MATERNAL BEHAVIOUR AND THE EVOLUTION OF CHEMICAL SIGNALLING BY OFFSPRING IN THE EUROPEAN EARWIG (FORFICULA AURICULARIA)

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Some words…

An individual that could exploit another and does not do so is an altruist, from Trivers 1971, cited by Zahavi 2007.

Quand, dans un Etat, vous ne percevez le bruit d'aucun conflit, vous pouvez être sûr que la liberté n'y est plus. Citations de Edouard Herriot

La tendresse du père est presque toujours en conflit avec les intérêts du chef. Mémoires d'Hadrien (1951) Citations de Marguerite Yourcenar

Les molécules sont d'une plasticité étonnante chez l'homme, alors que pour changer une loi, un regard social, il faut des livres et des livres, des conflits et des siècles. De la parole comme d'une molécule (1995) Citations de Boris Cyrulnik

Il n'y a de communication sans conflit. Flore Mas 2009

SUMMARY

Parental care provided to current offspring such as food and/or protection increases offspring development and survival which contributes to the parent's fitness. However parental investment by means of time and energy may also reduce future chance to reproduce and therefore entails parent's lifetime reproductive success. We expect parents to adjust their parental investment equally among their different offspring within and between broods in order to maximise their fitness. From the point of view of one current offspring, parental care should be rather positively biased towards itself compared to current or future siblings. Thus the different genetic interest over the duration and amount of parental investment is expected to lead to parent-offspring conflict. Resolution of this conflict may be achieved by the evolution of an offspring solicitation signal that regulates parental care as predicted by various theories and models. Although several empirical studies have supported the presence of offspring solicitation signals, mostly in birds, the origin and driving forces for the evolved signalling function have not been clearly demonstrated.

The European earwig, *Forficula auricularia*, displays facultative maternal care, i.e. offspring can survive in absence of a caring mother but have significantly higher survival when attended by a mother. Thus one can test the on-going selection for an offspring cue to evolve a function of solicitation signal, which is predicted to be condition-dependent and regulating maternal care. Chemical cues are the main means of communication in insects (i.e. pheromones), which also regulate reproductive physiology (i.e. hormones). Therefore I decided for my thesis to explore the possible evolution of chemical signalling by offspring in the context of maternal care in the European earwig.

In a first experiment, I manipulated the nutritional condition (low-food, LF, versus high-food, HF) of earwig first instar nymphs and extracted their cuticular hydrocarbons (CHCs). Caring mothers were presented to these different extracts or a solvent control (C) and their effects on maternal foraging as well as food provisioning to their brood were measured. By gas-chromatography coupled with mass-spectrum analysis, I found that nymphs of different nutritional state produce similar total amount of CHCs but they differ in the relative abundance of specific chemical compounds, particularly long-chain CHCs. Mothers exposed to offspring condition-dependent CHCs adjusted their maternal care behaviours. They foraged and later provisioned significantly more food to their brood when exposed to extract from HF. This first result demonstrated that CHCs of offspring are used as solicitation signals and that mothers may select for an offspring chemical signal of quality as opposed to a signal of need.

In a second experiment, I investigated the effects of offspring condition-dependent chemical signals on the maintenance of maternal care among broods and the distribution of maternal food within broods. Mothers were isolated from their 1st instar brood for 3 days and continuously exposed to chemical signals extracted from broods of experimentally manipulated nutritional state (HF, LF). After re-introducing mothers to their brood, a range of maternal behaviours were quantified. I found that earwig mothers groomed their offspring significantly more after exposure to chemical extract from HF brood in comparison with mothers exposed to extract from LF brood, which in turn displayed significantly more aggressive behaviour. Furthermore, I manipulated offspring individual nutritional condition

within the brood to evaluate the effect of offspring state on the within-brood food distribution. Within broods, poorly fed individuals received significantly more food than well-fed individuals. These contrasting results of offspring condition-dependent signals observed at the brood and individual levels suggest various selective pressures, such as scramble competition within brood and maternal selection among broods, shaping offspring solicitation signals.

Finally, to test whether offspring chemical signals can *per se* manipulate the lifetime reproductive success of mothers, I measured long term consequences of exposure to offspring chemical signals on mothers' residual fecundity. The probability to have a second clutch by females was not affected by offspring chemical signals. However, the predictability for females to lay a second clutch within a certain interval was significantly affected by the condition-dependent chemical signals produced by offspring. The date of laying a second clutch was highly related to date of first clutch laying/hatching and strongly predictable when females were exposed to extract from HF offspring. The importance of timing of the second clutch may be critical for seasonal species like *F. auricularia* in order to ensure offspring survival. This last result confirms the potential for offspring chemical signals to manipulate maternal future fecundity, yet mothers may actively select for this offspring signal of quality in their best interest in order to optimally adjust their investment between current and future broods.

In conclusion, I showed that earwig first instar nymphs produce CHCs that vary in their relative abundances depending on offspring nutritional state. Earwig mothers adjust their maternal care behaviours (food provisioning, grooming vs. aggressiveness) according to these condition-dependent CHCs. Higher food provisioning and more grooming by mothers exposed to HF brood extract suggest maternal selection for offspring chemical cues of quality. Finally, the potential of offspring chemical cues *per se* to influence future maternal fecundity confirms their evolving function as solicitation pheromone in the context of maternal care.

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ACKNOWLEDGMENTS

This thesis is first dedicated to my devoted Grand-mother "Alice" who cared all her life for her kin in which she invested all her energy and time even without solicitation from them. Maternal care in humans has extended beyond offspring nutritional independence, to provide them with the best social environment to successfully span their life.

This is also true for my Mother who always was attentive to my education and whom, as a biologist, was particularly interested in my progress in my scientific career.

To my Dad, who transmitted me his courage and willingness to achieve goals in life.

A special thanks to my advisor Mathias, who has been a really good supervisor, always encouraging me to develop my own ideas but also keeping me focused on the main line throughout. His strong theoretical background in family dynamics contributed to the success of my research and to its publication in high impact peer-reviewed journals. I hope that my discoveries on the chemical communication of the European earwig will contribute to unravel other mechanisms of family life of this great 'other social insect'.

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Sincerely, Flore.

GENERAL INTRODUCTION

Parental care has evolved independently several times in a wide diversity of species all across the animal kingdom, but the degree of parental investment varies greatly from egg care only to extended post-hatching parental care of new-born young (Clutton-Brock, 1991). Environmental conditions such as harsh environment, high levels of predation or competition or unpredictable resources are prime movers for the evolution of parental care (Wilson, 1975). Parental care consists of three main behaviours: protecting the egg or young, protecting vital resources or facilitating offspring feeding. In uniparental care, one parent cares: In most terrestrial arthropods and reptiles, it is the female while in fishes, it is generally the males(Gross, 2005; Gross & Sargent, 1985; Gross & Shine, 1981; Tallamy, 1984; Trumbo, 1996; Baylis, 1978;). In biparental care, both parents simultaneously care for young, as seen in most bird species (Clutton-Brock, 1991; Cockburn 2006). Anisogamy of propagule size or the rate of reproduction, along with mode of fertilization, are among the main causes of sex-biased parental care (Trivers, 1972; Maynard Smith, 1977). Internal fertilization often constrained the female in the role to care, while in most group uniparental male care is associated with external fertilization, but there are exceptions to these rules (e.g. in fish Gross & Sargent, 1985). Finally, egg-retention such as ovoviviparity and viviparity present in almost all mammals except the monotremes is an effective way to increase offspring survival but considerably constrains the number and size of eggs and is expected to have evolved from internal fertilization under harsh environments (Clutton-Brock, 1991).

Parental care is any form of behaviour that increases an offspring survival and thus parents' inclusive fitness (Clutton-Brock, 1991). Life-history theory predicts a trade-off in resources allocation between present and future brood for iteroparous species (Roff, 1992; Stearns, 1992). We expect natural selection to select for parental strategies that maximize an individual lifetime reproductive success (i.e. fitness) and optimize parental investment between current and future brood (Williams, 1966; Gross, 2005). But when parents stay in contact with their offspring after hatching, there is opportunity for offspring to influence parental investment. Trivers (1974), based on Hamilton's inclusive fitness principle, presented a verbal model demonstrating that offspring and parents should disagree on the amount of parental investment, that is, the amount and duration of parental care, leading to parent-offspring conflict (POC). Parents are normally equally related to all their offspring and thus are expected to allocate their parental investment equally among all offspring (between or within brood) to maximize their fitness. In contrast, offspring are only partially related to their siblings and to their parents, therefore, we expect natural selection to select for offspring that maximize their own share of parental investment. Thus, the optima of parental investment differ from the points of view of the parents and the offspring. Trivers (1974) suggested that offspring may evolve "psychological weapons" to influence parental investment. The tight bond between parents and offspring observed today, especially in vertebrates, may represent evolutionary stable strategies of parental care where parent-offspring conflict has been resolved. Several game theory models were suggested for the resolution of parent-offspring conflict. Parker and McNair (1979) suggested that scramble competition among sibs may have driven selection for an offspring begging signal to which parents respond passively. While Godfray (1991; 1995) proposed the 'honest signalling hypothesis' suggesting that parents have directly selected for an offspring trait that advertises their cryptic need so that parents can optimally adjust their parental investment according to the level of offspring signaling. Although both types of model proposed different routes of selection driving the resolution of POC, they both predict the evolution of an offspring begging/solicitation signal, which is condition-dependent and costly to produce and evolved because of its effect on parental investment (see review by Godfray & Johnstone, 2000; Parker et al., 2002; Royle et al., 2002).

The presence of offspring begging signals have been empirically thoroughly demonstrated in several species of birds (Kilner & Johnstone, 1997; Wright & Leonard, 2002) as well as mammals including humans (Hudson & Trillmich, 2008) but less explored in social insects, where other mechanisms of communication than vocal, visual or behavioural signals may be at play and yet unexplored. Despite the amount of literature on offspring begging signals, the evolution of such signals is still poorly understood (Royle et al., 2004; Kölliker et al., 2005). Most of the unresolved challenges lie on elucidating the origin of begging signals, the driving forces for their evolution (i.e, scramble competition, parental selection) and how ultimately they influence family dynamics (Parker et al., 2002). The difficulty to directly and exclusively manipulate offspring begging signals without influencing offspring inherent condition or their behaviour has hampered our understanding of the origin and evolution of solicitation signals (Royle et al., 2004). Current begging signals and parental care behaviours observed today in nature are the results from long term coevolution of parent-offspring interactions and possibly coadaptation of the two traits in some species (Kölliker et al., 2005; Smiseth et al., 2008). Thus, species for which interactions between parents and offspring are already tightly tuned and well-coordinated (i.e. parents automatically respond to obvious offspring begging signal) may not offer the conditions to experimentally test selection on offspring cues to evolve solicitation signals. The need to study new species with more ancestral characteristics such as facultative parental care where offspring can survive without care but do better in presence of parental food provisioning, for instance, may allow to directly and empirically test predictions of the different models about the driving forces leading to the evolution of a begging signals as the outcome of POC resolution (Smiseth et al., 2003).

One such system are earwigs (Dermaptera) and insect order where at least one parent stay and have contact with their hatching offspring. The experimental work of this PhD-thesis was carried out in the European earwig *Forficula auricularia* (Dermaptera: Forficulidae) that displays maternal care to first instar nymphs. The biology and life-cycle of the common earwig has been early described by Fulton (1924), Lamb (1976; 1975) and Vancassel (1980; 1984). Briefly, adult females and males mate during summer and in autumn females dig a nest into the soil where they lay a clutch from 40 to 60 eggs (25 % and 75 % quartile from pers. data). Females generally mate with several mates (i.e. multiple paternity), which could have later some consequences for sib-competition and kin selection within the brood (Guillet, 2000; Dobler & Kölliker, 2010). Females overwinter with their clutch which they keep clean of parasites and fungal infections by regularly cleaning them with their mouthparts possibly applying some anti-fungal secretions (Costa, 2006). During this time, females stay inside the nest and live on their fat reserves. Then when temperature increases and daylight extends in early spring, hatching of eggs is triggered and is generally completed over 48 hours (pers. obs). Mothers generally stay in the nest with their nymphs for the first two days and start external nocturnal foraging on the third day. They bring back ingested food to the nest and feed their nymphs individually by mouth-to-mouth regurgitation until the brood disperses, usually after nymphs molting to second instar (Lamb, 1976; Lamb, 1976). In absence of a mother, earwig nymphs are fairly independent, can already self-forage and may as well join another nest with a mother (Lamb, 1976; Kölliker & Vancassel, 2007). Populations of *F. auricularia* living in temperate zones are iteroparous and can have up to two clutches of eggs in a reproductive season (Vancassel, 1984; Vancassel & Quris, 1994). The maintenance of nymphs with the females was experimentally shown to delay and to reduce the production of a second clutch (Vancassel & Foraste, 1980). Therefore the life-history and biology of the common earwig suggest the presence of a potential genetic conflict of interest between mothers and offspring, at least for the first clutch, over the amount and duration of maternal care as formulated by Trivers (1974) and predicted by the POC theory. The empirical work by Kölliker (2007) on the costs and benefits of the family life in the common earwig demonstrated the differential survival of offspring when mothers were either absent or present (offspring had a significant higher survival percentage till 4th instar when a mother was present) and thus confirmed the potential conflict of interest over maternal investment. The presence of such mother-offspring conflict opens the door for investigation on the evolution of an offspring solicitation signal regulating maternal care behaviour in the common earwig.

In the present work, I explored the evolution of a communication system between mothers and nymphs of *Forficula auricularia* as outcome of mother-offspring conflict over maternal investment. To address this evolutionary question, I structured my research as followed:

Chapter 1: I reviewed the different forms of maternal care and offspring begging behaviours so far identified in social insects and suggested the hypothesis that chemical cues in offspring may have been co-opted in the context of maternal care to serve as condition-dependent offspring cue selected for the evolution of an offspring solicitation signal. I discussed how chemical signals can not only have short term effect on behaviours (i.e releaser) but also affect the physiology of the receiver and result in long term consequences (i.e. primer). I proposed a hypothetical mechanism of maternal care regulation through the co-evolution of maternal internal chemical cues (i.e., hormones) and offspring external chemical cues (i.e., pheromones).

Chapter 2: My first experiment consisted in demonstrating the existence of offspring chemical cues that are condition-dependent and to which mothers respond in *F. auricularia*, according to predictions of resolution models. I manipulated offspring nutritional state and extracted their cuticular hydrocarbons (CHCs) to which mothers were then exposed. I measured the behavioural effects of offspring chemical extracts on the amount of maternal food foraged and later provisioned to the brood. Using techniques of Gas-Chromatography coupled with Mass-Spectrometry, I analyzed, identified and quantified the chemical compounds extracted from nymphs to suggest chemical candidates for the offspring solicitation pheromone.

Chapter 3: In a second experiment, I tested the long term effect of offspring signals on the maintenance of maternal care behaviours. After separation from their offspring, females were maintained exposed to chemical extracts from broods of different nutritional state over three days. Females and their original brood were then regrouped and mothers' behaviours were observed for one hour. I also specifically investigated food distribution among nymphs of different nutritional state within the brood in order to find whether sib-competition is taking place and how it may affect maternal investment and the evolution of solicitation signals.

Chapter 4: Finally in the last experiment, I tested whether or not offspring chemical signals could ultimately influence the residual fecundity of females. Again mothers separated from their first brood were maintained daily exposed to chemical extracts from broods of different nutritional state or a solvent control during nine days. I measured the long term consequences of offspring chemical signals on the production of a second clutch. Timing and amount characteristics to second clutch/brood were compared among the three exposure treatments. This is the first time that an experiment measures the effect of an offspring solicitation signal on mother's residual fecundity and thus demonstrates the critical assumption of POC theory about the potential for offspring solicitation signal to manipulate parents' future reproduction.

To conclude, I summarized my empirical results and discussed the adaptive values of an offspring chemical signal and maternal investment observed in *F. auricularia.* By analyzing the divergent conflict of interest within a family and their fitness consequences, I discussed how control by either the mother or the offspring could explain the different patterns of care observed in Dermaptera. Finally, I mentioned how interactions among individuals within a family group can lead toward more cooperative behaviours along with complex regulatory systems of communication.

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Notes on some words

- In all my text, I used the term "signal" following the definition from Maynard-Smith & Harper (2003, p.3), which define it as: "any act or structure which alters the behaviour of other organisms, which evolved because of that effect, and which is effective because the receiver's response has also evolved".

- I used indistinguishably begging and solicitation

- "clutch" refers to the group of eggs and "brood" to the group of nymphs.

- *Forficula auricularia* is called commonly either the European earwig or the common earwig.

Chapter 1

MATERNAL CARE AND OFFSPRING BEGGING IN SOCIAL INSECTS: CHEMICAL SIGNALLING, HORMONAL REGULATION AND EVOLUTION

Flore Mas & Mathias Kölliker

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Abstract

Post-hatching maternal care such as food provisioning and protection has evolved several times in insects, allowing offspring (larvae, nymphs) to interact with their mothers and potentially influence their investment. The evolutionary conflict over the duration and amount of parental care is thought to promote the evolution of offspring begging behaviours either as honest signals of need or as competitive signals with the potential to manipulate parents into investing more. In most social insects, parental care is not obligatory and may represent a less derived state than in vertebrate systems making them more appropriate to test ancestral conditions for the evolution of begging signals. Here, we review forms of maternal care in insects ranging from protection to food provisioning and evidences of offspring begging behaviours influencing maternal care, including condition-dependent chemical cues produced by offspring that may turn out to be solicitation pheromones. Since behavioural parent-offspring interactions are embedded in the reproductive and developmental physiology of mother and offspring, we stress the need for behavioural studies to be complemented by physiological measurements which will allow us to understand better the nature of conflict resolution. We propose a hypothetical mechanism of maternal care regulation by direct internal chemical signals (i.e., hormones) and indirect external chemical signals (i.e., solicitation pheromones) influencing maternal reproductive physiology and future reproduction. Social insect species and the integrated study of behavioural interactions and physiological/reproductive consequences may represent promising new experimental systems for direct tests of the evolution of begging signals, complementing current research on parent-offspring conflict.

Keywords: begging behaviours; chemical cues; juvenile hormone; maternal care; parent-offspring conflict; pheromone; signal; social insects.

Introduction

In insects, post-hatching parental care has evolved independently in at least 10 orders (Zeh & Smith 1985). The most common form of post-hatching parental care is the protection of young against predators, but active parental food provisioning to offspring also occurs in a number of species (Tallamy & Wood 1986). The benefit of post-hatching care for offspring survival has been experimentally shown, for instance, in the burrower bug, *Sehirus cinctus* (Heteroptera: Cydnidae; Kight 1997), burying beetles, *Nicrophorus spp.* (Coleoptera; Eggert et al. 1998; Smiseth et al. 2003) and in the European earwig, *Forficula auricularia* (Dermaptera; Kölliker 2007). For parental care to evolve and be maintained, however, this benefit has to outweigh the cost of care because by providing care parents forego the opportunity to produce additional offspring (Trivers 1974; Clutton-Brock 1991). Consistent evidence for such a cost of care has been experimentally demonstrated in several insect species where females caring for offspring delay and/or reduce their future reproductive output relative to non-caring females (Tallamy & Denno 1982; Vancassel 1984; Hunt & Simmons 2004; Agrawal et al. 2005; Kölliker 2007).

When parents care after hatching, offspring have the potential to interact behaviourally with them and influence the duration and amount of costly care. This potential for reciprocal parent-offspring interactions provides opportunity for parent-offspring conflict to arise and drive the evolution of offspring begging signals. This parent-offspring conflict over parental investment arises because each offspring is twice as related to itself as it is to a sibling, whereas parents are equally related to each of their offspring. It generates selection favouring traits that allow offspring to increase their share of the parental resources. Trivers (1974) postulated the evolution of behavioural means by which offspring can manipulate parents into providing more than they should. A large number of explicit evolutionary game-theoretical models subsequently confirmed that the conflict can indeed lead to the evolution of conspicuous and costly offspring begging (Parker & Macnair 1979; Godfray 1991, 1995; reviewed in Parker et al. 2002; Royle et al. 2004). In these models, two major scenarios for conflict resolution have been considered: scramble models assumed that sibling rivalry drives the evolution of competitive begging to which parents respond passively (Parker & Macnair 1979) and honest-signalling models assumed that parents actively select for offspring begging signals that 'honestly' reflect their true need for parental resources (Godfray 1991, 1995).

Considerable empirical research has been done to test predictions of these models and distinguishing between the two scenarios experimentally has turned out to be a major challenge since both models qualitatively predict the same outcomes (reviewed in Kilner & Johnstone 1997; Royle et al. 2004; Smiseth et al. 2008). The strong experimental focus on bird systems has resulted in a taxonomic bias in our understanding of the evolution of begging signals (Smiseth et al. 2003). Altricial young birds are completely dependent on parents to get food, and they produce highly specialized and conspicuous multi-component begging displays (Kilner & Johnstone 1997; Budden & Wright 2001). Thus, albeit excellent for studying conflict resolution, birds may represent an evolutionary state that is too derived to test parent-offspring conflict theory fully, in particular with regard to the ancestral conditions under which begging originated (Smiseth et al. 2003). A difficulty of such evolutionarily derived systems is that the scope for experimentally testing the fitness consequences of variation in offspring begging and parental provisioning is limited; in addition, the measured fitness consequences reflect the dependence of offspring development on parental provisioning. Social insects as experimental systems may here be critical complements to current research in parent-offspring conflict.

In most social insects, post-hatching parental care is provided by females (Tallamy 1984) and is not prerequisite for offspring to survive. They may therefore represent a less derived state of parent-offspring interactions. For instance, in earwigs and burying beetles, offspring can either self-forage or obtain food from parents (Vancassel 1984; Smiseth & Moore 2004). Thus, social insects are potentially powerful experimental systems for better understanding the ancestral conditions for the evolution of begging signals from a non-signalling state and their influence in the regulation of parental care (Smiseth et al. 2003). Except for eusocial insects where offspring are sealed in cells and are completely dependent on worker care, the other social insects have been relatively little studied (Costa 2006) and mechanisms of provisioning and begging are still not sufficiently well documented. Nevertheless, there is increasing indirect and direct evidence for offspring influencing maternal care, and begging behaviours have now been described in a number of species, for instance in burying beetles (Rauter & Moore 1999).

Chemical signalling is the most important mode of communication between individuals in insects and it has been widely studied in several orders, mainly in the contexts of sexual selection, social recognition and regulation (Wyatt 2003). However, the study of chemical communication in the context of parent-offspring interactions in social insects has remained fairly unexplored and could reveal important new mechanisms of maternal care regulation. For instance, recent experiments have shown that condition-dependent offspring chemical cues influence caregiver food provisioning in burrower bugs, *S. cinctus* (Kölliker et al. 2005b, 2006) and bumblebees, *Bombus terrestris* (den Boer & Duchateau 2006). Also, honeybee, *Apis mellifera*, larvae produce a brood pheromone that influences care behaviours of workers, suggesting a possible role of the brood pheromone as a begging signal. The brood pheromone not only influences worker behaviour but also has lasting consequences for their physiology (Le Conte et al. 1995, 2001).

Maternal care in other social insects has been mainly studied in the context of female reproductive physiology and the hormones maintaining maternal care behaviours (reviewed in Nijhout 1994; Trumbo

2002). Specific hormones in females, notably juvenile hormone (JH), regulate the trade-off between care for current offspring and the production of new eggs/clutches (Vancassel et al. 1984; Rankin et al. 1995a; Scott et al. 2001; Scott & Panaitof 2004). Since the evolutionary resolution of parent-offspring conflict implies, on a behavioural level, coevolution of offspring begging signals and maternal responses, and, on a physiological level, coevolution of maternal reproductive physiology and offspring developmental physiology, considerations of how behaviours and physiology interact to generate the observed patterns of parente offspring interactions may be particularly revealing to understand better the evolutionary resolution of parent-offspring conflict (see also Wright & Leonard 2002; Lessells 2008).

Our aims in this review on maternal care and offspring begging in social insects are to (1) provide a comprehensive summary of mother-offspring interactions, (2) review the evidence for offspring begging behaviours that influence the level of maternal care, and (3) discuss the evolutionary implications of offspring signals, and in particular chemical signals, in the behavioural regulation and physiological maintenance of maternal care in social insects. Our review highlights the need for further research on mechanisms of maternal care and offspring begging in social insects, and especially on offspring chemical signals and female reproductive physiology.

Mother-offspring interactions

Care by females (i.e. maternal care) is the most frequent form of parental care in insects (Tallamy & Brown 1999). Thus we focus here on insect mother-offspring interactions, although our arguments would be valid for father-offspring interactions in species with paternal or biparental care. Table 1 provides an overview of different forms of post-hatching maternal care behaviours observed in various insect orders and which we classified into two main categories: protection (which can be behavioural, chemical or physical) and food provisioning to offspring (specifying whether the resource is provided to the brood as a whole or allocated to individual offspring). We also list the evidence for offspring influences on these maternal behaviours, including offspring begging signals. For comprehensive reviews of parental care in insects, see Zeh & Smith (1985) and Tallamy & Wood (1986).

As mentioned before, the most frequent form of post-hatching maternal care in social insects is protection against predators (Tallamy & Wood 1986). For instance, females of shield bugs *Elasmucha spp.* (Hemiptera) stay in the vicinity of their offspring and, when threatened, sit on top of their offspring and fan their wings to deter predators (Kaitala & Mappes 1997). The benefit of maternal protection to offspring has been tested in several species by removing tending mothers and measuring offspring survival. For instance, when thornbug treehopper, *Umbonia crassicornis*, mothers were experimentally removed, wasp attacks were successful in 94% of cases, compared to an attack success rate of only 23% when offspring were attended by mothers (Cocroft 2002). Other social insects lay their eggs in a safe place such as a nest, a burrow, a gallery, a tunnel or a ball. When the eggs hatch, the females (sometimes with a contribution of their male mate) stay to defend the entrance of the burrow by behavioural displays and, also, with defensive secretions released from specialized glands as, for example, in some beetles (Table 1). Keeping offspring in a protected area may have contributed to the evolution of food provisioning (Trumbo 1996) owing to the associated reduction in the cost of provisioning (i.e. in order to provision, parents have to leave their offspring temporarily unprotected which is less risky if they are in a burrow). Depending on the predator species, predators may also pose a direct threat to the tending female, making offspring protection a costly investment. Hence, according to parent-offspring conflict theory, offspring protection may be considered a form of parental investment where offspring may be selected to influence and maintain the protective tendency of the mother beyond her own optimum (Cocroft 2002). Offspring begging signals have rarely been studied in the context of protection against predation, and conflict resolution models may need to be adjusted to incorporate this functional aspect of care.

The other form of maternal care, food provisioning, is observed in fewer insect species but represents the typical form of care considered in conflict resolution models and has been mainly addressed in bird studies. By providing food to their offspring, mothers may suffer not only from higher risks of predation while foraging but also increased energy expenditure. In addition, food provisioning may decrease the female's own energy intake because, by sharing food with her offspring, she might accumulate fewer resources required for future reproduction. The benefit of enhancing offspring development and survival must have exerted strong selection favouring the evolution of mechanisms that ensure adequate food provisioning to offspring. In some species, females progressively provision food to the entire brood (Field 2005). For instance, the mother brings food back to the brood, as in burrower bugs (Kight 1997), or leads offspring to the food source and facilitates access to the food by pre-processing it as in stink bugs and treehoppers (reviewed in Tallamy & Wood 1986). In these cases, the benefit of maternal food provisioning is simultaneously shared among all offspring (Lazarus & Inglis 1986). Individual offspring of a given brood may compete among each other for access to the provided food, but this competition is not directed at the mother and cannot lead to competitive begging signals. Nevertheless, begging signals may also evolve because of between brood competition, selecting for cooperative begging signals that allow offspring of the same brood collectively to influence the rate of maternal food provisioning to the brood (Johnstone 2004).

Table 1. Forms of posthatching maternal care and offspring cues and behaviours in social insects (blank cells represent unstudied areas)

Insect order	Species	Posthatching maternal care		Offspring cues and behaviours	Source
		Protection*	Food provisioning†		
Blatodea	German cockroach, *Blatella germanica*	B	Brood	Aggregation pheromone	Brossut 1983; Nalepa & Bell 1997
	Wood roach, *Cryptocercus punctulatus*	P	Individual		Nalepa 1984, 1990
	Pacific beetle cockroach, *Diploptera punctata*	P, C	Individual	Alarm pheromone	Roth & Stay 1958
Coleoptera	Bessbug, *Odontotaenius disjunctus*	P	Brood	Stridulation	Schuster & Schuster 1997
	Dung beetle, *Oniticellus cinctus*	P	Brood	Chemical cues deposited on the ball	Halffter 1997
	Cephalodesmius spp.	P	Brood		Monteith & Storey 1981
	Coprini, *Copris* spp.	P	Brood		Klemperer 1982, 1983a, b
	Burying beetle, *Nicrophorus vespilloides*	B	Individual	Swaying behaviour and tactile stimuli	Fetherston et al. 1990; Eggert & Müller 1992, 1997; Rauter & Moore 1999; Smiseth & Moore 2002; Trumbo 2007
	Bark beetle, *Monarthrum* spp.	P	Brood	Aggregation pheromone	Kirkendall et al. 1997
	Engraver beetle, *Ips pini*	C			Reid & Roitberg 1994
	Ambrosia beetle, *Xyleborus* spp.	P	Brood		Kirkendall et al. 1997
	Desert beetle, *Parastizopus armaticeps*	P	Brood		Rasa 1998, 1999
Dermaptera	Common European earwig, *Forficula auricularia*	P, B, C	Individual	Antennal contact	Lamb 1976b; Vancassel & Foraste 1980; Vancassel 1984; Eisner et al. 2000
	Doru taeniatum	P, B, C	Individual		Rankin et al. 1996; Eisner et al. 2000
	Hump earwig, *Anechura bipunctata*	P, B, C	Individual		Vancassel & Foraste 1980; Vancassel 1984
	Anechura harmandi	P, B, C	Individual		Kohno 1997
	Striped earwig, *Labidura riparia*	P, B, C	Individual		Vancassel 1977; Caussanel et al. 1986; Rankin et al. 1995b
	Ring-legged earwig, *Eurobellia annulipes*	P, B, C	Individual		Klostermmeyer 1942; Rankin et al. 1996
Embioptera	Web spinner, *Antipaluria urichi*	P	Brood		Edgerly 1997
	Embia ramburi	P	Brood		Ledoux 1958
Hemiptera	Burrower bug, *Sehirus cinctus*	P, B, C	Brood	Solicitation pheromone (?)	Sites & McPherson 1982; Kight 1997; Kölliker et al. 2005b 2006
	Sehirus bicolor	P, B, C	Brood		Costa 2006
	Grey or parent bug, *Elasmucha* spp.	B	Brood	Alarm pheromone	Melber et al. 1980; Kudô 1990, 2000
	Stink bug, *Antiteuchus triperus*	B	Brood	Defence pheromone	Borges & Aldrich 1992
	Southern green stink-bug, *Nezara viridula*	B	Brood	Aggregation pheromone	Lockwood & Story 1985, 1986, 1987
	Treehopper, *Membracidaes* spp.	B	Brood	Alarm pheromone, secrete honey dew	Nault et al. 1974
	Publilia reticulata	B	Brood		Bristow 1983
	Thornbug treehopper, *Umbonia crassicornis*	B	Brood	Substrate-borne vibrational signals, alarm pheromone	Nault et al. 1974; Wood 1976; Cocroft 1996, 1999, 2001, 2002;
	Lace bug, *Gargaphia solani*	B	Brood	Defence & alarm pheromone	Tallamy & Denno 1981; Tallamy 1985; Parr et al. 2002;
Orthoptera	Ground weta, *Hemiandrus* spp.	P, B	Brood		Gwynne 2004
	Short-tailed cricket, *Anurogryllus muticus*	P	Brood		West & Alexander 1963

*Protection: P=physical (nest, burrow, gallery tunnel, brood ball, etc.); B=behavioural (i.e. defence display, guarding); C=chemical (defensive pheromone, aggregation pheromone, alarm pheromone).

†Food provisioning directed to the brood (i.e. bring food back to the nest, facilitation of feeding by guiding offspring to food source, processing the food) or to an individual (regurgitation through mouth-to-mouth contact, body secretions).

In other species, females directly allocate food to individual offspring, mostly through direct regurgitation by mouth-to-mouth contact. In these species, an individual offspring can directly influence the likelihood of obtaining food from the mother by begging and competing with siblings. For instance, earwig females have been observed in nature to make several foraging trips between the food sources and their burrow to provision food to their offspring (Lamb 1976a, b). Direct mouth-to-mouth transfer of food (i.e. regurgitation) between mother and offspring as a mechanism of maternal food provisioning was suspected for a long time (Fulton 1924; Lamb 1976a) and recently experimentally confirmed in the European earwig (Staerkle & Kölliker, in press). In burying beetles, one of the rare cases of biparental care (Fetherston et al. 1990; Eggert & Müller 1997), larvae can either feed on the preprocessed carcass or obtain food directly from either the female or the male by regurgitation (Smiseth & Moore 2002).

All these forms represent different mechanisms of maternal care which can have differential implications for the resolution of parent-offspring conflict (Johnstone 2004; Smiseth et al. 2008). Comparisons among them are valuable because they may shed light on how the dynamics of mother-offspring interactions evolved and influenced the evolution of begging signals. The diversity of these mechanisms observed among social insects may provide a promising arena to explore the relation between mechanisms of parent-offspring interactions and the evolution of begging signals also from a phylogenetic perspective (Smiseth et al. 2008). Yet, behavioural mother-offspring interactions have not been broadly studied in social insects and correspondingly few begging behaviours have been documented (Table 1). Clearly, more experimental research is needed to understand insect mother-offspring interactions better. Finally, behavioural interactions are embedded in the physiological state of the interacting individuals (West- Eberhard 2003). For instance, maternal food provisioning is expected only when females are in a physiological state that maintains their tendency to care as opposed to a physiological state that triggers egg production for the subsequent clutch (Vancassel 1984; Rankin et al. 1995c, 1997; Trumbo 1997; Scott et al. 2001). Thus, the evolution of parent-offspring interactions also requires the evolution of the relevant underlying reproductive and developmental physiology. In insects, JH is well known to play a role in the physiological maintenance of maternal care tendencies (see above) as well as vitellogenin synthesis and ovarian development (Nijhout 1994), and to be involved in the regulation of juvenile development (i.e. by repressing moult). A recent study also showed that JH may affect begging signals in burying beetles, albeit indirectly through an influence on larval growth (Crook et al. 2008). Thus, the critical role of JH in the reproductive and developmental physiology of most social insects makes it a good candidate for a proximate factor regulating mother-offspring interactions in social insects, although the exact physiological details of JH action still remain to be elucidated.

Exceptionally well-studied systems in the context of insect caregiver-offspring interactions, both on a behavioural and a physiological level, are eusocial insects (the honeybee in particular). In most eusocial insects, larvae are sealed in brood cells and are completely dependent on nurses for food, as are bird chicks in their nest. Whether a larva becomes a reproductive queen or a worker is usually determined by the quantity and quality of food it receives from the nurses (Wilson 1971; Wheeler 1986). Therefore, if larvae are able to influence their food provisioning, they may, in part, be able to determine their own development to become a worker or a queen (Bourke & Ratnieks 1999; Kaptein et al. 2005; den Boer & Duchateau 2006). Although workers and larvae are usually more genetically related (owing to the haplodiploid reproductive system) than parents and offspring, conflict between larvae and worker over food provisioning akin to parent-offspring conflict may also have driven the evolution of worker food-provisioning mechanisms and larval begging signals.

Offspring begging Behaviours

Begging for protection

In several hemipteran species, nymphs have specialized dorsoabdominal glands to store chemical cues which are released by nymphs when in danger. These chemical cues elicit a defensive response from the mother and are defined as alarm pheromones (Roth & Stay 1958; Nault et al. 1974; Tallamy & Denno 1981; Kudô 1990, 2000). In the subsocial thornbug treehopper, offspring produce a substrate-borne vibrational signal in addition to chemical signals, both of which trigger maternal defensive behaviours (Cocroft 1996, 1999, 2001). In the case of the vibrational signal, the activity of several offspring is required to trigger the defensive response from the mother (Cocroft 1996, 1999). Protection is a shared maternal investment so not only the signalling individual but also all the other siblings located in the vicinity benefit from the maternal protection. Therefore, competition between siblings to signal more than the others is not expected to evolve as it will benefit everyone. Instead, begging for protection is expected to be an honest signal of risk of predation. However, Cocroft (2002) showed that individuals close to the mother have a better chance of survival than individuals at the edge. Thus, differential survival may promote selection in offspring for manipulative signals potentially allowing individual nymphs to monopolize more protective positions close to the mother, but this hypothesis remains to be tested. The high cost of predation must have exerted a strong selection for efficient communication that can be expressed in a flexible way, highly conditional on predator, shaping the evolution of chemical signals (e.g. offspring alarm pheromones).

Begging for food

In contrast to the need for protection, the need for food is the typical proximate context in which parent-offspring conflict has been studied. The typical experimental design usually consists of a manipulation of offspring nutritional state by food deprivation or satiation, and the measurement of the level of offspring begging (reviewed in Kilner & Johnstone 1997; Budden & Wright 2001; Wright & Leonard 2002; Royle et al. 2002). The currently best studied insect system where offspring begging behaviour reflecting hunger has been explored in some detail is the burying beetle. Larvae of the genus Nicrophorus raise their head and wave their legs in the direction of the parents and, when in contact, tactile stimuli from their legs on the parent's mouthparts trigger food regurgitation (Rauter & Moore 1999; Smiseth & Moore 2002). Smiseth & Moore (2004) counted the times larvae were seen raising their head while waving their legs or touching the parent under different food treatments and they showed that food-deprived larvae spend more time raising their head than control larvae, clearly showing condition-dependent expression of this behaviour as would be required for a begging signal.

In addition to begging behaviours, a different sensory modality for the evolution of insect parent-offspring interactions has been proposed, and evidence supporting this hypothesis reported, in the burrower bug, *S. cinctus*. Kölliker et al. (2005b, 2006) experimentally varied the nutritional conditions of nymphs and later measured food provisioning of mothers when exposed to crude cuticular extracts (Kölliker et al. 2005b) and volatile chemical cues (Kölliker et al. 2006) emitted by these nymphs. Females exposed to chemical cues (both extracts and volatiles) obtained from nymphs in poor nutritional conditions provided significantly more food than females exposed to chemical cues obtained from nymphs that had access to *ad-libitum* food. Chemical analysis of the nymphal volatiles showed a significant quantitative difference in the blend of monoterpenes emitted by nymphs raised on the two food treatments. Thus, as predicted for the postulated solicitation pheromone (i.e. a chemical begging signal; Kölliker et al. 2006), offspring chemical cues were condition dependent and females responded correspondingly by adjusting their food provisioning. This example supports the hypothesis that social insect offspring can produce chemical cues that elicit maternal provisioning, providing information on offspring nutritional condition. Given the chemosensory predisposition of insects in general, parent-offspring conflict may have often led to the evolution of condition-dependent chemical begging signals in offspring. Solicitation pheromones may therefore be generally expected among the social insects (see also below for eusocial insects). However, it is still unclear whether cuticular chemicals are passive cues directly transferred from food processing to the cuticle or active signals that have evolved for their signalling function and which offspring can strategically adjust to influence maternal food provisioning (Kölliker et al. 2006). As expected for burrower bugs where females provide food to the whole brood, these results show that the overall signal produced by the brood can influence the rate of maternal food provisioning. Yet in other species with direct food provisioning to individual offspring (e.g. through regurgitation), individual chemical begging signals may be expected as well. More studies exploring offspring's chemical signals to caring parents are needed.

Among eusocial insects, several larval begging signals have been documented. For instance, in Vespidae wasps, larvae use acoustic signals by scraping their mandibles on the wall of their cells, and the scraping intensity seems to be related to larval hunger level (Ishay & Landau 1972; Ishay & Schwartz 1973; reviewed in Matsuura & Yamane 1984). Hölldobler et al. (1978) observed that in ants, such as *Formica sanguinea* and *Solenopsis invicta*, larvae appear to solicit food from workers by swaying their head and mandibles, similar to the begging display of burying beetle larvae. Kaptein et al. (2005) experimentally showed that the intensity of this swaying behaviour in larvae of the ponerine ant *Gnamptogenys striatula* is related to nutritional condition. Hungry larvae sway significantly more than well-fed larvae, confirming its function as a hunger signal. In honey bees, Huang & Otis (1991) experimentally deprived larvae for 2e6 h and measured the amount of food provisioned by nurse bees. They found that deprived larvae receive more food than well-fed larvae, suggesting a correlation between level of hunger and allocation of food through some unknown larval cue or behaviour. Finally, there is also evidence for chemical signalling between larvae and workers in bee colonies, although this evidence has, to our knowledge, not been interpreted functionally as chemical begging signals. Le Conte et al. (1990, 1995) showed that extract of cuticular chemicals from bee larvae is correlated with larval age, and that it induces different quantity and quality of food provisioning by the nurses. This blend of chemical compounds (i.e. a blend of 10 aliphatic esters, Le Conte et al. 1995) on the larva's cuticle was accordingly termed the 'brood pheromone'. After experimental bioassay with different synthetic chemicals similar to the ones present in the blend, one specific compound, the methyl linoleate, applied on the cell of larvae increased the amount of royal jelly deposited by workers (Le Conte et al. 2001). The brood pheromone in general and this specific chemical compound in particular, thus represents a prime candidate for a solicitation pheromone. Further research on the functional significance of the brood pheromone, including experimental tests of condition-dependent emission (i.e. whether compound quantity depends on larval hunger), is required, however. A recent experimental study in bumblebees showed that larval cuticular chemical cues are in fact condition dependent and thus support the hypothesis of chemical hunger signals produced by larvae that influence food provisioning of workers (den Boer & Duchateau 2006).

Evolution of a chemical begging signal

Chemical cues represent particularly interesting potential begging signals in the study of the resolution of parent-offspring conflict in social insects for several reasons: (1) they represent signals of the evolutionarily most ancient sensory modality (Wyatt 2003); (2) they are involved in communication for several insect species in a broad range of functional contexts (Vander Meer et al. 1998); and (3) pheromones (external signals) and hormones (internal signals) may often use similar or related molecular pathways (Tillman et al. 1999; Bellés et al. 2005) facilitating both their evolution and maybe our capacity to understand the details of their expression. Because the expression of both maternal behaviour and offspring begging is likely to be regulated by both physiological mechanisms with an underlying genetic basis and environmental influences, the investigation of chemically mediated mother-offspring interactions may turn out to be particularly fruitful. Thus, in the following discussion, we focus on chemical signals, but vocal or other signals may have analogous implications.

From a cue to a signal

The possibility of a begging signal evolving from a pre-existing condition-dependent non-signalling cue raises several important questions with respect to the definition of signals and the evolutionary process resolving parent-offspring conflict. Maynard Smith & Harper (2003, page 3) defined a signal as 'any act or structure that alters the behaviour of other organisms, which evolved owing to that effect, and which is effective because the receiver's response has also evolved'. Hence a signal implies a coevolutionary history between the signaller's behaviour and the receiver's response (as in 'honest-signalling' models). Experimental data showing an effect of offspring chemical cues on mother's food provisioning are now available, but there is as yet no evidence that the offspring condition-dependent chemical cues evolved for the function of soliciting food (Kölliker et al. 2006). Exposure of mothers to the compounds identified and confirmation that both the offspring cues and the female response are confined to the functional context of caring would be required to demonstrate that the cue evolved for the specific effects. Alternatively, the begging signal may have evolved for the purpose of influencing maternal provisioning, but the mother's response did not evolve (i.e. as in scramble models), and may rather represent a sensory bias (Ryan 1990; Endler & Basolo 1998). In this case, the offspring signal may have manipulative potential. The following examples stress the need for careful consideration of these alternative scenarios in parent-offspring interactions. Experiments in earwigs and burying beetles showed that continuous replacement of older offspring by younger offspring maintains care by mothers and delays future reproduction, suggesting an effect of age-dependent offspring stimuli on the mother's reproductive state and future reproduction (Vancassel et al. 1987; Scott & Panaitof 2004). Females may thereby adaptively respond to offspring age-specific stimuli by maintaining their tendency to care (i.e. maternal control scenario; Godfray 1991). Alternatively, these results could also be interpreted as evidence that offspring produce a cue that has at least the potential to manipulate the mother's reproductive physiology (i.e. offspring control; Parker & Macnair 1979). Some chemical cues have been shown to have a primer effect on the physiology of the receiver (see below) which, owing to their lasting effects, may manipulate the receiver's behaviour against its own interests (Wyatt 2003). A good example is the previously mentioned brood pheromone of honeybee larvae which acts as a releaser pheromone with transient effects on worker foraging and food provisioning (Le Conte et al. 1990, 1995; Pankiw 2007), but also as a primer pheromone with a lasting effect on worker physiology, delaying their behavioural and physiological, transition from nurses to foragers (Le Conte et al. 2001). Le Conte et al. (2001) also showed that the brood pheromone may in part influence worker JH titre, which contributes to this behavioural transition in eusocial hymenoptera (Robinson & Vargo 1997).

Hypothetical mechanism of maternal care regulation

Regulation in analogy to the chemical control of worker behaviour in honeybees, we propose the hypothesis that offspring of social insects produce a 'solicitation pheromone' that may act as a releaser pheromone inducing mothers to provide food as first suggested by Kölliker et al. (2005b). Since JH mediates the amount and duration of maternal care, any influence by the offspring on the mother's JH titre through a solicitation pheromone raises questions about the resolution of parent-offspring conflict in insects in terms of the interplay of behavioural interactions and physiological mechanisms (Fig. 1). If offspring can directly influence the mother's JH titre, they may gain substantial control over maternal care, potentially influencing her trade-off between current and future reproduction. Therefore, experimental research on begging signals should not only look at transient behavioural responses by parents, but also monitor physiological changes with potential lasting consequences for maternal reproduction. One problem in studying insect hormones for now remains the lack of a powerful and reliable methodology to measure and quantify JH (Schooley et al. 2005). Despite the increasing evidence for condition-dependent offspring cues to which insect mothers respond, the causal compounds remain to be chemically identified and experimentally tested in social insects (Kölliker et al. 2006). The finding of such chemical signals will allow direct manipulation of offspring begging by exposing mothers continuously to the offspring signal to test the critical and previously untested prediction that offspring, by producing a begging signal, can affect maternal reproductive physiology and reproductive output. Owing at least partly to the difficulty of experimentally manipulating behavioural begging displays, current evidence of the effects of begging on parents is fully based on short-term behavioural responses with transient effects on maternal physiology and reproduction (Scott & Panaitof 2004). Thus, important predictions from parent-offspring conflict theory remain to be tested, and social insects and solicitation pheromones may provide a promising system once more research has been done in this area.

The evolution of an honest versus manipulative signal is at the core of parent-offspring conflict theory (Trivers 1974; Godfray 1995; Kilner & Johnstone 1997; Royle et al. 2004). One may argue that a begging signal with a priming effect on maternal physiology would be mostly in the interests of the offspring by maintaining parental investment in the present brood at the expense of future broods for the parents. Meanwhile, the evolutionary interests of mothers may be best served if the effect of offspring signals has transient (i.e. releaser-type) effects on their behaviour, allowing the mother to respond flexibly to variation in offspring conditions. Therefore, the integrative approach of studying begging signals and female reproductive physiology deserves further research which could provide a direct test of the power of offspring begging to control maternal care. Physiological studies focusing particularly on the endocrine regulation of maternal care and offspring begging in social insects (Scott & Panaitof 2004; Crook et al. 2008) as well as in birds (Groothuis & Ros 2005; Quillfeldt et al. 2006) already provide promising results. Here, and beyond the current focus of deposition of maternal hormones in the eggs affecting offspring development (e.g. Müller et al. 2007), we suggest the physiological approach should be expanded to incorporate indirect effects of offspring solicitation on maternal physiology.

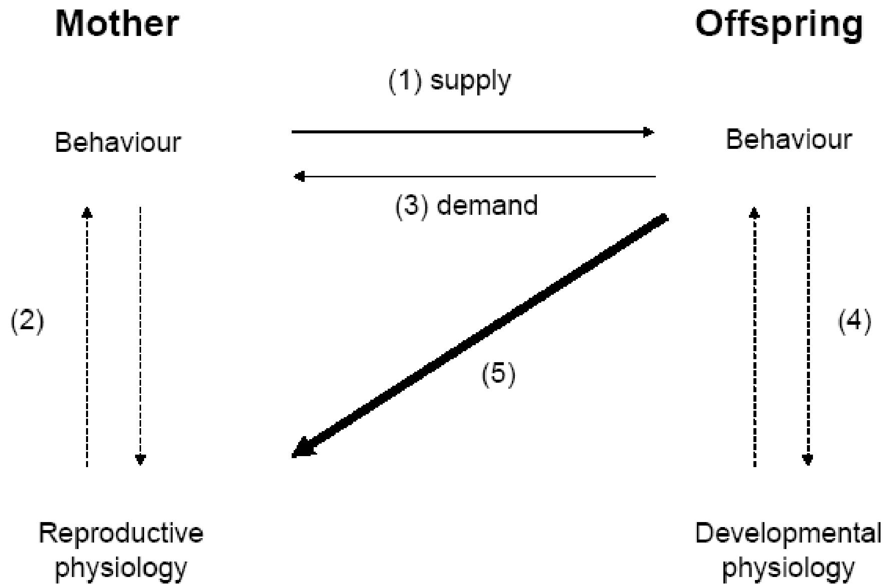

Figure 1: Schematic illustration of the regulation of maternal care in social insects. Mothers provide care to offspring (resources supply - arrow 1) that is influenced by mother's reproductive physiology (in part regulated by the juvenile hormone) (arrows 2). Similarly, the effect of offspring begging signals (resources demand) on maternal provisioning (arrow 3) is modulated by offspring developmental physiology (varying with age, nutritional state, juvenile hormone) (arrows 4). Arrow 5 describes a hypothetical direct priming effect of offspring signal on mother's physiology.

Conclusion

Social insect species show a wide range of forms of post-hatching parental care, usually provided by females, including protection and/or food provisioning directed to the whole brood and/or to individual offspring. Thus, they offer the potential to study the differential effects of parent-offspring interactions on the evolution of begging signals and the maintenance of maternal care. Offspring stimuli that signal need for protection have been described in several insect species (offspring alarm pheromones being the most common ones), but hardly studied from the perspective of parent-offspring conflict. Conversely, begging behaviours for food are less well known in social insects and only the tactile begging display of burying beetle larvae has been well investigated experimentally (Smiseth & Moore 2004). Yet, the difficulty of directly manipulating behavioural displays has prevented experimental tests of certain predictions of parent-offspring conflict theory with regard to lasting influences of offspring begging on maternal reproductive physiology and reproductive output. An in depth study of chemical mother-offspring interactions and the identification of solicitation pheromones in insects (Kölliker et al. 2005b, 2006; den Boer & Duchateau 2006) might be a promising way to find systems where these predictions of parent-offspring conflict theory could be tested. The usually rather short and discrete generations of these insects thereby facilitate the quantification of variation in lifetime reproductive success.

Furthermore the tendencies of both mothers to care and offspring to beg seem to be at least partly regulated by JH (Crook et al. 2008). Such a common hormonal mechanism of offspring and parental trait is consistent with predictions of evolutionary models of supply-demand coadaptation that predict a common inherited basis to (i.e. a genetic correlation between) parental provisioning and offspring begging (Wolf & Brodie 1998; Agrawal et al. 2001; Lock et al. 2004; Kölliker et al. 2005a). The focus on chemical communication in the context of mother-offspring interaction may offer a new perspective of research to test proximate mechanisms of maternal care regulation and the evolutionary function of offspring begging signals with regard to scramble and honest signalling models. Further research in chemical signalling and hormonal regulation of offspring begging and maternal provisioning is now needed to substantiate the currently scarce, but promising, data on parental care and offspring begging in social insects.

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Chapter 2

A CHEMICAL SIGNAL OF OFFSPRING QUALITY AFFECTS MATERNAL CARE IN A SOCIAL INSECT

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Abstract

Begging signals of offspring are condition-dependent cues that are usually predicted to display information about the short-term need (i.e. hunger) to which parents respond by allocating more food. However, recent models and experiments have revealed that parents, depending on the species and context, may respond to signals of quality (i.e. offspring reproductive value) rather than need. Despite the critical importance of this distinction for life history and conflict resolution theory, there is still limited knowledge of alternative functions of offspring signals. In this study, we investigated the communication between offspring and caring females of the common earwig, *Forficula auricularia*, hypothesizing that offspring chemical cues display information about nutritional condition to which females respond in terms of maternal food provisioning. Consistent with the prediction for a signal of quality we found that mothers exposed to chemical cues from well-fed nymphs foraged significantly more and allocated food to more nymphs compared with females exposed to solvent (control) or chemical cues from poorly fed nymphs. Chemical analysis revealed significant differences in the relative quantities of specific cuticular hydrocarbon compounds between treatments. To our knowledge, this study demonstrates for the first time that an offspring chemical signal reflects nutritional quality and influences maternal care.

Keywords: begging signal; chemical communication; parent–offspring conflict; signal of quality; cuticular hydrocarbons; *Forficula auricularia*

Introduction

Food provisioning is usually an essential form of post-hatching parental care enhancing offspring development and survival (see Clutton-Brock 1991). The amount and duration of parental investment is influenced by a conflict between parents and offspring (Trivers 1974), and the evolution of offspring begging signals is predicted based on an evolutionary resolution of this conflict (Parker et al. 2002b). Begging signals are supposed to reflect a cryptic short-term need (i.e., hunger) of offspring that parents use to adjust their food allocation (Godfray 1991). Despite a large number of experimental studies confirming the general function of offspring begging as a signal of short-term need (Kilner & Johnstone 1997; Budden & Wright 2001), recent studies also demonstrate that begging signals vary also with the social environment (brood size), physiological condition (age and size) and past experience (learning) of offspring (for e.g. Price et al. 1996; Lotem 1998; Cotton et al. 1999; Parker et al. 2002a; Grodzinski et al. 2008). Furthermore, the focus on vocal and postural signals of bird chicks that were clearly shown to signal a short-term need may have resulted in an underestimation of other forms of cues signalling an offspring quality and influencing parental care. Godfray (1991, 1995) modelled 'need' as the marginal fitness gain for the parent from investment of extra resources in individual offspring. It is usually assumed that this definition of need is equivalent to variation in short-term nutritional condition, because one unit of food would have a higher value for a hungry than for a satiated offspring. But the theoretical definition of need can also be interpreted as the amount of food required to reach independency, hence reflecting long-term physiological state (Price et al. 1996). Offspring in better physiological condition (quality) represent higher reproductive value for parents because the expected investment required to successfully raise offspring to independence is lower (Haig 1990; Davis et al. 1999; Jeon 2008). Experimental studies trying to disentangle short-term and long-term needs and their effects on begging signals have often manipulated offspring hunger and the composition of broods in terms of offspring age (for e.g. Lotem 1998; Cotton et al. 1999; Smiseth & Moore 2007). Because age is often confounded with competitive ability, it is not clear from these studies if parental food allocation is the result of parental choices or scramble competition (Royle et al. 2002). Nevertheless, a few recent studies in birds have revealed the presence of offspring cues that signal quality such as beak coloration (Saino et al. 2000), plumage coloration (Lyon et al. 1994) or UV reflectance (Jourdie et al. 2004; Bize et al. 2006; Tanner & Richner 2008).

Some social insect species display facultative parental care and offspring are not completely dependent on parental food (see Mas & Kölliker 2008). Such species provide a unique opportunity to study behaviours or cues that may reflect more ancestral conditions for the function of evolved begging signals (Smiseth et al. 2003; Smiseth & Moore 2004). In the present study, we tested the currently still insufficiently explored hypothesis that social insect offspring produce condition-dependent chemical cues that carers use to allocate their investment (Kölliker et al. 2005; den Boer & Duchateau 2006; Kölliker et al. 2006; Mas & Kölliker 2008) using the common earwig, Forficula auricularia (Dermaptera) as our experimental system. Female earwigs regurgitate food individually to nymphs (Staerkle & Kölliker 2008) and nymphs can survive without maternal care, albeit at a lower rate (Kölliker 2007; Kölliker & Vancassel 2007). Under the hypothesis of a signal of need, earwig females were predicted to provide more food after exposure to chemical cues of food-deprived nymphs. Conversely, under the hypothesis of a signal of quality, they were predicted to provide more food after exposure to chemical cues of well-fed nymphs. No a priori prediction could be made with regard to the effect of nymph condition on the chemical cue profiles because these were unknown and explored here for the first time.

Material & Methods

Earwig husbandry

We collected adult common earwigs from a natural population located in Gommiswald, Switzerland (see Kölliker 2007) in early summer (June–July) 2007.We set-up groups of approximately 30 males and 30 females for mating in glass aquaria (20 x 30 x 20 cm) with humid sand as substrate and ad libitum food consisting of either vegetables and fruits (carrots and apples), or flower pollen (Swiss

Extract, Bonadoz, CH) and bird food (Beo, Vitakraft) provided twice a week in alternating order. The aquaria were kept in a climate chamber at a constant temperature of 20 : 15°C and 50 per cent humidity with controlled photoperiod of 14 : 10 hours (day : night). In early August, 100 females were individually set-up in small Petri-dishes (10 x 2 cm) with humid sand and offered a piece of a half-cut plastic tube as shelter and kept under a new photoperiod of 8 : 16 hours to accelerate egg-laying. When egg-laying was observed, females were transferred with their clutch at 5°C and 50 per cent humidity in a dark chamber to terminate diapause of the eggs.

Experimental design

When eggs hatched, between 9 and 12 weeks later, females with their brood were transferred in new Petri-dishes (10 x 2 cm) with humid sand and a shelter to a 16 : 8 hours and 20 : 15°C photoperiod-temperature regime for the experiment. Females and their brood were randomly assigned to be used as either biosource or bioassay broods.

Biosource broods were used for chemical extraction of the chemical cues of nymphs, whereas bioassay broods were used for the behavioural tests. Biosource broods were standardized to 25 nymphs and set-up without a mother. In six cases out of 33, nymphs of different broods were combined to generate a biosource brood in order to maximize the use of hatching clutches per day. Whether extracts came from a single or mixed origin broods had no significant influence on the results (all $p>0.28$). All biosource broods received *ad libitum* food (pollen pellets; Kölliker 2007) from days 1 to 3 and were assigned randomly to two food treatments: the low-food (LF) treatment broods did not get any food from day 4 to 5, the high-food (HF) treatment broods were provided with food during this time. Thus, 2 days of food deprivation differentiated the LF and HF biosource broods. On the morning of day 6, LF and HF biosource broods were frozen at -20°.

After thawing, 20 nymphs randomly selected from each biosource brood were immersed together in 1 ml of solvent (n-heptane, Rotisolv 99 per cent pure; Carl-Roth AG, Reinach, Switzerland) for 10 min. From this total cuticular extraction, three times 300 ml were transferred in three cleaned glass vials (2 ml, Sigma-Aldrich, Buchs, Switzerland). Two samples of 300 ml (each one equivalent to 6 nymphs) were concentrated down to 100 ml under a light nitrogen stream for their later use during the behavioural experiments. The third sample was stored at K80° for later chemical analysis using a gas-chromatograph coupled to a mass-spectrometer (GC/MS). We used 100 ml of pure solvent (n-heptane) for controls (C). To avoid pseudo-replication, each extract was used only once in the behavioural assays.

Bioassay broods were set-up with a standardized 20 nymphs per brood and their mother. They were provided with food daily from day 1 to 4. To standardize the nutritional condition of the bioassay nymphs, the food was removed on day 5, 24 hours before the behavioural test.

Exposure & behavioural experiments

On day 6, females from bioassay broods were removed from their brood and set-up in an exposure chamber. Exposure chambers consisted of a small Petri-dish (3.5 x 1 cm) painted in black with openings cut on the side that could be opened or closed. This exposure chamber was placed as an artificial nest burrow inside a medium-sized Petri-dish (10 x 2 cm) with humid sand. To reduce the effect of handling and habituate the females to the experimental procedure, females were transferred to the exposure chamber and back to their brood on the previous day 2 and 4. Prior to the start of the experiment, one extract (100 ml of either LF, HF or solvent) was applied to a filter paper disc (diameter 55 cm). After complete evaporation of the solvent, the impregnated filter paper was inserted in the exposure chamber. At the start of the experiment, bioassay females were enclosed for 30 min in the exposure chamber to expose them to the experimental treatment (volatile and non-volatile chemical cues of the extracts). The origin of the extracts (LF, HF or C) was selected from a randomization list and not known by the experimenter during the test. After 30 min of exposure, the exposure chamber was opened and the females were allowed to forage on pollen pellets coloured with blue food-dye (synthetic E-131; patent blue V; Werner Schweizer AG, Switzerland) used as colour marker of female provisioning (Staerkle & Kölliker 2008).

After one hour of access to the food, females were returned to their brood and allowed to interact with their nymphs for an hour. This procedure (30 min exposure C1 hour foraging) was repeated a second time for each female using a second sample (100 ml) from the same total extract used in the first exposure and a new blue-dyed food pellet. This second session simulated a second foraging trip between the nest and the food source. This procedure differed from previous studies (Kölliker et al. 2005; den Boer & Duchateau 2006; Kölliker et al. 2006) in that it experimentally separated female exposure from her provisioning. Thus, it ensured that only mothers, and not the provisioned nymphs, were exposed to extracts, preventing a possible confounding exposure effect on nymph behaviour. After the second exposure session, females were returned to their brood, and they were left to interact overnight. On the subsequent morning (approx. 15 hours after the second exposure), we scored the total number of nymphs and the number of nymphs with green gut content (mix of blue dye and the yellow pollen). The pollen pellets females could feed on were weighed on a Mettler Toledo AT261 balance before and after each of the two experimental sessions. To standardize the food weight measurements, the food pellets were dried overnight at 70° after use in the experiments and cleaned of sand grains (repeatability of measurements: 99.9%; $F_{9,49}=7e5$, $p<0.0001$). The difference of weight of the same pellet before and after the exposure session provided us with a measurement of total food consumed by mothers after being exposed to extract of nymphs (Kölliker 2007). All behavioural experiments took place between 1500 and 1800 hours from 16 November 2007 to 12 February 2008 in a dark room at room temperature. Two red lights were used during short handling periods. Earwigs were not disturbed by the red lights and their nocturnal activity started as soon as the room was darkened (F. Mas 2008, personal observation).

Chemical analysis

The third sample from each total extraction of biosource broods was used for GC/MS analysis. We added 200 ng of an internal standard (n-octadecane, C18H34; Sigma-Aldrich, St Louis, MO) to each sample and concentrated down to 50 ml with a light stream of nitrogen. We injected 2 ml of each concentrated extract in a GC/MS (Hewlett-Packard 6890 coupled to a Hewlett-Packard 5793). The extract was carried through a DB-5 capillary column (30x0.25 mm ID and 0.25 mm film-thickness, J&W Scientific, Folsom, CA) with a helium flow rate of 1 ml minK1. The injector temperature was 250° and was operated in split/splitless mode. The temperature program started at 70°C for 2 min and reached a maximum of 320°C at a rate of 5°C minK1 where it was held for 5 min. Chromatograms were analysed using ChemStation software (Hewlett-Packard, Agilent Technologies). The identity of peaks was determined based on their retention time and mass-spectrum using the NIST 98 compound library as a reference, and guided by the preliminary identification of nymph cuticular compounds by (Liu 1991). Two peaks (C23.2 and 9-C23.1) were not well separated on the column, so we integrated both peaks together. We used the letters 'x' and 'y' to indicate different albeit unidentifiable positions of double-bonds or methyl-group. We estimated the absolute quantity of each compound by dividing the peak area by the area of the internal standard, and multiplying by 200 ng.

Statistical analysis

One bioassay family was discarded because of high offspring mortality in the brood (50% died). We carried out the statistical analysis using R v. 2.7.0 (R Development Core Team 2008) and SAS (SAS 1999). Sample sizes varied slightly between models due to technical problems for two measurements of food weight. Measures of maternal provisioning (food quantity foraged and proportion of nymphs fed) were analysed using generalized linear models with the extract treatment (LF=18, HF=15, C=17) entered as factor. The normality and homoscedascity of the residuals were verified for each model and planned contrasts were defined in the models.

For the statistical analysis of the chemical compounds, we used the absolute quantities calculated from the internal standard. Several compounds were not normally distributed, even after transformation, thus we conducted a Wilcoxon rank test to compare chemical quantities between the two food treatments (LF versus HF). To control for multiple testing, we provide the standardized effect size as well as p-values obtained with the false discovery rate correction (Nakagawa 2004). The chemical compounds were further analysed in a multivariate analysis of variance (MANOVA) with compound quantity as the dependent variable, the brood as the subject, peak identity as the within-subject factor, and treatment as between-subject factor. All p-values are two-tailed.

Results

Effect of exposure to nymphs' extracts on maternal care behaviours

As predicted if condition-dependent chemical cues affect maternal food provisioning, the exposure treatment had a significant effect on the total quantity of food eaten by mothers during the two sessions (figure 1a; one-way ANOVA, r 2Z0.15, $F_{2,45}$=3.98, p=0.025). Mothers exposed to HF-extracts consumed significantly more food than mothers exposed to LF-extracts (contrast 'HF–LF' t=2.21, p=0.032) or just the solvent (contrast 'HF-C' t=2.65, p=0.011).

The exposure treatment also significantly affected the proportion of nymphs with green guts (figure 1b; GLM with binomial error distribution, a logit link function and Williams correction for overdispersion: $F_{2,47}$=6.812, p=0.033), demonstrating an effect of the exposure treatment on how females allocated food among the nymphs. Mothers exposed to HF-extracts provided food to significantly more nymphs than mothers exposed to LF-extracts (contrast HF-LF z=2.552, p=0.011) but not compared to solvent (contrast HF-C z=1.581, p=0.11).

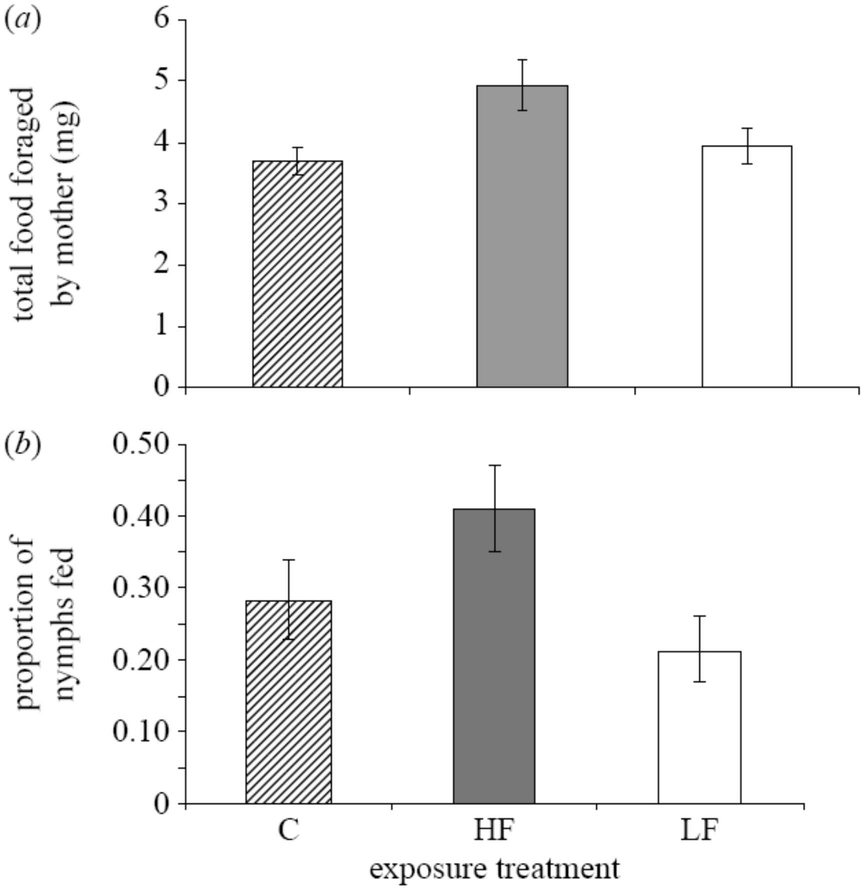

Figure 1. Effect of exposure treatment on maternal behaviours (C = solvent, HF = high-food nymph extract or LF = low-food nymph extract): (*a*) Mean total food foraged by mothers (in mg). (*b*) Mean proportions of nymphs fed by mothers. Error bars represent standard errors.

Effect of food treatment on offspring cuticular hydrocarbons

From the total cuticular extractions of the biosource broods, we identified 21 peaks that each represented at least 1 per cent of the total abundance found on the cuticle. The chemical compounds were all hydrocarbons ranging from 13 to 31 carbons. Nymphs from LF and HF treatment had a qualitatively similar chemical profile with hydrocarbons from the alkene class being the most abundant compounds independent from the treatment effect (74.4±0.9%) and alkanes as the second most prevalent class (19.3±0.9%) before methyl-branched hydrocarbons (6.2±0.45%; see figure 2a–c). There was no significant difference between treatments in the overall absolute quantities of all cuticular hydrocarbons (CHCs) but there was a significant interaction of treatment and peaks (repeated MANOVA, between treatment effect: $F_{1,33}=0.0358$, p=0.85, treatment*peak interaction: $F_{19,15}=2.53$, p=0.037). Three peaks in particular (two alkanes and one alkene) were strongly affected by the food treatment in terms of standardized effect size (r 2) and statistical significance (adjusted p-value): heptacosane (n-C27: $r^2=0.22$, W=238, p=0.003), nonacosene (x -C29.1: $r^2=0.30$, W=259, p=0.003) and hentriacontane (n-C31: $r^2=0.35$, W=267, p=0.001). Pentacosane (n-C25) also showed a notable effect size with a significant raw (but not adjusted) p-value ($r^2=0.12$, W=221, raw-p=0.024, adjusted-p=0.12). These four compounds were all found in higher amounts in HF extracts as compared to LF nymphs extracts, resulting in an overall larger proportions of alkanes among the CHCs profile of HF nymphs (% alkanes: HF=21.4±1.2, LF=17.2±1.2, W=217, p=0.035).

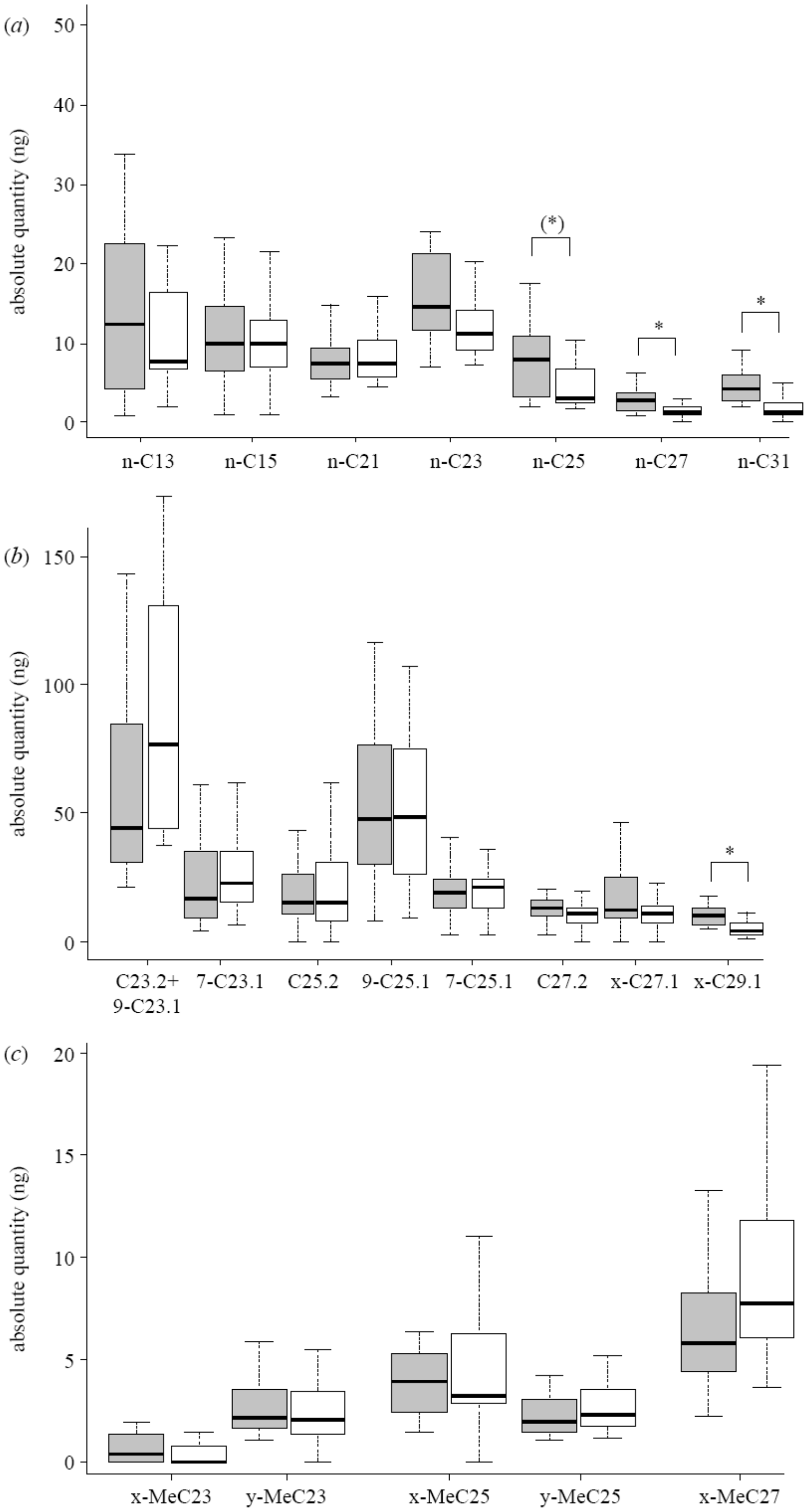

Figure 2. Box-plots of the absolute quantities (in ng) of hydrocarbons found on nymph's cuticle between the two food treatments: in grey HF, in white LF. The line represents the median of the sample, the box provides the upper and lower quartiles and the whiskers show 1.5 times the interquartile range. The scale of each graph was adjusted to the highest peak present in each class. (*a*) alkanes, (*b*) alkenes and (*c*) methyl-branched hydrocarbons. Asterisks show significant differences ($p < 0.05$) between HF and LF based on a Wilcoxon signed-rank test after adjustment for multiple testing. Asterisks in bracket indicate significant raw *p*-values but non-significant adjusted *p*-values.

Discussion

Our experiment confirmed the presence of chemical communication between earwig mothers and their offspring in the context of maternal care as previously hypothesized (Mas & Kölliker 2008). Our results demonstrate that offspring secrete a blend of hydrocarbons on their cuticle that vary with their nutritional condition and that mothers respond by adjusting their food provisioning. Mothers foraged for significantly more food and regurgitated to more nymphs after being exposed to chemical cues from HF nymphs as compared to LF nymphs. The positive maternal response to chemical cues from well-fed offspring supports the prediction for a signal of quality rather than a signal of need. While signals of quality have recently been described for visual cues in birds, this is to our knowledge the first demonstration of a chemical signal of quality expressed by offspring in a social insect.

The few studies that also explored chemical communication between carers and offspring (larvae or nymphs) in social insects have reported variable responses to condition-dependent offspring signals that, in contrast to our study, support the evolution of a signal of need. For instance in burrower bugs (*Sehirus cinctus*), Kölliker et al. (2005, 2006) showed that caring mothers increased their rate of food provisioning when exposed to volatiles from LF nymphs compared to HF nymphs or a solvent control. The major difference that may explain their contrasting results is that burrower bug mothers do not regurgitate food directly to an individual offspring but provide seeds to the entire brood (Sites & McPherson 1982). Thus, conflict between parent and offspring is expected to happen between different broods only and not among sibs of the same brood (Kölliker et al. 2005) which may shape differently the evolution of an offspring begging signal (Trivers 1974; Parker & Macnair 1978). In several species of eusocial insects, the foraging activity of the colony was also shown to be affected by chemical stimuli produced by the brood. For instance, in bumble-bees (*Bombus terrestris*), workers fed significantly more to larvae experimentally sprayed with extracts from food-deprived larvae compared to control larvae or larvae sprayed with extract from fed larvae (den Boer & Duchateau 2006). In honeybees (*Apis mellifera*), whole hexane extracts of the brood was also shown to affect the foraging activity of workers (Pankiw et al. 1998; Pankiw 2007). It was suggested that bee foragers used this brood pheromone to estimate the level of need of the brood (Pankiw et al. 1998; Pankiw 2007). However, Dreller & Tarpy (2000) demonstrated that volatile cues from hungry young brood were not sufficient to stimulate foraging, but their results suggested that maybe non-volatile chemical cues communicated by direct contact may play a role. Workers of eusocial insects are generally reproductively inactive so the cost of food provisioning is expected to be low compared with the social benefit of raising larvae that will become future workers. This could explain why eusocial insect workers respond to a signal of need rather than quality. Only in the context of larvae with the potential to become queens, we may expect selection from workers for an offspring signal of quality. These examples highlight the importance of the social organization and the ecology of the species in the evolution of an offspring begging signals and the resolution of carer offspring conflicts.

The chemical analyses in our study show that the CHC profiles of earwig nymphs carry information about their nutritional state. Higher relative amounts of alkanes characterized well-fed nymphs (HF) compared to poorly fed nymphs (LF). Hydrocarbons are the most commonly found compounds on insect cuticles and have been shown primarily to serve as protection against desiccation (Blomquist et al. 1987). CHCs vary with ecological and social environments and insects biosynthesize most of them, although some may be secreted directly from the diet on the cuticle (Blomquist & Jackson 1973; Tillman et al. 1999; Blomquist & Vogt 2003). Interestingly, the variation in alkane ratios has been shown to reflect the environmental conditions in which ant workers were living (Wagner et al. 2001) and used by workers to discriminate task-specific workers (Greene & Gordon 2003) or also between nest-mates of a same colony against intruders (Liang & Silverman 2000). These examples illustrate how condition-dependent CHCs, and particularly alkanes, have been co-opted and evolved a secondary signalling function in various social contexts. Our finding of condition-dependent offspring CHC profiles in earwig nymphs suggests that particular CHC compounds may have been selected and co-opted for a secondary signalling function as an offspring signal of quality to their caring mothers. In the future, manipulative studies with individual synthetic CHCs and blends of these compounds should be conducted to provide direct evidence and confirm this signalling function.

Finally, in species where there is a high juvenile mortality, we may expect parents to preferentially feed offspring of higher reproductive value in order to maximize their fitness return on parental investment (i.e. Haig 1990). Kölliker (2007) reported a high juvenile mortality between first instar nymphs and the adult stage in the common earwig. Maternal investment that is allocated to nymphs of low condition associated with low likelihood to survive appears maladaptive for mothers. Since maternal investment has been demonstrated to be costly in this species (Kölliker 2007), we expect selection on mothers to maximize their return on investment by favouring offspring of good quality. Therefore, if specific CHCs of nymphs correlate positively with their nutritional condition as previously discussed, mothers may preferentially feed nymphs displaying them and thus drive the selection for a chemical signal of quality.

In conclusion, our results support the hypothesis that chemical communication evolved in the context of maternal care to regulate food provisioning in the social species *F. auricularia* and that mothers may select for an offspring chemical signal of quality. The current evolutionary theory on parent–offspring conflict resolution has generally assumed that offspring begging signals advertise need but the recent discoveries of offspring signals of quality in different species with parental care suggest that the resolution of conflict may have led to a multitude of signals of different forms (visual, chemical and vocal) and different functions (need, quality) depending on the ecology and social context in which offspring–carer interactions evolved.

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Chapter 3

DIFFERENTIAL EFFECTS OF OFFSPRING CONDITION-DEPENDENT SIGNALS ON MATERNAL CARE REGULATION IN THE EUROPEAN EARWIG

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Abstract

Parent–offspring conflict theory predicts the evolution of offspring solicitation signals that can influence the amount and/or the duration of parental investment. Short-term effects of offspring solicitation signals on parental food provisioning have been widely demonstrated, but persistent effects of offspring signals on the maintenance of parental care have been rarely studied. Also, the relation between the amount of care provided to the brood and how it is distributed among individual offspring within a brood is not well enough understood. Here, we investigated in the European earwig (Forficula auricularia) the effects of offspring condition-dependent chemical signals on the maintenance of maternal care among broods and the distribution of maternal food within broods. Mothers were isolated from their brood for 3 days and continuously exposed to chemical signals extracted from broods of experimentally manipulated nutritional state. After re-introducing mothers to their brood, a range of maternal behaviours were quantified. We found that earwig mothers groomed their offspring significantly more after exposure to chemical extract from high-food brood in comparison with mothers exposed to extract from low-food brood, which in turn displayed significantly more aggressive behaviour. Furthermore, we manipulated offspring individual nutritional condition within the brood to evaluate the effect of offspring state on the within-brood food distribution. Within broods, poorly fed individuals received significantly more food than well-fed individuals, probably due to scramble competition. These results show that earwig nymphs express multi-component condition dependent signals and behaviours differentially affecting maternal care provisioned to the brood and the distribution of care within broods.

Keywords: parent-offspring conflict, scramble competition, parental care, chemical communication, begging, *Forficula auricularia*

Introduction

A substantial body of empirical research has demonstrated the role of offspring condition-dependent signals in regulating parental care (see for reviews, Kilner and Johnstone 1997; Royle et al. 2002; Wright and Leonard 2002). Most of these studies manipulated the short-term nutritional condition (hunger) of offspring and, hence, tested shortterm, potentially transient, effects of offspring signals on the amount of food provisioned by parents. Fewer studies, particularly on colouration and UV-reflectance in birds (Lyon et al. 1994; Price and Ydenberg 1995; Saino et al. 2000; Bize et al. 2006; Tanner and Richner 2008) and recently on a chemical signal in an insect (Mas et al. 2009), also showed that condition-dependent signals not always function as signals of short-term "need" but sometimes reflect a longer-term nutritional effect as an offspring signal of "quality" or reproductive value. Thus, offspring signals may contain a range of information influencing parental investment in terms of the amount and/or maintenance and duration of care. In some mammals, the use of olfactory cues produced by the offspring was shown to affect the maintenance of maternal care (reviewed in Lévy and Keller 2009). In an earlier paper, we proposed that in insects, offspring solicitation pheromone may evolve to influence maternal physiology and the maintenance of care in the offspring's best interest (Mas and Kölliker 2008). Few empirical studies in insects measured the effect of offspring on the maintenance or duration of care, and these focussed on offspring age (e.g. Caussanel 1970; Klemperer 1983; Kight 1997). But it has been rarely demonstrated that an offspring signal per se can influence the maintenance of parental care behaviours.

Although parental food provisioning seems at first controlled by parents, some models and empirical studies have shown that it can also be controlled by offspring which, through scramble competition, may exaggerate their signal to attract resources from their passively responding parents (Parker and Macnair 1979; Kacelnik et al. 1995; Cotton et al. 1999; Parker et al. 2002). Competition between interacting offspring in a brood may influence food distribution within the brood, but coordination of signalling among offspring may also act in a cooperative manner by increasing the overall amount of food provided to the brood by parents (Leonard and Horn 1998; Krebs 2001; Leonard and Horn 2001a; Mathevon and

Charrier 2004; Bell 2007; Horn and Leonard 2008). Understanding how potentially different offspring condition-dependent signals affect both the maintenance of care and the distribution of resources within the brood can thus provide insight into conflict resolution.

In this study, we tested if offspring condition-dependent signals and behaviours differentially affect the maintenance of maternal care and the distribution of food within broods in the European earwig, Forficula auricularia. This species displays characteristic maternal behaviours, such as brood defence, grooming (see Fulton 1924; Lamb 1976b) and food provisioning by trophallaxis (Staerkle and Kölliker 2008) until nymphs disperse. Recently, Mas et al. (2009) demonstrated an offspring chemical signal of quality based on cuticular hydrocarbons. Exposure of mothers to chemical cues extracted from well-fed nymphs lead to higher food provisioning to the brood than exposure to extracts from poorly fed nymphs or just solvent. Here, we tested if these recently discovered brood chemical signals of quality can also affect the longer-term maintenance of maternal care behaviours. Females were isolated for 3 days from their nymphs (i.e. mimicking dispersal of nymphs) and continuously exposed to chemical extracts of either high-food brood (HFB), low-food brood (LFB) or just the solvent (control C). If brood chemical signals of quality contribute to maintaining care in their mothers, we predicted that despite separation from their brood maternal behaviours should be maintained more when females were continuously exposed to chemical extracts from HFB. In addition, we manipulated individual nutritional condition of offspring within a same brood to test whether food distribution was random or whether it differed between offspring of different nutritional condition. In case of non-random food distribution, we were particularly interested in the direction of the effect of individual condition on the within-brood food distribution.

Material& Methods

Husbandry

The earwigs used in our experiments originated from a population located in Gommiswald Switzerland) and were caught in summer 2008. Husbandry conditions and general handling up to the hatching of clutches followed the protocols described in detail by Kölliker (2007) and Mas et al. (2009). For logistic reasons, that is, to spread out egg-laying and hatching, we split the lab population in two sub-populations that were set up either in July or October under a short-day photoperiod regime to trigger egg-laying (photoperiod/temperature: 8:16h / 20:15°C (day–night) and 50% relative humidity). At hatching, broods were randomly assigned to be used either for chemical extraction or to be kept with their mothers in family groups to be used in the behavioural tests. All hatched broods were transferred in new petri dishes (10×2 cm) with humid sand and a shelter and were kept for the entire experiment under a long-day photoperiod regime of (photoperiod/temperature 16:8h / 20:15°C and 50% relative humidity).

Broods for chemical extraction

The broods assigned for chemical extraction were standardised to 40 nymphs on day 1 after hatching by mixing two or three broods together and setting them up without a mother. On the first and second day, all broods received ad libitum food in the form of pellets of pollen (Kölliker 2007; Mas et al. 2009). On days 3 and 4, broods were randomly assigned to a food treatment, either broods continued to receive ad libitum food resulting in (HFB) or broods were not provided with food for the two successive days resulting in LFB. On day 5, LFB and

HFB were frozen at −20°C. The 40 nymphs from each biosource brood were together immersed in 800 µl of n-heptane (Rotisolv® 99% pure; Carl-Roth AG, Reinach, Switzerland) for 5 min. For later use for female exposure, 600 µl from these crude cuticular extracts were transferred in new glass vials (2 ml, Sigma-Aldrich, Buchs, Switzerland). To avoid contamination, all the used glassware was cleaned three times each with three different solvents (heptane, acetone, dichloromethane; Carl-Roth AG, Reinach, Switzerland).

Broods for behavioural assays

The broods assigned for behavioural assays were formed from only one family and broods were culled to 20 nymphs and kept with their mother. They were provided with food daily from day 1 till day 4 after hatching. On day 5, mothers and nymphs were separated from each other and females were allocated randomly to the chemical exposure treatments. See Fig. 1 for an illustration of the experimental design.

- Mothers exposure treatment: On day 5, mothers were set up in a new petri dish (10×2 cm) which included an exposure chamber. This exposure chamber consisted of a small petri dish (3.5×1 cm) with openings cut on the side that could be opened or closed by simply turning the lid of the petri causing minimal disturbance. The exposure chamber was lined with a disc of filter paper (3.5 cm diameter). From days 5 to 7, mothers were randomly assigned to an exposure treatment: they were either exposed to HFB extract, LFB extract or the solvent n-heptane (control C). Each mother was daily exposed to 100 µl of extract/solvent (equivalent of five nymphs). The extract/solvent was applied on the filter paper and allowed to dry for 30 min before the female was introduced to the exposure chamber. To ensure exposure, mothers were enclosed inside the exposure chamber for 3 h after which they were allowed to move in and out of the exposure chamber during their nocturnal active period. The likelihood that mothers were found inside or outside the exposure chamber on each day before renewing the extract was independent of the exposure treatment (GLM binomial (in/out): exposure treatments F2,88=0.07, p=0.92, day F2,169=1.32 p=0.27; exposure treatments × day, F4,169=0.49, p=0.74) confirming that none of the solvent/extracts were overly repulsive or attractive. During these three consecutive days of exposure, mothers had no access to food in order to ensure that mothers would not accumulate extra food which they could not provide to their offspring. On day 8, after 3 h of exposure, mothers were provided for 1 h with pellets of yellow pollen dyed with blue food dye as a marker (patent blue food dye; Werner Schweizer AG, Richterswil CH) resulting in green coloration (see Staerkle and Kölliker 2008).
- Offspring condition treatment: During the 3 days of separation from their mothers, each brood was split in half and kept in separate petri dishes (3.5×1 cm) resulting in

split-brood of an average of 9.42±0.08 nymphs. One half of the brood received ad libitum food in the form of pellets of pollen during the first 2 days (high-food individuals, HFI) whereas the other half did not receive any food (low-food individuals, LFI). Because these split-broods would be gathered again later during the experiments, we randomly marked either all the HFI or LFI on their abdomen with a red marker pen (Potaco A.quip) in order to differentiate them. On day 7, both groups were deprived of food in order to standardise their short-term condition (i.e. variation in gut content) and increase their motivation for maternal food on day 8 when the behavioural experiment took place. The LFI and HFI groups from each family were gathered again together in a new petri dish (10×2 cm) on the morning of the behavioural assays with the mother.

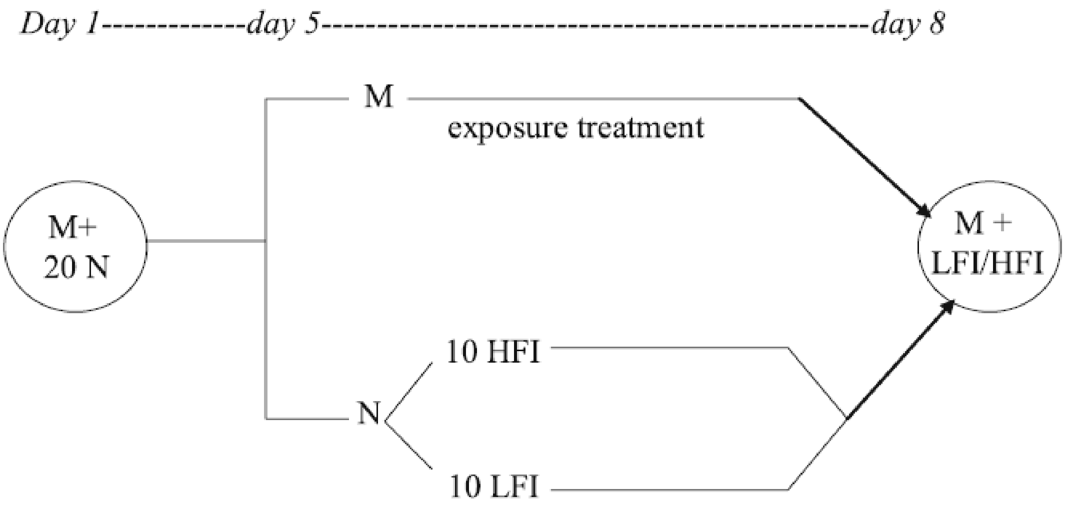

Fig. 1 Schematic figure of experimental setup. From days 1 to 4, mothers (*M*) and 20 nymphs (*N*) are kept together. On day 5, mothers are separated from their brood and randomly assigned to an exposure treatment: either extract from high-food brood (*HFB*) or extract from low-food brood (*LFB*) or pure heptan solvent as control (C). Also on day 5, the 20 nymphs are split in half and assigned to a condition treatment: ten nymphs on high-food treatment (*HFI*) and ten nymphs on low-food treatment (*LFI*). On day 8, mothers and their original brood, consisting of HFI and LFI, are gathered again. Maternal care behaviours were quantified during 1 h observation and mothers with their brood were let overnight together

Behavioural assay on day 8

Assay 1: Maintenance of maternal behaviours

In order to measure the effect of continuous exposure to brood chemical signals or the control solvent on the maintenance of maternal behaviour, we rejoined mothers with their

original brood 3 days after separation and let them interact over 1 h. During this hour, we used a scan sampling observational method (one scan observation every 5 min; i. e. 12 scans per replicate) to record and quantify behaviours. We considered the following behavioural categories as maternal behaviours directed to offspring: (1) antennal contact with the nymphs, (2) mouth-to-mouth contact (this could either describe a mother trying to regurgitate food to

a nymph or a nymph trying to trigger maternal regurgitation) and (3) grooming (female manipulating a nymph's body with her mouth parts). To obtain a measure of Fig. 1 Schematic figure of experimental setup. From days 1 to 4, mothers (M) and 20 nymphs (N) are kept together. On day 5, mothers are separated from their brood and randomly assigned to an exposure treatment: either extract from high-food brood (HFB) or extract from low-food brood (LFB) or pure heptan solvent as control (C). Also on day 5, the 20 nymphs are split in half and assigned to a condition treatment: ten nymphs on high-food treatment (HFI) and ten nymphs on low-food treatment (LFI). On day 8, mothers and their original brood, consisting of HFI and LFI, are gathered again. Maternal care behaviours were quantified during 1 h observation and mothers with their brood were let overnight together offspring rejection by mothers, we further quantified maternal aggression towards nymphs. We considered as aggressive behaviour when females were lifting their cerci upward in direction of a nymph, displaying the typical threat display of earwigs (Fulton 1924; Eisner 1960) or when shaking their abdomen while moving all over the petri dish to get rid of a nymph sitting on them (F.M., personal observation). Other female behaviours like scrubbing ventrally on the surface of the petri dish or digging close to the nymphs and carrying sand away could have been considered as maternal protection behaviours but since they were not obviously directed to or performed for the nymphs, we did not include them in the category of maternal behaviour. Finally, the most frequent behaviours such as exploring, self-grooming or resting were considered to be not specifically related to maternal care and although recorded, they were excluded from our statistical analysis. As a measure of mother–nymph aggregation, we scored at each scan if the closest group of two or more nymphs were within one female body length or not. Throughout these behavioural observations, the observer was blind with regard to the exposure treatments the mothers had been exposed to. Note that irrespective of the exposure treatment, the females always interacted with a brood consisting of approximately ten HFI and ten LFI nymphs and that the behavioural scores (grooming, antennal contact, mouth-to-mouth contact and aggression) in the scan samplings were not differentiated if they occurred between the mother and the HFI or the LFI nymphs.

Assay 2: Food distribution within brood

After this observational hour, mothers were separated from the nymphs again for 1 h and allowed to forage on a second pellet of blue-dyed pollen. This step was considered to mimic a

foraging trip. In order to assess the quantity of food eaten by mothers, pellets of food were weighed before and after each foraging trip with a Mettler Toledo AT261 balance with an accuracy of 0.001 mg. To control for effects of absorbed ambient humidity, the pellets were dried in the oven at 70°C prior to weighing. Finally, mothers were transferred back with their nymphs and allowed to interact overnight. The next morning (approximately 15 h later), provisioning to the brood was assessed by counting the total number of nymphs with green gut content and discriminating between LFI and HFI for food distribution within the brood (recognisable based on the marking; see above). Nymphs are fairly transparent and nymphs with food intake could be identified by their green gut content. Because only mothers had access to the green food, the proportion of nymphs with green gut content could be used as a measure of maternal provisioning (see Mas et al. 2009).

Statistical analysis

A total of 95 females with their brood were used in the behavioural assays. Four families were excluded from the statistical analysis because of female mortality before the end of the experiments resulting in a sample size of 91. Females in the different exposure treatments did not differ in body mass (one-way ANOVA: $F_{2,89}$=1.02, p=0.36), clutch size (one-way ANOVA: $F_{2,89}$=0.88, p=0.41) or brood size at hatching (non-parametric Kruskal–Wallis: H=4.38, p=0.11) confirming proper randomization of treatment groups. The scores of maternal behaviours obtained from the scan observations were summed up for each mother to obtain frequencies of each behaviour over 60 min. Given that most maternal behaviours in these scans showed skewed distributions and were rarely observed on average in the scans, we used generalised linear models (GLM) with a quasi-Poisson error distribution and a log-link function to test for differences in these maternal behaviours between the exposure treatments (extract from HFB or LFB or the solvent C). In order to take into account potential trends

over time during the observation period, we further performed an analysis where behaviours were summed over four successive periods of 15 min each. We then used a generalised linear mixed model (GLMM) with a quasi- Poisson error distribution and a log-link function, with the frequencies of the behaviour as the dependent variable, time (0–15, 15–30, 30–45 and 45–60 min) as a covariate, female identity as random factor and type of behaviours, exposure treatment and the interaction of exposure treatment with time as fixed factors.

To test the distribution of food within broods, we conducted a GLMM with a quasi-binomial error distribution and a logit function, number of nymphs with versus without green gut as odds ratio for the dependent variable, female identity as random factor, total food consumed by mother as covariate and nymph condition (HFI, LFI) as fixed factor. We used the statistical software

Results

Effect of brood chemical signals on maintenance of maternal care behaviours

Earwig mothers spent most of their time performing non maternal activities such as exploring, self-grooming or resting (mean ± S.E., 70±2% of total time). Behaviours considered as maternal or aggressive occurred in 28±2% and 2±0.7% of scan observations, respectively. Among these, there was a significant effect of the exposure treatment on maternal grooming of nymphs (GLM: $\chi2$=0.79, df=89, p=0.036) and aggressive behaviours against their nymphs (GLM: $\chi2=1.72$, df=89, p=0.003; Fig. 2). As predicted, mothers that were exposed to extracts from HFB groomed their nymphs significantly more often than mothers exposed to the solvent C (contrast: t=1.94, p=0.030) and more, though not significantly, than mothers exposed to extract from LFB (contrast: t=2.20, p=0.055). Mothers exposed to extracts from LFB displayed significantly more often aggressive behaviours toward their nymphs compared to mothers exposed to extracts from HFB (contrast: t=2.31, p=0.023) or to solvent (contrast: t=2.31, p=0.023), for both of which almost no aggression was observed. The exposure treatment had no significant effect on the other maternal behaviours such as antennal contact (GLM: $\chi2=10.29$, df=89, p=0.32) and mouth-to-mouth contact (GLM: $\chi2=13.19$, df=89, p=0.25).

Mothers spent most of their time in the vicinity of their offspring irrespective of the exposure treatments (frequency of observations where at least two nymphs were within less than

one female body distance=95%, H=0.14, p=0.93). When analysing the temporal dynamics of expression of behaviours over 60 min, maternal behaviours showed no clear trends over time (GLMM: behaviours × time $F_{3,1343}=2.07$, p=0.10), and there was no significant interaction between time and exposure treatments with regard to either the summed frequency of all the maternal behaviours (GLMM: behaviours × time × exposure treatments $F_{6,1343}=1.35$, p=0.23) or the frequency of any specific maternal behaviour (all p values >0.3).

Effect of offspring condition on within-brood food distribution

Only 32 out of 91 tested broods had nymphs with green gut content. The incidence of occurred provisioning did not differ significantly among the three exposure treatments (GLM: $\chi2=67.95$, df=88, p=0.99). Among the broods where provisioning occurred, the proportion of nymphs with food intake was not significantly affected by the exposure treatment ($F_{2,28}=0.26$, p=0.77) nor by an interaction between treatment and individual nymph condition ($F_{2,28}=1.18$, p=0.32). For further analysis of within-brood food distribution among HFI and LFI nymphs the broods from the three exposure treatments were pooled.

The proportion of nymphs that received maternal food in a brood was significantly and positively correlated with the amount of food foraged by the mother ($F_{1,30}$=11.22, p=0.002). Analysing the effect of nymph nutritional condition on within-brood food distribution revealed that significantly more nymphs from the low condition (LFI) had green gut contents as compared to nymphs from the high condition (HFI; $F_{1,31}$=10.86, p=0.003).

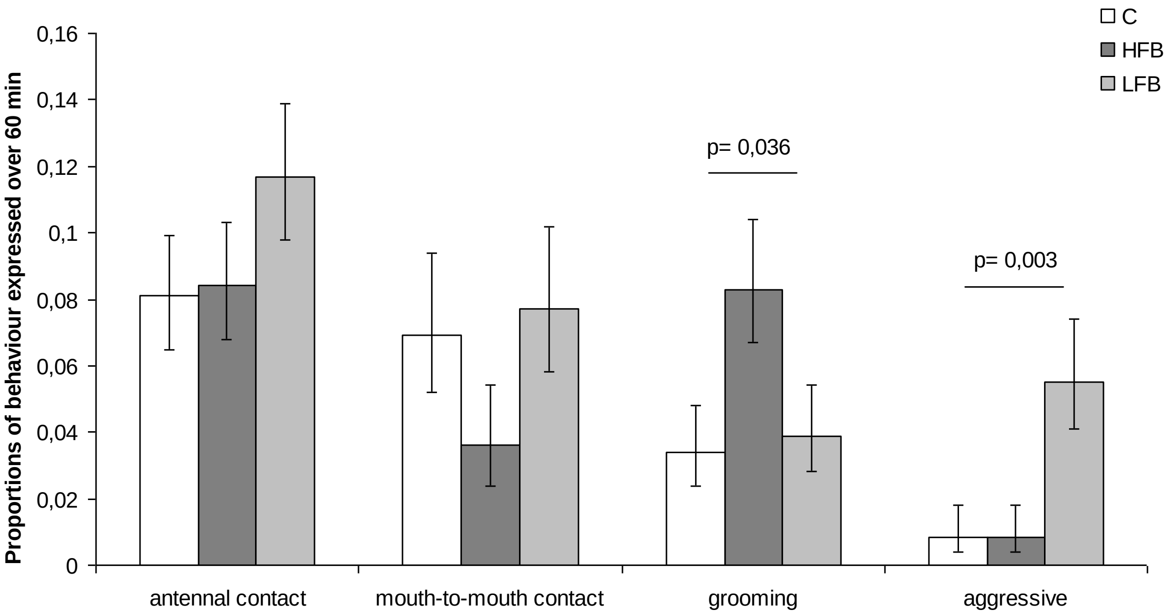

Figure 2: Frequencies for behaviours considered maternal (antennal contact, mouth-to-mouth contact and grooming) or aggressive expressed over 60 min after 3 days of separation and exposure treatment (extract from HFB or LFB or a solvent control C). Means and standard errors were obtained by back transforming the estimates from the Poisson regression model to the linear scale.

Discussion

Maternal behaviours and offspring signals affecting them co-evolve as interacting phenotypes (Kölliker et al. 2005; Smiseth et al. 2008), partly driven by selection from a conflict of interest between mothers and offspring over parental investment (Trivers 1974). Selection on parent– offspring interactions can arise due to effects of offspring signals on the amount and/or the duration of maternal care (Trivers 1974). We previously showed an effect of short term (1 h) exposure of females to condition-dependent brood chemical signals (cuticular hydrocarbons) on the amount of maternal food provisioning to nymphs (Mas et al. 2009). The present study demonstrates an additional function for condition-dependent brood chemical signals in maintaining specific maternal behaviours such as grooming and in affecting aggression towards offspring. The direction of effects are consistent between the two studies, with more care performed by females exposed to extracts from HF broods than LF broods, albeit with regard to different categories of maternal behaviour (food provisioning; Mas et al. 2009 and grooming/reduced aggression; this study).

Brood chemical signals and maintenance of maternal care

Although the function of grooming in maternal care is not immediately obvious, grooming clearly suggests that females tolerate physical proximity of nymphs, while aggression is indicative for the opposite effect. In earwigs, females are tolerant to their offspring during the maternal period but also display aggressive behaviours toward nymphs in particular after their dispersal (in *F. auricularia*, Lamb 1976b; and *Labidura riparia*, Vancassel 1977; Vancassel et al. 1987; Radl and Linsenmair 1991). Thus, the enhanced grooming by females exposed to HF extracts and the higher aggression by females exposed to LF extracts combined suggest a differential effect of condition-dependent chemical signals on the maintenance of care. By staying tolerant to their offspring, offspring can stay in proximity to their mothers and potentially gain protection benefit against predator attacks for longer, even at an age when food provisioning becomes less critical. Conversely, by behaving more aggressively toward nymphs, females exposed to cues indicating poor offspring quality may promote early dispersal of offspring and thus save maternal investment for her second brood. It remains to be tested in future studies whether the same chemical compounds from the total cuticular extract are responsible for the effects on provisioning and the other maternal behaviours.

The other behaviours that we considered as maternal like antennal contact and mouth-to-mouth contact did not differ significantly between the exposure treatments. Some of these results may at least partly be due to low statistical power, and our results on the maintenance of maternal behaviour may generally be rather conservative. The method of scan sampling used to screen several families at the same time may have underestimated the frequency of these types of maternal behaviours because they were relatively rare events and lasted for short time compared to behaviours like resting or cleaning (F.M., personal observation).

The previous study on earwig food provisioning (Mas et al. 2009) was based on short-term exposure of earwig mothers to extracts, that is, the immediate response in terms of food provisioning after exposure to condition-dependent offspring signals for 1 h (Mas et al. 2009). In the present study, it is conceivable that the low frequency and amount of provisioned food, and the lack of significant effect of exposure treatment, may in part be explained by the 3 days of separation of mothers from their brood, and the older age at which provisioning was quantified (day 8: this study; day 6: Mas et al. 2009). Food provisioning in earwigs is highest on days 3 and 4 after hatching and decreases afterwards (Kölliker 2007). Because with age earwig offspring start to leave the nest with their mothers for foraging (Lamb 1976a, b; F.M. and M.K., personal observation), maternal protection against predators may become relatively more important than maternal food provisioning.

Maternal care in F. auricularia has been reported to last until nymphs disperse (Lamb 1976b; Lamb 1976a). Vancassel and Foraste (1980a) showed that the presence of young nymphs maintains earwig maternal care behaviour, which can be empirically extended by preventing the nymphs from dispersing (Vancassel and Foraste 1980b). We demonstrated that the presence of condition-dependent brood chemical signals is sufficient to exert a persisting influence on some maternal behaviours, which might be correlated with the duration of care. Similar olfactory effects on maternal behaviour have been demonstrated in mammals (reviewed in Lévy and Keller 2009). For instance in sheep, females remain maternal after mother–young separation when olfactory cues are available (Poindron and Le Neindre 1980) and washing the lamb prevents acceptance behaviour while aggressive behaviour increases (Lévy and Poindron 1987). In general, in mammals, it is well understood how offspring signals act on the regulation of neuroendocrine factors and/or hormones mediating the expression of maternal behaviours (see Bridges 2008). These mechanisms are less studied in insects but a certain number of empirical studies have shown the effect of, yet unidentified, stimuli from offspring that regulate juvenile hormone titers of parents (i.e., in the ring-legged earwig, *Euborellia annulipes* Rankin et al. 1997; in burying beetle, *Nicrophorus* spp. Panaitof and Scott 2004; Scott and Panaitof 2004; Trumbo and Robinson 2008). Juvenile hormone is known to regulate development and reproduction in insects (Nijhout 1994) and is of particular importance in the regulation of parental care. In our study, we showed that chemical signals from offspring earwig maintained some maternal behaviours like grooming, which could potentially happen through an effect on the mother's physiological state, but this remains to be confirmed. If offspring chemical signals per se can influence the expression and maintenance of maternal behaviours such as grooming or aggression, offspring may potentially extend the duration of care provided by a mother in their best interest and at a cost for mother's future reproduction.

Offspring condition and within-brood food distribution

Independent from the exposure to the brood chemical signals, significantly more LFI than HFI nymphs received maternally provisioned food. Our experiment does not allow us to directly discriminate between an active choice from mothers to allocate food preferentially to LFI and the outcome of enhanced efforts of LFI nymphs to outcompete HFI nymphs in a scramble for maternally provisioned food. In the former case, our results would be consistent with a maternally selected honest signal of need. However, it would appear contradictory if earwig mothers selected for a signal of quality at the level of the brood (food provisioning, Mas et al. 2009; grooming/low aggression; this study), and for a signal of need at the level of the individual offspring within a brood. It is more likely that scramble competition between sibs drives the dynamics of food distribution within earwig broods, where competition can be harsh (as indicated by frequent siblicide and cannibalism, Dobler and Kölliker 2010).

Competitive asymmetries between offspring resulting from age or size differences, for instance, are expected to regulate scramble competition and several experimental studies on a variety of species showed that older and bigger offspring, presumably in better condition, received more maternal food than the other sibs (in birds, Price and Ydenberg 1995; Cotton et al. 1999; in burying beetles, Smiseth et al. 2006; Smiseth et al. 2007; Smiseth and Moore 2008). However, if competitive asymmetries are low and variation in nutritional need is large, a unit of food for offspring in poor condition (LFI) will be more beneficial than for high condition (HFI) offspring. LFI offspring are expected to have a higher payoff for competing more intensely (Parker et al. 2002), which in turn could promote higher competitive efforts and/or solicitation by LFI nymphs (Parker et al. 2002; Royle et al. 2002). Smiseth and Moore (2007, 2008) showed that in asynchronous broods of burying beetles, food-deprived or junior larvae spent more time begging than satiated or senior larvae. In our experiment, nymphs were all aged the same and the benefit of receiving food was probably higher for LFI than HFI despite the fact that the 2 days of food treatments might have had an effect on nymphs' growth and resulted in size differences. Thus, the most likely explanation for our result that food distribution was biased towards LFI nymphs is through an effect of condition scramble competition. Condition-dependent non-signalling components of offspring behaviour (Lotem 1998) like positioning towards the mother or activity (personal observation) may lead to dynamics of food distribution within broods that can differ from the maternally selected brood signal.

Maternal care regulation and sibling interactions: a hypothetical scenario

Our results on maternal behaviours and food distribution raise the possibility of frequency-dependent effects of condition-dependent offspring behaviours on a signal perceived by the female at the level of the brood in *F. auricularia*. In a brood where there are a majority of nymphs in good condition (HFI), the overall chemical signals emanating from the entire brood would provide a signal of high brood quality (HFB) to mothers promoting maternal care. Conversely, in a brood where a majority of nymphs is in poor condition (LFI), mothers would perceive an overall brood chemical signal of low quality (LFB), which could result in reduction of maternal care to the brood, including the few HFI nymphs present in the brood. With such a maternal response at the level of the brood, the interest of HFI may be less divergent with the interest of LFI because of a mutual benefit of maintaining mothers to care. Thus, when mothers respond to offspring signals of quality (i.e. reproductive value) at the level of the brood, but allow offspring to scramble over food distribution within broods, selection may favour more cooperation among siblings because, under diminishing returns of food intake on condition, there is an added benefit of having siblings in better condition. Studies reporting interactions similar to the ones reported here were on acoustic offspring signals in birds (tree swallow, *Tachycineta bicolor* Leonard and Horn 2001b; and black-headed gulls, *Larus ridibundus* Mathevon and Charrier 2004; Horn and Leonard 2008), and banded mongoose, *Mungos mungo* (Bell 2007). In these species, individual offspring in a brood/litter not only beg at higher intensity when hungry and when competing with siblings, but they also adjust the calls to each other, resulting in a collective signal enhancing overall food provisioning to the entire brood. Another example of a brood signal was reported in the treehopper *Umbonia crassicornis*, where the offspring synchronise vibrational signals from all offspring, even the ones further from the predator, which in turn facilitates mothers to locate predators and to defend their brood (Cocroft 1996; Ramaswamy and Cocroft 2009). These arguments and examples indicate that offspring solicitation signals may sometimes combine competitive and cooperative components depending on how competitive interactions within broods affect brood signals that the caregiver uses to allocate investment between broods (Parker et al. 2002; Wilson and Clark 2002; Johnstone 2004; Forbes 2007).

In summary, our study on the European earwig demonstrates that condition-dependent brood chemical signals can have persistent effect on the maintenance of maternal behaviours over time. Consistent with previous results on maternal food provisioning to the brood (Mas et al. 2009), an increased in maternal grooming after exposure to extracts from high-food brood supports the hypothesis that earwig mothers may select for a brood chemical signal of quality. Condition-dependent effects on the distribution of food within broods differed from the effects of the brood chemical signal on maternal behaviours, suggesting an important role of multi-component offspring signals and behaviours differentially affecting amount and duration of care.

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Chapter 4

AN OFFSPRING SIGNAL OF QUALITY AFFECTS THE TIMING OF FUTURE PARENTAL REPRODUCTION

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Abstract

Solicitation signals by offspring are well known to influence parental behaviour, and it is commonly assumed that this behavioural effect translates into an effect on residual reproduction of parents. However, this equivalence assumption concerning behavioural and reproductive effects caused by offspring signals remains largely untested. Here, we tested the effect of a chemical offspring signal of quality on the relative timing and amount of future reproduction in the European earwig (*Forficula auricularia*). We manipulated the nutritional condition of earwig nymphs and exposed females to their extract, or to solvent as a control. There were no significant main effects of exposure treatment on 2nd clutch production, but exposure to extracts of well-fed nymphs induced predictable timing of the 2nd relative to the 1st clutch. This result demonstrates for the first time that an offspring signal *per se*, in the absence of any maternal behaviour, affects maternal reproductive timing, possibly through an effect on maternal reproductive physiology.

Keywords: parent-offspring conflict, offspring begging, chemical communication, *Forficula auricularia*, parental care.

Introduction

It is well known that solicitation signals by offspring influence parental behaviour and are condition-dependent [1], which is consistent with predictions of models for the evolution of parent–offspring interactions [2–6]. A core assumption of all these models is that offspring signals not only affect the behaviour of parents, but that they can also affect their residual fecundity. The well-documented purely behavioural effects on parents may be transient and merely reflect short-term behavioural dynamics [7,8]. Only if offspring signals have the potential to affect residual parental reproduction can selection favour exaggeration and manipulative potential (as under conflict: [6]), and/or the co-adaptation with parental sensitivities [5]. Evidence for such an effect comes from a recent cross-fostering experiment in the canary (*Serinus canaria*) showing a negative correlation between the solicitation of foreign nestlings and the subsequent clutch size produced by foster females [9]. Theory more specifically assumes that the fitness effects of offspring signals on parents are equivalent to (or a direct function of) the behavioural effects (see [10] for discussion of 'investment ESS' versus 'food ESS'), that is, offspring signals should have no effect on parental residual reproduction in the absence of expressed care behaviour. This equivalence assumption has to our knowledge not been previously tested and may not apply if offspring signals evolved direct influences on maternal reproductive physiology (i.e. 'primer effect'; [8,11]). The potential for direct offspring influences on maternal reproduction is of evolutionary interest, because it modifies selection on offspring and parental behaviours. A hypothetical offspring signal that suppresses future reproduction by parents would eliminate any evolutionary cost of parental resource provisioning in terms of residual reproduction, disrupting the link between provisioning and investment [8]. Direct testing of this assumption requires manipulation of offspring signals independent of other traits, and measurement of effects on parents without the expression of care behaviours, both of which were difficult in the past.

Here, we provide such an experimental test in the European earwig (*Forficula auricularia*). We tested the direct effect of a previously described offspring signal of quality [12] on maternal residual fecundity in the absence of expressed maternal care. Female earwigs produce one or two clutches in their lifetime [13], provide maternal care for few weeks after hatching and adjust maternal behaviour to a condition-dependent mix of cuticular hydrocarbons (CHC) produced by the nymphs on their cuticle [12,14]. This system allowed us to experimentally expose females to the isolated offspring signal in the absence of expressed maternal behaviour.

Material & Methods

Adult earwigs were collected in Dolcedo (IT) in July 2009 and kept in the laboratory in mixed-sex groups of 30 males and females with ad libitum food [15]. Upon egg laying, the date and size of 1st clutches were noted and the eggs with their tending mothers placed at 158C in complete darkness. At hatching, the date and number of hatched nymphs were recorded and families were placed at 14 L : 10 D and a constant temperature of 20°C. Females and 15 of their one-day-old nymphs were transferred for four days to new Petri dishes (10 x 2 cm) with humid sand as substrate and ad libitum food. On day 5, females were separated from their nymphs and set-up in new Petri dishes containing a small exposure chamber [12]. Females were then randomly assigned to an exposure treatment. They were exposed either to extract from high-food (HF) brood (n = 42), extract from low-food (LF) brood (n = 42) or pure

solvent as control (C) (n = 43) during nine days. Extracts were obtained following the methods by Mas et al. [12] based on groups of usually 35 nymphs in experimentally manipulated nutritional condition. For each female, an extract from an independent group of nymphs was used (see the electronic supplementary material for more details).

On day 14, the females were transferred to a new Petri dish and provided with food twice a week until 2nd clutch egg-laying. The laying date and size for 2nd clutches, their hatching date and offspring numbers were recorded. For consistency, we included only those 2nd clutches that were laid within 60 days after hatching of the 1st clutch (representing extreme values; [15]). Note that inclusion of the nine later 2nd clutches did not qualitatively alter our results.

Our laboratory population is maintained under an artificial seasonality including 'winter', 'spring/summer' and 'autumn', simulated by photoperiod and temperature differences [15]. Thus, we could record temporal patterns of oviposition with regard to this seasonality as measures of the timing of breeding under laboratory conditions.

Statistical analysis

Statistical analysis was performed using R statistics software v. 2.11.1 (http://www.r-project.org) and JMP v. 8.0.2 [16]. The effect of exposure treatment on female reproductive parameters (frequency, timing and size of 2nd clutches/broods) was tested using logistic regression (LR) and analysis of covariance (ANCOVA) models, respectively. For each model, we included the exposure treatment as fixed factor, and the 1st clutch parameter corresponding to the analysed 2nd clutch parameter as covariate, and the interaction between treatment and the covariate. Female pronotum area (i.e. a measure of structural size) was never significant and removed from the models. When the residuals of a model were not normally distributed, a Johnson Su transformation [17] was applied.

Results

From the 127 tested females, 56 laid a second clutch within 60 days of hatching of the 1st clutch. There were no significant main effects of exposure treatment on the frequency (LR-Chi^2= 1.51, $p = 0.47$), the size (number of eggs: $F_{2,50} = 2.13$, $p = 0.12$; number of hatchlings: $F_{2,50} = 1.79$, $p = 0.17$) or the timing (egg laying date: $F_{2,50} = 0.04$; $p = 0.96$, hatching date: $F_{2,49} = 0.03$, $p = 0.97$) of the 2nd clutch (see the electronic supplementary material, table 1 for summary of life-history data according to treatment).

However, the exposure treatment significantly affected the relative timing of 2nd clutch production through an interaction with hatching date of the 1st clutch (interaction exposure treatment _ 1st clutch hatching date: $F_{2,50} = 6.35$, $p = 0.003$; figure 1). This effect was due to a strong positive, and statistically significant, relationship between the dates for 2nd and 1st clutch production in females exposed to extracts from HF broods (slope (±s.e.): 0.39 (±0.05); r2 = 0.78; F1,17 = 57.82, p , 0.001; figure 1b) and lack thereof in females exposed to solvent only (C; slope (±s.e.): 0.14 (±0.07); r2 = 0.16; F1,21 = 3.96, p = 0.06; figure 1a) or extracts from LF brood (slope (±s.e.): 0.06 (±0.05); r2 = 0.09; F $_{1,15}$ = 1.51, p = 0.24; figure 1c).

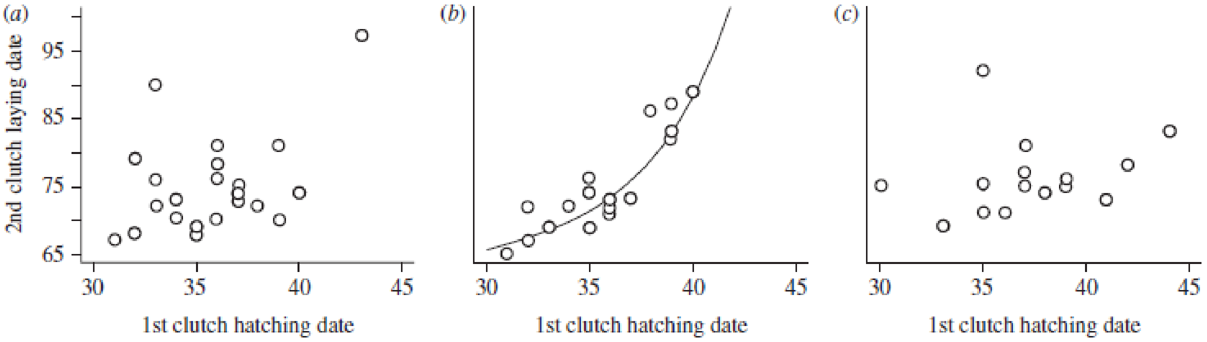

Figure 1. Relationship between 2nd clutch laying date and 1st clutch hatching date after exposure to (*a*) solvent (control females 'C'); (*b*) extract from high-food nymphs (HF exposure treatment); and (*c*) extract from low-food nymphs (LF exposure treatment). In (*b*) the solid line corresponds to the fit from our model (back-transformed model prediction: $r^2 = 0.78$; $p < 0.001$). Day 0 on the axes refers to 12 November 2009.

Discussion

Our study demonstrated that the condition-dependent cuticular extracts of earwig nymphs per se, and in the absence of other offspring traits or maternal behaviour, differentially affect the relative timing of future reproduction of their mothers. The nature of effect was more complex than anticipated: there were no significant main effects of exposure treatment on the frequency, size or timing of 2nd clutch production. Rather, the extracts of high-condition (HF) nymphs induced highly predictable maternal timing of 2nd relative to the 1st clutch. In this treatment, early females consistently laid their 2nd clutch soon after the first hatched, whereas late females delayed their 2nd clutch substantially. Conversely, females exposed to solvent (C) or extract of low-condition (LF) nymphs showed no significant predictability in the relative timing of 2nd clutches.

This pattern may be explained by a hypothetical scenario of co-adaptation [5] between the chemical offspring signal of quality and seasonal variation in the reproductive value of maternal behaviour for nymphs in 1st versus 2nd clutches. Early in the season, earwig nymphs experience less dense populations, less competition and risk of cannibalism and more time for development and mating before winter [13,18]. For 1st clutch nymphs in good nutritional condition survival may, under these circumstances, be sufficiently high even with shorter maternal care. But late in the season, longer maternal care for these high condition nymphs may be particularly valuable to further enhance their survival prospects. Hence, it may pay females with late 1st clutches to care for longer and delay the 2nd clutch. This relationship may be less or absent if offspring are in poor condition (our LF treatment) or in the absence of information on offspring condition (our control group C). A comparable, albeit purely behavioural, seasonal effect of an offspring signal of quality on parental food allocation was found in Alpine swifts (*Apus melba*) and starlings (*Sturnus vulgaris*) [19]. When offspring UV reflectance was manipulated, early breeding parents preferentially fed offspring with experimentally reduced UV reflectance, while late breeding parents favoured to feed control offspring with full UV reflectance. Whether in earwigs the apparent seasonal variation in the effect of nymph CHC on the timing of 2nd clutch production in *F. auricularia* reflects variation between early and late females in their responsiveness to a fixed chemical signal of quality, seasonal variation in the signal of quality emitted by nymphs of early versus late clutches, or both will have to be investigated in the future.

We previously showed that the main effects of earwig chemical signal of quality is on maternal behaviours such as provisioning [12], grooming and aggression [14], and that maternal behaviour (i.e. exposure to and interaction with nymphs) has a main delaying effect on the timing of 2nd clutches [15]. The present study demonstrates that offspring signals can have a direct influence on maternal reproductive physiology (i.e. through a primer effect; [8,11]), which differs from the behavioural effects. Primer effects of offspring signals on caregivers have only rarely been studied. The only other example for such effects that we are aware of is the brood pheromone in bees (*Apis mellifera*) which alters the timing of developmental transition from the nurse to the forager stage in workers [20,21].

This study shows that the equivalence assumption of behavioural and reproductive effects of offspring signals on parents does not necessarily hold. By affecting parameters of future reproduction of mothers directly, rather than indirectly through resource provisioning, the offspring signal shortcuts the link between the signal, care provisioning and the evolutionary cost of parental behaviour. Such an offspring signal may alter the selection operating on care provisioning, which in turn may have important consequences for the evolution of family interactions.

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Electronic Supplementary Material

An offspring signal of quality affects the timing of future parental reproduction.

Flore Mas*& Mathias Kölliker

Material &Methods

Chemical extraction: At the time broods were culled to 15 nymphs and set-up with their mother for behavioural test, the surplus individuals from different families were gathered and mixed in groups of usually 35 nymphs. They were kept in petri-dishes (10 x 2 cm) with humid sand. For two days all groups received ad-libitum food as pellet of pollen, before being randomly attributed to a food treatment: they either received ad-libitum food (HF) or nothing (LF) for a further 48 hours see (Mas *et al.* 2009). Then all groups were frozen at -20°C and all the nymphs from either LF or HF groups were immersed in 700 µl of solvent (n-heptan, Rotisolv® 99% pure; Carl-Roth AG, Reinach, Switzerland) for 10 minutes. From these total cuticular extractions, 600 µl were transferred in new glass vials and used for the female exposure treatment. Slight nymph mortality occurred in 29 extraction broods resulting in 0.4 more nymphs (or 1%) extracted on average in the LF than HF extracts (t=2.44, p =0.017). However, the number of nymphs per extract was unrelated to our measurements (all p >0.1) and cannot explain our results.

Female exposure: For female exposure, each exposure chamber was lined by a filter paper disc to which 100µl of one chemical extract (HF, LF or solvent) was applied daily from day 5 to 8, and every other day till day 13. Filter papers were allowed to dry for 30 minutes before female exposure. In total, females were exposed to 6 times 100µl (each extracts equivalent to mean±s.e 4.9±0.01 nymphs/exposure or a total extract equivalent to 29.6±0.07 nymphs). They had access to food overnight on days 8 and 12 and food consumption was measured by calculating the difference in dried food weight before and after. The exposure treatment had no significant effect on food consumption during these two nights ($F_{2,121}$=0.33, p =0.720).

Results

Table 1 presents and summarizes the frequency and means of the measured reproductive parameters for the 2nd clutches across the three treatments.

Table 1.Reproductive parameters of 2nd clutches across the three exposure treatments.

Life-history traits By treatments	**C**	**HF**	**LF**
Frequency nr/total	22/43	18/42	16/42
Laying date [25%-75% percentile]	[70-78.25]	[70.5-82.25]	[73.25-77.75]
Clutch size (mean±sd)	37.13±11.05	39.22±8.73	41.75±9.43
Hatching date [25%-75% percentile]	[89-99]	[89.75-103.25]	[93.25-99.75]
Brood size (mean±sd)	30.0±13.6	29.9±10.5	33.7±11.0

The laying date of the first clutch on the 11/12/2009 was taken as reference day 0. Clutch size refers to the number of laid eggs, and brood size to the number of hatched nymphs

The present work has demonstrated the existence of a chemical communication in *F. auricularia* and its functional use evolving in the context of maternal care. I first demonstrated that offspring earwig secrete cuticular hydrocarbons that vary in relative quantities depending on their nutritional state. Mothers exposed to these condition-dependent chemical cues foraged significantly different amount of food which later they provisioned to different numbers of nymphs within the brood. Chemical signals from broods in good nutritional state (high-fed, HF) triggered higher amount of food foraged and provisioned by mothers suggesting selection for an offspring chemical signal of quality and not need. Also, mothers continuously exposed to chemical signals from high fed nymphs significantly groomed more as opposed to mothers exposed to chemical signals from low fed broods which behaved significantly more aggressively towards their nymphs. Finally, I showed that offspring chemical signals can *per se* influence the timing of second clutch production by females and thus potentially manipulate females' residual fecundity. My results support that earwig mothers may be controlling their maternal investment and may actively select for an honest chemical signal of offspring quality that they use to optimally adjust their maternal investment.

Conflict resolution has led to the evolution of communication which requires the co-evolution of both the signaller and the receiver for which signal and response respectively become adaptive and specific to the context in which they evolve. In the light of parent-offspring conflict theory and resolution models, I discuss the co-evolution and the adaptive values of offspring solicitation pheromone and maternal investment in *F. auricularia*.

Co-evolution of caring mothers and their soliciting offspring

The adaptive value of offspring solicitation pheromone

The production of cuticular hydrocarbons (CHCs) originally evolved as a chemical barrier against body water loss when arthropods moved from sea life to life on land (Blomquist & Bagnères 2010; Blomquist et al. 1987). The quality and quantity of CHCs are genetically determined but also vary with environmental conditions such as temperature and humidity, social context, physiological state and phenotypic characteristics (age, sex, size) (Howard & Blomquist 2005). Since the discovery of the sex pheromone of *Bombyx mori* by Butenandt in 1959, tremendous effort has been done on demonstrating how CHCs evolved a secondary signalling function in different context of interactions between individuals of the same species (pheromone) or among different species (kairomone) (Blomquist & Bagnères 2010; Wyatt 2003). For instance, CHCs have been shown to be used in communication between nest mates of colonies in eusocial insects in order to discriminate kin from non-kin, or between sexual partners to discriminate and attract the opposite sex for mating or also between different aged individuals to differentiate castes associated behaviour (see for recent reviews Blomquist & Bagnères 2010; Howard & Blomquist 2005). But the use of chemical cues in the context of mother-offspring interaction during maternal care has rarely been investigated (see chapter 1). The evolution of a solicitation signal by offspring has been predicted by game theory models of POC resolution (signalling models, Godfray 1991; Godfray 1995; scramble models, Macnair & Parker 1979; Parker & Macnair 1979) and was largely experimentally demonstrated in birds (Wright & Leonard 2002). Theory predicts that offspring solicitation should be condition-dependent, that parents should respond to them by adjusting their parental investment accordingly and signals should be costly to ensure honesty/reliability (see for reviews Godfray & Johnstone 2000; Parker et al. 2002; Royle et al. 2004). In my experimental work, I have demonstrated that in the social insects *F. auricularia* nymphs produce CHCs that vary with their nutritional state and which influenced female maternal care behaviours. Chemical signals from high-fed (HF) brood increased the total food foraged by mother and later available for distribution among nymphs within the brood (chapter 2, figure 1). Chemical signals from low-fed (LF) brood triggered similar amount of maternal food provisioned by the females as the solvent control. Thus only chemical signals from HF nymphs had a significant positive effect on maternal food provisioning. Offspring in high nutritional condition (HF) did not produce higher total amounts of CHCs, but rather they produced different relative abundance of specific hydrocarbon compounds as compared to LF offspring (see chapter 2, figure 2 and appendix table x1&2). This suggests that chemical solicitation signals are continuous and their effect on maternal care appears discontinuous, maybe mediated by a response threshold (i.e. triggered above a certain concentration of specific peaks). HF nymphs produced higher relative amounts of longer chains (alkanes: n-C25, n-C27, n-C31 and an alkene C29 with a double bond in an unidentified position) as compared to LF nymphs. Biosynthesis of long-chain hydrocarbons generally requires more complex enzymatic processes for their synthesis compared to shorter chain hydrocarbons (Blomquist et al. 1987). Thus long chain hydrocarbons may be more costly to produce than shorter chains, and hence they provide honest information on individual nutritional state. Nevertheless, it is difficult to gauge the metabolic cost of offspring solicitation signals such as CHCs in insects or vocal calls of nestling birds (Kilner & Johnstone 1997; Moreno-Rueda 2007) relative to basic metabolic cost. Although game theory models predict that offspring signal should be costly, it has been claimed that cost-free signals could also evolved among relatives when interests are shared (Maynard Smith 1991) or when the benefits are higher than the cost of production (Grafen 1990), or when discrete signals are at a pooling equilibrium (Bergstrom & Lachmann 1997; Bergstrom & Lachmann 1998; Lachmann & Bergstrom 1998). Also, Kölliker et al. (2010) recently demonstrated that antagonistic co-adaptation between maternal care behaviour and offspring solicitation signal can lead to an evolutionary stable strategy even when offspring signal is cost free. Offspring signal may evolve through the exploitation of parental sensory bias as often observed for secondary sexual traits (Arnqvist 2006; Ryan 1990). Thus, more and more models argue that costs of signalling are not essential for the resolution of parent-offspring conflict. Nevertheless if signals are cost-free or low cost, stability of the communication system between parents and offspring may be at risk from exploiting cheaters (i.e. non-signalling individuals) in the phase of direct competition within the brood. In Chapter 2, I found that food provisioning was positively affected by offspring nutritional state reflected by the quality of the overall brood chemical signals. Conversely at the level of the individual nymph, food distribution was negatively correlated with offspring nutritional state (chapter 3). At the individual level, natural selection may favour selfish behaviours that monopolize maternal resources and scramble competition among siblings in different needs that value differently maternal food is expected. However, the shared benefits of increasing maternal food provisioned at the level of the brood may promote cooperation among siblings to signal (see Wilson & Clark 2002). If HF offspring do not signal or there are too many LF individuals in the brood, the overall brood chemical signal is of low quality resulting in reduced maternal care and increased competition for the reduced maternal food provisioned. Cannibalism is a common phenomenon in earwig families, in particular (but not exclusively) under conditions of food depletion (Dobler & Kölliker 2010). By cannibalizing a sibling, individuals may reduce competition for maternal food and enforce cooperation in signalling to maintain mothers to care. Whether HF nymphs eliminate sibs in lower condition or conversely LF nymphs eat HF nymphs and increased their nutritional state, in both cases it will result in an overall increase of brood signal quality and maintained maternal care. Siblicide in earwig may have evolved as mechanism to reduce sib competition like in birds (Mock & Parker 1997) or as punishment against exploiters like workers policing in eusocial insects (Ratnieks et al. 2006; Wenseleers et al. 2004; Wenseleers & Ratnieks 2006). Thus the evolution of an offspring solicitation pheromone that increases maternal investment to the current brood (i.e. food provisioning, grooming) appears adaptive for every offspring in the brood as it increased their survival and either the cost of signalling or mechanisms of control and coercion may ensure that the strategy becomes evolutionary stable. Yet, the persistence of self-foraging as alternative strategy to signalling strategy suggests that the latter behavioural strategy may be still under selection and has not yet reached an ESS in *F.auricularia* (see Dobler & Koelliker 2009). Solicitation by earwig offspring may contain multiple components such as individual behaviours influencing food distribution within the brood while the overall chemical signals from the brood affecting total food provisioned by mothers. Both scramble competition within the brood and maternal selection between the broods may take place and shape the evolution of offspring solicitation signals and maternal care regulation in *F. auricularia*

Further work:

The numerous and complex hydrocarbons found on the cuticle of offspring earwig that co-vary with their nutritional state would require further detailed analysis along with bioassay tests to identify the really specific CHCs in the bouquet that trigger the exclusive and specific maternal responses observed so one can define the offspring solicitation pheromone. A first step would be to couple a GC with and electro-antennogram (EAG) to be able to detect the compounds in the bouquet which elicit a difference in the polarity of the antennal nerves confirming the existence of receptors for these specific compounds. After identifying such compounds, the use of synthetic compounds in behavioural tests could confirm the function of these compounds in the specific context of maternal care. Finally, by manipulating ratios and various concentrations, one would be able to better understand their action. This is a long strenuous work, which was not under the scope of this thesis. Refer to Blomquist & Vogt (2003) for techniques to elucidate pheromones.

The adaptive value of maternal responses

My results from the first experiment (chapter 2) showed that females respond to offspring chemical signals by adjusting their short-term maternal behaviour. Mothers foraged more and provided food to more nymphs in the brood after being exposed to extract from HF broods compared to LF broods. The preferential allocation of food to nymphs when receiving cues from broods in good condition (HF) suggests selection for an offspring signal of quality either actively driven by mothers or as outcome of scramble competition. The large brood size combined with low rates of offspring survival till adult stage, estimated at 40% under lab condition and probably even lower in nature (Kölliker 2007) supports that investing in broods that are already in good conditions may be less risky for females and ensure their returns on investment. In this context, the identified offspring chemical signals represent offspring expected reproductive value and may be actively selected by females to maximise their inclusive fitness. In addition, in partial begging species like *F.auricularia,* offspring nutritional state is the result of maternal food provisioning but also self-foraging. Mothers are not in full control of offspring nutritional state and therefore the only way to assess their cryptic nutritional state is to rely on a condition-dependent signal. Under high food availability, nymphs may easily find food by themselves resulting in an overall high quality signal from the brood. While under low food condition, nymphs may not be efficient in self-foraging resulting in a brood signal of low quality. Condition-dependent offspring chemical signals may thus also reflect how well offspring can survive in the current environmental conditions which may be used by females to take decision on future investment. In chapter 4, I found that mothers that were exposed to offspring signal of good quality (HF) during their first reproduction had a higher predictability to lay a second clutch within a constant interval than females exposed to LF signals from their first brood. If offspring from the first brood produce chemical signals of good quality, they may have good chance to survive and thus mothers may take few risks to loose their current investment if they start a new clutch. Conversely, if offspring chemical signals reflect low condition, it may be similarly risky for mothers to keep caring for current clutch or starting a new one. Some theoretical models have also predicted similar outcomes of parental resources allocation depending on offspring state (age, size, need) and the environment in order to maximise their lifetime reproductive success (e.g. Davis et al. 1999; Jeon 2008). Under limited resources, models predicted that parents should always bias their resources toward older offspring offering greater reproductive value than younger offspring. These models corroborate with my results although authors assumed that parents have full control of food allocation irrespective of solicitation signal by their offspring. In conclusion, when earwig mothers perceive an overall brood chemical signal of low quality, reduction of maternal care (low food provisioning and more aggressive behaviour) may be adaptive for mothers which then save their energy for a future clutch when conditions get better (i.e., maximize residual reproductive success). Female responsiveness to offspring chemical signals of quality seems therefore adaptive in a seasonal environment where females should optimally adjust and balance their maternal investment between current and future broods (see also Grodzinski & Lotem 2007).

Further work:

An experiment where mother's would be exposed to chemical signal from either HF or LF brood under different food availability during their first reproduction and by measuring maternal investment in the second brood would confirm the plasticity and adaptive strategy of maternal investment according to the quality of offspring and the environmental conditions.

"Just as an ethologist can manipulate the behaviour of an animal by stimulating it appropriately, so can another animal" Krebs & Dawkins (1984)

Maternal control or offspring manipulation?

The adaptive values of a signal and its response are not often obvious, particularly when there is a conflict of interest as in parent-offspring interactions. Parents may select for an offspring cue that reflect offspring cryptic condition in order to maximize their parental investment. Conversely, maternal strategy may become evolutionarily fixed at a sub-optimal state for mothers if offspring evolved the capacity to manipulate mother's maternal behaviour through a priming effect. Maternal behaviours are generally exclusively expressed during the caring period just after parturition/hatching. Maternal care is usually characterized by a high level of tolerance towards offspring, which decreases after dispersal. The particular neuroendocrinological and physiological states in which mothers are while caring are partly maintained by constant feedback with offspring cues, a physiological phenomenon well studied in mammals (Bridges 2008). I suggested in chapter 1 that the co-evolution and co-adaptation of interacting caring mothers and their signalling offspring may lead potentially to the evolution of an offspring signal directly affecting mothers' physiology which primes them to care. From chapter 2, I concluded that mothers may select for an offspring chemical signal of quality which would suggest that mothers have full control on their investment. Yet in chapter 3, I demonstrated that offspring signals have a persistent influence on the expression of specific maternal behaviours, such as grooming and aggression. In chapter 4, I showed that by continuously exposing mothers to offspring chemical signals, offspring signals can influence mother's residual reproductive success by influencing timing of second clutch production. By selecting an offspring signal of quality, mothers may better adjust their future investment considering current offspring quality. Therefore, it seems that offspring solicitation signals serves best the interest of mothers that can balance their maternal investment between current and future offspring. Yet, it is not evident to assess whether it would be in the interest of the offspring to extract more maternal investment if they reduce also their indirect benefit through inclusive fitness of new siblings. Finally, individuals expressed both traits (demand and supply) at different time of their life (young and adult) and thus antagonistic selection across life-stages may favour coadaptation of the strategies that maximize an individual's lifetime fitness (Kölliker et al. 2010; Kölliker et al. 2005).

Further work:

To confirm whether offspring solicitation chemical signals evolved a priming effect, one could test whether offspring chemical signals influence or not juvenile hormone titres of females. Experimental work focusing on the physiology and the hormonal level (JH titers) of mothers would provide a direct assessment of the proximate mechanisms regulating maternal care. The present techniques of radioimmunoassay or LC-MS were not yet enough precise and repeatable to be able to measure JH titers of individual females and required pooling several females together in order to get one sample enough concentrated for measurement (see for comparisons of techniques the thesis by Westerlund 2004). Therefore the sacrifice of several females to get enough JH needs large lab population to test these mechanisms.

Family conflicts and life-history, examples from Dermaptera order

Species like the European earwig, *F. auricularia,* or the burying beetle *Nicrophorus vespilloides* display facultative maternal care with partially begging offspring (Smiseth et al. 2003). These species represent intermediate stage in the evolution of begging between non-signalling self-sufficient offspring like precocial species and signalling offspring completely dependent on parental food like altricial species. These are, therefore, especially interesting for observing and testing selection on offspring solicitation signal and how parental care regulation is coevolving. But comparative studies of closely related species contribute also to our understanding of the long-term evolutionary effects of family conflict on life history traits. The range of forms and duration of parental care observed in diverse and closely related species may be explained by the different trajectories of co-evolution of interacting parents and their offspring. In the order Dermaptera, we can observe diverse level of maternal investment: from really short time, 1 or 2 days in *Labidura riparia,* to extended maternal care, e.g. in *Anechura bipunctata* (Vancassel 1984; Vancassel & Foraste 1980; Vancassel & Foraste 1980). In the hump earwig *Anechura harmandi*, females even become the meal of their offspring (i.e., matriphagy) (Kohno 1997; Suzuki et al. 2005). Ecological conditions have been usually proposed as explanations for the observed level of maternal investment (Kohno 1997; Vancassel 1984). Species living in harsh environment (e.g., high altitude or flooding area) with food scarcity usually display extended maternal care while species in temperate zones have shorter maternal care duration (Tallamy & Wood 1986). But ecological studies did not integrate the evolutionary context of parent-offspring conflict as discussed in this research, and thereby may have underestimated the influences of offspring signal on patterns of maternal care. Tallamy & Browm (1999) suggested that in arthropods semelparity may often have evolved from iteroparous species as a consequence of extended maternal care. Iteroparous females may have been influenced by highly demanding offspring and co-adaptation may have led to the loss of several clutches for maximizing only one.

Further work:

A comparative analysis of the chemical signals produced by offspring from different species of Dermaptera would be interested to do and see how they correlate with level of maternal investment observed for these species. These results will confirm whether there has been some selection on chemical signalling associated with increased level of maternal investment as general offspring solicitation signal in Dermaptera. Nevertheless, different chemical compounds may be found to have evolved a signalling function depending on the ecology of the species.

To conclude, the present research has demonstrated the evolution of an offspring chemical signal used as solicitation pheromone in the context of maternal care in the European earwig. The evolution of communication among individuals with diverging interest is capital for conflict resolution. Particularly chemical communication has been shown to have evolved in various contexts of social interactions and present in all forms of life, from plants to animals. Communication allows coordination of action such as social foraging but also reduces overt conflicts like in sexual competition for instance. As signallers and receivers need to co-evolve in order to communicate efficiently, communication could be interpreted as a cooperative behaviour. However, exploitation by cheaters is always the risk and mechanisms of recognition, coercion and punishment need sometimes to evolve as well to maintain stability of the strategy. Some of these mechanisms have been demonstrated in the present study organism. Therefore, the European earwig as one of the "other insect society" (Costa 2006) has proven to be a particularly interesting study species that offers ancestral traits such as facultative maternal care and partially begging offspring to study the origin and evolution of maternal care. The discovery of offspring chemical signals from this research allows now to test, manipulate and observe easily in the lab the dynamic of family life and to address further questions on the evolution of complex social behaviours.

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Table.x1 Relative percentage of each identified compounds per extract of 20 nymphs (%)

peak number	**retention time**	**compound**	**HF**		**LF**	
			mean	**s.e**	**mean**	**s.e**
1	12.89	n-C13	4,40	0,69	3,96	0,53
2	17.89	n-C15	3,72	0,43	3,61	0,43
3*	24.52	IS (n-C18)	3,98	0,35	3,75	0,27
4	30.29	n-C21	2,68	0,17	2,73	0,15
5-6	33.41	9-C23:1+C23:2	20,30	2,58	28,41	3,05
7	33.51	7-C23:1	6,98	0,93	8,56	0,99
8	33.84	n-C23	5,25	0,31	4,20	0,29
9	34.42	x-MeC23	0,26	0,07	0,21	0,09
10	35.02	y-MeC23	0,90	0,08	0,83	0,12
11	36.55	C25:2	5,84	0,66	6,40	1,42
12	36.63	9-C25:1	18,65	1,98	16,96	2,02
13	36.74	7-C25:1	7,39	0,87	6,12	0,75
14	37.01	n-C25	2,69	0,32	1,53	0,20
15	37.54	x-MeC25	1,56	0,20	1,59	0,31
16	38.12	y-MeC25	0,79	0,09	0,98	0,14
17	39.58	C27:2	4,97	0,75	4,26	0,62
18	39.63	x-C27:1	4,91	0,60	3,86	0,63
19	39.98	n-C27	1,03	0,12	0,52	0,06
20	40.45	x-MeC27	2,35	0,28	2,99	0,32
21	42.42	x-C29:1	3,62	0,25	1,63	0,18
22	45.05	n-C31	1,62	0,15	0,61	0,10
23	*45.52*	*cholesterol*	*0,10*	0,05	*0,03*	0,02

Table.x2 Absolute quantities per nymphs (ng)

peak number	**compound**	**HF**		**LF**	
		mean	**s.e**	**mean**	**s.e**
1	n-C13	14,20	*2,69*	12,92	*2,41*
2	n-C15	11,57	*1,75*	11,31	*1,65*
3*	IS				
4	n-C21	7,92	*0,74*	8,43	*0,81*
5-6	**C23-2+9C23**	59,99	*8,71*	86,41	*11,08*
7	7-C23:1	22,04	*3,81*	26,21	*3,72*
8	n-C23	15,50	*1,31*	12,64	*1,20*
9	x-MeC23	0,81	*0,24*	0,67	*0,32*
10	y-MeC23	2,72	*0,36*	2,60	*0,43*
11	C25:2	17,57	*2,60*	20,19	*4,22*
12	9-C25:1	56,53	*8,17*	51,53	*7,04*
13	7-C25:1	22,27	*3,23*	18,31	*2,24*
14	n-C25	8,17	*1,07*	4,89	*0,89*
15	x-MeC25	4,70	*0,77*	4,82	*0,92*
16	y-MeC25	2,29	*0,25*	3,05	*0,50*
17	C27:2	13,87	*1,64*	14,30	*3,41*
18	x-C27:1	16,29	*2,81*	11,75	*2,48*
19	n-C27	2,97	*0,34*	1,61	*0,24*
20	x-MeC27	6,63	*0,84*	9,12	*1,05*
21	x-C29:1	10,66	*1,09*	5,27	*0,81*
22	n-C31	4,65	*0,50*	1,92	*0,36*
23	cholesterol	0,21	*0,10*	0,09	*0,06*
	TOTAL	**301,58**	***25,51***	**308,05**	***23,51***

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EDUCATION

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Title: Maternal behaviour and the evolution of chemical signalling by offspring in the European earwig (*Forficula auricularia).*
Advisor: Dr. Mathias Kölliker
- Extraction and identification of cuticular hydrocabrons with GC-MS
- Behavioural assay to test mother's response and understand maternal care regulation
- Long-term effect of offspring signal on female's fecundity

M.Sc. in Animal Behaviour - Université Paris Sud XI, Orsay, FRANCE **2002- 2003**
Title: Speciation and mating choice between two sister species of Drosophila
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- Comparison of courtship behaviours
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B.Sc. in Biology – Université Laval, Québec, CANADA **1999- 2002**

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TECHNICAL SKILLS

Languages: French native; English fluent; Spanish good; German (niveau B1).

Computer: Pack Office (Word, Excel, Power Point), Access, Endnote, R statistical software, Jump, Observer 3.0, SIG Arcview 3.2 (basic), Adobe Photoshop.

Techniques: Behavioural assays, olfactometer, Gas-chromatography and Mass-spectrometry, SPME, Radio-telemetry, colour analysis, video analysis, statistical analysis.

OTHER ACTIVITIES

- Peer-reviewer for: Animal Behaviour, American Naturalist, Biology Letters, Tropical Zoology, Ecological Entomology.

- Teaching: course assistant in behavioural ecology, co-supervise student research projects

- Volunteer work: wildlife monitoring and radio-tracking in a private reserve in South Africa; deer monitoring (kilometrix index) for the National Office of Forestry (FR), conservation management of salamanders and toads with the Regional Natural Park of Chevreuse (FR), administrative tasks for Birdlife France and WWF France and Canada.

Publications:

- Mas F. & Kölliker M. (2011). An offspring signal of quality affects the timing of future parental reproduction.**Biology Letters.** doi: 10.1098/rsbl.2010.1094
- Mas F. & Kölliker M. (2011). Differential effects of brood chemical signals and individual offspring condition on maternal care in the European earwig. **Behavioural Ecology & Sociobiology.** 65: 341-349. doi: 10.1007/s00265-010-1051-8
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Conferences:

- 2009: ESEB, Torino & ISCE, Neuchatel : "A chemical signal of offspring quality affects maternal care in a social insect"- poster
- 2008: 4th meeting in Ecology and Behaviour, Toulouse : "Maternal care and offspring begging in the common earwig *Forficula auricularia*". Talk
- 2007: International Society of Chemical Ecology, Jena: "Regulation of maternal care by chemical communication in insects". Talk

Grants:

- Emilia Guggenheim-Schnurr Stiftung for 4 months of research (Jan- April 2010)
- Price award for best Poster, International Society of Chemical Ecology (ISCE) meeting in Neuchâtel, Switzerland (2009)
- Travel grant award for the International Society of Chemical Ecology (ISCE) meeting in Jena, Germany (2007)

Press released:
BBC One show, March 2010
BBC News, 13 May 2009: "Earwigs sniff out best babies"
Discovery News, Sept 2008: "Baby bugs bully parents for food and protection"