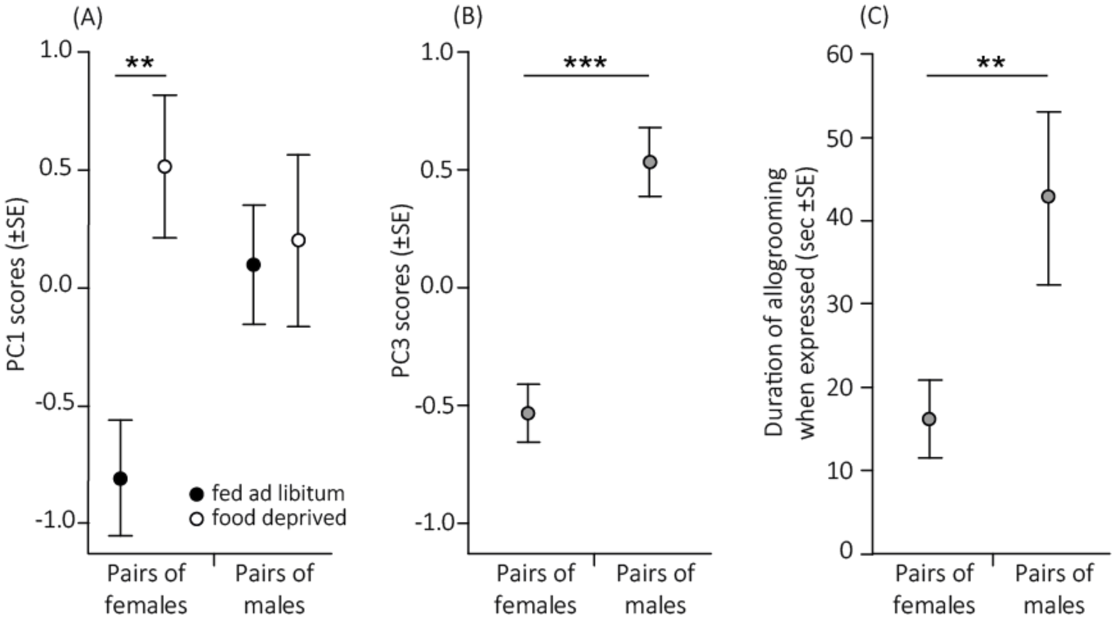

**Figure 3.3.** Influence (A) of sex and food deprivation on the negative link between the durations of self-directed behaviors and aggregation (negative and positive values of PC1 scores, respectively), (B) of sex on the duration of aggression (PC3 scores) and (C) of sex on the duration of allogrooming in the pairs where it has been expressed at least once over the 45 min of observation. ** $p < 0.01$; *** $p < 0.001$

Because allogrooming and aggressions have not been observed in any of the pairs (Figure 3.2B), we also tested whether relatedness, food deprivation and sex shaped the presence or absence of these behaviors. The occurrence was used instead of frequency (i.e. number of times it occurred), because of the unbalanced data set. The occurrence of allogrooming was unaffected by the three tested factors (Relatedness: LR $\chi^2_1$ = 1.24, P = 0.265; Food deprivation: LR $\chi^2_1$ = 0.09, P = 0.763;

Sex: LR $\chi^2_1 < 0.01$, $P = 0.984$; Interactions: all $P > 0.375$). When allogrooming was observed at least once, this behavior lasted significantly longer in male pairs than in female pairs (Figure 3.3C; GLMM on log-transformed duration, LR $\chi^2_1 = 6.28$, $P = 0.012$), but was not influenced by the other factors (Food deprivation: LR $\chi^2_1 = 0.08$, $P = 0.778$; Relatedness: LR $\chi^2_1 = 0.34$, $P = 0.562$; Interactions: all $P > 0.329$).

Aggression occurred more often in pairs of males than females (19 out of 38 and 7 out of 38 pairs, respectively; LR $\chi^2_1 = 7.66$, $P = 0.006$) but was not shaped by the two other factors (Relatedness: LR $\chi^2_1 = 0.18$, $P = 0.673$; Food deprivation: LR $\chi^2_1 = 0.05$, $P = 0.816$; Interactions: all $P > 0.384$). Neither relatedness nor food deprivation affected the duration of aggression in male pairs in which this behavior has been observed (GLMM on log-transformed duration; Relatedness: LR $\chi^2_1 = 0.01$, $P = 0.920$; Food deprivation: LR $\chi^2_1 = 0.11$, $P = 0.746$; Interaction: LR $\chi^2_1 = 0.46$, $P = 0.500$). Such an analysis could not be conducted in pairs of females, as aggression was too rare in these groups. The expression of aggressive behaviors and allogrooming were independent. In particular, the duration of aggression was not correlated with the duration of allogrooming when the two behaviors were expressed (Spearman rank test, n = 32; $r_s = -0.07$, $S = 5829.56$, $P = 0.710$) and the occurrence of allogrooming was independent of the occurrence of aggression (allogrooming was found in 10 of the 26 pairs [38.5%] in which aggression occurred and in 22 of the 50 pairs [44.0%] in which aggression never occurred; $\chi^2_1 = 0.22$, $P = 0.643$).

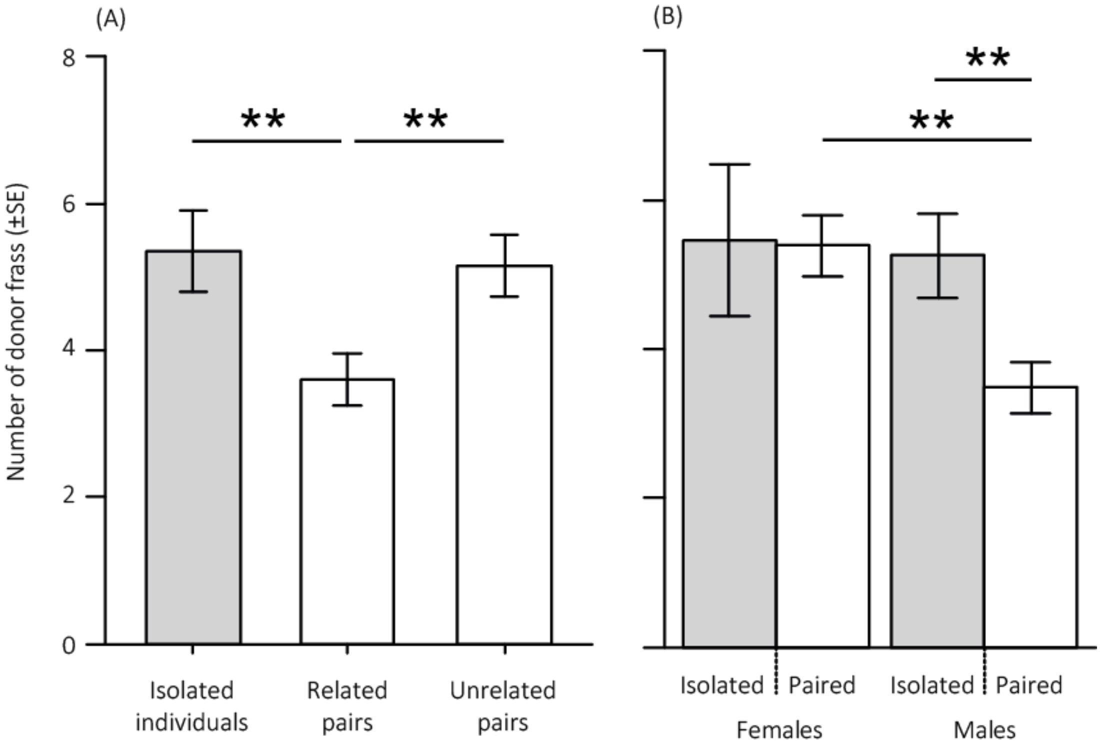

**Figure 3.4.** Influence of (A) relatedness and (B) group sex on the number of frass produced by the donor individuals and counted after 24h. Isolated treatments (controls) are in grey. ** p < 0.01

The number of frass pellets found in the arenas after 24h was higher in unrelated compared to related pairs (Figure 3.4A; Table 3.2) and higher in female than in male pairs (Figure 3.4B; Table 3.2). To disentangle whether these differences reflect an increase or a decrease in frass pellets between the treatments, we compared the corresponding pellet numbers to the ones in the control treatments (i.e. isolated individuals). We found lower pellet numbers in related pairs compared to isolated individuals (Figure 3.4A; LR $\chi^2_1 = 8.31$, $P = 0.004$), but found no difference between unrelated pairs and isolated individuals (LR $\chi^2_1 = 0.42$, $P = 0.520$). Less frass was counted when males were kept in pairs than in isolation (Figure 3.4B; LR $\chi^2_1 = 5.27$, $P = 0.022$), but there was no difference between paired and isolated females (LR $\chi^2_1 = 0.02$, $P = 0.895$). In arenas with isolated males and females, similar numbers of frass pellets were found after 24h (LR $\chi^2_1 < 0.01$, $P = 0.961$).

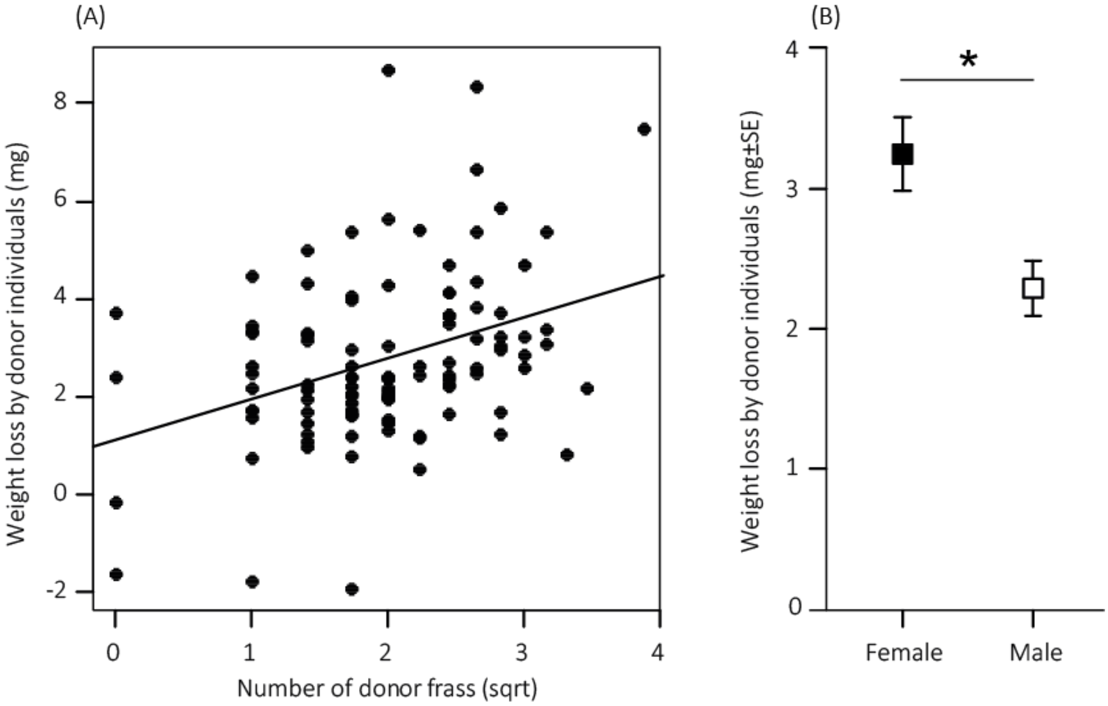

Figure 3.5. Influence of (A) frass production and of (B) sex on the absolute weight loss of donor individuals. ** $p < 0.01$

We then asked whether the reduced number of frass pellets in related pairs and in male pairs was due to lower defecation rates by donor individuals or increased frass consumption by recipient individuals (i.e. higher allo-coprophagy). In line with the first hypothesis, we found that the weight loss by donor individuals over 24h was positively correlated with the number of frass pellets (Figure 3.5A; $r = 0.36$; LR $\chi^2_1 = 11.09$, $P = 0.0008$) and that this correlation was not influenced by sex (interaction between frass number and sex: LR $\chi^2_1 = 1.08$, $P = 0.298$). However contrary to the prediction that relatedness favors frass consumption, we found that relatedness did not influence the correlation between donor weight loss over 24h and frass pellets (LR $\chi^2_1 = 0.03$, P = 0.857). Overall, donor females lost significantly more weight than donor males over 24h (Figure 3.5B; LR $\chi^2_1 = 4.34$, P = 0.037), which was independent of the number of frass pellets, and frass number was independent of relatedness (LR $\chi^2_1 = 0.21$, P = 0.648) or food deprivation (LR $\chi^2_1 = 0.45$, P = 0.504). Finally, the weight lost by recipient individuals over 24h was negatively correlated with the number of frass they had produced ($r = 0.19$; LR $\chi^2_1 = 4.60$, P = 0.039), but not affected by the number of frass produced by the donor individuals (LR $\chi^2_1 = 1.24$, P = 0.266) or by an interaction between these two frass productions (LR $\chi^2_1 < 0.01$, P = 0.941).

## DISCUSSION

This study revealed that pairs of adult earwigs mostly expressed behaviors that were either self-directed (self-grooming, resting and walking) or aggressive, but also showed behaviors that offer scope for cooperation, e.g. allogrooming. Sex shaped the expression of these behaviors, with pairs of males exhibiting longer aggressions and longer bouts of allogrooming (when it occurred) than pairs of females. Furthermore, food deprivation influenced the behaviors of females, but not of males: Females exhibited more self-directed behaviors than aggregation when previously fed *ad libitum*, whereas the opposite behavioral pattern occurred when females were food deprived. Relatedness and/or familiarity did not affect the expression of allogrooming and aggressions, demonstrating that these behaviors are unlikely to shape the genetic composition of earwig groups. Nevertheless, relatedness reduced the number of frass pellets produced by the donor individuals over 24h, which was also lower in pairs of males than females.

Our results demonstrate that unisexual pairs of *F. auricularia* adults expressed allogrooming, an active form of behavioral interaction also expressed by offspring during family life (JM personal observation ;Falk et al. 2014). In adults, the duration of this behavior was limited, but its relatively frequent occurrence over only 45 minutes of observation suggests that allogrooming is a common behavior. Across social animals, this behavior is known to provide important benefits to both donors and recipients by enhancing immune functions, favoring social bonding or improving nestmate recognition in eusocial insects (Rosengaus and Maxmen 1998; Zamma 2002; Boulay et al. 2004; Dunbar 2010; Hamilton et al. 2011; Turgis and Ordon 2012; Konrad et al. 2012; Semple et al. 2013). In *F. auricularia*, these benefits could be sex-specific, as we found that allogrooming lasted longer in pairs of males than in pairs of females (when it occurred at least once over the 45 minutes of observation). However, aggressions were also more frequent and lasted longer in pairs of males than females, a result in line with the male-male competition over female access (Radesäter and Halldórsdóttir 1993b; Forslund 2000). Such an apparent discrepancy is unlikely to reflect that allogrooming is a form of or precludes aggression between males (e.g. Harrison 1965; Radford and Plessis 2006), as the duration and occurrence of these two behaviors were independent. Further studies should investigate the function (benefits and/or costs) of allogrooming between adult earwigs, for instance by manipulating the presence of pathogens in the rearing environment (e.g. Konrad et al. 2012).

An additional sex-specific effect was found regarding the influence of food deprivation on the negative association between activity (self-grooming and walking) and rest (resting in group). In particular, food deprived females were less active than females fed *ad libitum*, whereas food deprivation did not shape such a negative association in males. The effect of food deprivation on female activity received contrasting support across a wide range of group-living animals, ranging from a reduction in mating activities (e.g. in the Japanese beetle *Popillia japonica*; Tigreros and Switzer 2008), over no effect on locomotor activity (e.g. in rats; Genn et al. 2003), to an increase in locomotor activity (e.g. in *Drosophila melanogaster;* Bell et al. 1985). In earwigs, this sex-specific effect of food deprivation is likely to reflect the generally strong importance of resource availability for female fitness, as compared to the strong importance of mating opportunities for male fitness (Bateman 1948). In line with this idea, it has been suggested that *F. auricularia* females in a poor condition produce smaller clutches than females in good condition (Meunier et al. 2012), but such an effect on male reproductive success remains to be further explored.

Whereas relatedness did not affect any of the measured behaviors, it reduced the number of frass produced by the donor individuals. In insects, frass production has multiple roles, such as to deter natural enemies, to attract conspecifics and potential mating partners, or to exchange nutrients and microbial fauna through allo-coprophagy (reviewed in Nalepa et al. 2001; Weiss 2006). In *F. auricularia*, frass is unlikely to be used against predators, as adult defense mostly relies on the use of the forceps and on the spray of chemical repellents (Eisner 1960; Gasch et al. 2013). Similarly, frass alone has been shown to have no direct effect on conspecific attraction in *F. auricularia* (Hehar et al. 2012). Although allo-coprophagy occurs in earwigs and has recently been shown to be higher in related as compared to unrelated pairs of *F. auricularia* offspring (Falk et al. 2014), our results are unlikely to reflect a kin-directed form of allo-coprophagy in pairs of adults. Indeed, this hypothesis would predict that the negative correlation between the number of donor frass counted after 24h and the absolute weight lost by these individuals is stronger in related as compared to unrelated pairs, which is not supported by our analyses. Another hypothesis is that lower frass production in related pairs is a kin-directed form of cooperation, which limits the exposure of relatives to frass-growing pathogens and predators/parasites attracted by the frass (Albouy and Caussanel 1990; Weiss 2006). However, the large quantity of frass commonly found under the shelters hosting earwig groups (Suckling et al. 2006; JM personal observation) makes this hypothesis very unlikely to solely drive the reported variation in frass production. A third hypothesis is that frass release is positively associated with the level of stress endured by *F. auricularia* adults. Such an effect is well-known in mammals, where stress stimulates gastrointestinal activity and thus feces production (Moberg 2000). In line with this hypothesis, we found a smaller number of frass in related pairs than in isolated individuals (i.e. controls), isolation being an important source of stress in group-living animals (e.g. Heidbreder et al. 2000; Lihoreau et al. 2009). However, frass number was also smaller in pairs of males than females, even if the former exhibited more aggressive behaviors. Disentangling whether frass release in adult pairs reflects social stress and/or provides benefits to the surrounding individuals will be explored in future experiments.

To conclude, we demonstrated that *F. auricularia* adults mostly express self-directed along with (albeit more rare) social behaviors when encountering a conspecific individual of the same sex. Variation in the relatedness and/or familiarity between, as well as the nutritional needs of one of these individuals did not modify the expression of allogrooming or aggressive behaviors. Across animal species, the nature and frequency of social interactions is known to possibly vary with the size and the composition of the groups (Sumpter 2010). Because groups of earwig adults vary in terms of sex ratio and size (from pairs to several dozen of individuals, JM personal observation), our findings call for further studies testing the importance of group size and sex composition on the expression of social behaviors in this species. However, by using the same experimental set-up than the one presented here, a recent study demonstrated that *F. auricularia* nymphs maintained in pairs did express frequent cooperative behaviors (e.g. allogrooming and trophallaxis) and that relatedness between the two nymphs shaped such expressions (Falk et al. 2014). This reveals that the expression of adult cooperation possibly depends on different parameters than the ones shaping the expression of offspring cooperation (e.g. more sensitive to group size) and more generally, that different socially-derived benefits might shape the evolution of early family life and adult group living in this species. Further studies should investigate the importance of social (e.g. group density and sex-ratio) and environmental (e.g. pathogen exposure) parameters on the expression of allogrooming and aggression in groups of adults, as well as on the role of these two behaviors on the evolution of adult group living.

## ACKNOWLEDGEMENTS

[removed for privacy purposes]

## SUPPLEMENTARY MATERIAL

**Table S3.1.** Correlations between the durations of the six recorded behaviors. The higher part of the diagonal indicates the coefficients of correlation (spearman rank correlations), while the lower part indicates the associated *p*-values.

| | **Allogrooming** | **Resting in group** | **Self-grooming** | **Walking** | **Resting alone** | **Aggression** |
|---|---|---|---|---|---|---|
| Allogrooming | | rs = 0.22 | rs = 0.00 | rs = 0.07 | rs = -0.29 | rs = -0.11 |
| Resting in group | p = 0.056 | | rs = -0.49 | rs = -0.34 | rs = -0.19 | rs = -0.24 |
| Self-grooming | p = 0.976 | **p < 0.0001** | | rs = 0.29 | rs = -0.35 | rs = -0.09 |
| Walking | p = 0.565 | **p = 0.003** | **p = 0.011** | | rs = -0.10 | rs = 0.14 |
| Resting alone | **p = 0.012** | p = 0.106 | **p = 0.002** | p = 0.401 | | rs = 0.12 |
| Aggression | p = 0.356 | **p = 0.039** | p = 0.425 | p = 0.215 | p = 0.295 | |

# CHAPTER 4

# When earwig mothers do not care to share: parent-offspring competition and the evolution of family life

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*Manuscript in preparation*

# ABSTRACT

Kin competition is often considered a central process in social evolution, because it can reduce and sometimes entirely negate the benefits of cooperation among relatives. Surprisingly, however, the potential impact of kin competition between parents and their offspring on the evolution of family life has been largely overlooked. Here, we examined the occurrence and consequences of mother-offspring competition over food access in the facultatively caring European earwig *Forficula auricularia*. We showed that maternal presence under food restriction triggered a competition between mothers and offspring that manifested itself in a trade-off between their respective weight gains. This competition drastically reduced offspring survival, but positively affected the mothers' relative investment into their subsequent clutch. In contrast, the body size of the surviving adult offspring was independent of the intensity of competition, but generally reduced under maternal presence. Notably, the competition was most intense in clutches tended by mothers with a low initial weight. Overall, these results demonstrate that mothers competed with their offspring to maintain their body condition and/or to shift their investment to future reproduction. More generally, our findings reveal that parent-offspring competition can counteract the benefits of (facultative) care, and thus hamper the evolution of family life in resource-poor environments.

*Keywords:* insect; orphaning; parental care; environmental conditions

# INTRODUCTION

The association of offspring with their tending parents after birth or hatching (defined as family life; Clutton-Brock 1991) is a rare, but taxonomically widespread phenomenon in nature (Royle et al. 2012b) that can only evolve if the fitness benefits of family interactions outweigh the costs of a prolonged association of the family members (Alonso-Alvarez and Velando 2012). The fitness benefits of family life predominantly derive from the expression of parental care (e.g. in the form of nest construction, brood attendance or food provisioning ;Costa 2006; Alonso-Alvarez and Velando 2012; Wong et al. 2013) which ultimately increases the survival and/or quality of tended juveniles (Klug et al. 2012). By contrast, the costs of family life typically result from kin competition (West et al. 2002) among family members for limited resources and reproduction (Krause and Ruxton 2002). While kin competition is known to affect key life-history traits such as sex allocation (Frank 1985), dispersal (Le Galliard et al. 2003), and aggressive behavior (West et al. 2001), it remains unclear whether its expression can affect and/or involve both offspring and their parents during family life. Hence, it is essential to shed light upon the conflicting parties engaged in competition to understand the cost-benefit ratio – and more generally the evolution – of family life (Klug and Bonsall 2010; Klug et al. 2012).

Somewhat surprisingly, kin competition during family life only received close empirical and theoretical scrutiny in studies on sibling rivalry (Mock and Parker 1997; Roulin and Dreiss 2012), while the occurrence of competition between parents and offspring has been neglected and its impact on the evolution of parental care remains unknown. This oversight conceivably results from a historical bias towards studying parental care in altricial species (Clutton-Brock 1991; Royle et al. 2012b), in which the scope for parent-offspring competition during family life is limited by the low foraging capabilities of juveniles. However, juveniles are not fully dependent on parental resources in precocial species (i.e. parental care is facultative) and the early onset of offspring foraging might put them into direct competition with their caring parents – a scenario that likely prevailed during early stages of the evolution of family life (Smiseth et al. 2003b; Kölliker 2007). In such situations, parent-offspring competition could diminish the benefits of parental care, and the costs of intense competition might ultimately render parental presence (Scott and Gladstein 1993; Ward et al. 2009; Boncoraglio and Kilner 2012) and family life maladaptive altogether (Meunier and Kölliker 2012a).

The intensity of competition typically depends on the environmentally determined availability of limited resources (Wilson 1975b). Hence, harsh environments could have a profound impact on parent-offspring competition and the evolution of family life (Wilson 1975b; Klug et al. 2012). The nature of this impact, however, remains controversial. On the one hand, harsh conditions should favor the evolution of parental care and thus family life, because then the benefits of care are likely to be substantial (Wilson 1975b; Clutton-Brock 1991). On the other hand, such environments are expected to exacerbate the costs of care (such as an increased energy loss or an elevated predation risk (Alonso-Alvarez and Velando 2012), and the limited availability of resources may not only favor shifts in parental investment from current to future reproduction (Clutton-Brock 1991; Klug and Bonsall 2007), but also increase parent-offspring competition and ultimately hamper the evolution of care and family life. In line with this hypothesis, the prolonged presence of fathers has been shown to reduce larval body weight and survival under food limitation in the burying beetle *Nicrophorus vespilloides*, a species with biparental care in which both parents might come into competition with their offspring as they feed on the carcass employed for breeding (Scott and Gladstein 1993; Ward et al. 2009; Boncoraglio and Kilner 2012). Moreover, food restriction in the European earwig *Forficula auricularia* has recently been shown to counteract the benefits of maternal care (Kölliker and Vancassel 2007; Kölliker 2007) to an extent that renders maternal presence – and hence family life – detrimental to offspring survival (Meunier and Kölliker 2012a), a finding that might reflect the outcome of kin competition between offspring and their tending mother.

In this study, we investigated the occurrence, as well as potential causes and consequences of mother-offspring competition under harsh conditions (emulated by food restriction) in the European earwig *F. auricularia*. In this precocial insect, mothers provide extensive forms of care to their mobile offspring (called nymphs) for several weeks after hatching (Lamb 1976b). Maternal care includes the protection and grooming of nymphs, as well as their provisioning with food (e.g. through regurgitation; Lamb 1976a; Staerkle and Kölliker 2008), and is even expressed by mothers in a bad nutritional condition (chapter 2). However, post-hatching maternal presence and care are not obligatory for offspring survival (Kölliker and Vancassel 2007; Kölliker 2007), as nymphs can forage independently soon after hatching (relying on the same food resources than their mother; Beall 1932; Lamb 1976a) and may even obtain food from their siblings (Falk et al. 2014; chapter 1 and 2). As a result, the cost-benefit ratio of maternal presence in *F. auricularia* generally depends on the particular environmental conditions experienced by mothers and nymphs during the period of family life (Kölliker 2007; Meunier and Kölliker 2012a; chapter 5).

To assess the occurrence of mother-offspring competition, we raised nymphs under food restriction either together with or without their mother, and investigated whether offspring survival was reduced when mothers consumed food and thus restricted offspring feeding (or *vice versa*). We then examined whether the condition of the mothers and/or their nymphs at hatching determined the intensity of the putative competition, as well as whether this intensity affected the mothers' future reproduction and the morphology of their (surviving) first-brood offspring. If mother-offspring competition occurred, we would expect that offspring survival under maternal presence (but not under maternal absence) is negatively affected by a trade-off between the weight gains of mothers and their nymphs during family life (reflecting the mutual restriction of food consumption). Moreover, we predicted that mothers exhibiting a low body weight would compete more intensely – and thus curtail their offspring's survival more extensively - than mothers exhibiting a high body weight. Similarly, groups of nymphs with low weight were predicted to compete more intensely with their mother to ensure their own survival. Finally, we expected that intense competition might benefit mothers and the surviving (high-quality) offspring, for instance by enabling them to increase their investment into future reproduction and their final body size, respectively.

## MATERIAL AND METHODS

### *Laboratory rearing and experimental design*

We investigated the occurrence and potential repercussions of mother-offspring competition under harsh conditions in 128 female earwigs and their first brood. The females had been collected in July and August 2013 in Mainz, Germany and were reared under standard laboratory conditions (adapted from Meunier et al. 2012; see supplementary material for details) until they produced their first clutch. On the first day after egg hatching, we haphazardly distributed a random subset of 32 nymphs (original brood size: 49.6 ± 1.1 nymphs; mean ± se) among two equally sized groups. The potential for mother-offspring competition over food access was manipulated by raising one of these groups together with their mother (maternal presence-, or *MP-group*) and the other group without their mother (maternal absence-, or *MA-group*). After 16 days, mothers were removed from the MP-groups to mimic natural family disruption (Meunier et al. 2012) and to determine their investment into the production of a second (and final) clutch. In parallel, the MP- and MA-nymphs were maintained in their groups and their survival and morphology were measured upon adult emergence. Note that 16 of the 128 initially employed families were excluded from our analyses because the mother died during family life.

Harsh environmental conditions were emulated according to an established protocol (Meunier and Kölliker 2012a) by providing MP- and MA-groups with a restricted amount of an artificial diet (composition detailed in chapter 1) every six days for the duration of three days, respectively. This six-day cycle was initiated on the first day after hatching and repeated until the juveniles reached adulthood. The amount of food the groups received at a time was increased stepwise from 60 mg (until the end of family life) to 120 mg (until day 31) and finally to 240 mg (until adulthood; cf Meunier and Kölliker 2012a). This feeding regime successfully established scope for mother-offspring competition, as the amount of food provided during family life was more often fully consumed before removal in MP- than in MA-groups (in 81 vs. 25 % of cases; paired Wilcoxon signed rank test: $V = 4656$, $P > 0.0001$). We also manipulated the conditions experienced by mothers after family life to investigate the effect of food availability during that period on their investment into a second clutch. To this end, isolated mothers either received 60 mg of food in a continuation of the above detailed six-day cycle (low food-, or *LF-treatment;* n = 54 mothers), or an *ad libitum* amount that was renewed twice per week (high food-, or *HF-treatment*; n = 58 mothers). Isolated mothers and all groups of young nymphs were kept in medium Petri dishes (Ø = 9 cm) throughout the experiment. On day 31, groups of older nymphs were transferred into large Petri dishes (Ø = 14 cm). All Petri dishes contained humid sand as substrate and a plastic tube as shelter.

### *Measurements*

We determined the occurrence and intensity of mother-offspring competition by testing whether maternal weight gains that came at the expense of offspring weight gains reduced offspring survival (see in *statistical analyses* below). To this end, we measured the relative weight changes of mothers and nymphs (used as a proxy for maternal and nymphal food consumption, respectively), as well as the survival rates of nymphs at the end of family life and upon adult emergence in all remaining 112 families (see above). The weights of mothers were determined by weighing each mother on the first day after egg hatching, as well as at the end of family life. Similarly, the average weights of nymphs were determined by weighing all nymphs of a family on the first day after hatching, as well as all surviving nymphs of a group at the end of family life, and then dividing these weights by the corresponding number of weighed nymphs. The *relative* weight changes were subsequently calculated by subtracting the weight measured at the beginning of family life from the weight measured at the end of family life, and then dividing this difference by the first weight measured. All mothers and nymphs were weighed to the nearest 0.01 mg using a microscale (model MYA5; PESCALE, Bisingen, Germany). The survival rates of offspring were determined by first counting the nymphs that survived until day 16 or until adulthood in each group, and then dividing this number by the number of nymphs initially distributed to that group, respectively. Note that two nymphs per group were removed six days after hatching to conduct an independent experiment (data not shown). These nymphs were not considered in the calculation of survival rates.

We also determined the consequences of the suspected mother-offspring competition on the future (and final) reproductive effort of mothers, as well as on the morphology of the surviving, grown-up offspring. To this end, we checked all 112 isolated mothers daily for oviposition over a period of 100 days, and assessed (1) the occurrence of egg deposition and - where applicable - (2) the length of the inter-clutch interval and (3) clutch size, i.e. the number of eggs produced within three days after the onset of egg-deposition (Meunier and Kölliker 2013). The inter-clutch interval was defined as the number of days between isolation from the first brood and the deposition of the second clutch (Kölliker 2007). Conversely, the morphology of adult offspring was assessed by measuring two fitness-relevant morphological traits - eye distance (as a proxy of body size) and forceps length (Radesäter and Halldórsdóttir 1993a) - in the first male and female adult that emerged in each group (males can easily be distinguished from females based on the shape of their forceps). Overall, we measured 192 males and 198 females [at least one male emerged in 92 (100), and at least one female in 94 (104) of the 112 MP- (MA-) groups]. All morphological measurements were taken under $CO_2$-anesthetization to the nearest 0.001 cm using a camera coupled to a stereo microscope (Leica DFC425, Leica Microsystems Ltd, Heerbrugg, Switzerland) and operated with the Leica Application Suite 4.5.0. software.

### *Statistical analyses*

Establishing the occurrence of mother-offspring competition over food access requires to demonstrate that offspring fitness under maternal presence is reduced – and maternal fitness possibly increased – when mothers restrict offspring feeding through their own food consumption (or vice versa). Accordingly, the first step of our analysis was to capture a measure of the mutual restriction of feeding, as is potentially provided by a trade-off between the relative weight gains of mothers (mean ± SD: 0.138 ± 0.093) and their nymphs (mean ± SD: 0.859 ± 0.289). To extract this trade-off, we entered the weight gains of mothers and nymphs (after scaling to unit variance) into a principal component analysis (PCA). Note that this step was crucial, as weight changes may not only reflect the influence of extrinsic factors such as competition, but also family-specific intrinsic (genetic) factors that, for instance, determine how effectively mothers and offspring transform food into body weight (Wilson and Nussey 2010). The PCA yielded two principal components (PCs) that reflected a positive association between relative maternal and nymph weight gains on the one hand (PC1), and a separate and independent trade-off between these weight gains on the other hand (PC2, see results).

The second step in establishing the occurrence of mother-offspring competition was to test whether our measure of the mutual restriction of feeding (i.e. PC2, see above) affected offspring fitness only under maternal presence. Note that this is not inevitably the case, since the trade-off between weight gains reflected by PC2 could alternatively affect offspring survival irrespective of maternal presence (indicating, for instance, that it reflects an intrinsic correlation between maternal and offspring traits), or not at all (indicating that it fails to capture a biologically meaningful dimension of our data). To discern these possibilities, we first used a generalized linear mixed model (GLMM) with binomial error distribution to test whether offspring survival (entered as odds ratio) was affected by the two PCs, maternal presence (MP or MA), the time of measurement (at the end of family life or upon adult emergence) or any of their interactions. An observation-level variable and Family-ID were entered into the model as random effects to account for overdispersion (Harrison 2015) and the common origin of nymphs in the MP- and MA-groups, respectively. Because a triple-interaction between the time of measurement, maternal presence and one of the PCs shaped offspring survival (Table S1; Wald $\chi_1^2 = 8.01$, $P = 0.0047$), we split the data set by time of measurement and fitted two GLMMs with the same set of random effects and explanatory variables (but the time of measurement) within each subset. The effects of the putative competition on offspring morphology were tested in two separate linear mixed models (LMM), in which the two PCs, maternal presence, and adult sex were entered as explanatory variables, and either eye distance or forceps length (corrected for eye distance; see supplementary material for details) as response variable. Both models were fitted using Family-ID as random effect.

In the third step of our analysis, we tested in two generalized linear models (GLMs) and one linear model (LM) whether the putative mother-offspring competition affected the mothers' future reproduction. Each of these models included the two PCs and the availability of food after family life (HF and LF) as explanatory variables. The GLMs were fitted with a binomial error distribution (corrected for overdispersion) and using the occurrence of 2nd clutch production or the relative investment of mothers into the production of their 2nd clutch (entered as odds ratio of 2nd to 1st clutch eggs) as response variable. Conversely, the LM was fitted using the length of the inter-clutch interval as response variable. Note that this LM and the GLM analyzing the relative investment of mothers into 2nd clutch production were fitted on the subset of those mothers that eventually produced a second clutch. In the fourth and final step of our analysis, we investigated the determinants of mother-offspring competition. To this end, we fitted a LM in which the PC reflecting a trade-off between maternal and offspring weight gains was entered as response variable, and maternal weight at hatching and the average nymph weight at hatching (corrected for the initial weight of the mother; see supplementary material for details) as explanatory variables. An analysis of the effects of the weights of mothers and nymphs at hatching on the PC reflecting the joint weight gains is given in the supplementary material.

All statistical analyses were performed with the statistics software R version 3.0.3 (http://www.r-project.org/). Mixed model analyses were implemented using the R package *lme4.* Significance levels of effects in these models were determined using the R packages *car* (*Anova* function) and *lmerTest* (*summary* function). All statistical models initially included all possible interactions between the tested variables. Model selection was then conducted via stepwise deletion of non-significant interactions (all $p < 0.05$). Models were checked for homogeneity of variance and normality of residuals before and after each step of the model selection procedure. Note that our results do not qualitatively change if the PCA is computed using absolute instead of relative weight changes.

## RESULTS

The PCA performed on the relative weight gains of mothers and nymphs during family life provided two orthogonal principal components (PCs). Both maternal and offspring weight gains loaded positively onto the first component (*PC1*; loadings of both variables = 0.707; variance explained: 52.8%), which thus reflects joint weight gains of mothers and nymphs. In contrast, maternal weight gain loaded positively (loading = 0.707) and offspring weight gain negatively (loading = -0.707) onto the second component (*PC2*; variance explained: 47.2%), indicating that PC2 represents a trade-off between the weight gains that might reflect mother-offspring competition. High values of PC2 hence indicate that mothers gained weight at the expense of their offspring, whereas low values indicate that offspring gained weight at the expense of their mother.

**Table 4.1. Offspring survival.** Influences of maternal presence and the two principal components - reflecting the joint weight gain of mothers and nymphs during family (PC1) and a separate trade-off between these weight gains (PC2), respectively - on the survival of nymphs (A) upon adult emergence and (B) at the end of family life. Significant P-values are given in bold print ($^{\S}$ values as indicated before removal of the interaction from the model).

| Offspring survival | (A) upon adult emergence | | (B) at the end of family life | |
|---|---|---|---|---|
| | Wald $\chi_1^2$ | P | Wald $\chi_1^2$ | P |
| Maternal presence (MP) | 54.90 | **< 0.0001** | 5.11 | **0.0238** |
| PC1 | 7.79 | **0.0053** | 1.03 | 0.3093 |
| PC2 | 11.66 | **0.0006** | 0.46 | 0.4999 |
| MP : PC2 | 6.99 | **0.0082** | $0.75^{\S}$ | $0.3858^{\S}$ |

In line with the expectation that mother-offspring competition manifests itself as the effect of a trade-off between maternal and offspring weight gains under maternal presence only, we found that PC2 scores and maternal presence interacted in shaping the survival of nymphs until adulthood (Table 4.1A, Figure 4.1A). Under maternal presence, nymph survival was negatively affected by PC2 scores (Estimate ± SE: -0.315 ± 0.073, z = -4.286, P < 0.0001), indicating that nymph mortality increased when mothers gained weight at the expense of their offspring during family life. In contrast, the survival of nymphs raised without their mother was independent of the PC2 scores (Estimate ± SE: -0.072 ± 0.066, z = -1.099, P = 0.2720). Irrespective of this trade-off, the survival of nymphs until adulthood increased with joint weight gains of mothers and nymphs in both MP- and MA-groups (PC1 scores; Table 4.1A, Figure 4.1B). None of these effects were detected within the first weeks after egg hatching, as the survival of nymphs at the end of family life – albeit slightly lower under maternal presence (Mean ± SE: 87.7 ± 1.5%) than maternal absence (Mean ± SE: 90.9 ± 1.4%) - was overall high and independent of the two PCs (Table 4.1B).

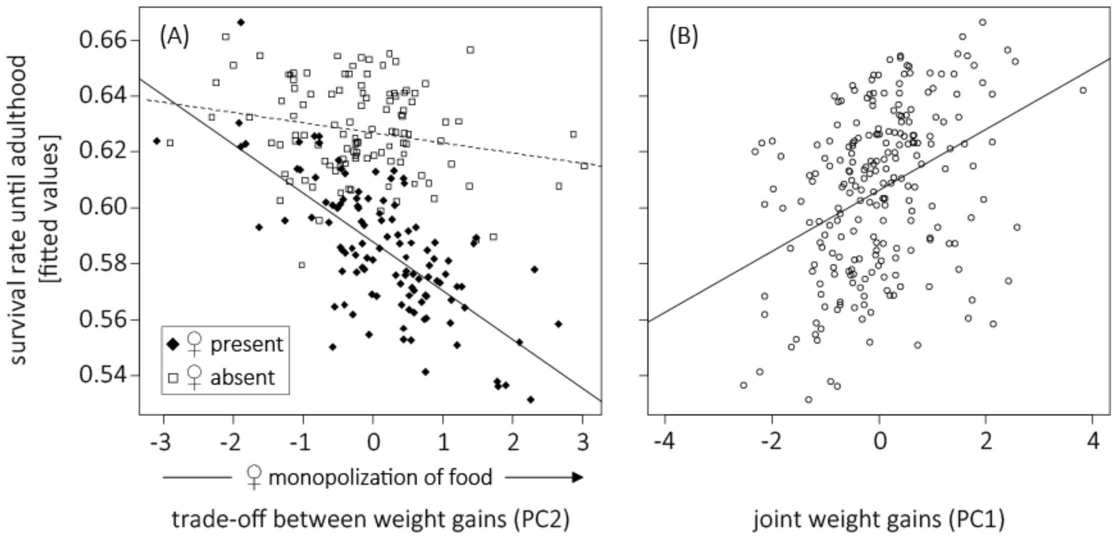

**Figure 4.1. Offspring survival until adulthood.** Influence of (A) the trade-off between the weight gains of mothers and their nymphs (PC2) on the survival rate of offspring raised together with or without their mother, and (B) general increase of offspring survival with the joint weight gains of mothers and their nymphs (PC1).

Interestingly, the 17 mothers that had an overall positive effect on long-term offspring survival under our harsh experimental conditions featured lower PC2 scores than the remaining 95 mothers (two sample t-tests; PC2 scores: t = -2.695, P = 0.0076; PC1 scores: t = -0.727, P = 0.4683), indicating that they gained less weight at the expense of their offspring. Finally, maternal weight at hatching negatively affected the PC2 scores (Estimate ± SE: -49.699 ± 11.805, t = -4.210, P < 0.0001), revealing that initially light mothers gained more weight at the expense of their offspring than initially heavy mothers (Figure 4.2). In contrast, the PC2 scores were independent of the average nymph weight at hatching (Estimate ± SE: 853.013 ± 528.006, t = 1.616, P = 0.1090).

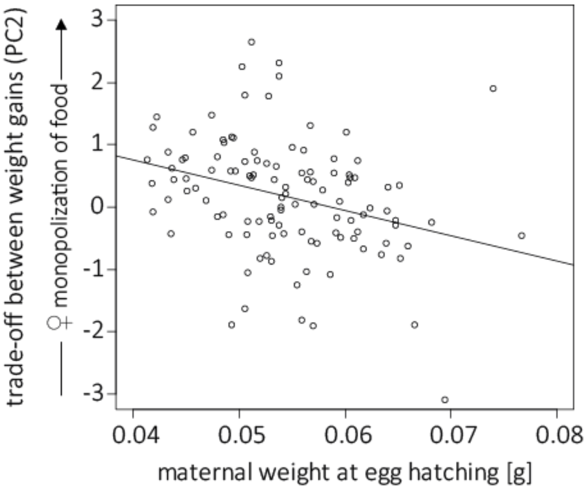

**Figure 4.2.** Influence of the initial maternal weight on the trade-off between the weight gains of mothers and their nymphs during family life.

**Table 4.2. Maternal investment into second clutch production.** Influences of the joint weight gains of mothers and nymphs during family life (PC1), the trade-off between these weight gains (PC2), and the food availability after the end of family life (high food or low food) on (A) the likelihood of 2nd clutch production, (B) the length of the inter-clutch interval, and (C) the relative investment into 2nd clutch production by tending mothers. Significant P-values are given in bold print.

| | (A) Likelihood of production | | (B) Inter-clutch interval | | (C) Relative investment | |
|---|---|---|---|---|---|---|
| | LR $\chi_1^2$ | P | $F_1$ | P | $F_1$ | P |
| PC1 | 1.30 | 0.2550 | 0.54 | 0.4656 | 0.69 | 0.4102 |
| PC2 | 0.80 | 0.3725 | 0.05 | 0.8303 | 5.76 | **0.0240** |
| Food availability | 4.37 | **0.0365** | 4.14 | **0.0466** | 3.31 | **0.0429** |
| Model type \| sample size | GLM \| 113 | | LM \| 62 | | GLM \| 62 | |

Overall, 61 of the 112 mothers (54.5 %) produced a second clutch after isolation from their first-brood nymphs. The trade-off between maternal and nymphal weight gains during family life (as represented by the PC2 scores) did not affect the likelihood of 2nd clutch production (Table 4.2A) or the length of the inter-clutch interval (Table 4.2B), but influenced the relative investment into the 2nd clutch (Table 4.2C). Specifically, the relative investment increased with the PC2 scores (Estimate ± SE: 0.044 ± 0.019, t = 2.400, P = 0.0197; Figure 4.3), illustrating that mothers produced more 2nd clutch eggs if they had gained a lot of weight at the expense of their first-brood offspring. By contrast, the availability of an *ad libitum* (rather than restricted) amount of food after family life decreased the inter-clutch interval (Table 4.2B), and increased both the likelihood of (Table 4.2A) and the mothers' relative investment into 2nd clutch production (Table 4.2c). Finally, the PC1 scores did not influence any of the measured 2nd clutch traits (Table 4.2A-C).

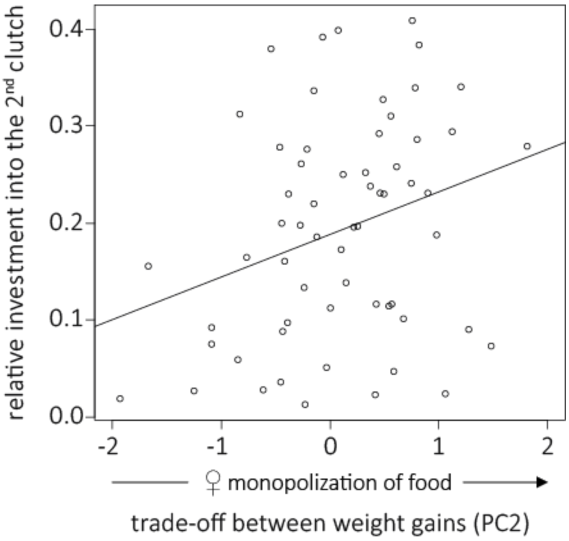

**Figure 4.3.** Influence of the trade-off between the weight gains of mothers and their nymphs during family life on the relative investment of mothers into 2nd clutch production.

**Table 4.3. Morphology of the surviving adult offspring.** Influences of maternal presence, offspring sex, and the two PCs reflecting the joint weight gains of mothers and nymphs during family life (PC1) and the independent trade-off between these weight gains (PC2) on (A) the eye distance and (B) the (corrected) forceps length of the surviving adult offspring. Significant P-values are in bold print.

| | (A) Eye distance | | (B) Forceps length | |
|---|---|---|---|---|
| | Wald $\chi_1^2$ | P | Wald $\chi_1^2$ | P |
| Maternal presence | 6.16 | **0.0131** | 0.69 | 0.4075 |
| Sex | 59.51 | **< 0.0001** | 659.51 | **< 0.0001** |
| PC1 | 7.34 | **0.0068** | 0.21 | 0.6507 |
| PC2 | 3.03 | 0.0816 | 2.41 | 0.1203 |

The eye distance of adult offspring was overall smaller when they grew up together with (Mean ± SE: 1.340 ± 0.005 mm) rather than without their mother (Mean ± SE: 1.354 ± 0.005 mm; Table 4.3A), and overall larger in females (Mean ± SE: 1.370 ± 0.005 mm) than in males (Mean ± SE: 1.324 ± 0.004 mm; Table 4.3A). Moreover, adult eye distance decreased with increasing PC1 scores (Estimate ± SE: -0.0097 ± 0.0034, $t_{142.8} = -2.868$, $P = 0.0048$), but was independent of PC2 scores (Table 4.3A). By contrast, the corrected forceps length of the surviving adults was – albeit overall larger in males (Mean ± SE: 0.240 ± 0.017 mm) than in females (Mean ± SE: -0.233 ± 0.012 mm) – independent of maternal presence and both PCs (Table 4.3B).

## DISCUSSION

Kin competition often reduces and sometimes even entirely negates the benefits of cooperation among relatives (West et al. 2002). Surprisingly, however, the potential role of kin competition between parents and their offspring in the evolution of parental care has been largely overlooked. Here, we demonstrated that maternal presence under food restriction triggered a mother-offspring competition in the European earwig *F. auricularia*. This competition manifested itself as the effect of a trade-off between the relative weight gains of mothers and their offspring during family life, and dramatically reduced long-term offspring survival. The intensity of this competition was determined by the weight of the mother at egg hatching, and positively affected the future reproductive investment among those mothers that produced a second clutch. By contrast, joint weight gains of mothers and their offspring during family life positively affected the long-term survival of juveniles independent of maternal presence, suggesting that these weight gains reflect intrinsic factors such as the (genetic) quality of family members (Wilson and Nussey 2010). Finally, we showed that maternal presence curtailed the adult body size of those offspring that overcame the lethal consequences of the competition with their mother.

We showed that the survival of offspring until adulthood was substantially reduced if mothers gained weight at the expense of their offspring during family interactions. Notably, the negative effect of this trade-off was only evident in offspring that grew up with their mother, and emerged *after* the end of family life. This demonstrates that mothers competed with their offspring by consuming portions of the limited amount of food available, and thereby indirectly limited the long-term survival prospects of their progeny, e.g. by increasing the likelihood of offspring starvation or by triggering high levels of siblicide (Dobler and Kölliker 2010). Interestingly, the (short-term) survival of offspring *at* the end of family life was independent of mother-offspring competition and of the joint weight gains, but overall reduced under maternal presence. This finding might have reflected filial cannibalism, for example aimed at increasing the survival prospects of the remaining juveniles (Forbes and Mock 1998; Klug and Bonsall 2007). Alternatively, the presence of a tending mother might have increased sibling competition (Gardner and Smiseth 2011; Wong et al. 2014a), or mothers might have exhibited behaviors that are maladaptive under laboratory conditions (such as the burying of food, presumably to prevent microbial growth; see chapter 5). This notwithstanding, our results reveal that mother-offspring competition can substantially diminish the long-term survival prospects of precocial juveniles. These results are unlikely to be an artefact of the prevention of offspring dispersal in our experimental setup, as food limitation does not accelerate nymph dispersal in *F. auricularia* (Wong and Kölliker 2012). Hence, our findings overall suggest that local competition between parents and their offspring might counteract the benefits of facultative care (Scott and Gladstein 1993; Ward et al. 2009; Boncoraglio and Kilner 2012), and thus impede the transition to – and the maintenance of – social life in resource-poor environments.

Our results revealed that the body weight of the mothers, but not that of their offspring, affected the intensity of mother-offspring competition. Mothers with a low weight at egg hatching gained more weight at the expense of their offspring – and thus reduced the offspring's long-term survival prospects more substantially – than initially heavier mothers. This result indicates that the extent of mother-offspring competition is subject to maternal control (Smiseth et al. 2008; Hinde et al. 2010). Interestingly, the fact that the strength of competition did not differ between clutches of particularly heavy and light nymphs also emphasizes that mothers did not adjust the extent of competition in relation to the perceived reproductive value of their offspring, for instance by showing more self-restraint in feeding when tending high compared to low quality nymphs (Kilner and Hinde 2012). Overall, these findings suggest that the (genetic) quality and/or the condition of mothers at egg hatching (see also Koch and Meunier 2014) determine how mothers react to food limitation (e.g. by affecting their responsiveness to offspring solicitation behaviors; Grodzinski and Johnstone 2012) and thus whether they compete with their offspring. Here, mothers of low quality or bad condition might have favored somatic maintenance over self-restraint (McNamara and Houston 1996; Dall and Boyd 2002; Alonso-Alvarez and Velando 2012), either to safeguard their ability to perform crucial parenting behaviors such as predator defense (Bateson 1994), or in an attempt to shift their investment from their current to future offspring (Thorogood et al. 2011; chapter 2).

In line with the hypothesis of a condition-dependent shift of maternal investment, the intensity of mother-offspring competition positively affected the relative investment into 2nd clutch production among those mothers that produced another clutch. By contrast, the likelihood of 2nd clutch production and the length of the inter-clutch interval were independent of mother-offspring competition. Note that these effects were independent of food availability after family life, even if *ad libitum* access to food resources overall benefitted mothers by shortening the inter-clutch interval and by increasing the likelihood of – and the relative investment into – 2nd clutch production. Together, these results first indicate that the decision whether to produce a second clutch and (where applicable) its size might be regulated independently. For example, mothers might only be able to re-invest resources acquired during family life if the resource availability after family life allows them to reach a resource-threshold that triggers egg production (Drent and Daan 1980; Weimerskirch 1992). Second, these findings reveal that mother-offspring competition does not necessarily trigger a shift in maternal investment from current to future reproduction (Klug et al. 2012). This supports the conjecture of an additional motivation for this competition (e.g. the maintenance of maternal condition to perform crucial parenting behaviors such as predator defense; Bateson 1994), which in turns highlights that parent-offspring competition should not uncritically be taken as evidence for parent-offspring conflict (*sensu* Trivers 1974). In particular, the scope for conflict might be limited if mothers re-invest the competitively acquired resources into their current brood (e.g. by raising their ability to repel predators during family life) and thereby offset the costs of competition for 1st brood juveniles. Finally, the extent of joint weight gains during family interactions did not influence any of the measured 2nd clutch traits, a finding in line with the assumption of their determination by (fixed) intrinsic factors.

In contrast to its influence on offspring survival and the mothers' relative investment into 2nd clutch production, the intensity of mother-offspring competition did not affect the size and forceps length of offspring that survived until adulthood. This result suggests that this competition is unlikely to reflect an *indirect* mechanism of adaptive brood reduction by mothers. Specifically, mother-offspring competition does not seem to have handicapped low-quality (*marginal*) offspring to an extent that favored the survival and development of higher-quality (*core*) offspring, as the average size of maternally-tended offspring should have been larger than that of non-tended juveniles in this scenario (Simmons 1988; Mock and Parker 1997; Roulin and Dreiss 2012). Nevertheless, our results showed that adults were overall smaller when they had been raised with their mother, a negative effect of maternal presence that has already been found under favorable conditions (featuring unrestricted food access) and that might result from maternal behaviors that are maladaptive under laboratory conditions (see above and chapter 5). Our results also revealed that males were overall smaller but exhibited longer forceps than females, confirming the sexual dimorphism of these morphological traits (Radesäter and Halldórsdóttir 1993a; chapter 5). Finally, we showed that body size generally decreased with joint weight gains of mothers and nymphs. This result is presumably due to the larger number of nymphs alive in broods that had featured high (as compared to low) joint weight gains during family life, and thus likely reflects the negative effect of sibling competition on the body size of the surviving adults in large compared to small broods.

In conclusion, we demonstrated that local competition between parents and their offspring can drastically reduce offspring fitness in facultatively caring species. Parent-offspring competition might thus not only diminish the benefits of a prolonged association of parents with their offspring (Scott and Gladstein 1993), but even impede the evolution of family life altogether. This finding illustrates that parental presence can be associated with costs for the tended offspring (Scott and Gladstein 1993; Meunier and Kölliker 2012a; chapter 5) that are usually masked by the benefits of parenting behaviors in the wild (Costa 2006; Wong et al. 2013), but that emerge whenever the (laboratory) conditions prevent these benefits from taking effect. While parents might be able to reduce the costs of local competition with their offspring under natural conditions, the behavioral changes necessary to do so are likely themselves costly. For example, parents might increase their foraging range (West et al. 2002), but will then likely suffer from a higher predation risk (Alonso-Alvarez and Velando 2012). Our results thus generally stress the crucial role of environmental factors such as resource availability and predation pressure in the early evolution of social life. Importantly, our findings also provide a diachronic perspective on social evolution: they suggest that competition between parents and their offspring should decline with an increasing reliance of offspring on parentally provided resources, a process that in turn is known to increase the scope for competition *among* offspring (Gardner and Smiseth 2011). The transition from facultative to obligatory forms of family life might hence be accompanied by a shift in the type of competition that most profoundly affects family interactions.

## ACKNOWLEDGEMENTS

[removed for privacy purposes]

## SUPPLEMENTARY MATERIAL

### Animal rearing

We followed a previously established rearing protocol (see Meunier et al. 2012), but modified the housing temperatures (originally adapted to a population from Dolcedo, Italy) to match the requirements of our local population. In particular, the 128 females used in our experiment were a haphazardly chosen subset of 660 females that had been field-caught together with an equal number of males in July and August 2013 in Mainz, Germany. The females were initially kept under spring/summer conditions (12:12h light/dark, 20:18°C and 60% humidity) in groups of 30 individuals with an equal number of males in plastic terrariums (37 x 22 x 25 cm) to stimulate (uncontrolled) mating. Each terrarium contained humid sand as ground material and an egg carton and plastic tubes as shelters. An artificial diet mainly consisting of carrots, wheat germ, pollen and cat food (detailed composition see in chapter 1) was provided *ad libitum* twice a week. After three months, the females were isolated individually in Petri dishes (9 cm diameter) and transferred to autumn conditions (constant darkness and 15°C, 60% humidity) to initiate the deposition of their first clutch of eggs. Three weeks after female isolation, the ambient temperature was first decreased to 10°C and - after another three weeks - to 5°C, thus simulating winter conditions. After one month at 5°C, the temperature was first increased to 10°C and - after another three weeks - to 15°C. Each female received an *ad libitum* amount of the artificial diet twice a week until egg deposition (females generally do not consume food during egg care; Kölliker 2007). Upon egg hatching, females and their first brood of offspring were transferred to the above detailed spring/summer conditions and entered the experiment. Note that females were transferred back to autumn conditions (constant darkness and 15°C, 60% humidity) for the production of their 2$^{nd}$ clutch of eggs.

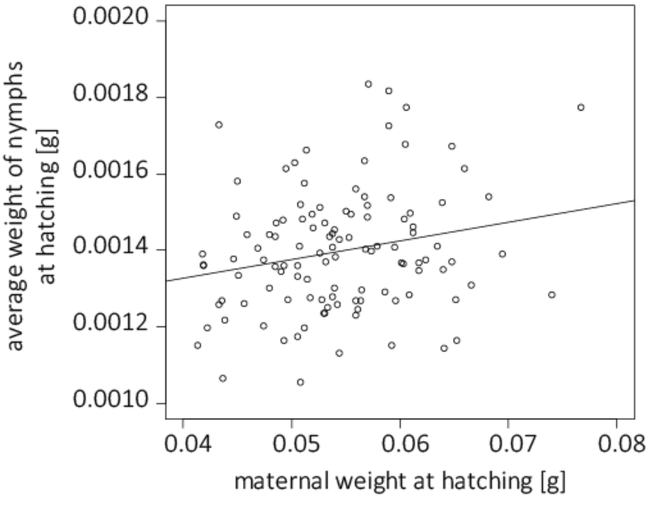

**Figure S4.1.** Increase of the average weight of nymphs at hatching with the initial weight of their mother.

## Nymphal and maternal weight at hatching

The average weight of nymphs at hatching increases with the initial weight of their mother (LM: Estimate ± SE: 0.006 ± 0.002, t = 2.631, P = 0.0097; Figure S4.1). We hence calculated a 'corrected nymph weight at hatching by extracting the residuals from a linear model (LM) that was fitted using the average weight of nymphs at hatching as (continuous) response variable and the weight of their mother at that time as (continuous) explanatory variable.

## Initial weights and family quality (PC1 scores)

To determine whether the PC1 scores were affected by the (average) weight of nymphs or their mother at hatching, we fitted a linear mixed model (LMM) in which the PC1 scores were entered as (continuous) response, and maternal presence (MP or MA, bimodal), the corrected average initial weight of the nymphs (continuous), the initial weight of their mother (continuous), and all interactions as explanatory variables. Family-ID was entered as random effect to account for the common origin of nymphs in the MP- and MA-groups, respectively. The PC1 scores were generally lower under maternal presence (Mean ± SE: -0.1608 ± 0.0985) than under maternal absence (Mean ± SE: 0.1608 ± 0.0937; Wald $\chi_1^2$ = 23.269, P < 0.0001), and decreased with female weight at egg hatching (Figure S4.2A; Estimate ± SE: -50.710 ± 11.588, $t_{109.0}$ = -4.376, P < 0.0001) and the corrected average nymph weight at egg hatching (Figure S4.2B; Estimate ± SE: -0.289 ± 0.080, $t_{109.0}$ = -3.611, P = 0.0005) across MP- and MA-groups.

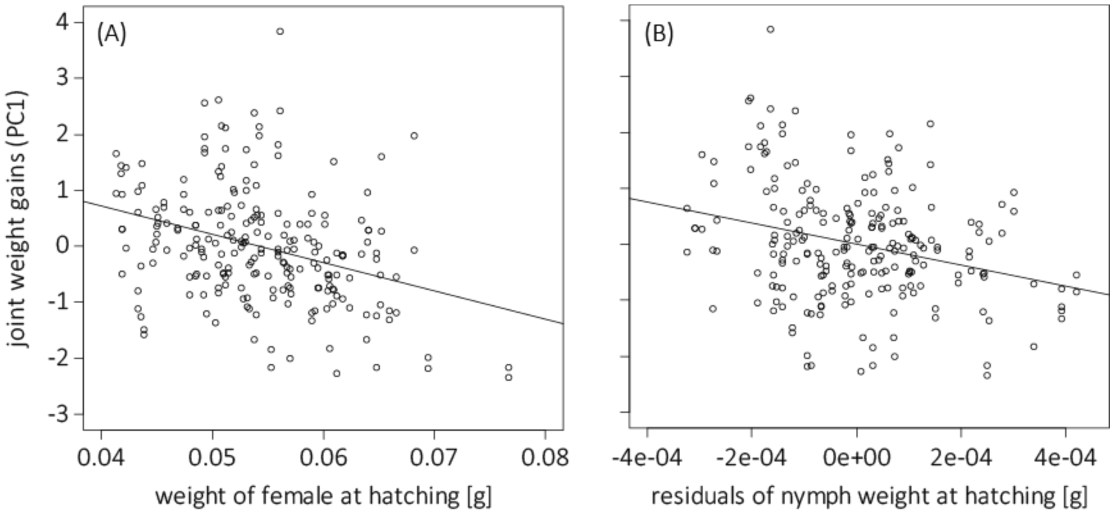

**Figure S4.2. Joint weight gains and initial weights.** Decrease of PC1 scores (reflecting a joint weight gain of mothers and nymphs during family life) with (A) the weight of the female at hatching, and (B) the average weight of nymphs at hatching.

## Calculation of the corrected forceps length

Forceps length is generally correlated with eye distance (Pearson's r = 0.22; LMM Estimate ± SE: 1.0810 ± 0.2413, $t_{388.00}$ = 4.480, $P < 0.0001$; Figure S4.3). Hence, we calculated a 'corrected forceps length' by extracting the residuals of a linear mixed model (LMM) that was fitted using forceps length as (continuous) response variable, eye distance as (continuous) explanatory variable, and Family-ID as random effect (to avoid pseudo-replication).

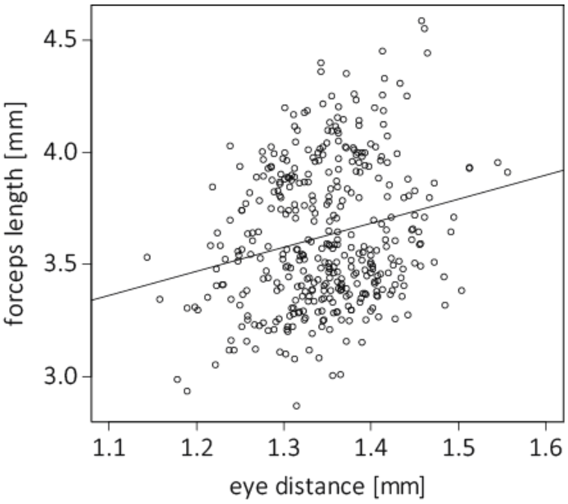

**Figure S4.3.** Increase of the (uncorrected) forceps length with the eye distance of adult offspring.

**Table S4.1. Offspring survival.** Influences of maternal presence, the time of measurement (at the end of family life or upon adult emergence), and the two principal components reflecting joint weight gains of mothers and nymphs during family life (PC1) and the trade-off between these measures (PC2) on offspring survival. Significant P-values are given in bold print.

| | Offspring survival | |
|---|---|---|
| | Wald $\chi_1^2$ | P |
| Maternal presence (MP) | 34.40 | **< 0.0001** |
| Time of measurement (TM) | 1203.73 | **< 0.0001** |
| PC1 | 4.77 | **0.0290** |
| PC2 | 2.82 | 0.0931 |
| MP : TM | 5.01 | **0.0252** |
| MP : PC1 | 0.69 | 0.4075 |
| TM : PC1 | 0.14 | 0.7114 |
| MP : PC2 | 0.99 | 0.3188 |
| TM : PC2 | 11.99 | **0.0005** |
| PC1 : PC2 | 0.48 | 0.4888 |
| MP : TM : PC2 | 8.01 | **0.0047** |

# CHAPTER 5

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

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[Ω] Authors contributed equally to the work

*Based on*

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## ABSTRACT

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

*Keyword:* family life; inheritance; insect; orphaning; parental care; social evolution

## INTRODUCTION

Family life is a common phenomenon in nature and is usually associated with substantial fitness benefits for offspring. These benefits mostly derive from the expression of parenting behaviors (Royle et al. 2012b; Klug and Bonsall 2014) such as nest construction, brood/juvenile attendance or food provisioning (Royle et al. 2012b; Wong et al. 2013), and are thus contingent on parental presence. Consequently, parental loss, e.g. due to clutch desertion or premature mortality, has been predicted to entail severe fitness costs for offspring that may ultimately select for the maintenance of family life across generations. In line with this prediction, short-term costs of parental loss have been reported in a large set of taxonomically diverse species, in which it is typically associated with a reduction in growth and/or survival rates of juveniles (Foster et al. 2012; Smiseth et al. 2012; Andres et al. 2013; Klug and Bonsall 2014). Importantly, other studies also showed that parental loss can entail long-term and transgenerational costs by hampering the mating success of adult offspring and diminishing the level of care they express towards their own descendants (Harlow and Suomi 1971; Champagne and Curley 2009; Klug and Bonsall 2014). For instance in rats, females that had experienced long periods of maternal loss as pups exhibited low levels of care towards their own offspring, which in turn also exhibited lower levels of care as F2 adults (Gonzalez et al. 2001; Fleming et al. 2002).

Somewhat surprisingly, the long-term and transgenerational effects of parental loss have only been studied in altricial vertebrates, in which juveniles exhibit limited foraging capabilities and thus heavily rely on parental resources (Francis et al. 1999; Weaver et al. 2004; Maestripieri 2005). However, investigating the occurrence of these effects in precocial invertebrates, in which juveniles exhibit early foraging capabilities and consequently only facultatively rely on parental resources (Trumbo 1992; Kölliker 2007), could provide crucial information on the early evolution of parental care. Indeed, transgenerational costs could be a key promoter of the maintenance of family life when parental loss has limited (if any) short-term costs in terms of offspring survival, a scenario that applies to precocial systems and likely prevailed in the early evolution of family life (Falk et al. 2014). Furthermore, studying the consequences of parental loss in precocial invertebrates could help to determine whether the tight association between parental care and offspring survival (as is found in altricial vertebrates) is a prerequisite for the expression of transgenerational costs, thus shedding light on the importance of these costs in the multiple forms of family life.

Here, we used a series of two experiments encompassing three generations of individuals to investigate the short-term, long-term and transgenerational effects of maternal loss in the European earwig *Forficula auricularia*. In this precocial insect, mothers provide care to their eggs over winter and, after hatching, to their mobile juveniles (called nymphs; Meunier et al. 2012;

Koch and Meunier 2014). Post-hatching maternal care lasts for several weeks and takes multiple forms including nymph grooming and food provisioning through regurgitation (Meunier and Kölliker 2012b; Kölliker et al. 2015). Although the early foraging capability of nymphs allows them to survive in the absence of a tending mother, maternal loss has been shown to entail short-term costs under sub-optimal food quality, as it reduces the survival rate of nymphs until their fourth and last developmental instar (Kölliker 2007). Conversely, a second study showed that maternal loss enhances nymph survival rate when food quantity is limited, a result possibly due to a mother-offspring conflict over restricted food access (Meunier and Kölliker 2012a).

In our first experiment, we investigated the short-term effects of maternal loss on the newly produced offspring. To this end, we reared nymphs with or without their mother under *ad libitum* food conditions and then monitored their development and survival rates until adulthood, as well as measured body size and forceps length in adults (two known fitness-related traits; Tomkins and Simmons 1998; Rantala et al. 2007). If maternal care was a key component of offspring development and survival, we would expect that maternally deprived adults emerge earlier due to developmental stress (Meunier and Kölliker 2012a), survive less well and exhibit smaller body and forceps sizes compared to maternally tended adults. In our second experiment, we used the adults produced in the first experiment to determine whether maternal loss had long-term and transgenerational effects on the expression of maternal care.

The expression of maternal care is generally known to reflect phenotypic and/or genetic traits of the caring mother, as well as maternally-inherited and paternally-inherited traits expressed by the tended juveniles, e.g. through maternal effects and epigenetic modifications (Walling et al. 2008; Curley et al. 2011; Meunier and Kölliker 2012b). We therefore conducted a full-factorial cross-breeding/cross-fostering experiment, in which we mated maternally-deprived and –tended females with maternally-deprived and –tended males, and cross-fostered the resulting eggs to a foster mother of the same or a different experimental group. We then measured the reproductive output of these families and determined the level of maternal care expressed by the foster mothers. If maternal loss had long-term negative effects, we would expect maternally deprived mothers to exhibit a lower reproductive output and to express lower levels of maternal care. If maternal loss had transgenerational effects, we would expect the genetic origin of nymphs to affect the expression of care by foster mother.

# MATERIAL AND METHODS

## *Experiment 1: Short-term effects of maternal loss*

### Experimental design

The short-term effects of maternal loss were tested by rearing nymphs from 80 families of the European earwig *F. auricularia* with or without their mothers. These families descended from 80 females and 73 males that were collected in a natural population in Dolcedo, Italy in September 2012 and kept under standard laboratory conditions throughout the experiments (details in Koch and Meunier 2014). Upon egg-laying, females were isolated in Petri dishes (9 cm diameter). One day after egg hatching, approximately 40 nymphs per brood (original brood size: mean ± SE = 61.2 ± 1.3) were set up in a new Petri dish either with their mother (maternally tended, n = 40 families; mean brood size = 39.6 ± 0.29) or without their mother (maternally deprived, n = 40 families; 39.3 ± 0.35; Figure 5.1A). Fourteen days later, all tending mothers were removed from the maternally tended groups to mimic natural family disruption (Meunier and Kölliker 2012b). Both maternally deprived and maternally tended nymphs were subsequently transferred to larger Petri dishes (14 cm diameter) to allow their development until adulthood. At adult emergence, males and females of each family were separated to avoid inbreeding and ensure virginity (Meunier and Kölliker 2013). All Petri dishes contained humid sand as ground material, a plastic tube as a shelter and an *ad libitum* amount of laboratory food, which mainly contained carrots, flower pollen and dry cat food (detailed composition in Meunier et al. 2012).

### Measurements of life-history traits in F1 individuals

We measured the developmental time and survival rate of F1 nymphs at each developmental instar, as well as the eye-distance (a proxy of body size) and the forceps length of the resulting adults. Developmental time was defined as the day at which the first nymph within a brood was observed to molt into the next instar (newly molted individuals stay whitish for one day), a measurement known to predict the developmental time for the whole brood (Gómez and Kölliker 2013). Survival rates were measured for each developmental instar by counting the number of offspring alive three days after the first molted individual had been observed. For the 1st instar, nymph survival was measured on day 10 after egg hatching. Finally, the average eye distance and forceps length of two haphazardly chosen male and female adults per family were measured to the nearest 0.001 mm using a camera coupled to a binocular microscope (Leica DFC425, Leica Microsystems Ltd., Heerbrugg, Switzerland) and the software *Leica Application Suite 4.5.0*. Note that one or two individuals were removed at each nymphal instar to conduct another experiment not presented here. These removed nymphs were excluded from survival rate calculations.

### *Experiment 2 - Long-term and transgenerational effects of maternal loss*

#### Experimental design

The long-term and transgenerational effects of maternal loss were tested using three successive steps: (1) cross-breeding maternally deprived and maternally tended adults, (2) cross-fostering the resulting eggs to foster mothers of either the same or a different experimental background than their biological mother, and finally (3) splitting the resulting families into two groups, one of which stayed with the foster mother whereas the other was orphaned (Figure 5.1B). The cross-breeding was conducted by pairing 78 females with 78 unrelated males to obtain 19 to 20 replicates of each of the four possible combinations of maternally deprived and maternally tended adults (Figure 5.1B, Table S5.1). These adults were haphazardly chosen among the ones obtained from experiment 1. Note that two families did not produce enough adults and could thus not be used in experiment 2. Mating pairs were maintained under standard laboratory conditions for two months, after which females were isolated to allow egg laying (Meunier and Kölliker 2012a).

The resulting eggs were cross-fostered on average 5.7 ± 0.7 (± SE) days after they had been laid. The cross-fostering was conducted to obtain the 8 possible combinations of experimental backgrounds of the parents (maternally deprived or maternally tended), i.e. by controlling for the background of the genetic mother (gMother) and the genetic father (gFather) of the transferred eggs, as well as for the background of the recipient mother (then called foster mother, fMother) and the male who was paired with the foster mother (fFather) (Figure 5.1B). Females of the European earwig readily accept foreign eggs (Meunier and Kölliker 2012b), thus allowing us to ensure that foster mothers were always unrelated to the eggs they tended. After cross-fostering, all foster females and their adopted eggs were kept under standard laboratory conditions until egg hatching. Note that 6 of the 78 females were excluded from the cross-fostering, because they either did not produce any eggs or produced them too early/late to conduct a cross-fostering (details in the supplementary material).

Finally, the split-clutch was conducted to determine whether maternal attendance could mask the transgenerational effects of maternal loss on offspring life-history traits. One day after egg hatching, the nymphs of each of the cross-fostered families were attributed to two equally-sized groups: one group was tended by the foster mother for 14 days (then called maternal-presence) whereas the other group was raised without a mother (then called maternal-absence; Figure 5.1B). At day 14 after egg hatching, the foster females were discarded from the experiment and the nymphs maintained under standard laboratory conditions until they reached adulthood.

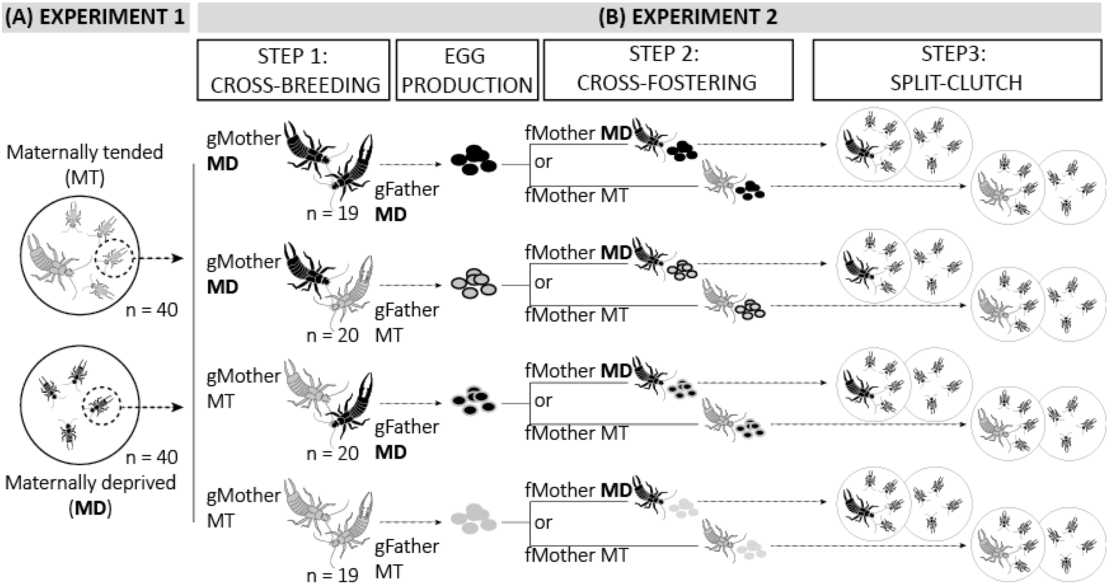

Figure 5.1. Experiments investigating the (A) short-term as well as (B) long-term and transgenerational effects of maternal loss. Transgenerational effects could be mediated by the experimental background of the genetic mothers (gMother) and genetic fathers (gFather) of the offspring, as well as the one of their foster mother (fMother). Grey individuals have been maternally tended (MT), whereas black ones have been maternally deprived (MD).

### Measurements of maternal care by F1 adults and of life-history traits in F2 individuals

We measured the long-term and transgenerational effects of maternal loss on the expression of three forms of maternal care (in the maternal-presence groups). The first form was (1) clutch defense, which reflected the females' willingness to protect their clutch of eggs or nymphs from predator attacks. Note that under natural conditions, mothers typically stay on or in the vicinity of their clutch to defend it against predators (JM, pers. comm.). It was measured on day 10 after egg-laying (egg defense) and on day 4 after hatching (nymph defense), by standardly poking each female on the pronotum with a glass capillary (one poke per second) and then recording the number of pokes required until she moved more than two body lengths away from her clutch. The second form of maternal care was (2) clutch desertion, which showed how long females abandoned their clutch after being chased away by a simulated predator attack. Under natural conditions, temporary clutch abandonment can be costly for females, as it allows predators - including conspecifics - to consume the clutch of eggs or nymphs. It was measured by recording the time the female took to return to her clutch of eggs or nymphs after the end of the clutch defense measurement. First, we observed that females are very aggressive when they tend their clutch of eggs/juveniles and that they extensively use their forceps against small predators like pseudo-scorpions and other insects (including male earwigs).

Finally, the third form of maternal care was (3) food provisioning, which revealed how much food earwig mothers provided to their nymphs. In brief, the process involved four standard (cf. Meunier et al. 2012) and successive steps that started at day 6 after hatching. They consisted in (i) food depriving mothers and nymphs for 24h, (ii) isolating females for 1h while offering them *ad libitum* green-colored pollen pellets (Hoyer and DEKO BACK), (iii) re-assembling each female with a standardized number of 15 (or all if less than 15 available) of her foster nymphs for 15h and finally (iv) calculating the proportion of nymphs with green colored gut. This measurement was not conducted in clutches with less than 5 nymphs. The nymphs from the maternal presence groups not used in these tests, as well as all nymphs from the maternal absence groups, were isolated under the same conditions. Once the green-colored nymphs had been counted, the maternal-presence group was reassembled and all groups were provided with *ad libitum* laboratory food and maintained under the standard conditions. Due to the requirement of at least 5 surviving nymphs per clutch to reliably measure food provisioning and time constraints associated with the simultaneous measurements of multiple forms of maternal care across clutches, we used 61 (85%) of the 72 cross-fostered clutches to measure clutch defense, 59 (82%) to measure desertion time and 47 (65%) to measure food provisioning (sample sizes in Table S1).

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

### *Statistical analyses*

The short-term effects of maternal loss were analyzed using a series of Generalized Linear Mixed Model (GLMM) and Linear Mixed Models (LMMs). In the first models, nymph survival rate (GLMM) or nymph developmental time (LMM) at each instar was entered as the response variable, whilst maternal loss (bimodal; maternally tended or deprived, respectively MT or MD) and each developmental instar (continuous) were used as explanatory variables. The family ID was entered as a random effect into these two models, because each family group was measured at each instar. In the other models, the mean eye distance (LMM) or the forceps length corrected for body size (see ESM for calculation; LMM) was entered as the response variable, and maternal loss and sex as explanatory factors. The family ID was also entered as a random effect, because each family group provided values for males and females.

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

Finally, offspring developmental time and survival rate until adulthood were analyzed in two separate steps. In the first step, the effects of gMother, gFather and fMother on offspring developmental time (LM) and survival rate (GLM) were tested in the split-clutches tended by a foster mother (i.e. in the maternal-presence groups only). Because fMother was never significant in these tests (Table S5.2), we pooled the two levels of this factor and, in a second step, compared offspring developmental time and survival rates of maternal-presence and maternal-absence groups. To this end, we tested whether gMother, gFather and/or the presence of a tending mother (yes or no) affected either the developmental time (LMM) or the survival of nymphs until adulthood (GLMM). The ID of the original clutch was entered as a random effect into these two models, because split-clutches with and without a tending mother were used.

Statistical analyses were conducted using the software R 3.1.3. All interactions between explanatory factors were tested in each model, which were then simplified stepwise by removing the non-significant interactions (all $p > 0.12$). All GLM(M)s were fitted with a binomial error structure corrected for overdispersion. Proportion data (e.g. survival and developmental rates) were entered into these models using the *cbind* function. Note that fFather was not entered into any statistical model because there was no specific prediction based on this effect for the different measurements (see also Meunier and Kölliker 2012b) and this ensured an average of 7.5 replicates for each of the tested combinations (Table S5.1).

## RESULTS

### *Experiment 1: Short-term effects of maternal loss*

Contrary to the predicted short-term costs of maternal loss, we found that maternal loss positively shaped two morphological traits in adult offspring (Figure 5.2). Specifically, maternally-deprived adults had wider eye distances (Figure 5.2A; Likelihood ratio (LR) $\chi^2_1 = 5.75$, $P = 0.016$) and longer forceps (corrected for eye distance; Figure 5.2B; LR $\chi^2_1 = 6.83$, $P = 0.009$) than maternally-tended adults. Moreover, eye distance was overall wider for females (Mean ± SE = 1.42 ± 0.006 mm) than for males (1.39 ± 0.006 mm; LR $\chi^2_1 = 8.25$, $P = 0.004$), and was not shaped by an interaction between maternal loss and sex (LR $\chi^2_1 = 1.25$, $P = 0.264$). The statistical model on the corrected forceps length reported no main significant effects of sex (LR $\chi^2_1 =0.36$, $P = 0.547$) or of the interaction between maternal loss and sex (LR $\chi^2_1 = 3.58$, $P = 0.058$).

Maternal loss did not influence nymph survival rate (Figure 5.2C; LR $\chi^2_1 = 0.05$, $P = 0.826$) and developmental time (Figure 5.2D; LR $\chi^2_1 = 0.45$, $P = 0.502$) until adulthood. Not surprisingly, the nymphs survival rate decreased with time (LR $\chi^2_1 = 919.19$, $P < 0.0001$; Model estimate ± SE = -0.81 ± 0.04), while the nymphs' developmental time increased with developmental instar (LR $\chi^2_1 = 6543.81$, $P < 0.0001$; Estimate ± SE = 17.04 ± 0.30). However, nymph developmental instar did not interact with maternal loss to shape these two measurements (Survival rate: LR $\chi^2_1 = 0.53$, $P = 0.465$; Developmental time: LR $\chi^2_1 = 0.002$, $p = 0.963$).

**Table 5.1. Long-term and transgenerational effects of maternal loss on the expression of maternal care by adult offspring.** Maternal care was measured towards clutches of eggs or nymphs (Clutch type), except for food provisioning, which was only expressed towards nymphs. Significant p-values are in bold. LR stands for Likelihood ratio. Note that non-significant interactions are reported to allow comparison among models, but their removal did not qualitatively change the results.

| | (A) Clutch defense | | (B) Clutch desertion time | | (C) Food provisioning | |
|---|---|---|---|---|---|---|
| | LR $\chi^2_1$ | P | LR $\chi^2_1$ | P | LR $\chi^2_1$ | P |
| Clutch type (CT) | 26.35 | **<0.0001** | 0.64 | 0.424 | - | - |
| fMother | 1.31 | 0.253 | 4.62 | **0.032** | 5.24 | **0.022** |
| gMother | 0.89 | 0.346 | 0.23 | 0.634 | 0.87 | 0.351 |
| gFather | 0.85 | 0.357 | 0.04 | 0.850 | 0.03 | 0.853 |
| CT:gMother | 3.95 | **0.047** | 0.09 | 0.758 | - | - |
| gMother:gFather | 0.46 | 0.498 | 0.62 | 0.431 | 4.78 | **0.029** |

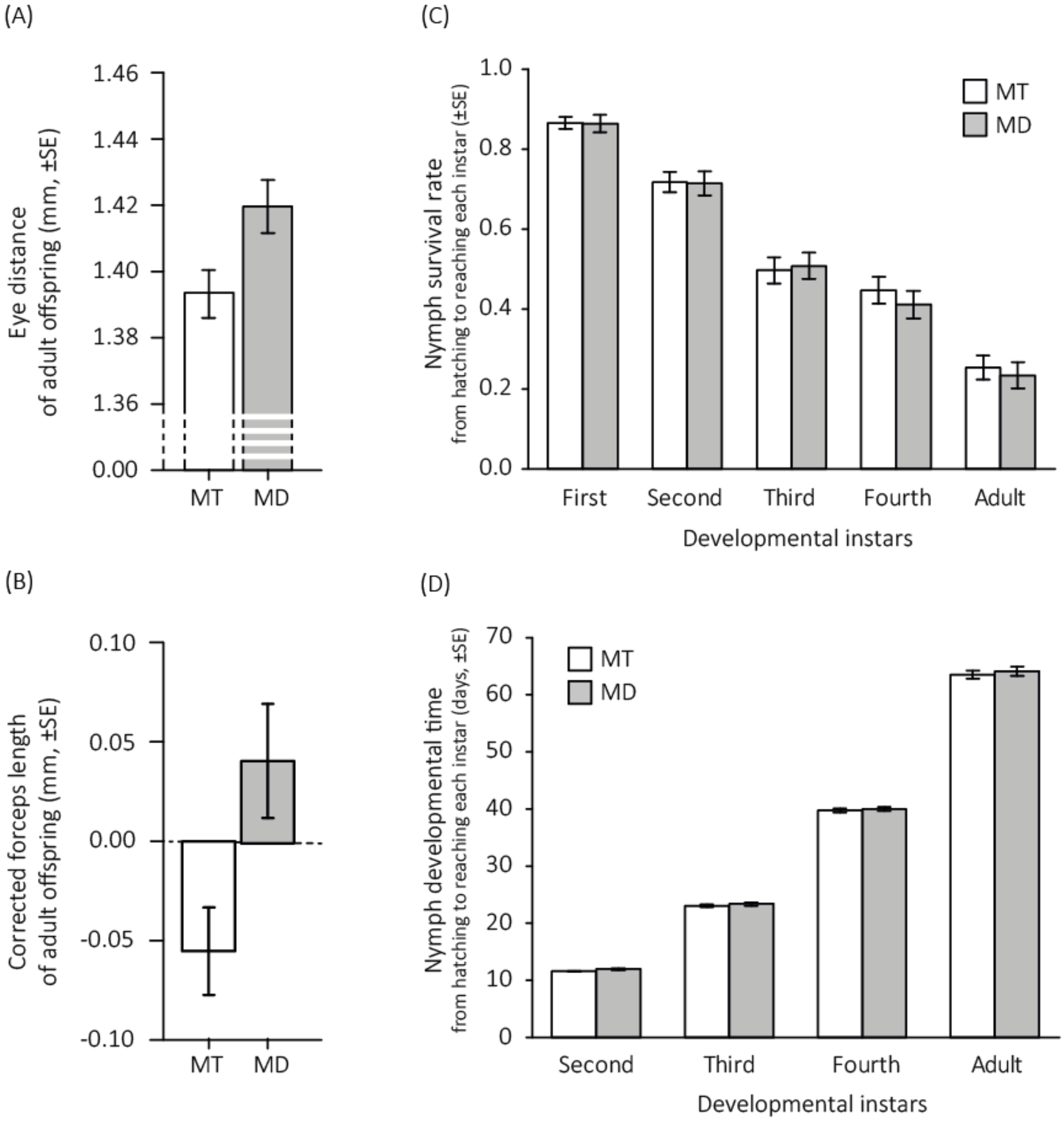

**Figure 5.2. Short-term effects of maternal loss on the (A) mean eye distance, (B) corrected forceps length, (C) survival rate and (D) developmental time of offspring.** Nymphs were either maternally tended (MT) or maternally deprived (MD) during the 16 days following their emergence.

### *Experiment 2: Transgenerational effects of maternal loss*

Maternal loss affected the expression of three forms of maternal care by adult offspring through long-term effects in the caring mother, as well as through inherited, parent-of-origin specific effects expressed in the nymphs (Table 5.1). First, maternally-deprived foster mothers abandoned their clutch for an overall longer period of time than maternally-tended foster mothers (Table 5.1B, Figure 5.3A), an effect that was independent of the genetic parents of the offspring and of the age of the clutch (i.e. eggs or nymphs, Table 5.1B). Second, maternally-deprived foster mothers provisioned overall more nymphs with food than maternally-tended foster mothers (Table 5.1C, Figure 5.3B). Food provisioning was also shaped by an interaction between the experimental backgrounds of the two genetic parents of the tended nymphs (Table 5.1C). Nymphs of maternally-tended males received more food when they had been produced by maternally-deprived compared to maternally-tended females (Figure 5.3C; Estimate ± SE = 1.25 ± 0.59; $t_{48}$= 2.11, P = 0.041), whereas the experimental background of the females had no effect on food provisioning when nymphs were sired by maternally-deprived males (Estimate ± SE = -0.54 ± 0.60; $t_{48}$ = -0.90, P = 0.374). Finally, maternally-deprived mothers produced nymphs, but not eggs, that were overall less defended by the caring foster mothers (Figure 5.3D; Interaction in Table 5.1A; Nymph stage: Estimate ± SE = -0.51 ± 0.25; $t_{67}$ = -2.04, P= 0.045; Egg stage: Estimate ± SE = 0.12 ± 0.24; $t_{67}$ = 0.52, P = 0.606). Note that all but two measurements of maternal care were independent of each other (Table S5.3). Only egg defense and the duration of egg abandonment were overall positively correlated (Spearman correlation test, $r_s$ = 0.32, P = 0.009).

Table 5.2. Transgenerational effects of maternal loss on the reproductive output of the resulting adult offspring. The significant p-value is in bold.

| | Number of nymphs at egg hatching | | Mean weight of nymphs at egg hatching | | Development time of nymphs until adulthood | | Survival rate of nymphs until adulthood | |
|---|---|---|---|---|---|---|---|---|
| | LR $\chi^2_1$ | P | LR $\chi^2_1$ | P | LR $\chi^2_1$ | P | LR $\chi^2_1$ | P |
| gMother | 1.43 | 0.232 | 1.02 | 0.312 | 6.19 | **0.013** | 1.04 | 0.307 |
| gFather | 0.33 | 0.568 | 0.08 | 0.776 | 0.73 | 0.394 | 0.33 | 0.564 |
| fMother [a] | 0.32 | 0.570 | >0.01 | 0.991 | - | - | - | - |
| Maternal presence [a] | - | - | - | - | 0.73 | 0.394 | 0.2 | 0.655 |

[a] Because fMother did not influence post-hatching traits measured in nymphs tended by a foster mother (Table S5.2), this factor was pooled to form a new factor describing the presence or absence of mothers after hatching, then called “maternal presence”.

Maternal loss influenced only one of the four measurements taken on nymphs and none of the three measurements taken on eggs produced by the adult offspring. Nymphs produced by maternally-tended females reached adulthood in 72.13 ± 0.67 days (Mean±SE), which was significantly longer than the 69.25 ± 0.51 days required by nymphs produced by maternally-deprived females (Table 5.2). By contrast, the experimental background of the genetic parents had no effect on the number of eggs (gMother: LR $\chi^2_1$ = 0.08, p = 0.782; gFather: LR $\chi^2_1$ = 0.06, p = 0.806; Interaction: LR $\chi^2_1$ = 0.20, p = 0.657), mean egg weight (gMother: LR $\chi^2_1$ = 0.16, p = 0.688; gFather: LR $\chi^2_1$ = 0.92, p = 0.337; Interaction: LR $\chi^2_1$ = 0.77, p = 0.380) and hatching success (gMother: LR $\chi^2_1$ < 0.01, p = 0.999; gFather: LR $\chi^2_1$ = 0.05, p = 0.827; Interaction: LR $\chi^2_1$ = 2.07, p = 0.150). The experimental background of the parents also had no effect on the number and mean weight of nymphs at egg hatching (Table 5.2) or their survival until adulthood (Table 5.2). Notably, the presence of a foster mother after egg hatching did not influence nymphs' survival rates and developmental times (Table 5.2).

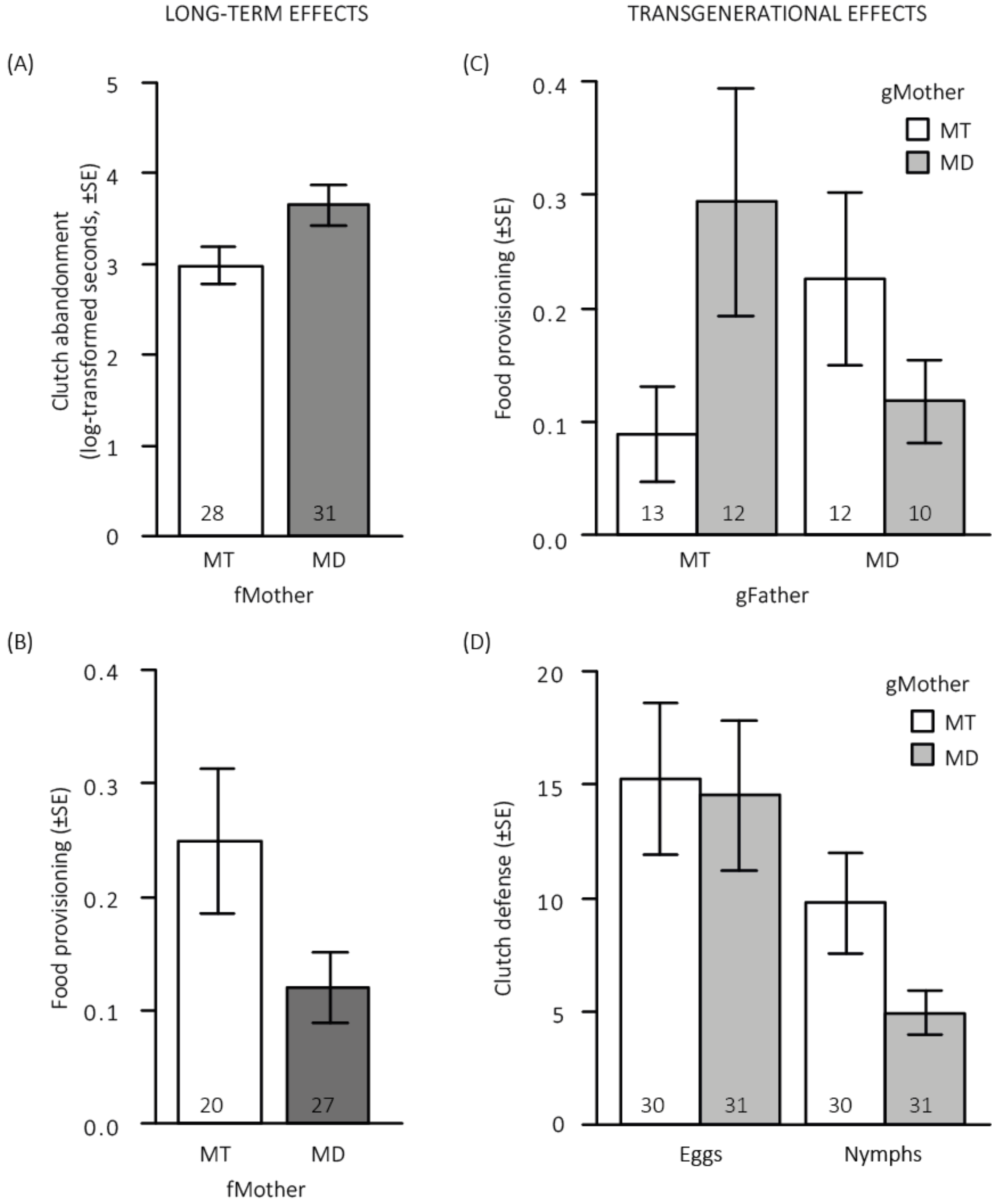

**Figure 5.3. Long-term and transgenerational effects of maternal loss on the expression of (A) clutch abandonment, (B, C) food provisioning and (D) clutch defense by adult offspring**. MT stands for maternally tended and MD for maternally deprived. Sample sizes are at the bottom of each bar.

## DISCUSSION

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

Somewhat surprisingly, we found that maternal loss did not affect nymph development and survival, but yielded adult offspring of larger body and forceps sizes, two morphological traits associated with fitness benefits in *F. auricularia* (Tomkins and Simmons 1998; Rantala et al. 2007). These apparent short-term benefits of maternal loss obtained under laboratory conditions contrast with the short-term costs typically expected under natural conditions (e.g. Kölliker and Vancassel 2007; Andres et al. 2013). Previous studies already revealed that maternal presence reduces nymph survival when families had restricted food access (Meunier and Kölliker 2012a), but increased nymph survival when families had access to low quality food (Kölliker 2007). Here, the effects of maternal presence under *ad libitum*, high quality food could reveal an increased expression of sibling rivalry when juveniles have access to maternal resources, as proposed in a recent model (Gardner and Smiseth 2011). Alternatively, these effects could result from maternal behaviors that benefit offspring under natural conditions, but directly or indirectly hamper their development under laboratory conditions. For instance, mothers often cover food in the vicinity of the nest, presumably to prevent microbial development (J Kramer & J Meunier, pers. observation). This might have been costly for juveniles in the absence of pathogens, as it might have restricted their access to food. This notwithstanding, both scenarios emphasize that parental presence can be associated with costs for the tended offspring (see also Meunier and Kölliker

2012a), which emerge when the (laboratory) conditions do not allow (variation in) the benefits of parental care to be revealed. These findings overall indicate that as long as parental care only has limited effects on offspring development and survival (a scenario that presumably prevailed in the early evolution of family life), it is likely that the emergence and maintenance of parental investment into post-hatching care mostly relies on the benefits of parenting behaviors that enable offspring to better cope with environmental constraints, such as limited food access or the presence of pathogens and predators (Royle et al. 2012b; Wong et al. 2013).

Contrary to the above effects, maternal loss lowered the expression of maternal care by adult offspring. Females reared without mothers abandoned their juveniles for longer and provisioned them with less food than females reared with tending mothers. To the best of our knowledge, the long-term, negative effects of maternal loss on the expression of maternal care have only been reported in altricial vertebrates (Harlow and Suomi 1971; Gonzalez et al. 2001; Fleming et al. 2002; Maestripieri 2005). In these species, the effects of maternal loss typically result from a disrupted learning process (Oostindjer et al. 2011) and/or from induced hormonal/neurobiological changes during juvenile development. For instance, temporary maternal loss is known to alter the brain development of juvenile rodents and primates, which in turns disturbs hormonal and neurobiological processes in adults and then hampers their expression of parental care (e.g. Champagne and Curley 2005; Keverne 2014). In altricial insects like honey bees and dung beetles, the absence of brood care is also known to delay the development of sensory and integrative brain centers in juveniles (e.g. antennal lobes and mushroom bodies; Farris and Sinakevitch 2003; Farris 2013). However, the link between these developmental changes and the expression of care remains unknown. Our results thus call for further studies that test the effects of maternal loss on hormonal/neurobiological traits in juveniles of (precocial) invertebrates. Moreover, they suggest that effects of learning (cf. Giurfa 2015) on the expression of parenting behaviors might not be restricted to vertebrates. Finally, the occurrence of negative effects of parental loss in a precocial insect demonstrates that their expression is not restricted to species (such as altricial vertebrates) in which parental care is obligatory for offspring survival. Note that the larger body and forceps size of maternally deprived adults are unlikely to be the main drivers of these long-term negative effects on maternal care, as a previous study showed that large *F. auricularia* females express more rather than less maternal care (including food provisioning) than small females (Meunier et al. 2012).

Importantly, the effects of maternal loss on the expression of maternal care were not only mediated by parental effects reflecting the experimental background of the caring mothers, but also by effects of the background of the parents that produced the nymphs. On the one hand, nymphs of maternally-tended females molted into adults at a greater age than nymphs of maternally-deprived females. On the other hand, nymphs produced by maternally-deprived females were less well defended by foster mothers, but – when fertilized by maternally-tended males - received more food from foster mothers than nymphs produced by maternally-tended females. These results are overall in line with studies demonstrating that parent-of-origin effects inherited to and expressed by juveniles are key components of parental care (Hager and Johnstone 2003; Walling et al. 2008; Curley et al. 2011; Meunier and Kölliker 2012b). Interestingly, our findings also reveal that these effects can be acquired from the early social environment of the future parents (e.g. through epigenetic modifications; Danchin et al. 2011), showing the central importance of this phenomenon in the evolution of parental investment into care in a precocial insect. Under our experimental conditions, these effects did not translate into the production of high quality nymphs by maternally deprived females, as we found no parent-of-origin effect of maternal loss on the mean weight and survival rate of these nymphs. However, the accelerated developmental time of the offspring of maternally-deprived females could be indicative of parent-of-origin effects that increase the level of sibling rivalry among these offspring (Kölliker 2007; Meunier and Kölliker 2012a). On a proximate level, the transgenerational effects of maternal loss on nymph defense could be mediated by a parent-of-origin effect on the offspring's chemical signatures. These signatures are known to mediate behavioral interactions between earwig mothers and nymphs as well as among nymphs, to be flexible over time, and to determine the amount of food provisioned by mothers (Mas et al. 2009).

To conclude, our study demonstrates that maternal loss during earwig family life may entail short-term benefits regarding adult morphology, but is associated with transgenerational costs that are mainly mediated through the expression of parental care. These contrasting effects stress the importance of encompassing the short- and long-term effects of parental presence when estimating the costs/benefits ratio of mother-offspring interactions for offspring. More generally, the surprising short-term benefits of maternal loss obtained under laboratory conditions shed light on the importance of (natural) environmental constraints on the net benefits gained by juveniles through parental care and on their role in the evolution of family life (Wong et al. 2013). Finally, our data reveal that the early social environment of juveniles is a key determinant of parental investment across generations, as it shapes both the behavior they later adopt as parents and the behaviors they transmit to their own offspring. Hence, this study overall suggests that environmental constraints and transgenerational effects of parental loss are keystones in the emergence and maintenance of ancestral forms of family life, in which parental care has limited effects on offspring development and survival (Falk et al. 2014).

## ACKNOWLEDGEMENTS

[removed for privacy purposes]

## SUPPLEMENTARY MATERIAL

### Additional details on the statistical analyses of experiment 1

Because eye distance and forceps length were correlated in both males ($r^2 = 0.36$, LM slope ± SE = $3.65 \pm 0.68$, $t = 5.4$, $P < 0.0001$) and females ($r^2 = 0.25$, LM slope ± SE = $1.48 \pm 0.34$, $t = 4.33$, $P < 0.0001$), we calculated a "corrected forceps length" by extracting the residuals of two Linear Models (in males and females, separately) with forceps length as response variable and eye distance as explanatory variable. Furthermore, 40 out of the 80 families (20 MT and 20 MD) had been reared in Petri dishes that were not regularly cleaned (to measure its effect on immunity - results not shown). This parameter was thus first entered as an additional explanatory, fixed factor in the four tested models, in which it was never significant (Survival rate: LR $\chi^2_1 = 1.42$, $P = 0.232$; Developmental time: LR $\chi^2_1 = 1.12$, $P = 0.734$; Eye distance: LR $\chi^2_1 = 0.99$, $P = 0.318$; Forceps length: LR $\chi^2_1 = 1.45$, $P = 0.228$). As a consequence, and because investigating the effects of this parameter was out of the scope in the present study, this factor was secondarily removed from the statistical models. Note that keeping or excluding this parameter did not qualitatively change the presented results.

**Table S5.1. Combinations of gMother, gFather, fMother and fFather in experiment 2.** The table reports the number of replicates for each combination (N), as well as the sample size used for each analysis (Stats). The corresponding adults were either maternally deprived (MD) or maternally tended (MT).

| gMother | gFather | fMother | fFather | Life-history traits | | Clutch defense | | Clutch desertion time | | Food provisioning | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *N* | Stats | *N* | Stats | *N* | Stats | *N* | Stats |
| MD | MD | MD | MD | *5* | 10 | *4* | 8 | *4* | 8 | *3* | 6 |
| MD | MD | MD | MT | *5* | | *4* | | *4* | | *3* | |
| MD | MD | MT | MD | *4* | 8 | *4* | 8 | *4* | 8 | *2* | 4 |
| MD | MD | MT | MT | *4* | | *4* | | *4* | | *2* | |
| MD | MT | MD | MD | *5* | 10 | *4* | 9 | *4* | 9 | *4* | 7 |
| MD | MT | MD | MT | *5* | | *5* | | *5* | | *3* | |
| MD | MT | MT | MD | *4* | 9 | *2* | 6 | *2* | 6 | *2* | 5 |
| MD | MT | MT | MT | *5* | | *4* | | *4* | | *3* | |
| MT | MD | MD | MD | *4* | 8 | *3* | 7 | *3* | 7 | *4* | 8 |
| MT | MD | MD | MT | *4* | | *4* | | *4* | | *4* | |
| MT | MD | MT | MD | *5* | 9 | *4* | 8 | *3* | 6 | *1* | 4 |
| MT | MD | MT | MT | *4* | | *4* | | *3* | | *3* | |
| MT | MT | MD | MD | *4* | 9 | *3* | 7 | *3* | 7 | *3* | 6 |
| MT | MT | MD | MT | *5* | | *4* | | *4* | | *3* | |
| MT | MT | MT | MD | *4* | 9 | *4* | 8 | *4* | 8 | *4* | 7 |
| MT | MT | MT | MT | *5* | | *4* | | *4* | | *3* | |
| | | | TOTAL | | 72 | | 61 | | 59 | | 47 |

### Origin of the females excluded from the cross-fostering part of experiment 2

Indicated are the maternal (gMother) and paternal (gFather) origins of the 6 females that were excluded from the cross-fostering:

| | gMother | gFather |
|---|---|---|
| No eggs produced | maternally tended | maternally tended |
| No eggs produced | maternally tended | maternally deprived |
| No eggs produced | maternally deprived | maternally tended |
| No eggs produced | maternally deprived | maternally deprived |
| Eggs produced too early | maternally tended | maternally tended |
| Eggs produced too late | maternally deprived | maternally deprived |

Table S5.2. Effects of gMother, gFather and fMother on nymph developmental time and survival rate until adulthood in the maternal-presence groups.

| | Developmental time | | Survival rate | |
|---|---|---|---|---|
| | LR $\chi^2_1$ | P | LR $\chi^2_1$ | P |
| fMother | 0.16 | 0.689 | 0.11 | 0.738 |
| gMother | 3.39 | 0.066 | 0.96 | 0.327 |
| gFather | 0.19 | 0.666 | 0.14 | 0.704 |
| Interactions | - | > 0.05 | - | > 0.05 |

**Table S5.3. Correlation among the five measurements of maternal care.** The lower and upper diagonals present the correlation value and the p-values, respectively, obtained from spearman rank correlation tests. Significant p-values are in bold.

| | Egg guarding | Nymph guarding | Egg abandonment | Nymph abandonment | Food provisioning |
|---|---|---|---|---|---|
| Egg guarding | | p = 0.459 | **p = 0.009** | p = 0.799 | p = 0.811 |
| Nymph guarding | rs = 0.10 | | p = 0.880 | p = 0.632 | p = 0.508 |
| Egg abandonment | **rs = 0.32** | rs = 0.02 | | p = 0.499 | p = 0.778 |
| Nymph abandonment | rs = -0.03 | rs = 0.06 | rs = -0.09 | | p = 0.461 |
| Food provisioning | rs = 0.04 | rs = -0.11 | rs = 0.04 | rs = 0.12 | |

# CHAPTER 6

# The population determines whether and how life-history traits vary between reproductive events in an insect with maternal care

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*Based on*

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## ABSTRACT

The last reproductive event of a female is often associated with major changes in terms of both maternal and offspring life-history traits. However, the nature of these changes and the importance of population-specific environmental constraints in shaping their expression are difficult to predict and, as a consequence, poorly understood. Here, we investigated whether and how life-history traits vary between reproductive events and whether this variation is population-dependent in the European earwig *Forficula auricularia*. In this insect species, females produce up to two clutches during their lifetime and express extensive forms of maternal care. We conducted a common garden experiment, in which we measured 11 life-history traits of the first and second clutches of 132 females originating from three populations. Our results showed that clutch size was lower - and the level of care expressed towards juveniles higher - in second as compared to first clutches in all three populations. By contrast, we found a population-specific effect on whether and how the reproductive event shaped juvenile quality and a trade-off between egg developmental time and female weight at hatching. Overall, these findings emphasize that the last reproductive event of a female entails both positive and negative effects on various life-history traits of the female herself and her clutch of juveniles. Moreover, our study stresses the importance of population idiosyncrasies on the expression and nature of such cohort-specific effects.

*Keywords:* senescence; terminal investment; parental care; family life; semelparity

## INTRODUCTION

Life-history traits are crucial phenotypic variants that reflect how an organism allocates time and energy to optimize its development and maximize its reproduction (Roff 1992; Stearns 1992; Flatt and Heyland 2011). These traits can be divided in a great diversity of categories, including the reproductive capability and success of an individual (e.g. age and size at maturity, number and size of offspring), as well as its physiology (e.g. immunocompetence and senescence) and behaviors (e.g. aggressiveness, reproductive tactics and parental care; Southwood 1977; Brommer 2000; Reznick et al. 2000). Studying the determinants of life-history traits is thus a pivot of evolutionary biology and ecology, as it helps to better understand the evolutionary constraints that shape the multiple aspects of an individual's fitness and, more generally, the factors that drive the evolution of populations and species (Stearns 1992; Schluter 2001; Rundle and Nosil 2005).

Numerous factors are known to either positively or negatively influence an individual's life-history traits (Stearns 1992; Nylin and Gotthard 1998; Flatt and Heyland 2011). The nature of the effects of being produced during the late reproductive period of a female (thereafter called 'cohort'), however, remains controversial. On the one hand, offspring can benefit from being produced in a late cohort. This is mostly because parental effort into reproduction is predicted to increase when the parents' prospects for survival and future reproduction decline (the 'terminal investment' hypothesis; Williams 1966b; Clutton-Brock 1984; Javoiš 2013) and this might benefit offspring if the increase is allocated to parental care (rather than to the production of additional offspring). Over the last decades, numerous studies conducted across species and taxa provided empirical support for this prediction (Fox and Czesak 2000; Hasselquist and Nilsson 2009; Royle et al. 2012b; Santos and Nakagawa 2012). For instance, females of the North American red squirrels *Tamiasciurus hudsonicus* and the burying beetle *Nicrophorus orbicollis* have been shown to produce juveniles of better quality (and/or more juveniles) in late compared to early cohorts (Descamps et al. 2007; Creighton et al. 2009). Similarly, parents showed higher levels of parental care for late- as compared to early-produced juveniles in the collared flycatcher *Ficedula albicollis* and in *N. orbicollis* (Part et al. 1992; Creighton et al. 2009). On the other hand, offspring may also suffer from being produced in a late cohort. This cost often results from physiological constraints that hamper the expression of parental investment and thus ultimately decrease offspring quality (reviewed in Javoiš 2013). Maternal senescence is a well-known physiological constraint that negatively and specifically affects the juveniles of late cohorts, as aging females usually become unable to increase or even maintain their reproductive efforts (McNamara and Houston 1996; Nussey et al. 2013; Kowald and Kirkwood 2015). For instance, maternal and offspring body mass, weaning mass and the level of lactation have all been shown to decline with age due to physiological degeneration in the grey seal *Halichoerus grypus* (Bowen et al. 2006).

Importantly, (often population-specific) environmental constraints such as food limitation and pathogen presence can either promote or hamper the expression of a terminal investment and may thus have a substantial impact on the traits of late cohort offspring. For instance, low food intake has been shown to inhibit the expression of terminal investment by females of the Alpine chamois *Rupicapra rupicapra* (Mason et al. 2011), whereas food restriction and pathogen presence favored its expression in males of the yellow mealworm beetle *Tenebrio molitor* (Krams et al. 2015) and the blue-footed booby *Sula nebouxii* (Velando et al. 2006), respectively. Although environmental constraints often vary between populations, the occurrence of population-specific differences in the life-history traits expressed by individuals from early and late cohorts remains surprisingly poorly studied (see Mason et al. 2011; Javoiš 2013; Vincze et al. 2013).

In this study, we investigated whether maternal and offspring life-history traits vary between initial and terminal reproductive events in the European earwig *Forficula auricularia*, and whether the occurrence and nature of this variation are population-specific. In this insect species, females produce up to two clutches during their one-year lifespan: the initial clutch of eggs is generally produced in early-winter and hatches in the following early-spring, while the terminal clutch (when present) is produced in mid-spring and hatches in the following early-summer (Meunier et al. 2012). From the date of egg laying until several weeks after egg hatching, mothers provide extensive forms of care to their eggs and juveniles (called nymphs) that include the protection against predators and pathogens, as well as the provisioning of nymphs with food (e.g. through regurgitation; Lamb 1976a; Kölliker 2007; Koch and Meunier 2014; Boos et al. 2014; Diehl et al. 2015; Kölliker et al. 2015).

In a previous study focusing on differences between semelparous and iteroparous *F. auricularia* females, Meunier et al. (2012) showed that the 2nd clutches of iteroparous females were smaller and developed faster than their 1st clutches. However, it remains unknown whether this variation (1) shapes other key life-history traits of earwigs, such as maternal condition and the expression of maternal care and, more importantly, whether it (2) depends on the studied population. Here, we addressed these questions by comparing a total of eleven life-history traits measured in the 1st and 2nd clutches of 132 *F. auricularia* females sampled in three distant populations in Europe. These traits encompassed measures of clutch quantity and quality, maternal condition during post-hatching family interactions, as well as the expression of brood defense and food provisioning, two important forms of post-hatching maternal care (Meunier and Kölliker 2012b; chapter 5).

## MATERIAL AND METHODS

### Field sampling and laboratory breeding

Our experiment started with 696 *F. auricularia* individuals sampled in September 2014 in three populations located in Girona (Spain, n = 120 females and 118 males), Montblanc (Spain, n = 118 females and 107 males) and Vincennes (France, n = 119 females and 114 males; Figure 6.1). All these populations belong to the same *F. auricularia* genetic clade B (Wirth et al. 1998; M. Veuille and X. Espalader, unpublished data), and are subjected to different environmental conditions in terms of altitude, temperature and precipitation (see details in Figure 6.1). Note that neither of these populations corresponded to the population studied in Meunier et al. (2012).

Individuals from all three populations were maintained under standard laboratory conditions adapted from Meunier et al. (2012). This allowed the expression of inherited population-specific traits while controlling for plastic responses to the environment. The setup started by haphazardly distributing all field-sampled individuals of each population among large plastic containers to form groups of 58 ± 0.58 (mean ± SE) individuals encompassing a maximum of 30 males and 30 females. These groups were maintained at 20°C, 60% humidity, 14:10 h light: dark photoperiod (thereafter called summer conditions) to allow uncontrolled mating (Sandrin et al. 2015). Two months later, a random sample of 237 females (Girona: n = 71; Montblanc: n = 59; Vincennes: n = 107) was isolated to allow egg production. These females were maintained under complete darkness and 60% humidity, with a temperature of 10°C for 2 weeks, and then 5°C for three months to mimic winter conditions. Spring was subsequently simulated by first increasing the temperature to 10°C and one week later to 15°C. When the first nymph hatched, the corresponding family was transferred to and maintained under summer conditions (see above) to favor nymph development. Fourteen days later, each female was isolated to mimic natural family disruption and subsequently maintained under complete darkness to allow 2nd clutch production (Meunier et al. 2012). The laboratory rearing of 2nd clutch eggs and nymphs was similar to the above detailed rearing of the 1st clutches, except that eggs were maintained under summer temperatures. Among the 209 females that produced 1st clutch nymphs (28 females did not; Girona: n = 9; Montblanc: n = 5; Vincennes: n = 14), a total of 136 females also produced a 2nd clutch (see results) and were thus used in the present study. For a comparison of the life-history traits expressed by semelparous and iteroparous females, please see Meunier et al. (2012).

All large plastic containers (30 x 17 x 21 cm) contained humid sand as ground material and an egg cardboard as shelter. Isolated females and, upon hatching, their nymphs were set up in Petri dishes (diameters 8.5) containing humid sand as substrate and a plastic tube (cut in half) as shelter. All the tested individuals received an *ad libitum* amount of standard laboratory food (see composition in chapter 1). However, we did not provide food to the isolated females between egg laying and hatching, as they typically stop feeding during this period (Kölliker 2007). All temperature changes were implemented gradually over four days. Note that all 14 days old nymphs (of both 1st and 2nd clutches) were discarded from the experiment.

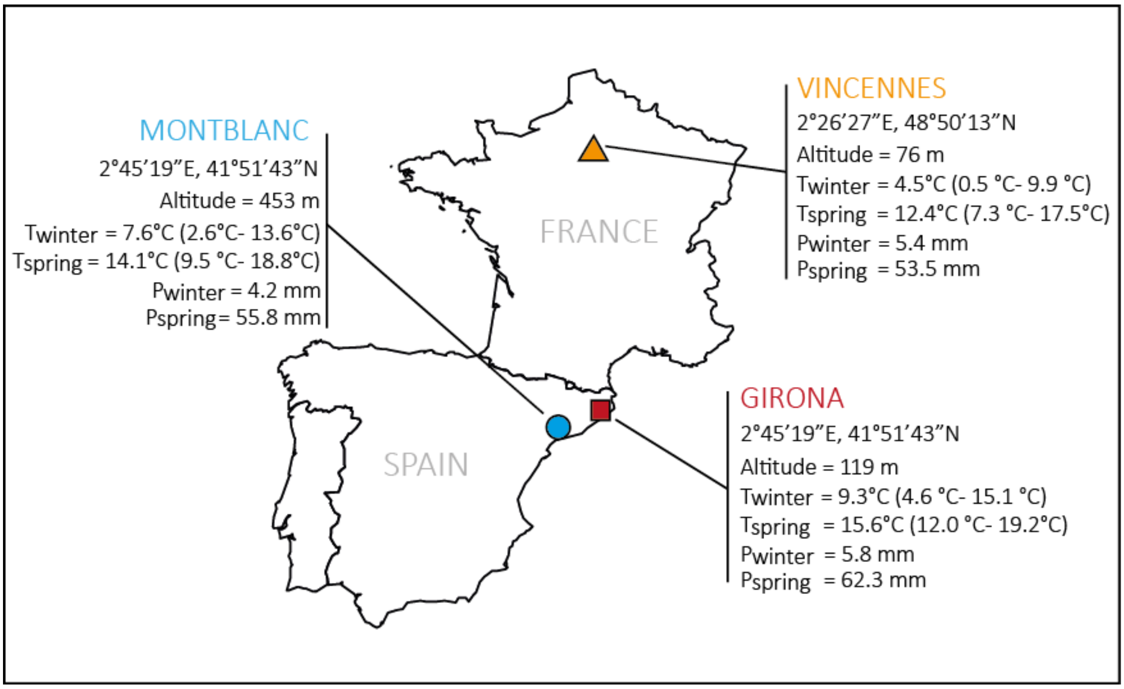

**Figure 6.1**. Location and climatic details of the three studied populations of *F. auricularia*. For each population, we provide the GPS coordinates and altitude, as well as the mean (minimum – maximum) temperatures and the mean precipitations recorded in winter and spring over the last 50 years. Information from the Worldclim data base (http://www.worldclim.org/).

### *Measurements of 11 life-history traits*

We investigated the effects of population and juvenile cohort on a total of 11 life-history traits reflecting clutch quantity and quality, maternal condition during post-hatching family interactions, and post-hatching maternal care. These measurements started with the (1) egg developmental time, which was defined as the number of days between the first day of egg laying and the first day of egg hatching. We then counted (2) the number of eggs produced within three days after the first egg laying, (3) the number of nymphs present one day after the first egg hatching and (4) the number of nymphs alive 14 days after hatching. We used different timespans for the first two countings, because *F. auricularia* females generally need up to three days to finish the deposition of their clutch of eggs, whereas egg hatching is well synchronized within each clutch and is generally completed over a single day (Koch and Meunier 2014). We also measured the (5) mean weight of nymphs one day and (6) 14 days after egg hatching. To this end, a group of

10 nymphs (or all nymphs if brood size was lower than 10) was haphazardly sampled in each brood and weighed to the nearest 0.01 mg using a microscale (model MYA5; PESCALE, Bisingen, Germany). We then recorded (7) the developmental time of nymphs from the first to the second developmental instar, which was obtained by counting the number of days between egg laying and the emergence of the first second instar nymph in each clutch. This measurement is known to reflect the developmental time of the entire clutch in *F. auricularia* (Gómez and Kölliker 2013). Finally, changes in maternal condition over the period of post-hatching family interactions were measured by weighing each mother (8) one day and (9) 14 days after the first egg hatching.

**Table 6.1.** Mean values of the 11 life-history traits measured in the three populations. For each trait, we provided the mean value, its standard error (SE), as well as the number of replicates in which it has been measured (N).

| | GIRONA | | | | MONT BLANC | | | | VINCENNES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | First clutch | | Second clutch | | First clutch | | Second clutch | | First clutch | | Second clutch | |
| | Mean±SE | *N* | Mean±SE | *N* | Mean±SE | *N* | Mean±SE | *N* | Mean±SE | *N* | Mean±SE | *N* |
| Egg developmental time (days) | 37.58 ± 2.33 | *52* | 15.10 ± 0.09 | *52* | 54.97 ± 1.87 | *39* | 14.64 ± 0.12 | *39* | 22.82 ± 0.83 | *45* | 15.38 ± 0.09 | *45* |
| Egg number | 68.63 ± 1.41 | *52* | 46.83 ± 1.21 | *52* | 58.95 ± 1.8 | *39* | 32.21 ± 1.65 | *39* | 41.22 ± 1.57 | *45* | 25.11 ± 1.41 | *45* |
| Nymph number day 1 | 63.44 ± 1.43 | *52* | 38.12 ± 1.96 | *52* | 54.03 ± 1.76 | *39* | 27.31 ± 1.77 | *39* | 31.53 ± 1.75 | *45* | 16.00 ± 1.3 | *45* |
| Nymph weight day 1 (mg) | 1.44 ± 0.03 | *52* | 1.38 ± 0.04 | *52* | 1.58 ± 0.04 | *36* | 1.42 ± 0.04 | *39* | 1.35 ± 0.04 | *45* | 1.49 ± 0.04 | *45* |
| Nymph number day 14 | 60.19 ± 1.56 | *52* | 36.87 ± 1.95 | *52* | 51.67 ± 1.66 | *39* | 26.21 ± 1.74 | *39* | 29.82 ± 1.64 | *45* | 14.22 ± 1.3 | *45* |
| Nymph weight day 14 (mg) | 2.83 ± 0.06 | *52* | 2.93 ± 0.09 | *52* | 3.04 ± 0.1 | *36* | 3.01 ± 0.11 | *39* | 2.60 ± 0.07 | *45* | 2.90 ± 0.08 | *45* |
| Nymph developmental time (days) | 12.73 ± 0.17 | *51* | 12.92 ± 0.21 | *51* | 12.06 ± 0.15 | *35* | 12.51 ± 0.22 | *39* | 13.18 ± 0.24 | *44* | 12.84 ± 0.19 | *43* |
| Mother weight day 1 (mg) | 58.79 ± 1.21 | *52* | 59.02 ± 1.38 | *52* | 51.22 ± 1.49 | *36* | 53.85 ± 1.46 | *38* | 44.78 ± 0.96 | *45* | 44.51 ± 1.01 | *45* |
| Mother weight day 14 (mg) | 71.90 ± 1.62 | *52* | 70.30 ± 1.6 | *52* | 64.35 ± 1.86 | *36* | 61.30 ± 1.72 | *38* | 55.32 ± 1.51 | *45* | 50.24 ± 1.39 | *45* |
| Brood defense | 21.42 ± 2.4 | *52* | 21.12 ± 2.46 | *52* | 17.97 ± 2.28 | *35* | 23.13 ± 3.17 | *38* | 12.11 ± 1.48 | *44* | 16.98 ± 2.65 | *43* |
| Food provisioning | 11.22 ± 1.48 | *51* | 11.00 ± 1.95 | *42* | 15.59 ± 2.61 | *36* | 13.35 ± 2.89 | *32* | 11.38 ± 2.41 | *44* | 17.54 ± 3.54 | *29* |

Post-hatching maternal care was estimated by measuring food provisioning and brood defense. (10) Food provisioning was measured in 102 clutches (a haphazard sample of 34 clutches was omitted from this measurements due to time constraints; see details in table 6.1) using a standard method relying on the fact that ingested colored food is visible through the partially transparent cuticle of 1st instar nymphs (Staerkle and Kölliker 2008; Kölliker et al. 2015; chapter 1). In brief, food was removed from each family on day 5 after hatching. Twenty-four hours later, mothers were isolated and had access to a green pollen pellet (naturally yellow-colored pollen colored with blue dye; Hochland Bio-Blütenpollen by Hoyer; Food die by DEKO BACK) for one hour. Afterwards, mothers were returned to a standardized number of 20 of their own nymphs (or all of their nymphs if the clutch had less than 20 nymphs; mean number of nymphs used in this test ± SE =

18.9 ± 0.21) to allow family interactions. Finally, the number of green-colored and non-green-colored nymphs was counted fifteen hours later using a stereomicroscope (Leica S8 APO, 10x). Note that food provisioning was not measured in clutches with less than five nymphs (n = 5) and that the level of food provisioning was independent of the number of recipient nymphs (Spearman correlation test, rho = -0.08, S = 23869, p = 0.577). During the food provisioning test, all left-over nymphs were maintained in their original Petri dish and provided with standard laboratory food. To follow the treatments detailed above, all unused families were starved for 24 h on day 5 and fed again on day 6.

Finally, (11) brood defense was assessed using a previously established method (chapter 5), in which each female was standardly poked on the pronotum with a glass-capillary (one poke per second) to record the number of pokes necessary to induce her running away beyond a distance of twice the female's body length. Brood defense was tested at day 4, day 8, and day 12 after hatching and the average of these three values was used as brood defense. The brood defense test was carried out under red light, as earwigs are nocturnal.

### *Statistical analyses*

We were first interested in testing the effects of population and the juveniles' cohort on the life-history traits of mothers and offspring. To control for possible non-independence among the 11 measured traits, we first conducted a Principal Component Analysis (PCA) to obtain non-correlated principal components (PCs) reflecting single or combinations of different life-history traits. In this PCA, the values of food provisioning were logit-transformed and the values of brood defense were log-transformed to comply with normal distributions. The PCA was conducted by scaling the data to unit variance and running a regularized iterative MFA method (with K-fold cross-validation) to handle the few missing values in the dataset (Lê et al. 2008; Husson et al. 2011). The resulting and selected PCs (Table 6.2) were then analyzed separately using Linear Mixed Models (LMMs), in which the population, the juveniles' cohort and their interaction were entered as fixed factors and female ID was used as a random factor (Table 6.3). In case of significant interactions between population and the juveniles' cohort (see results), pairwise comparisons among each combination were tested using Tukey HSD tests.

To determine the occurrence of an investment trade-off between 1st and 2nd clutches, we also tested whether the life-history traits measured in 2nd and 1st clutches were negatively (i.e. reproductive trade-off) or positively (i.e. quality-dependent reproduction) correlated, and whether the occurrence and nature of these correlations were similar across populations. To this end, we conducted a series of five General Linear models (LM), in which the 2nd clutch values of each of the five above PCs were used as a response variable, while the corresponding 1st clutch values, the population and their interaction were entered as fixed variables. All statistical analyses were conducted using R v 3.2.1 (http://www.r-project.org/) loaded with the packages *car*, *FactoMineR*, *missMDA*, *lmerTest* and *lsmeans*.

## RESULTS

Overall, 52 (83.8%) of the 62 females from Girona, 39 (72.2%) of the 54 females from Montblanc and 45 (48.4%) of the 93 females from Vincennes produced two clutches of nymphs (Pearson's Chi-squared test, $\chi^2_2 = 22.25$, $p < 0.0001$). These proportions were significantly smaller in Vincennes compared to both Girona ($\chi^2_1 = 20.00$, p-value < 0.0001) and Montblanc ($\chi^2_1 = 7.93$, p-value = 0.005), but comparable between the two Spanish populations ($\chi^2_1 = 2.32$, $p = 0.128$).

Table 6.2. Loadings of the five first Principal Component (PCs) reflecting single or combinations of eleven life-history traits. The traits having significant loadings on each PC are in bold.

| | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| Egg developmental time | 0.591 | 0.153 | **0.584** | 0.005 | -0.265 |
| Egg number | **0.931** | 0.032 | 0.129 | -0.045 | 0.004 |
| Nymph number d1 | **0.931** | -0.248 | 0.156 | -0.021 | 0.021 |
| Nymph number d14 | **0.923** | -0.243 | 0.151 | -0.031 | 0.016 |
| Nymph weight d1 | -0.071 | **0.859** | 0.066 | 0.013 | -0.238 |
| Nymph weight d14 | -0.092 | **0.859** | 0.019 | 0.204 | -0.142 |
| Nymphs dvpt time till 2nd instars | 0.005 | **-0.737** | -0.269 | 0.045 | 0.076 |
| Mother weight d1 | 0.591 | 0.396 | **-0.631** | -0.077 | 0.106 |
| Mother weight d14 | 0.667 | 0.439 | -0.497 | -0.086 | 0.093 |
| Food provisioning | -0.072 | 0.450 | 0.387 | -0.059 | **0.790** |
| Brood defense | 0.214 | -0.073 | -0.053 | **0.962** | 0.098 |
| Variance explained (%) | 34.5 | 24.7 | 11.6 | 9.0 | 7.3 |
| Cumulative variance explained (%) | 34.5 | 59.2 | 70.8 | 79.8 | 87.2 |

The PCA conducted on the life-history traits measured in the 1st and 2nd clutches of these 136 females provided 11 orthogonal principal components (PCs), of which we extracted the first five ones (total variance explained = 87.2%, Table 6.2). The first component (PC1) was highly and positively loaded with the number of eggs as well as with the number of nymphs at day 1 and day 14, therefore overall positively reflecting clutch size. The second component (PC2) revealed a positive association between the nymphs' weights on day 1 and day 14, as well as post-hatching developmental speed, which overall reflects nymph quality. Accordingly, high values of PC2 indicate that clutches consisted of heavy nymphs which quickly molted into the second developmental instar, whereas small values indicate clutches with light nymphs which required more time to develop into the second instar. The third component (PC3) revealed a trade-off between egg developmental time and the mother's weight at egg hatching. High values of PC3 thus represent clutches in which eggs required a long time to develop and mothers were light at egg hatching (probably because they spent more time caring for the eggs), whereas small values reflect clutches in which eggs developed fast and mothers were heavy at egg hatching. Finally, the fourth (PC4) and fifth (PC5) components were solely and positively loaded with brood defense and food provisioning, respectively.

**Table 6.3**. Influence of population, juvenile's cohort and their interaction on the five principal components (PC1 to PC5) based on the 11 measured life-history traits. Values obtained from LMMs. Significant p-values are in bold.

| | Population | | Juvenile's cohort | | Interaction | |
|---|---|---|---|---|---|---|
| | LR $\chi^2_2$ | P | LR $\chi^2_1$ | P | LR $\chi^2_2$ | P |
| PC1 | 281.6 | **<0.0001** | 797.3 | **<0.0001** | 38.6 | **<0.0001** |
| PC2 | 8.5 | **0.014** | 1.4 | 0.231 | 8.3 | **0.016** |
| PC3 | 33.3 | **<0.0001** | 209.5 | **<0.0001** | 90.1 | **<0.0001** |
| PC4 | 4.0 | 0.134 | 6.1 | **0.013** | 0.7 | 0.696 |
| PC5 | 5.5 | 0.064 | 10.1 | **0.002** | 5.5 | 0.064 |

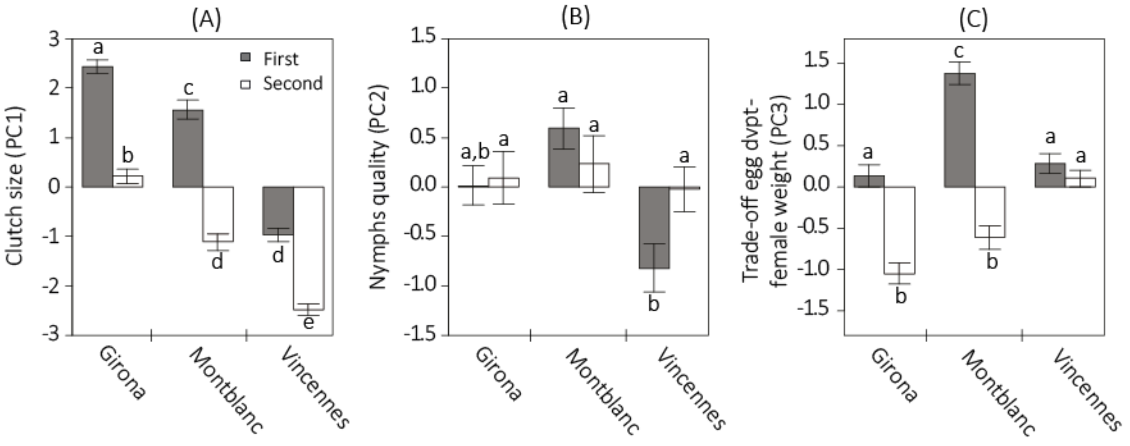

**Figure 6.2**. Interactive effects of population and the juveniles' cohort on the principal components reflecting (A) clutch size (PC1), (B) nymph quality (PC2) and (C) the trade-off between egg developmental time and the mother's weight at egg hatching (PC3). Values represent means ± s.e.m. Different letters indicate significant differences (see Table S6.2 for detailed values of pairwise comparisons).

An interaction between population and the juvenile's cohort significantly shaped PC1, PC2 and PC3 (Table 6.3). For PC1, pairwise comparisons within the statistical model showed that 1st clutches were overall larger than 2nd clutches within each population, whereas the interaction emphasized that this difference was smaller in Vincennes as compared to Girona and Montblanc (Figure 6.2A, Table S6.1). By contrast, the interactive effect of population and the juveniles' cohort on PC2 revealed that 1st clutch nymphs were of lower quality than 2nd clutch nymphs in Vincennes, but not in Girona and Montblanc (Figure 6.2B). Similarly, the significant interaction shaping PC3 showed a slower developmental time of eggs and a lighter weight of females at hatching in the 1st compared to the 2nd clutches in Girona and Montblanc, but not in Vincennes (Figure 6.2C). Note that the raw values of each trait can be found in Table 6.1.

The values of PC4 and PC5, which respectively reflected the level of brood defense and food provisioning, were overall higher in the 2nd compared to 1st clutches (Table 6.3 and Figure 6.3). However, they were independent of the population or of an interaction between population and the juvenile's cohort (Table 6.3).

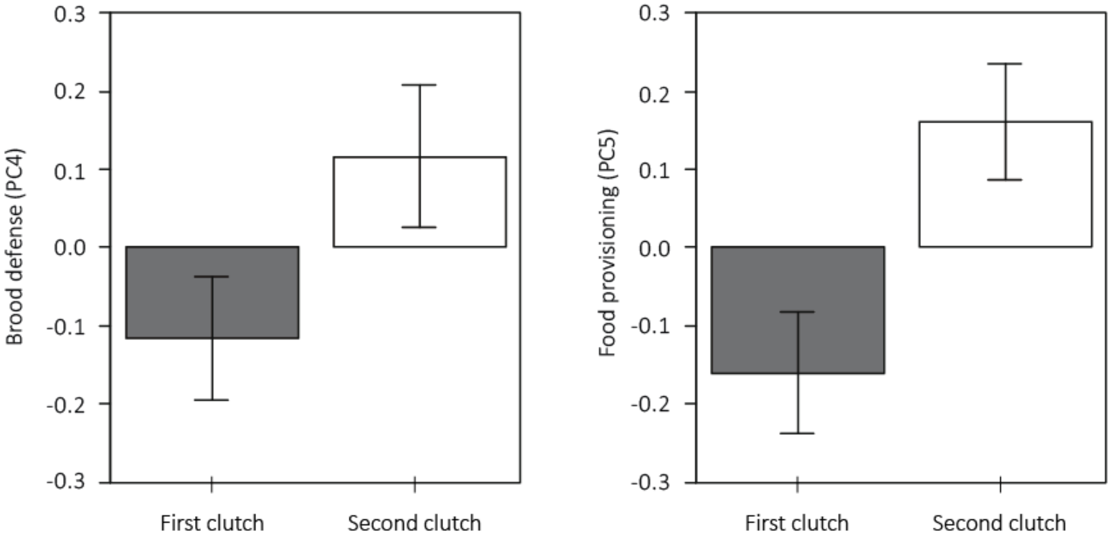

**Figure 6.3**. Influence of juveniles' cohort on the principal components reflecting brood defense (PC4) and food provisioning (PC5). Values represent means ± s.e.m.

Finally, we found consistencies (i.e. positive associations) across the two breeding attempts (Table 6.4) in terms of clutch size (PC1; Model estimate ± SE = 0.697 ± 0.039, t = 17.55, $P < 0.0001$), nymph quality (PC2; estim. = 0.375 ± 0.091, t = 4.13, $P < 0.0001$), the trade-off between egg developmental time and maternal weight at egg hatching (PC3; estim. = 0.335 ± 0.074, t = 4.55, $P < 0.0001$) and brood defense (PC4; estim. = 0.469 ± 0.092, t = 5.10, $P < 0.0001$), but not in terms of food provisioning (PC5; estim. = 0.086 ± 0.083, t = 1.04, P = 0.301; Figure 6.4). The occurrence and strength of these associations were independent of the population (interaction in Table 6.4). These statistical models also confirmed the results of the above analyses by showing that both clutch size and the trade-off between egg developmental time and the mother's weight at egg hatching observed in the 2nd clutches were population specific (Table 6.4, Figure 6.2).

**Table 6.4**. Influence of first clutch values, population and their interaction on the five PCs. Significant P-values are in bold.

| | Population | | 1st clutch values | | Interaction | |
|---|---|---|---|---|---|---|
| 2nd clutch values | F (2,130) | P | F (1,130) | P | F (2,130) | P |
| PC1 | 11.26 | **<0.0001** | 81.26 | **<0.0001** | 0.83 | 0.4395 |
| PC2 | 0.36 | 0.7002 | 16.91 | **<0.0001** | 0.11 | 0.8997 |
| PC3 | 35.18 | **<0.0001** | 40.27 | **<0.0001** | 0.01 | 0.9859 |
| PC4 | 0.54 | 0.5841 | 23.97 | **<0.0001** | 0.56 | 0.5711 |
| PC5 | 1.98 | 0.1428 | 1.00 | 0.3193 | 2.24 | 0.1102 |

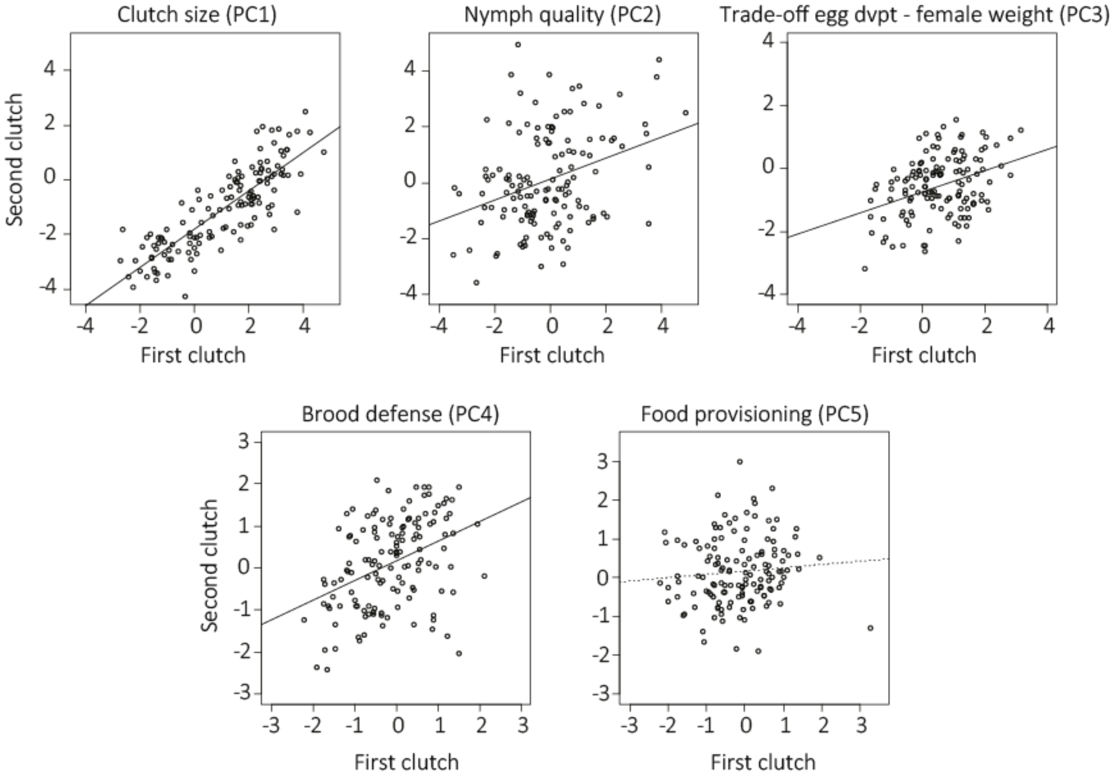

**Figure 6.4**. Association between PC-values of the first and second clutches across the three populations.

## DISCUSSION

This study overall shows that initial and terminal clutches of the European earwig *F. auricularia* exhibit differences in life-history traits, and that the occurrence and nature of these differences depend on the trait and/or the studied population. Specifically, we found that nymph quality was higher in the 2nd compared to 1st clutches of Vincennes females only, and that egg developmental time and female weight at hatching were longer and lighter, respectively, in initial compared to terminal clutches of females from Girona and Montblanc. By contrast, clutch size was lower and maternal care – i.e. brood defense and food provisioning – higher in 1st as compared to 2nd clutches in all three studied populations. Interestingly, the expression of all traits measured in the 1st clutches (but food provisioning) was positively associated with the expression of these traits in the 2nd clutches, and the occurrence and nature of these associations were independent of the studied population.

Across species and taxa, the expression of life-history traits often varies among populations. This is the case, for instance, for offspring mass and number in the scorpion *Centruroides vittatus* (Brown and Formanowicz 1995), for the juveniles' development and survival in the Mediterranean fruit fly *Ceratitis capitat* (Diamantidis et al. 2011), and for the level of parental care in the Kentish plover *Charadrius alexandrinus* and the snowy plover *C. nivosus* (Vincze et al. 2013). In earwigs, our results do not only demonstrate that each population is characterized by a specific proportion of females producing two clutches under identical (laboratory) conditions (see also Wirth et al. 1998; Meunier et al. 2012), but also that the population determines whether and how certain life-history traits differ between initial and terminal clutches.

Although females from the three populations were maintained under the same laboratory conditions, we found that their population of origin determined the effects of the juveniles' cohort on nymph quality and the trade-off between egg developmental time and female weight at hatching. This is important, as it reveals that these effects are not determined by the environmental conditions experienced during family life, but instead depend on the conditions experienced during female development and/or on (the conditions that have shaped) the evolutionary history of the population. In line with the first hypothesis, the quality of the environment experienced by *F. auricularia* juveniles during their development is known to affect the life-history traits of the resulting adults, for instance in terms of investment into 2nd clutch production and maternal care (Wong and Kölliker 2014; chapter 5). Conversely, harsh winter conditions have been suggested to select for a delayed production of 1st clutch eggs (Meunier et al. 2012), which may benefit females by shortening the developmental time of their 1st clutch eggs and thus allowing the mothers to exhibit a higher condition (i.e. a higher body weight) at hatching. Ultimately, such conditions may prevent differences in the trade-off between egg developmental time/female weight at hatching between 1st and 2nd clutches (which are always produced in spring, under favorable environmental conditions), and might thus explain its absence in females from Vincennes, the population with the coldest winter (Figure 6.1) and the latest production of 1st clutch eggs (Table 6.1). Whether our results are the outcome of the interactive or independent effects of these two processes remains to be further studied.

Contrary to the population-specific effects described above, we found that earwig females expressed higher levels of post-hatching care towards 2nd compared to 1st clutches in all three tested populations. Notably, this effect was present even if the females' weight at the beginning of family life (i.e. at egg hatching) was comparable between the two clutches (see Table 6.1). These results are overall in line with a terminal investment of *F. auricularia* females in terms of maternal care (Williams 1966b; Clutton-Brock 1984; Javoiš 2013), as found in the collared flycatcher *F. albicollis* (Part et al. 1992) and the burying beetle *N. orbicollis* (Creighton et al. 2009). Furthermore, these findings could indicate that the benefits of maternal care are more important for 2nd than 1st clutch nymphs and therefore select for higher expression of care towards late clutch nymphs across populations. In line with this idea, spring is often well advanced when 2nd clutch eggs hatch, so that the environmental conditions experienced by the resulting nymphs are likely to increase (as compared to 1st clutch nymphs) their exposure to pathogens, such as fungi, or their predators, such as other arthropods and even the 1st clutch nymphs (Dobler and Kölliker 2010). Irrespective of the mechanism mediating the increased level of maternal care towards 2nd clutch nymphs, the absence of a population-specific effect indicates that the environmental conditions experienced by females during 1st clutch family life (and egg care) are crucial in determining the subsequent expression of maternal care towards their 2nd clutch offspring. This is supported by recent studies showing that maternal condition at egg hatching determines the nature of sibling and mother-offspring interactions in *F. auricularia* (Wong and Kölliker 2012; chapter 1, chapter 2).

Interestingly, we found a reduction in size of the 2nd as compared to the 1st clutch in all three studied populations. This reduction is in line with an effect of senescence on female reproduction, but may also reflect an adaptive strategy of females. For example, the uncertainty of surviving until 2nd clutch production could favor a higher investment into 1st clutch production independent of female age. Such an effect of the perceived risk of death on female investment into reproduction has been nicely demonstrated in the burying beetle *Nicrophorus vespilloides*, in which an experimental activation of the immune system caused females to switch from reproductive restraint to terminal investment (Cotter et al. 2011). Interestingly, our findings in earwigs reveal that although clutch sizes were different, almost all measurements of life-history traits taken in the 1st clutches were positively correlated with the corresponding measurements in the 2nd clutches. This highlights that the overall reproduction of a female is tightly linked to her own quality irrespective of whether or not senescence shapes the size of 2nd clutches (Meunier et al. 2012). Further studies should be conducted to experimentally disentangle the (mutually non-exclusive) effects on maternal investment into egg production caused by senescence and/or the perceived risk of death on the one hand, and the effects on maternal care caused by terminal investment and/or reproductive strategy (see above).

To conclude, our study demonstrates that offspring cohort and population-membership interact in determining crucial life-history traits in the European earwig. We showed in a common garden experiment that population-membership affected the expression of two cohort-specific traits only (nymph quality and the trade-off between egg developmental time and female weight at egg hatching), suggesting an important role of past environmental conditions in their expression, but also indicating a limited role of such past conditions for the expression of maternal care. Although our results are overall in line with an effect of terminal investment on maternal care, and of senescence on maternal reproduction, our findings call for further experimental studies deciphering the independent or entangled action of these mechanisms (see, for example, Cotter et al. 2011). Finally, it is important to note that the direction and strength of the cohort-specific effects reported in this study should be interpreted with caution, as our laboratory conditions might have unwillingly favored individuals from certain populations. Nevertheless, our results demonstrate that even within a single genetic clade (namely the *F. auricularia* clade B; Wirth et al. 1998), population idiosyncrasies may have major effects on the expression of life-history traits associated with successive reproductive attempts. The question whether these idiosyncrasies reflect the capability of individuals to develop under specific laboratory conditions, the evolutionary history of the population, and/or the biotic/abiotic constraints experienced by the juveniles during their development remains open for further studies.

## ACKNOWLEDGEMENTS

[removed for privacy purposes]

## Supplementary Material

**Table S6.1.** Pairwise comparisons (contrasts) between populations in the statistical models on PC1, PC2 and PC3. P-values were corrected for multiple testing using the Tukey method. Significant p-values are in bold.

| | Comparison | estimate | SE | df | t-ratio | P-value |
|---|---|---|---|---|---|---|
| PC1 | Girona,First - MtBlanc,First | 0.867 | 0.211 | 192.6 | 4.11 | **0.001** |
| | Girona,First - Vincennes,First | 3.392 | 0.203 | 192.6 | 16.74 | **<.0001** |
| | Girona,First - Girona,Second | 2.209 | 0.121 | 133.0 | 18.29 | **<.0001** |
| | Girona,First - MtBlanc,Second | 3.538 | 0.211 | 192.6 | 16.78 | **<.0001** |
| | Girona,First - Vincennes,Second | 4.897 | 0.203 | 192.6 | 24.17 | **<.0001** |
| | MtBlanc,First - Vincennes,First | 2.525 | 0.218 | 192.6 | 11.60 | **<.0001** |
| | MtBlanc,First - Girona,Second | 1.343 | 0.211 | 192.6 | 6.37 | **<.0001** |
| | MtBlanc,First - MtBlanc,Second | 2.671 | 0.139 | 133.0 | 19.15 | **<.0001** |
| | MtBlanc,First - Vincennes,Second | 4.030 | 0.218 | 192.6 | 18.51 | **<.0001** |
| | Vincennes,First - Girona,Second | -1.182 | 0.203 | 192.6 | -5.83 | **<.0001** |
| | Vincennes,First - MtBlanc,Second | 0.146 | 0.218 | 192.6 | 0.67 | 0.985 |
| | Vincennes,First - Vincennes,Second | 1.505 | 0.130 | 133.0 | 11.59 | **<.0001** |
| | Girona,Second - MtBlanc,Second | 1.328 | 0.211 | 192.6 | 6.30 | **<.0001** |
| | Girona,Second - Vincennes,Second | 2.687 | 0.203 | 192.6 | 13.26 | **<.0001** |
| | MtBlanc,Second - Vincennes,Second | 1.359 | 0.218 | 192.6 | 6.24 | **<.0001** |
| PC2 | Girona,First - MtBlanc,First | -0.576 | 0.342 | 239.3 | -1.68 | 0.544 |
| | Girona,First - Vincennes,First | 0.835 | 0.329 | 239.3 | 2.54 | 0.117 |
| | Girona,First - Girona,Second | -0.079 | 0.258 | 133.0 | -0.31 | 1.000 |
| | Girona,First - MtBlanc,Second | -0.218 | 0.342 | 239.3 | -0.64 | 0.988 |
| | Girona,First - Vincennes,Second | 0.037 | 0.329 | 239.3 | 0.11 | 1.000 |
| | MtBlanc,First - Vincennes,First | 1.410 | 0.353 | 239.3 | 3.99 | **0.001** |
| | MtBlanc,First - Girona,Second | 0.497 | 0.342 | 239.3 | 1.45 | 0.694 |
| | MtBlanc,First - MtBlanc,Second | 0.358 | 0.298 | 133.0 | 1.20 | 0.837 |
| | MtBlanc,First - Vincennes,Second | 0.612 | 0.353 | 239.3 | 1.73 | 0.511 |
| | Vincennes,First - Girona,Second | -0.913 | 0.329 | 239.3 | -2.78 | 0.064 |
| | Vincennes,First - MtBlanc,Second | -1.053 | 0.353 | 239.3 | -2.98 | **0.037** |
| | Vincennes,First - Vincennes,Second | -0.798 | 0.278 | 133.0 | -2.87 | **0.053** |
| | Girona,Second - MtBlanc,Second | -0.139 | 0.342 | 239.3 | -0.41 | 0.999 |
| | Girona,Second - Vincennes,Second | 0.116 | 0.329 | 239.3 | 0.35 | 0.999 |
| | MtBlanc,Second - Vincennes,Second | 0.255 | 0.353 | 239.3 | 0.72 | 0.979 |
| PC3 | Girona,First - MtBlanc,First | -1.242 | 0.182 | 215.4 | -6.81 | **<.0001** |
| | Girona,First - Vincennes,First | -0.151 | 0.175 | 215.4 | -0.86 | 0.955 |
| | Girona,First - Girona,Second | 1.186 | 0.121 | 133.0 | 9.79 | **<.0001** |
| | Girona,First - MtBlanc,Second | 0.746 | 0.182 | 215.4 | 4.09 | **0.001** |
| | Girona,First - Vincennes,Second | 0.033 | 0.175 | 215.4 | 0.19 | 1.000 |
| | MtBlanc,First - Vincennes,First | 1.091 | 0.188 | 215.4 | 5.79 | **<.0001** |
| | MtBlanc,First - Girona,Second | 2.429 | 0.182 | 215.4 | 13.32 | **<.0001** |
| | MtBlanc,First - MtBlanc,Second | 1.988 | 0.140 | 133.0 | 14.21 | **<.0001** |
| | MtBlanc,First - Vincennes,Second | 1.275 | 0.188 | 215.4 | 6.77 | **<.0001** |
| | Vincennes,First - Girona,Second | 1.338 | 0.175 | 215.4 | 7.63 | **<.0001** |
| | Vincennes,First - MtBlanc,Second | 0.897 | 0.188 | 215.4 | 4.76 | **0.000** |
| | Vincennes,First - Vincennes,Second | 0.184 | 0.130 | 133.0 | 1.42 | 0.718 |
| | Girona,Second - MtBlanc,Second | -0.440 | 0.182 | 215.4 | -2.42 | 0.156 |
| | Girona,Second - Vincennes,Second | -1.153 | 0.175 | 215.4 | -6.58 | **<.0001** |
| | MtBlanc,Second - Vincennes,Second | -0.713 | 0.188 | 215.4 | -3.79 | **0.003** |

# CHAPTER 7

# Kin and multilevel selection in social evolution: a never-ending controversy ?

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*Based on*

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## ABSTRACT

Kin selection and multilevel selection are two major frameworks in evolutionary biology that aim at explaining the evolution of traits in a social context. However, the relationship between these two theories has been plagued by controversy for almost half a century and debates about their relevance and usefulness in explaining social evolution seem to rekindle at regular intervals. Here, we first provide a concise introduction into the kin selection and multilevel selection theories and shed light onto the roots of the controversy surrounding them. We then review two major aspects of the current debate: the presumed formal equivalency of the two theories and the question of whether group selection can lead to group adaptation. We conclude by arguing that the two theories can offer complementary approaches to the study of social evolution: kin selection approaches usually aim at identifying optimal phenotypes and thus often focus on the end result of a selection process, whereas multilevel selection approaches focus primarily on the ongoing selection process itself. The two theories thus provide different perspectives that might be fruitfully combined to promote our understanding of the evolution in group-structured populations.

Keywords: cooperation; altruism; group selection; levels of selection; inclusive fitness

## INTRODUCTION

Why should an individual cooperate to benefit others? This question pinpoints one of the central theoretical problems of sociobiology (Wilson 1975b; Sachs et al. 2004; Foster 2009). Cooperative behaviors such as altruism (an action that benefits others at one's own expense) would reduce the fitness of the performer relative to selfish individuals that do not perform the behavior, and hence should be selected against (West et al. 2007a; Wilson and Wilson 2007; West et al. 2011). However, this expectation is in striking contrast to the ubiquity of cooperation in nature, which occurs among 'simple' microorganisms (Foster 2010; Drescher et al. 2014) and within highly complex eusocial societies alike (Hölldobler and Wilson 1990).

Kinship and group selection are two key concepts of modern sociobiology that have been proposed to help resolve this apparent conundrum (Wilson and Wilson 2007; Foster 2009). Despite their common origin in the writings of Charles Darwin (cf. Gardner and Foster 2008), the developments of these two concepts in the modern kin and multilevel (or group) selection theories followed diverging paths and fueled a persisting and often heated debate about their relevance and usefulness in the study of social evolution (e.g., Maynard Smith 1964; West et al. 2008; Allen et al. 2013). Here, we provide a concise introduction to the two theories and the controversy surrounding them as well as highlight the complementarity of the approaches typically taken by their proponents. To this end, we first separately introduce the two theories and then point to the roots of the controversy. We subsequently review two important aspects of the current debate in more detail: the presumed formal equivalency of the two theories and the notion of group adaptation. We overall suggest that these issues illustrate the complementary nature of the perspectives offered by the kin selection and multilevel selection theories.

## KIN AND MULTILEVEL SELECTION THEORIES IN A NUTSHELL

### *Kin selection theory*

Interacting organisms may have an evolutionary incentive to help each other (or at least to hurt each other less) if they share genes, and the magnitude of this incentive should increase with the degree of relatedness between them; this is the central tenet of William D. Hamilton's *inclusive fitness theory* (Hamilton 1963; Hamilton 1964a; Hamilton 1964b; the term *kin selection theory* was coined by John Maynard Smith [1964] and is here used as a synonym for 'inclusive fitness theory' to comply with its conventional use). This tenet is encapsulated in a very simple form in *Hamilton's rule*, which states that a (gene for a) social behavior is favored by natural selection if $rb\text{-}c > 0$, where $c$ is the fitness cost to the individual performing the behavior, $b$ equals the fitness benefit to the recipient(s), and $r$ is the genetic relatedness between them (Hamilton 1963; Hamilton

1964a). The rule thus formalizes the realization that natural selection acts not only through direct effects of a behavior on the actor's own fitness (often measured as reproductive output) but also through indirect effects on the fitness of the actor's relatives (that have an above-average probability of sharing the actor's genes, including the one(s) that cause the social behavior in question; Foster 2009). Moreover, it provides a potential solution to the central problem of sociobiology, as it shows that even costly social behaviors can be favored by natural selection as long as the direct costs are outweighed by a sufficient amount of indirect benefit to sufficiently closely related individuals (Figure 7.1; Birch and Okasha 2015). Note, however, that the application of Hamilton's rule—and thus kin selection theory—is not restricted to altruistic behaviors: *rb* and *-c* represent, respectively, the indirect and the direct fitness consequences of *any* character of interest and hence can both be positive, negative, or zero (Gardner et al. 2011). Accordingly, they can also represent mutually beneficial (both fitness components positive), spiteful (both components negative), or selfish (direct component positive and indirect component zero or negative) behaviors.

Alongside Hamilton's rule, the concept of *inclusive fitness* is the second central element of kin selection theory. An organism's inclusive fitness is defined as the sum of its direct (Darwinian) and indirect fitness components (Figure 7.1). The latter is calculated as the relatedness-weighed sum of those effects on the fitness of other individuals for which the organism is causally responsible (Hamilton 1964a; Birch and Okasha 2015). Inclusive fitness is thus an actor-centric approach that examines how a focal individual influences its own fitness and that of its social partners (West and Gardner 2013). Hamilton (1964a) also suggested an alternative and (by now) increasingly used approach to account for direct and indirect fitness effects (e.g., Taylor and Frank 1996; Taylor et al. 2007; Marshall 2015): the *neighbor-modulated fitness* (sometimes referred to as *personal* or *direct* fitness). The two approaches differ in how the indirect component is conceptualized: in contrast to inclusive fitness, neighbor-modulated fitness is a recipient-centric approach and thus examines how social partners influence the fitness of a focal individual (West and Gardner 2013; Figure 7.1). The two approaches are usually seen as equivalent, as they predict the same overall response to natural selection (Gardner et al. 2011; but cf. Frank 1998; Frank 2013). The inclusive fitness approach, however, comes with one significant conceptual advantage: in principle, an individual is causally responsible for both its direct and indirect fitness and hence can control its inclusive fitness. Thus, natural selection might favor organisms that act as if they are attempting to maximize their inclusive fitness (Hamilton 1964a; Grafen 2006; Grafen 2014; Okasha et al. 2014; but cf. Lehmann and Rousset 2014a; Birch 2015). The possibility of conceptualizing individuals as maximizing agents (an optimality approach; Goodnight 2013) greatly facilitates the linking of theoretical and empirical research and has been central to the study of adaptation in behavioral and evolutionary ecology (West and Gardner 2013).

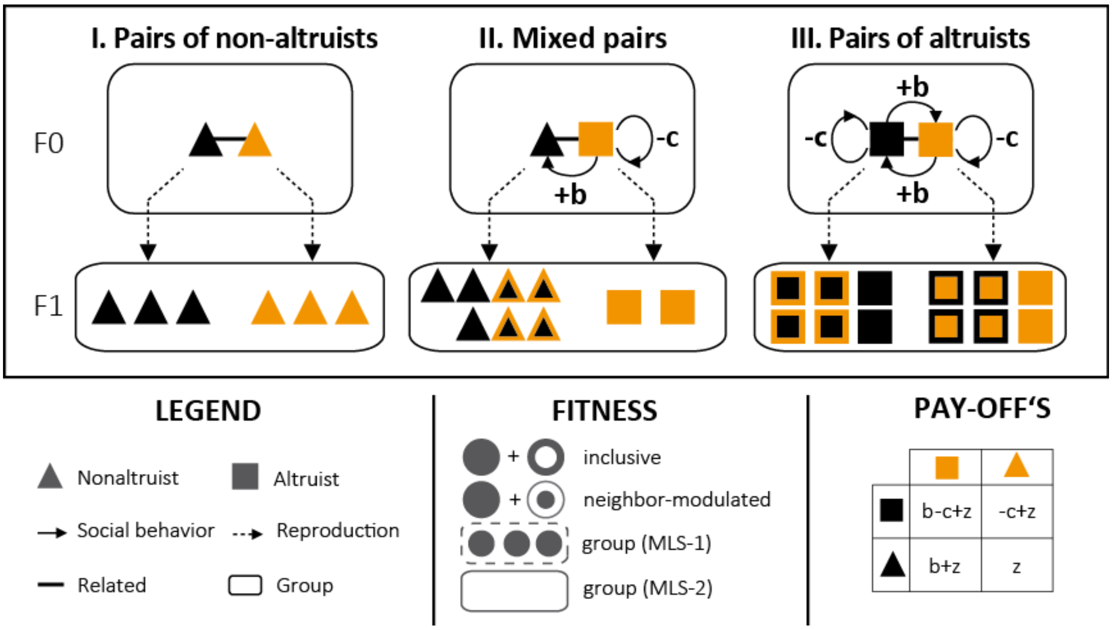

**Figure 7.1. Social interactions in a group-structured population.** In this example, non-altruists and altruists (symbolized by triangles and squares, respectively; see the *legend* for details) form (I) pairs of non-altruists, (II) mixed pairs, and (III) pairs of altruists. Altruists and non-altruists do not share the gene(s) causing the altruistic behavior but are overall related (i.e., genetically similar with respect to other traits). Upon reproduction, the (F1) progeny of each pair forms an independent group. Non-altruists do not express social behaviors and reproduce according to a baseline fitness (here arbitrarily fixed at z = 3). In contrast, altruists (unconditionally) confer a fitness benefit (b = 4) onto their partner at a cost (c = 1) to themselves. Although altruists incur a direct fitness cost, they benefit indirectly from assisting their partner and hence overall increase their inclusive fitness (see the *fitness box* for an illustration: inclusive fitness is composed of a direct [unicolored symbols] and an indirect [symbols framed in the same color] component). Similarly, their neighbor-modulated fitness is increased if they are assisted by their partner. However, altruists have a relative fitness disadvantage within mixed pairs because they increase the fitness of their non-altruistic partner at a cost to themselves and without receiving help in return (see *pay-offs box* for details). Hence, altruism can be disadvantageous even within groups of overall related individuals (precisely if they do not share the altruistic gene). In contrast, uniform pairs of altruists produce more offspring than mixed groups or groups of non-altruists (they hence have a higher group fitness in an MLS-1 framework; the group fitness in an MLS-2 framework corresponds to the number of groups produced and is here the same for all pairs). Positive assortment of altruists (e.g., due to limited dispersal) reinforces this 'group-advantageous' effect and can explain the evolution of cooperation on the long run.

Over the last five decades, kin selection theory has been extensively developed and generalized (e.g. Hamilton 1975; Michod 1982; Grafen 1985; Queller 1992a; Taylor and Frank 1996; Lehmann and Rousset 2014b; see Frank 1998; Marshall 2015 for book-length treatments) beyond the limited scope of Hamilton's original formalization (Hamilton 1963; Hamilton 1964a; Hamilton

1964b). For instance, Hamilton originally defined relatedness as a genealogical measure of shared ancestry (Hamilton 1964a) but quickly realized that this is only one (albeit by far the most frequent) way of generating the above-average genetic similarity among individuals that ultimately drives the evolution of many cooperative behaviors (Hamilton 1964b; Hamilton 1970; Hamilton 1975). In contemporary discussions, relatedness is accordingly more broadly defined (e.g. Frank 1998; Frank 2013) and encompasses any genetic similarity, regardless of whether it arose by common descent or by other means such as green-beard effects (Dawkins 1976; Gardner and West 2010). Moreover, the generalization of kin selection theory resulted in the development of general versions of Hamilton's rule (most notably on the basis of the Price equation; see below and Queller 1992a) that—unlike the original version—make no assumptions such as weak selection or additivity of fitness payoffs (see also Gardner et al. 2007; Gardner et al. 2011; Marshall 2011a). In these general versions, the cost-benefit parameters no longer denote simple fitness payoffs of social interactions but rather partial regression coefficients that quantify the overall statistical association among an organism's phenotype/genotype, its fitness, and the phenotype/genotype of its social partners (Birch 2014; Birch and Okasha 2015). Concomitant with its generalization, kin selection theory has become the dominating framework for the explanation of social behavior from an evolutionary viewpoint (e.g. Davies et al. 2012; Alcock 2013). Its most prominent empirical prediction, namely that social behavior should correlate with relatedness, has been supported across diverse taxa (the equally important impact of the costs and benefits received less attention, but see (Krakauer 2005; Refardt et al. 2013), and kin selection theory has greatly contributed to our current understanding of a variety of biological phenomena such as dispersal, reproductive skew, and queen-worker conflicts in eusocial insects (Bourke 2011a; Bourke 2014).

### Multilevel selection theory

The central tenet of multilevel (or group) selection theory conveys that selection not only acts on individuals but can act (simultaneously) on multiple levels of biological organization, including cells and/or groups (Okasha 2006). This view suggests that even if behaviors that benefit other individuals are selectively disadvantageous at the level of the individual, they might still evolve if they are advantageous at—and hence selected for on—a higher level of the biological hierarchy (e.g. on the group or colony level; Okasha 2006; Wilson and Wilson 2007). Altruism, for instance, is costly for the altruistic individual, but groups containing a higher proportion of altruistic individuals usually have a competitive advantage over groups that are composed mostly of selfish individuals (e.g., because altruistic groups are more productive or superior in direct confrontations). In such situations, altruism can evolve—driven by a process of selection *between* groups—even against the background of selection favoring selfishness *within* each group (e.g.

Wilson 1975a; Goodnight et al. 1992). This is the potential solution of the central problem of sociobiology from a multilevel perspective (Figure 7.1). The applicability of multilevel selection theory, however, is not limited to situations in which selection pressures on different hierarchical levels are opposing. Multilevel selection approaches more generally examine the direction and strength of (naturally occurring or experimentally applied) selection pressures on multiple hierarchical levels, investigate the mediators (e.g., indirect genetic effects; Wade 1978; Wolf et al. 1998; Bijma and Wade 2008) and magnitude of their respective contributions to total evolutionary change (Goodnight et al. 1992; Stevens et al. 1995; Eldakar et al. 2010), and explore effects of selection on group traits (Pruitt and Goodnight 2014).

Interestingly, there is currently no unanimously accepted formal theory of multilevel selection (e.g. Simon et al. 2013; Gardner 2015a; Goodnight 2015; Gardner 2015b) apart from the broad consent that defines group selection as natural selection based on the differential survival and reproduction of groups (Wade 1978; Sober and Wilson 1998; Wilson and Wilson 2007; but cf. Stevens et al. 1995). As a result, both individuals and groups can be found as focal units of attention (Figure 7.1) in multilevel selection approaches. Similarly, the definitions of *group fitness* and *group* vary among studies depending on whether they aimed at explaining the changing frequency of different types of *organisms* in a group-structured population or at explaining the changing frequency of different types of *group* in a meta-population of groups. In the first case, group fitness is often defined as the average (or total) fitness of its constituent organism (multilevel-selection-1, or *MLS-1*; Figure 7.1; Mayo and Gilinsky 1987; Damuth and Heisler 1988; Okasha 2004), and the group as a set of interacting individuals that influence each other's fitness, but not the fitness of individuals outside the group, with respect to a particular trait (*trait-groups*; Wilson 1975a; Sober and Wilson 1998; see Okasha 2006 for a discussion of this concept). In the second case, group fitness is defined as the expected number of offspring groups (*MLS-2*; individual fitness is then usually defined separately and on a different timescale; Mayo and Gilinsky 1987; Damuth and Heisler 1988; Okasha 2004). MLS-2 approaches often explicitly incorporate group-level events such as fission or extinction and thus assume that groups of individuals undergoing such group-level events can be identified in the population (Mayo and Gilinsky 1987). Groups are consequently more narrowly defined as geographically discrete, multigenerational, and reproductively isolated demes (Figure 7.1; Okasha 2006).

It is important to note that MLS-1 and MLS-2 approaches are not equivalent, as they relate to different natural processes (Okasha 2006; but cf. Gardner 2015a). In an MLS-1 scenario, groups 'merely' generate the population structure that affects the fitness of *individuals*. Hence, groups can propagate by producing individuals as long as these individuals form groups themselves at some stage of their life (e.g., after blending in a common mating pool). In contrast, groups need to reproduce in an ordinary sense in an MLS-2 scenario (i.e., by producing more groups; Okasha

2006). Though sometimes seen as fostering confusion (e.g. West et al. 2007b; Gardner 2015a), the difference between MLS-1 and MLS-2 also provides an intriguing diachronic perspective on 'major transitions' in evolution—and thus on the evolution of the biological hierarchy itself—as such transitions involve a temporal shift from MLS-1 (groups *of* individuals) to MLS-2 (groups *as* individuals; Michod 2005; Okasha 2005; Shelton and Michod 2010; Szathmáry 2015).

Despite its controversy-plagued history (see below), the multilevel selection theory has undergone a resurgence of interest in recent years (e.g. Wilson and Wilson 2007; Wilson 2008; West et al. 2008; Leigh 2010; Lion et al. 2011; Gardner 2015a; see Okasha 2006 for a book-length treatment). This is because it has provided novel perspectives on a variety of issues such as parasitic virulence (Kerr et al. 2006), cultural group selection in humans (that is often envisioned as the outcome of warlike confrontation; Boyd et al. 2003; Fehr and Fischbacher 2003), or the 'major transitions' in evolution (Okasha 2006; Szathmáry 2015).

## THE CONTROVERSY

### *A brief history*

The controversy surrounding the theories of kin and multilevel selection has a long and turbulent history (detailed in Sober and Wilson 1998; Okasha 2006; Foster 2009; Borrello 2010; Frank 2013). Until the second half of the last century, many biologists did not clearly distinguish between different levels of selection and it was often uncritically assumed that group selection would easily prevail over individual selection (e.g. Wynne-Edwards 1962) or that individual selection alone would foster adaptations 'for the good of the group' (e.g. Lorenz 1963). It was the rebuttal of these 'naïve' assumptions (though not of the theoretical plausibility of group-level thinking *per se*; cf. Wilson and Wilson 2007) that widely led to the rejection of group selection as a significant evolutionary force (Borrello 2005). Notably, kin selection theory—along with other theoretical frameworks such as evolutionary game theory (Maynard Smith and Price 1973) and selfish gene theory (Williams 1966a; Dawkins 1976) – was initially developed as an alternative to group selection (e.g. Hamilton 1963; Maynard Smith 1964), which likely contributed to an increasing polarization in disfavor of arguments based on group-level thinking (e.g. Dawkins 1976).

However, the demise of group selection was only temporary. Subsequent studies dropped the 'naïve' assumption of the unconditional superiority of group selection and instead acknowledged that selection within groups often undermines selection among groups (Wilson 1975a; Wilson 1977; Wilson 1980; Wilson 1987). Building on this premise, trait-group models suggested that group selection can drive evolutionary change even when opposed by within-group selection and that a periodical blending of groups (e.g., in a common mating pool) can prevent the seemingly inevitable fixation of selfish types within groups (Wilson 1975a; Wilson 1987). Moreover, empirical studies demonstrated that experimentally applied group selection can drive evolutionary change (e.g. Wade 1977; Craig 1982; Goodnight 1985; reviewed in Goodnight and Stevens 1997; Goodnight 2015), and argued that early models had restricted the applicability of group selection by deploying unrealistic assumptions, such as the notion that group and individual selection are always diametrically opposed (reviewed in Wade 1978; Wilson and Wilson 2007). Interestingly, Hamilton himself showed that multilevel selection was formally equivalent with his theory of inclusive fitness (Hamilton 1975; see next section), suggesting that the two theories simply outline different perspectives on the same natural processes. In some minds, this realization closed the debate, as the choice between the two theories seemed to have become a mere matter of personal taste. But far from it, the relationship between kin and multilevel selection remained controversial.

Over the last four decades, the group selection controversy has lost little of its initial momentum and continues to polarize opinions fueled by semantic debates (reviewed in West et al. 2007b; Wilson 2008; West et al. 2008) and, ultimately, the different implications the two theories seem to have for the evolution and self-perception of our own species (Shavit 2004; Shavit and Millstein 2008; Okasha 2010; see also Pinker 2012 and associated responses). Accordingly, some biologists contest the usefulness of multilevel selection in the study of social evolution in general (West et al. 2007b; West et al. 2008), whereas others call for a reframing of the theoretical foundations of sociobiology from a multilevel perspective (Wilson and Wilson 2007). Nevertheless, the focus of the controversy has shifted away from the question of whether group selection occurs at all, and now mainly revolves around (the consequences of) its presumed formal equivalence with kin selection theory and the question of whether group selection can lead to (group) adaptation. These are the two topics we are discussing below.

### *Kin and multilevel selection: formally equivalent theories?*

Most biologists consider kin and multilevel selection formally equivalent (e.g. Queller 1992b; Sober and Wilson 1998; Lehmann et al. 2007; Marshall 2011a; Frank 2013), but this view is not universally accepted and the number of dissenting voices has recently grown (e.g. Wilson and Hölldobler 2005; van Veelen 2009; Nowak et al. 2010; Traulsen 2010; van Veelen et al. 2012; Wilson 2012; Simon 2014; van Veelen et al. 2014). What, then, is the basis for the formal equivalency of the two approaches, and why is it still controversial?

On a practical level, the compatibility of kin and multilevel selection relies on the fact that both theories require positive assortment of (genetically) similar individuals for cooperative behaviors to evolve (Hamilton 1975; Débarre et al. 2014; Tekwa et al. 2015; see Griffin et al. 2004;

Queller 2004; Chuang et al. 2009; Kümmerli et al. 2009 for examples). From a multilevel perspective, positive assortment increases the scope for between-group selection, as it will make groups internally more homogeneous and thus reduce the potential for within-group selection (Figure 7.1; Okasha 2006). From a kin selection perspective, positive assortment ensures that costly social behaviors such as altruism are preferentially directed toward individuals that show the behavior themselves (Hamilton 1975). This directionality is crucial: altruistic traits are selectively disadvantageous even when directed at otherwise (genetically) very similar non-altruists (Figure 7.1) and thus can evolve only if altruists sufficiently often interact with other altruists, thereby increasing their *average* inclusive fitness over that of non-altruists (Hamilton 1975). In practical terms, the compatibility of kin and multilevel selection hence conveys that individuals expressing social behaviors (such as altruism) have a higher inclusive fitness than selfish individuals, whenever selection between groups is stronger than selection within them, and *vice versa* (Sober and Wilson 2011).

On a theoretical level, the compatibility of kin and multilevel selection is conventionally understood to predicate that group selection models can *always* be recast in terms of inclusive fitness (Gardner et al. 2007; Lehmann et al. 2007). This formal equivalency is usually inferred by using an equation developed by George C. Price (Price 1970; Price 1972) that expresses the intergenerational, population-level response to natural selection in a heritable trait as the covariance, taken over all individuals within the population, between an individual's trait and its fitness (here measured as its fecundity; e.g. Hamilton 1975; Queller 1992b; Marshall 2011a). The Price equation allows partitioning the evolutionary change into its direct and indirect components and can be used to derive Hamilton's rule (the kin selection approach; Hamilton 1970; Queller 1992a; Gardner et al. 2011; Marshall 2015). However, it also lends itself to partition the evolutionary change into effects at the individual and group levels (Price 1972; Hamilton 1975; Queller 1992b; Okasha 2006; Marshall 2011a; Marshall 2015). Hence, kin and multilevel selection are formally equivalent when formulated as alternative decompositions of the Price equation, as both approaches make it possible to correctly compute the total evolutionary change. The approaches differ merely in how this change is partitioned and thus offer different, potentially complementary ways of viewing evolution in structured populations (Hamilton 1975; Wade 1985; Queller 1992b; Marshall 2011a). Two points, however, deserve a closer examination: firstly, the above-described decomposition of multilevel selection applies only to scenarios of the MLS-1 type (as group fitness is defined as average individual-level fitness of group members; Okasha 2006). Secondly, the multilevel partitioning requires individuals to be nested in non-overlapping groups; the kin selection approach comes with no such requirement and thus is arguably more general within the Price framework (Hamilton 1975; Okasha 2006; Frank 2013).

Albeit most commonly used in theoretical studies, the Price framework is not the only approach to study multilevel selection. An alternative approach (that is often adopted in empirical studies; e.g. Eldakar et al. 2010; Moorad 2013; Weis et al. 2015) is offered by contextual analysis (Heisler and Damuth 1987; Goodnight et al. 1992; Okasha 2006). Like the Price equation, contextual analysis partitions the change due to natural selection (in MLS-1 scenarios; Okasha 2006) into individual and group effects; but unlike the Price equation, it detects group selection only if group effects on fitness remain even after controlling for individual effects. Contextual analysis thus accommodates a classic criticism against the multilevel partition of the Price equation, which can detect a component of between-group selection even in non-social contexts (i.e., when the evolution of the population can be predicted without taking group structure into account), and hence might not always accurately reflect the true causal effect of group selection (Heisler and Damuth 1987; Okasha 2006). However, contextual analysis has problems of its own, as it detects group selection when there is no variation in fitness among groups (e.g., because they all have the same productivity), but individual fitness depends on their ranking within the group (soft selection; Heisler and Damuth 1987; Goodnight et al. 1992; Okasha 2006; but cf. Goodnight 2015). Owing to these problems, it is still controversial which approach is better suited to study multilevel selection (e.g. Okasha 2006; Earnshaw 2015; Gardner 2015a; Goodnight 2015; Jeler 2015; Gardner 2015b; see also van Veelen 2005; Frank 2012; van Veelen et al. 2012). Note however that contextual analysis is formally very similar to modern kin selection models that are based on neighbor-modulated fitness. This supports the conjecture of an equivalency of kin and multilevel selection, as the two approaches seem inter-translatable even when multilevel selection is studied by using contextual analysis rather than the Price equation (Foster 2009; Goodnight 2013).

Though suggested by both the Price equation and contextual analysis, the formal equivalency of kin and multilevel selection remains controversial (e.g. van Veelen 2009; Traulsen 2010; van Veelen et al. 2012; Simon 2014; van Veelen et al. 2014). Most critics seem to reject (aspects of) the generalization of kin selection theory and instead contrast specific, narrowly defined formulations of kin selection with more general approaches to multilevel selection (e.g. Wilson and Hölldobler 2005; van Veelen 2009; van Veelen et al. 2012; Wilson 2012). For example, Wilson and Hölldobler (2005) rejected the broad definition of relatedness, arguing that it leads to a departure from the earlier and heuristically very useful narrow definition of kin selection. As a consequence, it can be argued that kin selection is only a special case of multilevel selection because relatedness (i.e., genetic similarity) can occur without strict kinship and hence evolution can occur by group selection in the absence of selection among narrow-sense kin (for instance, if group selection acts on green beards; Foster 2009). Similarly, Van Veelen and colleagues (van Veelen 2009; van Veelen et al. 2012; see also Traulsen 2010; van Veelen et al. 2014) rejected attempts to generalize kin selection and then contrasted multilevel selection with a specific (rather than a general) version of kin selection in which the cost-benefit parameters of Hamilton's rule denoted fitness payoffs (rather than partial regression coefficients). They showed that this kin selection approach can lead to incorrect predictions if the payoffs are non-additive (see also Queller 1984) and hence concluded that multilevel and kin selection are not equivalent. However, they compared a specific formulation of kin selection with their general formulation of multilevel selection and hence arguably could not refute assertions of the equivalency of the two theories that are based on general formulations of kin selection (cf. Birch and Okasha 2015).

Interestingly, these rejections of the general formulation of kin selection (van Veelen 2011; van Veelen et al. 2012; see also Marshall 2011b; Birch and Marshall 2014) relate to a more extensive debate that was initiated by a high-profile charge of Nowak, Tarnita, and Wilson against the value of inclusive fitness theory in explaining the evolution of eusociality (Nowak et al. 2010). This partly philosophical debate revolves around the question of whether the cost-benefit parameters in general formulations of Hamilton's rule allow a causal interpretation at all. As these parameters denote partial regression coefficients, they can depend on relatedness (Nowak et al. 2011) and population gene frequency, which can, for instance, lead to the counterintuitive result that a social behavior satisfies Hamilton's rule at a low, but not at a high, frequency (Allen et al. 2013). Whereas critics consequently deny the general formulations of Hamilton's rule any explanatory power and claim that they cannot accurately describe the evolutionary dynamics of any given system (e.g. Nowak et al. 2010; van Veelen 2011; Allen et al. 2013; Nowak and Allen 2015), others argue that they serve as a unifying principle that provides a super-ordinated framework for interpreting the results of otherwise-disparate models in a general terminology (e.g. Gardner et al. 2007; Gardner et al. 2011). Overall, this debate reveals that the formal equivalency of kin and multilevel selection (somewhat obviously) holds only if equally general formulations of the two theories are pitted against each other and that the issue at the heart of the debate really is the question of whether such general formulations make sense from a heuristic perspective (e.g. Nowak et al. 2010; Abbot et al. 2011; Gardner et al. 2011; West and Gardner 2013; Allen et al. 2013; Bourke 2015; Liao et al. 2015; Nowak and Allen 2015; see Birch 2014; Birch and Okasha 2015 for in-depth reviews). Indeed, the question of whether and when general versions of Hamilton's rule (and thus kin selection theory) provide a better/worse *causal* (rather than statistical) representation of the evolutionary process than the corresponding general approaches to multilevel selection (see also Conclusions section) might provide a fruitful avenue for future discussions.

Another line of reasoning against the equivalency of kin and multilevel selection suggests that even generalized formulations of kin selection cannot account for the long-term effects of events on the group level (Simon et al. 2013; Simon 2014; van Veelen et al. 2014). Group-level events such as fission, fusion, or extinction often occur asynchronously, and Simon and colleagues (Simon et al. 2013) recently suggested that group selection should consequently be thought of (and analyzed) as an asynchronous, continuous-time process that is shaped by the combined, long-term effects of such group-level events. On this basis, they argued that although kin and multilevel selection are often equivalent when only one time interval between two group-level events is analyzed, they would almost never be equivalent in a dynamical setting, because kin selection approaches could not account for the asynchronous nature of the group-level events (Simon et al. 2013; Simon 2014; see also Simon et al. 2012). Interestingly, they also suggest that the long-standing disagreement over the equivalency of kin and multilevel selection is based on oversimplified models of multilevel population dynamics and an inappropriate definition (via the Price equation) of group selection (Simon et al. 2013; Simon 2014): in its usual form, the Price equation traces the evolutionary change only over short periods (see above), assumes that all relevant processes such as reproduction or mass dispersion occur at a discrete set of time points, and is restricted to MLS-1 scenarios in which group-level events (other than mass dispersion) do not feature explicitly (Simon et al. 2013; Simon 2014). Thus, the Price equation (and contextual analysis) might be insufficient to capture all relevant aspects of the selection among groups.

Overall, the last word on the equivalency of kin and multilevel selection has surely not been spoken, as the partly philosophical character of the debate prevents it from being settled by theoretical or empirical results alone (cf. Birch and Okasha 2015). However, even if it would turn out that the equivalency is untenable in some situations, kin and multilevel selection will surely continue to occupy largely overlapping domains, leaving evolutionary biologists with both the blessing and curse of the existence of multiple theoretical frameworks to study social behavior.

### *Does group selection lead to group adaptation?*

One fundamental issue that triggered the initial rejection of group selection was the (then naïvely alleged) claim that it can foster group adaptation (i.e., promote the evolution of traits 'for the good of the group'; Wilson and Wilson 2007). Although an evolutionary response to *group selection* has by now been demonstrated in a variety of laboratory and field studies (e.g. Wade 1977; Muir 1996; Eldakar et al. 2009; Gordon 2013), the claim that it can foster *group adaptation* (or any adaptations at all) remains highly controversial (West et al. 2007b; West et al. 2008).

Recently, Pruitt and Goodnight (2014) reported that natural colonies of the social spider *Anelosimus studiosus* are characterized by a site-specific mixture of 'docile' and 'aggressive' individuals, and showed that experimentally constructed colonies with compositions mimicking the naturally occurring mixtures survived in the field but that colonies with deviating compositions perished. Experimental colonies with a perturbed composition that had survived at a 'foreign' site had shifted their composition toward a mixture that would have been optimal at their native site rather than toward the locally optimal mixture. Considering these results, Pruitt and Goodnight suggested that the composition of colonies differs between sites because of site-specific group selection and—as it is optimized to promote long-term colony survival at the native site—constitutes a group adaptation (Pruitt and Goodnight 2014). This latter conclusion, however, did not go unchallenged (Smallegange and Egas 2015; Grinsted et al. 2015; Gardner 2015c). Grinsted and colleagues (Grinsted et al. 2015) criticized that individual-level selection was not ruled out as an alternative explanation of Pruitt and Goodnight's results, and Smallegange and Egas (Smallegange and Egas 2015) indeed developed an environmental threshold model to explain Pruitt and Goodnight's observations at the individual rather than the group level. Finally, Gardner (Gardner 2015c) argued that colony composition is unlikely to maximize colony fitness and thus rejected the claim that the site specificity of colony composition constitutes a group adaptation.

Do the results of Pruitt and Goodnight (Pruitt and Goodnight 2014) thus provide no evidence for a group-level adaptation after all? The answer depends on the definition of 'group adaptation'. In a kin selection framework, adaptations are regarded as occurring at the level of the individual organism and to maximize an individual's inclusive fitness (Gardner and Grafen 2009; West and Gardner 2013; Gardner 2015c). By analogy, group adaptation is thus understood as a process that is driven by between-group selection and optimizes phenotypes for the purpose of group fitness maximization (Gardner and Grafen 2009; Gardner 2015c; see also Okasha and Paternotte 2012). This optimization process, however, is typically compromised by within-group selection because of conflicts among group members and thus will be favored by natural selection only if these conflicts are either absent (as for instance in clonal groups) or completely suppressed (Gardner and Grafen 2009; but cf. Kokko and Heubel 2011; Akçay and Van Cleve 2012), for example, through mechanisms such as fair meiosis (Leigh 1977; Frank 2003) or worker policing (Frank 2003; Wenseleers and Ratnieks 2006). Accordingly, group adaptations are expected to occur only rarely in nature, where their demonstration would require showing that within-group conflict is absent and group fitness is maximized (Gardner and Grafen 2009; Gardner 2015c). Pruitt and Goodnight (Pruitt and Goodnight 2014) did not assess within-colony conflict and thus arguably provide no conclusive evidence for group adaptation according to the above definition – a view that is embraced by all critics of their interpretation (Smallegange and Egas 2015; Grinsted et al. 2015; Gardner 2015c).

However, a different approach to group (and individual) adaptation is conventionally taken in a multilevel selection framework (e.g. O'Gorman et al. 2008; Sober and Wilson 2011; Pruitt and Goodnight 2015). In accordance with the kin selection framework, a process would be defined as group adaptation if the trait frequency evolves toward (or has settled down at) the group optimum (i.e., the trait frequency that is predicted to evolve when only between-group selection is at work). Likewise, a process would be defined as individual adaptation in both frameworks if the trait frequency is driven by within-group selection only and hence evolves toward (or has settled down at) the individual optimum. However, differences between the kin and multilevel selection frameworks emerge if within- and between-group selection are aligned or if the metapopulation evolves toward (or has settled down at) a *compromise* (i.e., an intermediate trait frequency; Sober and Wilson 1998; Sober and Wilson 2011; see also Foster 2009). In these situations, the (outcome of the) process would be called an individual adaptation in the kin selection framework, where adaptations are generally considered to occur at the level of the individual *irrespectively* of the strength of between-group selection (Gardner 2009; Gardner and Grafen 2009). In contrast, such compromises are often considered group adaptations in a multilevel selection framework, especially if they are (predominantly) driven by between-group selection (O'Gorman et al. 2008; Sober and Wilson 2011). Accordingly, while mechanisms of conflict suppression such as policing and punishment are a prerequisite of group adaptation in the kin selection framework, they are often considered group adaptations themselves in a multilevel selection framework (Wilson 1980; Wilson and Wilson 2007). In a reply to their critics, Pruitt and Goodnight adhere to this latter view and argue that the group-level trait 'colony composition' is shaped by site-specific group selection and hence constitutes a group adaptation (Pruitt and Goodnight 2015).

This recent debate about the implications of Pruitt and Goodnight's findings (Pruitt and Goodnight 2014) sheds light on a clear distinction between the kin and multilevel selection frameworks. The kin selection approach typically grants the individual priority as an evolutionary agent because it appears as an adaptive unit, and consequently allows a clear-cut distinction between individual and group adaptations even if selection acts on both the within- and between-group levels (Gardner 2009; Gardner and Grafen 2009; but cf. Sober and Wilson 1998; Foster 2009; Sober and Wilson 2011). In contrast, the multilevel selection approach allows such a clear distinction only in special cases (namely if selection acts only on one level) but places more emphasis on the fact that the realized frequency of a social trait is usually a compromise of different selection pressures (Sober and Wilson 2011; Pruitt and Goodnight 2015). Moreover, the multilevel selection approach allows for situations in which selection pressures on the individual and group levels are (at least to some extent) aligned. This might be the case where collective traits (e.g., the superstructure of water-proof rafts built by fire ants through self-assembly; Mlot et al. 2011) simultaneously promote the survival of the group and directly benefit the individuals within it. We suggest that both approaches may provide important insights into our understanding of social evolution and that, instead of focusing on their (semantic) differences, a more fruitful approach to the adaptiveness of social groups might be to ask how well adapted a particular social group is relative to the (theoretical) ideal of a conflict-free group (Foster 2009; see also Gardner and Grafen 2009; Queller and Strassmann 2009). Moreover, it is important to note that although the kin selection approach to group adaptation is more restrictive than the multilevel selection approach, both *in principle* allow for group adaptation. Thus far, the *formal* demonstration of group adaptation (i.e., a group-optimal and conflict-free outcome) according to Gardner and Grafen's kin selection-based definition (Gardner and Grafen 2009) is still pending. However, elaborate group-level traits such as the dance-language of honey bees (Seeley 1997) are good candidates that might live up to the definition of group adaptation within both frameworks.

## CONCLUSIONS

Kin and multilevel selection are two key concepts of modern sociobiology that provide different perspectives on the evolution of social behaviors. Unfortunately, these approaches are often pitted against each other in a seemingly endless (and largely semantic) debate that arguably impedes scientific progress (Okasha 2010) and prevents the benefits of the different perspectives from being harnessed.

Most biologists prefer kin selection over multilevel selection approaches as a matter of habit or personal taste (West et al. 2007b; West et al. 2008), and this preference seems partly justified as kin selection approaches have received more theoretical attention (and are hence highly versatile; Taylor and Frank 1996; Frank 1998) and have been put to work in more empirical applications (Bourke 2011a; Bourke 2011b; Birch and Okasha 2015; but note that the widespread acceptance of their formal equivalency implicates that empirical evidence for one theory cannot be used as evidence against the other). However, we believe that a bipartisan view on the kin and multilevel selection theories might ultimately prove more fruitful. After all, there might be situations in which one approach provides a more accurate representation of the causal structure of social interactions despite their (presumed) equivalency as statistical decompositions of evolutionary change (Okasha 2015; Birch and Okasha 2015). For example, it might be more *causally apt* to describe the selection pressures on a segregation distorter allele that has negative effects on the fitness of its bearer in terms of multilevel selection (i.e., as opposing selection pressures at the gene and individual levels). Conversely, it might be more *causally* apt to describe the selection on cooperative behavior in pairwise interactions between related individuals in terms of kin selection, especially if those pairs are ephemeral and form only for the duration of the social interactions (Birch and Okasha 2015). More generally, kin selection might provide a more accurate representation of the causal structure of social interactions than multilevel selection where fitness pertains to individuals in the first instance (and group fitness is a simple function of the fitness of its constituent individuals; see also Okasha 2006; Marshall 2015), whereas the opposite might be true where fitness pertains to the whole group in the first instance (and individual fitness is determined by group fitness; Okasha 2015). Such considerations of causal aptness might help to explain why multilevel selection was readily accepted for the study of major transitions (Okasha 2006; Szathmáry 2015) but only slowly establishes itself in the field of behavioral ecology, in which social interactions are often studied in ephemeral 'groups' of genealogical kin (Davies et al. 2012). It is noteworthy that considerations of causal aptness seem less clear in the case of eusocial systems (such as ants, termites, and some species of bees and wasps; cf. Okasha 2006) and that exactly these systems take center stage in the controversy surrounding the kin and multilevel selection theories (e.g. Wilson and Hölldobler 2005; Nowak et al. 2010; Abbot et al. 2011; Wilson and Nowak 2014; Bourke 2015).

Interestingly, kin and multilevel selection approaches might ultimately prove to be very useful exactly when applied to the same system. Kin selection analyses often follow an optimality ('adaptationist') approach and accordingly try to identify the phenotype(s) with the highest overall fitness to extrapolate where a population will eventually stabilize (Gardner et al. 2011; Goodnight 2013). The strength of kin selection (as a driver of evolutionary change over the course of one or multiple generations), however, is rarely reported (Goodnight 2013). In contrast, multilevel selection approaches often follow an 'evolutionary change' approach and examine how a population will change its current configuration (for example, depending on the strength of within- and between-group selection or in response to an applied selection pressure; Goodnight 2013; Goodnight 2015). The optimal phenotype, however, is typically not identified in multilevel selection studies (Goodnight 2013). The two perspectives opened up by the kin and multilevel selection approach, respectively, seem to be highly complementary (Goodnight 2013; Goodnight 2015). Experimental studies already began to harness the translatability of the two approaches (e.g. Chuang et al. 2009; Kümmerli et al. 2009; Chuang et al. 2010; Kümmerli et al. 2010). However, we still eagerly await studies that make use of the full potential of their complementarity by combining both the 'evolutionary change' and 'adaptationist' perspective and the methods that come along with them. We believe that such studies would go a long way toward gaining a deeper understanding of the processes that ultimately drive the evolution of social behaviors in structured populations.

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# PERSPECTIVES

## The early evolution of family life revisited

Jos Kramer

„If at first the idea is not absurd, then there is no hope for it.“

Albert Einstein (quoted in Des MacHale, 2002. *Wisdom*)

The intricacies of family life have been under close theoretical and empirical scrutiny for decades. Nonetheless, understanding the evolutionary causes promoting its origin and early evolution has remained an enduring challenge (Gross 2005; Royle et al. 2012a; Royle et al. 2016). The studies presented in this thesis indicate that this challenge persists because the evolutionary dynamics in non-derived family systems often differ substantially from the well-studied dynamics in their advanced counterparts. For instance, mechanisms such as sibling cooperation (chapter 1) and parent-offspring competition (chapter 4) might be crucial determinants of the fitness effects of early family life, and yet are only of limited relevance in advanced family systems (cf. Roulin and Dreiss 2012; Meunier and Kölliker 2012a; Falk et al. 2014). At the same time, mechanisms typically maintaining family life in advanced systems – such as the often fatal consequences of parental loss – can have less marked effects during early evolutionary stages (chapter 5). Early family systems are thus not just simplistic imitations of their more advanced counterparts, but rather reflect surprisingly sophisticated social environments (chapter 2) that have far-reaching effects on life-history characteristics (chapter 6), and might affect social behaviors well beyond the duration of family life (chapter 3). These unexpected complexities and their implications for the evolution of sociality (see also chapter 7) suggest that the challenge to understand the early evolution of family life is worthwhile of a comprehensive answer.

In this dissertation, we investigated the social behaviors and evolutionary mechanisms that shape the social life of the European earwig *F. auricularia*, a precocial insect species that recently emerged as a model system for the early evolution of family life (Kölliker 2007; Meunier and Kölliker 2012a; Falk et al. 2014; Diehl et al. 2015; Körner et al. 2016). The aim of this concluding section is not to provide a mere recapitulation of our diverse findings, since this would largely amount to a repetition of the detailed discussions in the different chapters of this thesis. Instead, I intend to integrate these findings into an emerging perspective on the evolutionary origin and subsequent consolidation of family life. This integration necessarily entails inferences about events in the evolutionary past, and hence is necessarily speculative at least to some extent. The present perspective should thus not be taken as the endpoint of a completed research project, but rather inform future studies and thus serve as a stepping stone towards a deeper understanding of the transition to sociality and the evolution of family life.

## *Family life – more than just parental care*

Family life is rarely discussed in the literature as anything more than the social environment in which parents provide – and offspring compete for – parental care (Clutton-Brock 1991; Royle et al. 2012b). This strong focus on parental care and directly related phenomena (such as sibling rivalry and parent-offspring conflict) is typically justified by the substantial impact of parental behaviors on the life-history and fitness of family members (Alonso-Alvarez and Velando 2012;

Klug et al. 2012). However, the centrality of parental care comes along with a crucial, but as yet little-appreciated drawback: it might inadvertently foster the erroneous equalization of the fitness effects of parental care with the (in reality more general) fitness effects of family life (see pp. 7-8). This misconception is severe, because it arguably deflects scrutiny of fitness effects that are not directly linked to – and thus could occur in the absence of – parental care. For instance, cooperative interactions among juvenile siblings have so far received little attention, despite their potential role in the evolution of family life (but see, for instance, Biedermann and Taborsky 2011; Yip and Rayor 2013; Falk et al. 2014). In this thesis, we showed that the cooperative food transfer among *F. auricularia* nymphs can potentially compensate for insufficient maternal food provisioning, suggesting that sibling cooperation could indeed affect the evolution of family-living (chapter 1, chapter 2). This finding thus highlights the need to account for the fitness effects of all types of behavior when assessing the costs and benefits of family life.

However, the equalization of the fitness consequences of parental care with those of family life is not only problematic because it deflects scrutiny of such potentially important behaviors. It is also disquieting because the usually highly asymmetrical fitness effects of parental care might conceal fitness effects of family life that are opposed to – and thus usually masked by – the benefits of parental care to offspring, or the costs of care for parents. For instance, the net benefits of parental care might mask that parental presence increases sibling rivalry (Smiseth et al. 2007; Gardner and Smiseth 2011), or triggers direct parent-offspring competition (chapter 4) and the expression of behaviors that are maladaptive under certain conditions (such as the covering of food in the absence of predators/pathogens; chapter 5). Conversely, the net costs of providing parental care might conceal direct fitness benefits of family life for parents (for instance, earwig mothers sometimes feed on the feces mediating the food transfer among their offspring; JK, personal observation). Differences in factors underlying such disregarded fitness consequences might be crucial in explaining why even closely related species exposed to comparable environmental conditions often show striking differences in their social organization (cf. Costa 2006; Trumbo 2012). Understanding the evolution of family life thus requires a complete picture of all factors affecting the short and long term fitness consequences of family-living. Albeit doubtlessly the most important, parental care is only one among many such factors.

### *The emergence of family life revisited*

The emergence of family life is typically envisioned to occur if certain environmental conditions and life-history characteristics (see pp. 9-10; Klug et al. 2012) favor the extension of pre-hatching parental care beyond the time of offspring emergence (e.g. Lack 1968; Clutton-Brock 1991; Smiseth et al. 2012). However, this standard account of the emergence of family life passes over the potential influence of social interactions among the newly emerged offspring, and is thus likely incomplete. In species without family life, offspring are predicted to disperse soon after hatching to avoid the impending competition with their siblings (West et al. 2001; West et al. 2002). The standard account for the emergence of family life accordingly implies that the extension of parental care beyond offspring emergence can at first only result in brief periods of family life. Longer periods of family-living would thus only arise secondarily, for instance if the benefits of offspring attendance and other early forms of parental care select for delayed offspring dispersal. However, benefits of cooperative interactions among the juveniles might offer an alternative (or additional) reason for offspring to delay the dispersal from their natal site. For instance, the possibility to share food with their siblings might give offspring an incentive to re-aggregate after independent foraging trips (chapter 1). In such situations, delayed dispersal due to sibling cooperation would allow for much longer periods of family life right from the beginning.

Intriguingly, the benefits of sibling cooperation can generally accrue independent of parental care, and might thus favor offspring aggregations even in the absence of parents. Congregations of juveniles (such as the semisocial larval societies of sawflies; (Costa 2006) might thus not only constitute an alternative evolutionary pathway to group formation (see pp. 6-7), but could actually precede the emergence of family life. In particular, semisocial juvenile aggregations could initially arise whenever the benefits of sibling interactions favor delayed dispersal, and might subsequently give rise to subsocial families if parents extend already existing pre-hatching forms of care beyond offspring emergence. This scenario suggests that species might not only exhibit both the subsocial and the semisocial pathway to group formation during different stages of their life cycle (chapter 3; see also Costa 2006). Rather, they might also follow the two pathways at different times in the course of their evolutionary history. From an offspring's point of view, family life is classically thought to evolve *despite of* the presence of competing siblings (cf. Mock and Parker 1997; Roulin and Dreiss 2012). However, the occurrence and potential role of sibling cooperation in the origin of family life suggests the interesting possibility that family life might rather emerge – or at least be initially favored – *because of* the presence of siblings.

### *The consolidation of family life revisited*

Following the emergence of family interactions, co-adaptation between parental and offspring traits is commonly predicted to promote the rapid consolidation of family life and foster the emergence of novel social behaviors (Kölliker et al. 2012; Uller 2012). However, the rich complexities resulting from the co-evolution of multiple social behaviors only recently moved into focus (e.g. Agrawal et al. 2001; Kölliker et al. 2005; Gardner and Smiseth 2011; Royle et al. 2016), and are thus not well understood. Here, I outline some of the implications of our results that might help to shed light onto the processes and evolutionary dynamics that shape the different evolutionary stages of the consolidation of family life.

### (1) Origin and loss of social behaviors

Novel social behaviors have been predicted to emerge rapidly after the onset of the consolidation of family life (Kölliker et al. 2012; Uller 2012). In line with this hypothesis, a recent theoretical model suggests that the evolution of parental food provisioning can prompt evolutionary changes in other components of care, and may trigger a coevolutionary feedback-loop that fosters progressively increasing levels of food provisioning and sibling rivalry (Gardner and Smiseth 2011; see also pp. 10). Such mutual reinforcement may lead to co-adaptation, and would thereby foster an increasing reliance of offspring on parental resources, as well as the evolution of novel and increasingly complex forms of care (Gardner and Smiseth 2011; Smiseth et al. 2012). However, the consolidation of family life might also lead to the repression or loss of certain types of family interaction (potentially explaining their rarity in altricial species). For instance, evolutionary conflicts between social partners generally reduce the likelihood that they engage in cooperative behaviors (Frank 1998; West et al. 2002). The increasing levels of sibling rivalry that evolve in concert with parental food provisioning might accordingly decrease the likelihood of cooperative interactions among juveniles, suggesting that some forms of sibling cooperation could be lost during the consolidation of family life (chapter 1). Likewise, the scope for parent-offspring competition should decreases (and the scope for parent-offspring conflict increase) as a result of the delay of offspring foraging that arises concomitantly with the increased reliance on parental resources (chapter 4). Overall, these findings illustrate that the consolidation of family life is a multifaceted process that may involve both the origin and loss of social behaviors.

### (2) The pathway towards advanced family systems

The consolidation of family life has been predicted to favor a unidirectional and hardly reversible trend towards increasingly complex family systems that is fueled by the mutual reinforcement and co-adaptation of parental and offspring traits (Wilson 1975b; Gardner and Smiseth 2011; Royle et al. 2016). However, simple family systems are abound across taxa, and phylogenetic comparisons among invertebrates suggest that family life has been regularly lost due to high costs of parental care (Tallamy and Schaefer 1997; Lin et al. 2004; Filippi et al. 2009). These observations hence suggest that the evolutionary progression towards complexity is likely not inevitable. Intriguingly, they also raise the question which conditions could prevent this trend. We showed that parental loss can entail costs even in species featuring 'simple' family systems with non-obligatory care (chapter 5). This finding implies that high mortality rates of parents *during* family life might be a key factor counteracting its consolidation. This is because even though juveniles can survive the early death of their parents under facultative care, they will still suffer (non-lethal) consequences of parental loss such as an impaired development. High parental mortality rates might accordingly not only increase the likelihood that these negative consequences arise; instead, they might also select against the further consolidation of family life, since the concomitant deepened integration of parental care into offspring development would increase the conditional costs of parental loss. Moreover, high parental mortality rates might select for the maintenance of alternative survival strategies among juveniles (such as sibling cooperation; chapter 1 and 2). The mortality rate of parents could hence be crucial in determining whether the evolution of a given family system follows the trend towards increasing complexity.

Advanced family systems are often caught in a *parental trap* that enforces the maintenance of family life irrespective of its current adaptive value (Eberhard 1975). By contrast, less advanced forms of family life are apparently readily lost over evolutionary times (Tallamy and Schaefer 1997; Lin et al. 2004; Filippi et al. 2009). In the light of the above considerations, these findings might indicate the existence of a threshold of social complexity that determines whether family life is self-sustaining. Above this threshold, the phenotypic integration of parental care into offspring development would be tight enough to render parental care obligatory for offspring survival. Family life would then be beneficial to offspring irrespective of the external conditions, and could thus hardly ever be lost. By contrast, the integration of parental care into offspring development below this threshold would be sufficiently limited to enable offspring survival in the absence of the parents. In this situation, family life would (still) be facultative, and environmental conditions and the life-history characteristics affected by these conditions (such as the mortality rate of parents during family life; see above) would determine whether family life is maintained at its *status quo*, abandoned in favor of an alternative solitary lifestyle, or propelled towards the threshold that separates facultative from obligatory family systems. The existence of such a threshold would reconcile the current debate over the loss of parental care and family life (cf. Trumbo 2012), since it allows for the co-existence of self-sustaining as well as labile family systems. Finally, it would also leave scope for the theoretically expected unidirectional trend toward increasingly complex family systems – namely if the prevailing conditions are favorable and stable enough to promote an ever-increasing integration of parental and offspring traits.

### *Implications for social evolution*

The evolution of family-living does not only mark a transition from solitary to social life, but also constitutes the initial step in the major transition to eusocial societies (Szathmáry and Maynard Smith 1995; Bourke 2011a). Understanding the origin and consolidation of family life might thus help uncover universal processes that also shape other major transitions. In the course of such transitions, cooperation typically spreads among lower-level units (such as individuals) and replaces the initially prevailing conflicts between them (Bourke 2011a; see also chapter 7). The evolution of family life indeed shows evidence for both processes: parental care, a hallmark cooperative trait (Hamilton 1964a; Smiseth et al. 2012), greatly diversifies during the evolution of complex family systems. Conversely, the initially prevailing direct competition between parents and offspring might be progressively suppressed (chapter 4). However, the evolutionary dynamics of family life also indicate that not all forms of cooperation might be favored – and not all conflicts equally suppressed – during the consolidation of social groups. For instance, cooperation among juvenile siblings likely occurs frequently in facultative family systems, but is arguably rare in advanced systems with obligatory family life (chapter 1). Conversely, sibling rivalry and parent-offspring conflict (*sensu* Trivers 1974) typically increase during the evolution of complex family systems (Gardner and Smiseth 2011). This suggests that some conflicts characteristic of later stages in a major transition might arise from dynamics that shaped earlier stages of that transition. In more general terms, these considerations indicate that the increase in cooperation and the suppression of conflicts might be overall trends that need to hold true neither for all types of cooperation and conflict, nor for all stages of a major transition.

The various stages of a major transition broadly fall into two categories delineating the initial formation of *collectives* (such as groups) out of formerly independent *particles* (such as individuals) on the one hand, and the subsequent transformation of these collectives on the other hand (Bourke 2011a). This transformational phase entails the transfer of key (e.g. metabolic or reproductive) functions from the particle to the collective level (Szathmáry and Maynard Smith 1995; Bourke 2011a), and hence exhibits a striking parallel to the consolidation of family life. In both cases, an increasingly tight phenotypic integration ties the fate of single particles [offspring] closer and closer to the fate of the collective [family], eventually resulting in obligatory social life – that is the inability of particles [offspring] to survive alone. This parallel suggests that 'collective mortality' might have a crucial role in the transformational phase that corresponds to the role of parental mortality in the consolidation of family life (see above). In particular, the likelihood of a costly collapse of a facultative collective might influence whether the phenotypic integration among its constituent particles proceeds, and could thus ultimately determine whether the collective becomes obligatory for particle-survival. Like the shift from facultative to obligatory family life, the shift from facultative to obligatory collectives could occur when the life-history characteristics of the particles and the environmental conditions allow for the breaching of a threshold of social complexity (see above). Interestingly, the increasing phenotypic integration among the particles underlying this shift might also be paralleled by a shift from particle to collective-level selection (Okasha 2005; Shelton and Michod 2010). This change in the most relevant level of selection could in turn determine whether kin selection or multilevel selection approaches best describe the underlying evolutionary process (chapter 7; Okasha 2015). The different stages of the evolution of family life offer rich opportunities to investigate these possibilities. Exploring the intricacies of family life might thus be a good starting point to advance our understanding of the major transitions and the theoretical framework of sociobiology.

## *Conclusion*

The studies presented in this thesis demonstrate that facultative family systems can give rise to surprisingly sophisticated social environments. These environments are shaped by a unique set of behavioral mechanisms, and thus often differ profoundly from the social environments in obligatory family systems: sophisticated forms of parental care are paralleled by – and intertwined with – sophisticated forms of sibling cooperation; parents compete with their own offspring; and yet, offspring already suffer long-term costs of parental loss. However, the social behaviors expressed during family life do not only resonate in the behaviors expressed in the adult stage; they also affect life-history characteristics across populations, and their interplay ultimately shapes the evolution of family life and thus the transition from simple to complex family systems.

Despite their range, these findings can only offer a brief glimpse of the rich complexities characterizing facultative forms of family-living. I nevertheless dare to hope that this thesis offers a stepping stone toward a deeper general understanding of the early evolution of family life.

## *Outlook*

I conclude by highlighting three broad steps that could jointly propel this promising area of future research based on our findings. In particular, these steps are:

1. **Working out the mechanisms in laboratory experiments.** An in-depth understanding of the evolutionary origin and subsequent consolidation of family interactions presumes the knowledge of all mechanisms that shape the benefits and costs of family life. Experimental studies under standardized laboratory conditions are a pivotal step in uncovering and working out these mechanisms, because they allow for the controlled manipulation of individual parameters. Such studies can thus disentangle interdependencies among the multiple facets of family life, and identify the action of mechanisms that are masked under field conditions. Accordingly, such studies could reveal aspects of family life that are beneficial for parents [detrimental for offspring], but typically masked by the costs [benefits] of parental care under natural conditions. Moreover, such studies could investigate the role of additional forms of sibling cooperation (such as mutual grooming or coordinated begging) and their integration into the complex web of family interactions. Finally, careful experimental manipulation could reveal key environmental parameters and their effects on the costs and benefits of family interactions, and thus ultimately hint at their influence on the evolution of family life.

2. **Determining the relative importance of these mechanisms in field studies.** Despite their many advantages, laboratory studies by necessity fall short of reproducing all potentially relevant aspects of a natural environment. However, the relevance of a given mechanism for the evolution of family life can only be assessed conclusively under the natural conditions that shaped the evolution of this mechanism. While laboratory studies are suitable to work out the action of individual mechanisms, field studies are needed to determine the relative importance of these mechanisms in their natural context. Such studies could reveal the occurrence and fitness effects of mechanisms such as sibling cooperation and parent-offspring competition under natural conditions. Moreover, they could help to put these mechanisms into a greater context. For instance, parental care and sibling cooperation both depend on foraging opportunities, predation pressure, the prospects of future reproduction, and a variety of additional, tightly interwoven factors that cannot be simulated together under artificial laboratory conditions.

3. **Identifying universal mechanisms in comparative studies**. Laboratory and field studies on single species can work out the action of individual mechanisms, and determine their influence on the evolution of family life in that species under natural conditions. However, both approaches can only hint at the universal relevance of these mechanisms. By contrast, comparative studies involving broad phylogenetic comparisons can pin down the environmental conditions, ecological factors, and behavioral mechanisms that favor (or hinder) the evolution of family life across species. Comparative studies are hence crucial to identify general mechanisms that drive the evolutionary origin and subsequent consolidation of family life. They could, for instance, uncover the role of sibling cooperation for the early evolution of family life by assessing its frequency and its links to specific environmental conditions or forms of parental care across facultatively-caring species. Notably, such studies would be informed by laboratory and field studies that identified sibling cooperation as potentially relevant mechanism in the first place. A comprehensive research project on family-evolution should accordingly harness the complementarity of all three approaches. I should like to conclude by pointing out that the order Dermaptera would be a prime candidate for this endeavor: (pre-hatching) parental care seems to be virtually universal among the 1700 to 1800 earwig species, and a number of them seem to feature facultative family life (Costa 2006). The Dermaptera thus offer rich opportunities to study the transition from solitary to social life.

# AUTHOR CONTRIBUTIONS

Chapter 1. Jos Kramer, Julia Thesing, and Joël Meunier (2015) Negative association between parental care and sibling cooperation in earwigs: a new perspective on the evolution of family life? *Journal of Evolutionary Biology. 28, 1299-1308.*

JK and JM designed the experiment; JK and JT collected the data; JK and JM analyzed the data; JK wrote the first draft of the manuscript; JK, JT, and JM revised it until completion and responded to the reviewer comments.

Chapter 2. Jos Kramer and Joël Meunier (2016) Maternal condition determines offspring behavior toward family members in the European earwig. *Behavioral Ecology. 27, 494-500.*

JK and JM designed the experiment; JK collected and analyzed the data; JK wrote the first draft of the manuscript; JK and JM revised it until completion and responded to the reviewer comments.

Chapter 3. Charlotte Weiß, Jos Kramer, Kai Holländer, and Joël Meunier (2014) Influences of relatedness, food deprivation, and sex on adult behaviors in the group-living insect *Forficula auricularia*. *Ethology. 120, 923-932.*

CW, JK, KH, and JM designed the experiment; CW, JK, and JK collected the data; JM analyzed the data and wrote the first draft of the manuscript; all authors revised it until completion and responded to the reviewer comments.

Chapter 4. Jos Kramer, Maximilian Körner, Janina MC Diehl, Christine Scheiner, Aytül Yüksel-Dadak, Teresa Christl, Philip Kohlmeier, and Joël Meunier (in preparation) When earwig mothers do not care to share: parent-offspring competition and the evolution of family life.

JK and JM designed the experiment; JK, MK, JMCD, CS,AYD,TC, and PK collected the data; JK analyzed the data and wrote the first draft of the manuscript; all authors revised it until completion.

Chapter 5. Julia Thesing, Jos Kramer, Lisa K Koch, and Joël Meunier (2015) Short-term benefits, but transgenerational costs of maternal loss in an insect with facultative maternal care. *Proceedings of the Royal Society B: Biological Sciences. 282, 20151617.*

JT, JK, LKK, and JM designed the experiment; JT, JK, and LKK collected the data; JM analyzed the data. JK and JM wrote the first draft of the manuscript; all authors revised it until completion and responded to the reviewer comments.

Chapter 6. Tom Ratz, Jos Kramer, Michel Veuille, and Joël Meunier (2016) The population determines whether and how life-history traits vary between reproductive events in an insect with maternal care. *Oecologia. 182, 443-452.*

TR, JK, MV, and JM designed the experiment. MV collected the earwigs in the field. TR and JK conducted the experiment. TR and JM analyzed the data. JM wrote the first draft of the manuscript; all authors revised it until completion and responded to the reviewer comments.

Chapter 7. Jos Kramer and Joël Meunier (2016) Kin and multilevel selection in social evolution: a never-ending controversy? *F1000 Research, 5 (F1000 Faculty Rev): 776.*

JK and JM conceived the review; JK performed the literature search and wrote the first draft of the manuscipt; JK and JM revised it until completion and responded to the reviewer comments.

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# CURRICULUM VITAE

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