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ÉVOLUTION DE LA VIE DE FAMILLE CHEZ UN INSECTE PRÉCOCIAL

Importance des interactions sociales et de l'écologie

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Résumé

La vie de famille est un phénomène omniprésent dans la nature et constitue une étape clé dans l'évolution des sociétés animales. Comprendre les facteurs évolutifs qui ont permis son émergence et son maintien est donc fondamental pour étudier l'évolution de la vie sociale en général. A l'heure actuelle, un grand nombre d'étude se sont intéressées aux coûts et aux bénéfices des interactions sociales, et en particulier des soins parentaux durant la vie de famille et aux facteurs écologiques qui pouvaient influencer ces interactions. Cependant, la majorité de ces études se sont focalisées sur les espèces altriciales et eusociales chez qui la vie de famille est obligatoire pour la survie des jeunes et/ou permanente, créant une vision incomplète des processus responsables de l'évolution de la vie de famille dans les formes ancestrales de vie de groupe. Au cours de cette thèse j'ai exploré ce manque de connaissance en m'intéressant à l'évolution de la vie de famille chez un insecte subsocial précocial : Le forficule européen. Mon travail vise, dans un premier temps, à mieux comprendre les interactions mère-descendants et les bénéfices nets qu'elles apportent aux mères, puis à explorer l'impact de facteurs écologiques qui ont pu, peuvent ou pourront moduler ces les bénéfices de ces interactions.

Mes résultats montrent d'abord que chez le forficule européen, les soins maternels envers les œufs n'impliquent pas de discrimination de parentèle et que le cannibalisme filial partiel des œufs est une stratégie adaptative pour les femelles qui n'est pas lié à leur investissement dans les soins. Mes données suggèrent aussi que même si les femelles consomment les fèces de leurs juvéniles et peuvent en tirer des bénéfices, les juvéniles ne coopèrent pas de façon active avec leur mère en modulant la production de leurs fèces. Dans la deuxième partie de cette thèse, je souligne l'importance d'étudier les mécanismes de l'immunité sociale dans toutes formes de socialité pour mieux appréhender son rôle dans l'émergence de la vie de famille. Enfin, je montre que l'altération du microbiote intestinal par un antibiotique n'induit pas de modification dans l'expression des soins maternels alors qu'une exposition à une dose sublétale d'un pesticide pyréthrinoïde altère l'expression des soins maternels envers les œufs chez les femelles forficules.

Dans son ensemble, ce travail de thèse apporte une vision nouvelle de l'interaction mère-descendants et de l'impact de facteurs écologiques sous-estimés dans l'évolution de la vie de famille chez les insectes précociaux. Il ouvre ainsi de nouvelles perspectives d'étude sur l'évolution de la vie de famille au sein de ces formes ancestrales de vie sociale.

Abstract

Family life is a widespread phenomenon in animal kingdom and is a key step in the evolution of animal societies. Understanding the evolutionary factors that drove its emergence and maintenance is therefore fundamental to study the evolution of social life in general. Over the last decades, a large number of studies focused on the costs and benefits of social interactions, particularly parental care during family life, and the ecological factors that could influence these interactions. However, most of these studies focused on altricial and eusocial species in which family life is mandatory for the survival of the young and permanent, creating an incomplete view of the processes responsible for the evolution of family life in ancestral forms of group living. During this thesis I addressed this gap in knowledge by focusing on the evolution of family life in a precocial insect: the European earwig. My work first aimed to better understand mother-offspring interactions and the net benefits they bring to mothers and second, to explore the impact of ecological factors that have been, can or will be able to shape the benefits of these interactions for family members.

My results first show that in the European earwig, maternal egg care does not involve kin discrimination and that filial egg cannibalism is an adaptive strategy for females that is not linked to their investment in care. My data also suggest that although females consume the feces of their juveniles and may benefit from it, the juveniles do not actively cooperate with their mothers by modulating their feces production. In the second part of this thesis, I stress the importance of studying the mechanisms of social immunity in all forms of sociality to better understand its role in the emergence of family life. Finally, I show that the alteration of the intestinal microbiota by an antibiotic does not induce any modification in the expression of maternal care, whereas exposure to a sublethal dose of a pyrethroid pesticide alters the expression of maternal care towards the eggs in fortified females.

Overall, this thesis provides novel insights about the nature of the behavioral interactions occurring between mother and offspring, and about the impact of neglected ecological factors in the evolution of family life in precocial insects. This provides new perspectives for the study of the evolution of family life in these forms of social life.

SOMMAIRE

INTRODUCTION GÉNÉRALE

« Dans la vie, rien n'est à craindre, tout est à comprendre. »

Marie Curie

I-Évolution de la vie sociale

1-Les formes de vie sociale

La tendance des organismes à se rassembler pour former des groupes sociaux, appelée « socialité », est un phénomène omniprésent dans la nature. La socialité ne prend pas la même forme chez toutes les espèces et différentes formes plus ou moins complexes peuvent être observées. Dans le tableau 1 nous recensons ces formes de vie sociale dans un ordre de complexité croissant selon certains critères décrits en premier par Wilson en 1971 et expliqués dans ce paragraphe (Wilson, 1971). Il est important de noter qu'il existe plusieurs nomenclatures et que celle utilisée dans cette thèse correspond à celle décrite pour les formes de vie sociales chez les insectes (Tableau 1). Le degré de socialité le plus simple se retrouve chez les **espèces grégaires**, comme par exemple les criquets, et de nombreuses espèces de mammifères. Ces groupes sont formés par les individus issus de la même génération et sont généralement temporaires. Viennent ensuite les **espèces subsociales** qui forment des groupes familiaux composés d'un ou deux parents avec leurs petits. Les parents prodiguent des soins à leur descendants, ces soins pouvant être obligatoires pour la survie des jeunes (espèces altriciales) comme chez la plupart des mammifères et des oiseaux ou non-obligatoires (espèces précociales) comme on peut le retrouver chez le lièvre européen ou le scarabée nécrophore. Les **espèces coloniales**, quant à elles, désignent les espèces formant des groupes familiaux et partageant un site commun d'élevage. C'est notamment le cas de beaucoup d'espèces d'oiseaux marins qui se regroupent sur un même site de ponte pour se reproduire et s'occuper ensuite des petits. Si, en plus de partager un nid commun, les individus d'une même colonie coopèrent dans les soins aux jeunes, on parle alors d'**espèces communales**. Ce type de groupes sociaux se retrouve chez certaines espèces d'araignées et d'oiseaux ou encore chez les marmottes. Enfin, les groupes au degré de socialité le plus élevé correspondent aux **espèces eusociales,** comme les termites, les fourmis, et certaines espèces d'abeilles, de guêpes, de crevettes et de thrips et, chez les mammifères, les rats taupes nus. Ces groupes eusociaux, en plus de cumuler tous les critères décrits dans les formes précédentes (interactions entre individus, présence de soins parentaux, site d'élevage commun, coopération dans les soins aux jeunes), possèdent deux critères propres : (i) des individus qui sont spécialisés dans la reproduction et (ii) plusieurs générations d'individus qui se superposent au sein de la colonie (Wilson, 1971; Costa, 2006; Aron & Passera, 2009a).

Tableau 1: Classification des différents degrés de socialité en fonction de l'apparition de paramètres de complexité croissante. Tableau adapté de Aron and Passera 2009. Le « + » correspond aux caractères présents dans les différents degrés de socialité.

Degré de socialité	Attraction mutuelle	Soins parentaux	Site d'élevage commun	Coopération dans les soins aux jeunes	Individus spécialisés dans la reproduction
Solitaire					
Grégaire	+				
Subsocial	+	+			
Colonial	+	+	+		
Communal	+	+	+	+	
Eusocial	+	+	+	+	+

L'évolution de l'eusocialité, c'est-à-dire la transition entre les organismes vivant seuls et les organismes vivant en groupes sociaux complexes, est une étape majeure de l'évolution de la vie (Szathmáry & Smith, 1995). Bien que nos connaissances sur la diversité des formes de vie sociale dans la nature soient importantes (Bourke, 2011), comprendre l'évolution et en particulier l'émergence de la socialité reste encore à l'heure actuelle un défi en biologie évolutive. Afin de comprendre pourquoi les organismes s'associent et restent en groupe, il est important de comprendre quels bénéfices et quels coûts ils peuvent tirer individuellement de la coopération et de la vie de groupe.

2-Les bénéfices liés à la vie de groupe

Face aux contraintes écologiques, la vie sociale confère aux individus qui coopèrent de meilleures chances de survie et ainsi une meilleure valeur adaptative, aussi appelée « fitness » (Krause *et al.*, 2002). Cette coopération peut notamment baisser le risque de prédation, augmenter la probabilité d'acquérir des ressources alimentaires ou encore permettre une meilleure survie des jeunes. Face à des prédateurs, différents mécanismes de coopération peuvent être mis en place afin de diminuer le risque de prédation. La défense collective peut servir à prévenir les individus du groupe de l'attaque d'un prédateur grâce à des cris d'alarme, comme chez les marmottes *Marmota flaviventris* (Blumstein & Armitage, 1997). Elle peut permettre de chasser un prédateur avant que celui-ci n'attaque, comme chez la grive litorne *Turdus pilaris* (Andersson & Wiklund, 1978). Enfin, elle peut même permettre de lutter contre le prédateur si celui-ci décide d'attaquer, comme chez les buffles *Syncerus caffer* (Hayward & Kerley, 2005 ; Figure 1a). La coopération peut aussi augmenter l'efficacité dans la recherche et l'acquisition de nourriture. C'est le cas chez les grands prédateurs tels que les lycaons ou les lions (Creel, 1997), mais aussi chez les araignées sociales *Anelosimus eximius* et *Stegodyphus dumicola* (Figure 1b) qui effectuent une chasse coopérative pouvant mener à la capture de plus grosses proies (Avilés, 1997; Vakanas & Krafft, 2001). Enfin, chez les espèces communales et eusociales, la coopération dans les soins aux jeunes permet d'augmenter leur chance de survie. C'est le cas par exemple des geais de Floride *Aphelocoma coerulescens* ou des suricates *Suricata suricatta* (Figure 1c) où les jeunes adultes des reproductions précédentes ont un rôle d'assistant (« helpers » en anglais) et aident les reproducteurs dans la surveillance du territoire et l'apport de soins aux petits (Hailman *et al.*, 1994; Clutton-Brock *et al.*, 1998).

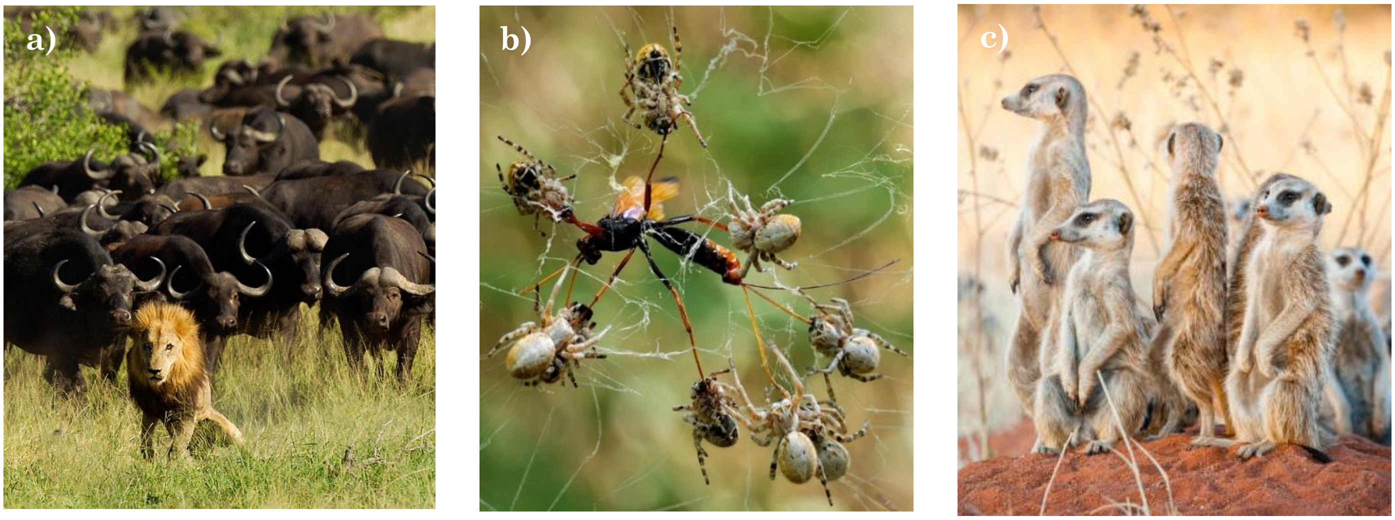

Figure 1: Exemples des bénéfices liés à la coopération dans la vie de groupe, a) défense collective chez les buffles *Syncerus caffer* contre l'attaque d'un lion (photo: L. Gregg), b) chasse coopérative chez l'araignée sociale *Stegodyphus dumicola* (photo: Wynand_uys) et c) soins aux jeunes par les assistants chez le suricate *Suricata suricatta* (photo: J. Parsons).

Si le niveau de coopération dans la vie sociale dépend des contraintes écologiques présentes dans l'environnement, il peut aussi être influencé par le lien de parenté génétique entre les individus. Ce paramètre a été formulé par Hamilton en 1964. Il propose notamment le concept de la « fitness inclusive » qui correspond aux bénéfices indirects qu'un individu va retirer lorsque celui-ci transmet ses gènes par le biais d'autres individus génétiquement apparentés – ce qui est souvent le cas au sein d'un groupe (Hamilton, 1964). Ainsi les individus d'un groupe auront davantage intérêt à coopérer avec des individus qui leur sont apparentés génétiquement qu'avec des individus non apparentés. Cette théorie dite de « sélection de parentèle » reste à l'heure actuelle une des pierres angulaires dans l'étude des sociétés animales et propose notamment d'expliquer des comportements jusqu'alors considérés comme altruistes comme la présence des assistants chez beaucoup d'espèces animales (Clutton-Brock, 2002), le sacrifice des abeilles lors de la défense de la colonie (Shorter & Rueppell, 2012) ou encore la présence d'individus stériles dans un grand nombre d'espèces eusociales (Queller & Strassmann, 1998). Il est cependant important de noter que même si la sélection de parentèle explique un grand nombre de comportements coopératifs au sein des groupes sociaux, celle-ci n'est pas un prérequis aux interactions coopératives qui peuvent aussi exister entre individus non apparentés dans de nombreux exemples (Clutton-Brock, 2009).

3-Les coûts liés à la vie de groupe

Bien qu'il soit évident que le fait de vivre en groupe apporte des bénéfices aux individus qui coopèrent, la vie sociale peut aussi être le théâtre de coûts non négligeables (Aron & Passera, 2009b). D'une part, ces coûts dérivent du fait que la sélection naturelle favorise toujours les individus présentant une meilleure fitness, même si cela se fait au détriment de celle des autres (Darwin & Wallace, 1958). Parce qu'une façon de maximiser sa fitness est d'essayer de monopoliser les ressources ou la reproduction, des phénomènes de compétition ou de conflits sociaux peuvent émerger chez les espèces vivant en groupe. Chez le babouin du Cape *Papio ursinus*, comme chez de nombreuses autres espèces de primates vivant en groupes composés de plusieurs males, des conflits ont lieu pour l'accès à la nourriture et aux femelles. Ces conflits peuvent prendre la forme de cris agressifs, de poursuites voire de combats entrainant parfois de graves blessures (Bulger, 1993; Kitchen *et al.*, 2003 ; Figure 2a). Chez les insectes eusociaux, bien que la production des descendants soit assurée par des individus spécialisés (c.à.d. les reproducteurs), des conflits pour la reproduction peuvent aussi avoir lieu (Heinze, 2004). Dans certaines sociétés de fourmis par exemple, des ouvrières (*i.e.* non spécialisées dans la reproduction) tentent de se reproduire en pondant des œufs non fécondés (ce qui donne des mâles), un système de « police » peut alors être mis en place pour détruire ces œufs et punir les ouvrières (Ratnieks & Visscher, 1989; Ratnieks & Wenseleers, 2005).

D'autre part, la promiscuité et le fort apparentement génétique des individus au sein d'un même groupe augmentent le risque d'infection et de propagation d'agents pathogènes (Schmid-Hempel, 1998; Pie *et al.*, 2004; Cremer *et al.*, 2007; Stroeymeyt *et al.*, 2014; Meunier, 2015). Les nombreuses épidémies humaines qui ont eu lieu au cours de l'histoire en sont de parfaits exemples. La plus marquante est surement celle de la peste noire, causée par la bactérie *Yersinia pestis,* qui a ravagé l'Europe au XIV^e siècle, provoquant la mort de 25 à 50 % de la population (Cohn, 2002). La plus récente est celle liée au coronavirus SARS-Cov-2 que nous rencontrons actuellement et qui a débuté à Wuhan en Chine fin 2019. Le virus s'est propagé en l'espace de quelques mois à l'échelle mondiale faisant plus de 800 000 morts (données du 22 Août 2020 à 15h30 ; Dong et al. 2020 ; Figure 2b)

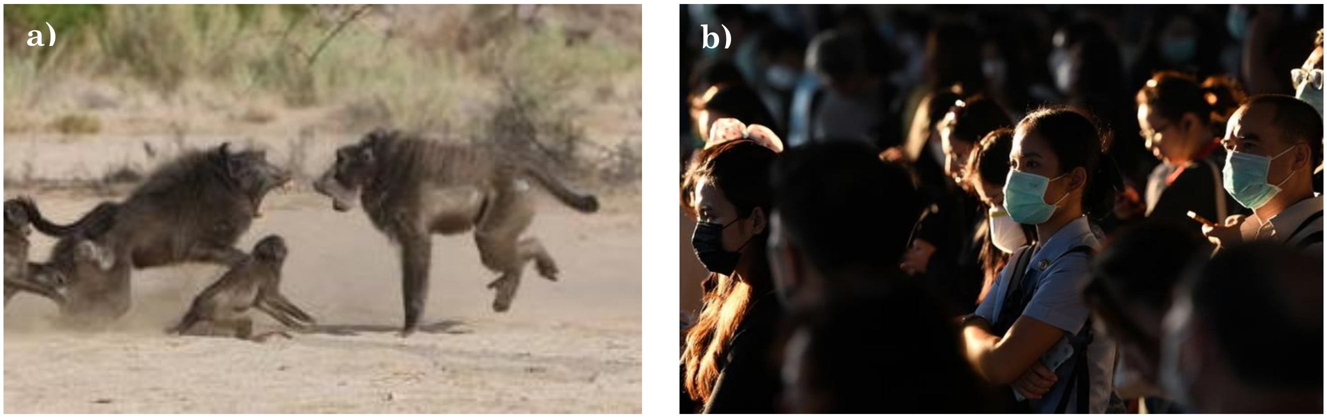

Figure 2: Exemple de coûts liés à la vie de groupe, a) combat entre mâles chez le babouin du Cape *Papio ursinus* pour l'accès à la reproduction (photo: E. Huchard), et b) pandémie chez l'homme provoquée par le coronavirus SARS-Cov-2 débutée en 2019 (photo: Reuters).

La vie sociale n'a pu évoluer que chez les espèces capables de limiter les coûts de la vie sociale, ce qui équivaut à des bénéfices nets supérieurs à zéro (Bourke, 2011). Ce rapport coûts/bénéfices est donc un mécanisme qu'il est essentiel de comprendre afin d'améliorer notre compréhension de l'évolution des sociétés animales.

4-Les transitions évolutives de la vie sociale

Les bénéfices et les coûts liés à la vie en groupe ne sont pas équivalents dans les différentes formes de vie sociales. Parce que les chercheurs portent un grand intérêt pour les formes de socialités les plus avancées, un grand nombre d'études a été réalisé chez les espèces eusociales, ce qui a permis d'enrichir nos connaissances sur les coûts et les bénéfices présents chez ces espèces. Cependant, très peu d'études se sont intéressées aux formes ancestrales de vie de groupe telles que les espèces subsociales. Or, il a été proposé que cette forme de vie sociale marque la première étape dans l'évolution de la socialité. Lin et Michener (1972) qui se sont intéressés à l'origine de la vie sociale, proposent que la transition initiale de l'état solitaire à celui de social pourrait avoir suivi deux grandes voies évolutives: **la voie parasociale** et **la voie subsociale** (Lin & Michener, 1972). Le chemin évolutif parasocial correspond au regroupement temporaire d'individus de même génération. Cette voie est centrée sur les bénéfices que peut apporter la coopération pendant la vie de groupe. Le chemin subsocial, quant à lui, correspond à l'association d'au moins un des parents avec sa descendance formant ainsi une unité familiale. Cette voie repose en particulier sur les bénéfices directs des soins parentaux pour les juvéniles qui devraient favoriser la vie de groupe en retardant la dispersion des jeunes de façon plus ou moins permanente. Ces jeunes pourraient alors par la suite coopérer avec leurs parents pour les soins envers leurs frères et sœurs (stade communal), ce qui aurait ensuite mené à une spécialisation des individus dans la reproduction au sein des sociétés eusociales (Boomsma & Gawne, 2018). Même si les deux scenarii ont pu mener à l'évolution des structures sociales avancées, le chemin subsocial semble le plus probable compte tenu des bénéfices des interactions sociales entre les membres d'une même famille, donc apparentés génétiquement, favorisant ainsi la fitness inclusive (Michener, 1969; Wilson, 1975; Bourke, 2011). **L'étude des bénéfices et des coûts liés aux interactions sociales au sein des unités familiales semble donc être primordiale pour comprendre l'évolution et le maintien des formes de vie sociale plus complexes (Clutton-Brock, 1991; Gross, 2005).**

II-Évolution de la vie de famille

1-Unité familial et interactions sociales

La vie de famille se définit par l'association d'un ou deux parents s'occupant d'un ou plusieurs descendants après leur éclosion ou leur naissance (Kramer & Meunier, 2019). C'est un phénomène commun dans le règne animal que l'on peut retrouver chez les mammifères, les oiseaux, les reptiles, les poissons mais aussi chez les invertébrés comme les annélides, les mollusques et les insectes (Clutton-Brock, 1991; Balshine, 2012; Wong *et al.*, 2013 ; Figure 3). Cette vie familiale est extrêmement variable au sein de ces différents taxa et même entre les différentes espèces. Elle peut par exemple varier en termes de composition, les groupes familiaux pouvant être composés d'un ou de plusieurs juvéniles associés à leurs deux parents ou uniquement à leur mère ou à leur père. Elle est également variable dans la persistance et l'importance des interactions sociales. La vie de famille peut, en effet, ne durer que quelques heures et être facultative chez les espèces subsociales précociales comme chez le colin de Californie ou le forficule européen (Kölliker, 2007). Elle peut également être permanente et obligatoire dans les sociétés eusociales des fourmis ou des termites (Hölldobler & Wilson, 1990; Costa, 2006; Koenig & Dickinson, 2016).

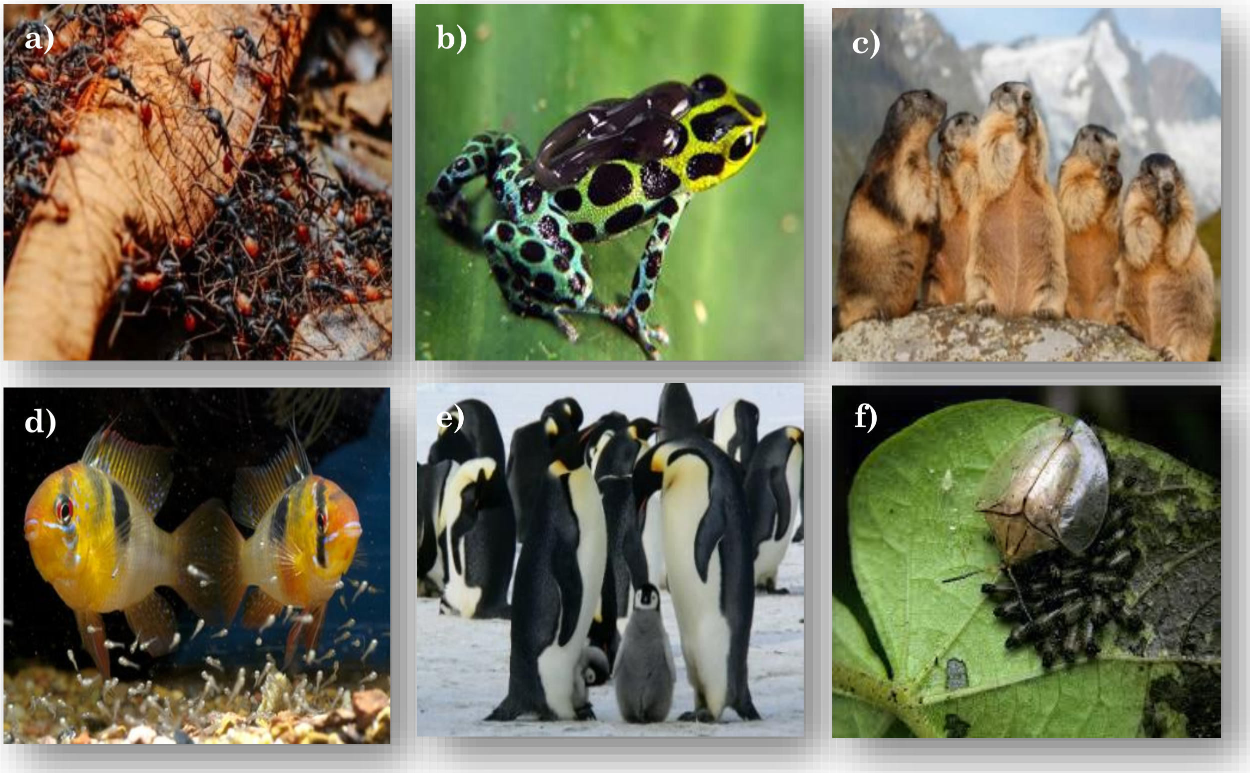

Figure 3: Exemples de la diversité de la vie de famille dans la nature à travers différentes espèces et différents taxa. Chez a) la fourmi légionnaire *Eciton burchellii* (photo: G. Gallice), b) la dendrobate *Ranitomeya variabilis* (photo: J.L Brown), c) la marmotte des Alpes *Marmota marmota* (photo: H. Toni), d) le cichlidé ramirezi *Mikrogeophagus ramirezi* (photo : wordpress.com, e) le manchot empereur *Aptenodytes forsteri* (photo: Pixabay), f) la chrysomelidae *Acromis sparsa* (photo: A. Anker).

L'unité familiale forme un environnement social dans lequel les interactions entre les individus engendrent, en plus des bénéfices et des coûts liés à la vie de groupe (voir section I-2 et I-3 de l'introduction), des multiples formes de coopérations et de conflits. Dans de nombreuses l'étude de la vie de famille est majoritairement associé à l'étude des bénéfices et des coûts liés aux soins parentaux (Clutton-Brock, 1991; Gross, 2005; Schrader *et al.*, 2015; Duarte *et al.*, 2016; Jarrett *et al.*, 2017)

Les soins parentaux sont définis, selon Royle et al. (2012), comme le regroupement de n'importe quelle expression du comportement parental permettant d'augmenter la fitness des descendants et qui ont été sélectionnés dans ce but (Clutton-Brock, 1991; Royle *et al.*, 2012). Ces comportements divers et variés peuvent se traduire par la construction d'un nid, l'approvisionnement en nourriture ou encore la défense contre d'éventuels prédateurs. Chez la mésange bleue *Cyanistes caeruleus* par exemple*,* les femelles nourrissent (Figure 4a) et protègent les oisillons des pathogènes en agrémentant le nid avec des plantes aromatiques aux propriétés antibactériennes et antifongiques (Mennerat *et al.*, 2009). A travers ces formes de soins, les parents minimisent les contraintes de l'environnement et apportent des bénéfices directs aux juvéniles en augmentant leur chance de survie et ainsi leur fitness. A l'inverse, l'expression des soins parentaux peut induire des coûts importants pour les parents comme un risque accru à la prédation et au parasitisme ou encore une altération de leur condition énergétique, ce qui résulte en général à une baisse d'investissement dans une reproduction future (Trivers, 1972; Daan *et al.*, 1990; Clutton-Brock, 1991; Royle *et al.*, 2012). Ainsi, chez l'araignée loup *Pardosa saltans*, en plus de coûts physiologiques (Ruhland *et al.*, 2016), le transport du cocon diminue la mobilité des femelles ce qui altère leur efficacité dans la capture de proie et augmente le risque de prédation (Colancecco et al. 2007, Figure 4b).

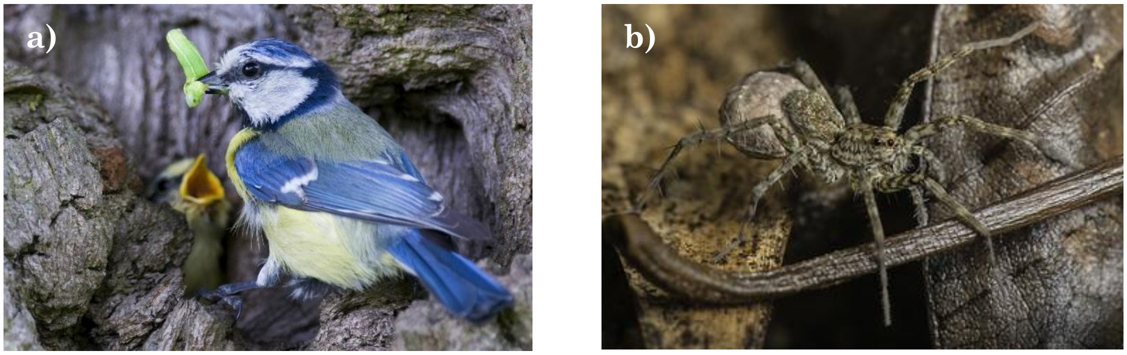

Figure 4: Illustration du bénéfice des soins parentaux pour les descendants a) chez la mésange bleue *Cyanistes caeruleus* qui approvisionnent ses oisillons avec de la nourriture et des coûts associés pour les parents (photo: alliance/dpa), b) chez l'araignée loup*, Lycosidae pardosa* où la mobilité de la mère est réduite par le port du cocon (photo: J. Tan).

Les soins parentaux impactent donc de façon importante la fitness des parents et des descendants (Alonso-Alvarez & Velando, 2012; Klug & Bonsall, 2014). Parce que la sélection naturelle favorise les individus présentant une meilleure fitness, et qu'une façon de maximiser sa fitness est de limiter les coûts ou d'optimiser les bénéfices liés à la vie de famille, les soins parentaux sont la source de conflits sociaux entre les membres de l'unité familiale (Parker *et al.*, 2002; Royle *et al.*, 2004). Ainsi, des conflits peuvent apparaître (i) entre les deux parents qui essayent, l'un comme l'autre, de réduire leur investissement dans les soins (Trivers, 1972; Steinegger & Taborsky, 2007; Meunier & Kölliker, 2012a; Smiseth, 2019), (ii) entre les juvéniles pour le monopole des soins parentaux (Mock & Parker, 1997; Roulin & Dreiss, 2012) et (iii) entre les parents et les juvéniles car la sélection favorise, d'un côté, les juvéniles qui demandent davantage de soins alors que, de l'autre côté, elle favorise les parents qui optimisent au mieux la quantité de soins qu'ils prodiguent (Trivers, 1974; Arroyo *et al.*, 2002; Maestripieri, 2002; Kölliker *et al.*, 2015).

2-Les limites de la vision traditionnelle de la vie de famille

A ce jour, beaucoup d'études se sont intéressées aux coûts et aux bénéfices des interactions sociales et notamment des soins parentaux au sein de la vie de famille (Schrader *et al.*, 2015; Duarte *et al.*, 2016). Cependant, la majorité de ces études ont été réalisées chez des espèces de mammifères, d'oiseaux ou d'insectes eusociaux présentant des caractéristiques altriciales, c'est-à-dire où les soins parentaux sont obligatoires pour la survie des descendants (Clutton-Brock, 1991; Stearns, 1992; Balshine, 2012). Or, comme les jeunes sont entièrement dépendants de l'expression des soins parentaux pour leur survie (Clutton-Brock, 1991; Starck & Ricklefs, 1998), les bénéfices de ces soins pour les jeunes sont dans tous les cas extrêmement importants et cela crée obligatoirement un biais dans notre compréhension de l'évolution de la vie de famille. Ainsi, la représentation des interactions familiales semble correspondre uniquement aux espèces ayant une vie familiale déjà évolutivement dérivée ce qui est une limite si on désire comprendre l'émergence de la vie de famille à un stade ancestral (Costa & Fitzgerald, 1996; Kramer & Meunier, 2019).

Chez les espèces précociales, les jeunes sont autonomes dès leur naissance et n'ont donc pas besoin des soins parentaux pour leur survie (Starck & Ricklefs, 1998). Ils sont donc par exemple en capacité de compenser l'absence des parents et/ou de leurs soins, ce qui permet d'avoir une autre vision des coûts et des bénéfices liés aux soins parentaux et aux autres interactions sociales durant la vie de famille (Smiseth *et al.*, 2003; Kölliker, 2007; Klug *et al.*, 2012; Kramer & Meunier, 2019). Ces espèces présentent une forme de vie sociale plus précoce et semblent donc être de bons modèles d'étude pour comprendre l'émergence et le maintien de la vie de famille. Des études récentes chez le forficule européen, un insecte précocial, ont notamment mis en lumière des interactions sociales jusqu'ici négligées au sein de la vie de famille. Ces études révèlent qu'en dehors des soins parentaux prodigués par les femelles (voir Encadré 1 pour plus de détails), les mères et leurs juvéniles rentrent en compétition pour le monopole des ressources alimentaires lorsqu'elles sont limitées et que cela occasionne des coûts importants pour les juvéniles (Meunier & Kölliker, 2012b; Kramer *et al.*, 2017). De plus, les juvéniles coopèrent entre eux et ce d'autant plus lorsque les mères sont déficientes dans l'expression de leurs soins (Falk *et al.*, 2014; Kramer *et al.*, 2015). **La mise en évidence de ces interactions a permis de montrer la complexité sous-estimée des interactions sociales dans la vie de famille chez les espèces précociales et appelle donc à continuer les recherches chez ces espèces afin de revoir notre vision de la vie de familiale. Plus précisément, étant donné la place importante des soins parentaux dans la vie de famille, il semble crucial d'étudier de nouveau le rôle de cette interaction chez les espèces aux formes ancestrales de vie de groupe.**

3-Reconsidération de l'interaction parent-descendants chez les espèces précociales

Discrimination de parentèle pendant les soins parentaux

Nous l'avons vu dans la partie précédente, les soins parentaux apportent des bénéfices importants aux descendants en diminuant les effets des contraintes environnementales et engendrent des coûts non négligeables pour les parents. Pour ces raisons, il est attendu selon la théorie de la sélection de parentèle (Hamilton, 1964) que les parents soient sélectifs dans la distribution de leurs soins et qu'ils les dirigent essentiellement envers leurs propres descendants. Ainsi, les femelles vont adapter leur comportement de soins et rejeter les petits qui ne sont pas les leurs chez le cerf élaphe *cervus elaphus* et de nombreux autres mammifères exprimant des soins parentaux (Lévy *et al.*, 2004; Corona & Lévy, 2015; Sauvé *et al.*, 2015). Néanmoins, la discrimination de parentèle n'est pas un mécanisme obligatoirement associé aux soins parentaux et des pressions de sélection peuvent aussi, à l'inverse, favoriser l'acceptation de descendants (œufs et juvéniles) étrangers et/ou contre-sélectionner le rejet des descendants étrangers (Penn & Frommen, 2010). En effet, si des mécanismes qui permettent de limiter la probabilité de rencontre avec des œufs ou des juvéniles d'autres femelles (comme la construction de nid) existent déjà ou si les soins parentaux sont peu couteux alors la discrimination de parentèle n'a que peu de chance d'évoluer (Tallamy, 2005; Klug *et al.*, 2012). L'existence de la discrimination de parentèle pendant les soins aux œufs dépend donc du type de soins prodigués par les parents, de l'environnement social des individus (forte densité de population) mais aussi de l'histoire évolutive de l'espèce étudiée (Penn & Frommen, 2010). Bien documentée chez les espèces altriciales, la discrimination de parentèle durant les soins parentaux reste peu étudiée chez les insectes précociaux et en particulier lorsque les soins sont dirigés envers les œufs. **Afin de mieux comprendre comment les soins maternels sont déterminés chez le forficule européen, nous avons, dans le premier chapitre de cette thèse étudié la discrimination de parentèle dans l'expression des soins envers les œufs.**

Le cannibalisme filial partiel des œufs pendant les soins parentaux

Pendant l’expression des soins parentaux, un comportement de cannibalisme de la part des parents envers leurs œufs ou leurs juvéniles peut être observé chez de nombreuses espèces de mammifères, de reptiles, de poissons et d’arthropodes (Polis, 1981; Elgar & Crespi, 1992). Consommer une partie de sa descendance, bien que cela puisse paraître de premier abord comme un comportement contre-intuitif pendant les soins parentaux, est considéré comme une stratégie adaptative car elle apporte de nombreux bénéfices aux parents et aux descendants restant. Ce cannibalisme dit « filial » et « partiel » permet par exemple aux parents du scarabée nécrophore *Nicrophorus Fabricius* de réduire la taille de la couvée en fonction de la quantité de nourriture disponible et ainsi de limiter la compétition future entre les juvéniles (Bartlett, 1987). Chez le poisson dard *Etheostoma squamiceps*, les pères consomment préférentiellement les œufs infectés par des microorganismes pathogènes ce qui réduit la probabilité d’infection du reste de la ponte (Bandoli, 2016). Enfin, le cannibalisme filial d’une partie de la descendance peut permettre de réduire la taille de la couvée ce qui permet aux femelles de réallouer des ressources énergétiques à la reproduction future et/ou à une meilleure qualité de soins à la couvée actuelle comme on peut le retrouver chez le hamster doré *Mesocricetus auratus* et de nombreuses espèces de poissons (Day & Galef, 1977; Sargent, 1992; Manica, 2002, 2004). Bien que le cannibalisme filial partiel de la descendance semble être un aspect important dans l’expression des soins parentaux chez de nombreuses espèces appartenant à différents taxa, la plupart des travaux théoriques et empiriques pour comprendre son évolution ont été réalisés chez les poissons (voir Manica, 2002). De plus, la signification évolutive du cannibalisme filial partiel, ainsi que son lien avec l’expression des soins parentaux, restent mal compris dans de nombreux systèmes. **Pour ces raisons, nous avons étudié les modalités de l’expression de ce comportement et son lien avec l’expression des soins maternels envers les œufs chez le forficule européen dans le second chapitre de cette thèse.**

Une interaction coopérative possible des descendants vers les parents ?

Les soins parentaux, parce qu'ils ont été principalement étudiés chez les espèces altriciales, ont longtemps été considérés comme la seule forme d'interaction coopérative entre les parents et les descendants (Kramer & Meunier, 2019; Socias-Martínez & Kappeler, 2019). Cependant, un nombre croissant d'études souligne que d'autres formes de coopération, jusqu'ici sous-estimées, peuvent se produire entre les membres de l'unité familiale et que les bénéfices liés à ces interactions pourraient avoir un rôle clé dans l'émergence de la vie de famille chez les espèces où les soins parentaux ne sont pas obligatoires pour la survie des juvéniles (comme chez les espèces précociales) (Kramer & Meunier, 2019). Parmi les formes négligées d'interactions sociales, il y a la présence de comportements coopératifs des descendants envers leurs parents. Or, des comportements de ce type ne sont pas rares dans les formes de vie sociale avancées, comme les espèces communales et eusociales (Brown, 1987; Taborsky, 1994). Chez ces espèces, les descendants, qui sont généralement des jeunes adultes, aident leurs parents pour diverses tâches comme la protection du territoire, la recherche de nourriture ou encore les soins aux jeunes (Hailman *et al.*, 1994; Clutton-Brock *et al.*, 1998). Chez les geais de Floride *Aphelocoma coerulescens* par exemple, les jeunes nés au début de l'année aident leurs parents dans la défense du territoire et les soins aux jeunes (Hailman *et al.*, 1994). Dans les colonies d'insectes eusociaux (Rubenstein & Abbot, 2017), les parents (les reines et parfois les rois) sont presque exclusivement nourris par leur descendante ouvrière (qui sont des jeunes dans les sociétés de termites ; Eggleton, 2011). Malgré l'importance de cette interaction, elle reste néanmoins inexplorée chez les espèces précociales alors que chez ces espèces les juvéniles sont autonomes dès leur naissance et on donc les capacités d'aider leurs parents. **Nous avons donc testé si cette interaction coopérative des juvéniles envers leur mère pouvait exister chez le forficule européen dans une troisième étude.**

4-Impact des facteurs écologiques sur les interactions sociales

Les bénéfices et les coûts liés aux interactions sociales au sein de la vie de famille ou de la vie de groupe peuvent généralement être modulés par des facteurs écologiques biotiques et abiotiques (Van Schaik, 1996; Hatchwell & Komdeur, 2000; Wong *et al.*, 2013). En effet, certaines contraintes écologiques comme le parasitisme, la pression de prédation, le climat ou la disponibilité en nourriture ont pu favoriser ou contre-sélectionner l'apparition des soins parentaux en modulant le rapport coûts/bénéfices qu'ils apportaient aux juvéniles et aux parents (Webb *et al.*, 2002; Lion & van Baalen, 2007; Klug & Bonsall, 2010; Pike *et al.*, 2016). Chez le lézard *Mabuya longicaudat*, c'est la prédation qui joue un facteur clé dans l'expression des soins maternels. Lorsque le prédateur des lézards adultes est absent de l'environnement, les femelles prodiguent des soins à leurs œufs, ce qui augmente le taux d'éclosion de façon drastique (Huang, 2006). Cependant, si le prédateur des femelles est présent dans le milieu, les femelles abandonnent leurs œufs car le risque de prédation est alors trop important (Huang, 2006). Chez la souris rayée *Rhabdomys pumilio,* c'est l'environnement abiotique qui module l'expression des soins paternels et la forme de vie sociale. Ainsi, dans les prairies humides où la température nocturne est modérée, les mâles ne participent pas aux soins et les nids sont très espacés entre les femelles (Schradin & Pillay, 2005). Les souris rayées dans cet environnement sont solitaires. A l'inverse, dans les régions semi-désertiques, les températures nocturnes sont plus fraiches, les nids des différentes femelles sont proches et permettent la coopération dans les soins. De plus, l'investissement du mâle dans les soins permet une croissance plus rapide des jeunes (Schradin & Pillay, 2005). Les bénéfices nets en termes de survie pour les juvéniles dans les zones semi-désertiques ont donc favorisé l'apparition des soins paternels chez cette espèce et une forme de vie sociale communale (Schradin & Pillay, 2004).

Les facteurs écologiques pouvant impacter les bénéfices et les coûts des interactions sociales sont nombreux et leurs effets dépendent de l'écologie de chaque espèce. Dans la littérature, la majorité des études sur le sujet s'est intéressée à l'influence du climat, de la pression de prédation et de la structure des habitats sur l'évolution des soins parentaux. Cependant, des facteurs jusqu'à maintenant négligés dans la littérature attirent de plus en plus l'attention. C'est le cas des agents pathogènes, des microorganismes du microbiote intestinal et des substances chimiques anthropiques comme les pesticides.

Interactions avec les pathogènes présents dans l'environnement

Au cours de son cycle de vie, chaque animal interagit avec un grand nombre d'agents pathogènes présents dans son environnement tels que des virus, des procaryotes unicellulaires, des bactéries, des helminthes et des champignons (Lu & St. Leger, 2016). Ces agents pathogènes peuvent engendrer des coûts importants sur la fitness des individus en altérant leur survie ou leur capacité de reproduction (Schmid-Hempel, 2014). Ces coûts pour les individus peuvent par extension moduler les coûts et les bénéfices de la vie sociale. En effet, la vie en groupe peut, d'un côté, être un terrain propice à la transmission des pathogènes car les contacts fréquents entre les individus et leur apparentement génétique fort augmentent le risque d'une propagation efficace des pathogènes (Pie *et al.*, 2004, 2005; Masri & Cremer, 2014; Stroeymeyt *et al.*, 2014). De ce point de vue, la présence de pathogènes dans l'environnement pourrait limiter les interactions familiales. D'un autre côté, de nombreuses études ont montré que le fait de vivre en famille (c.-à-d. en groupe) permettait aussi une meilleure protection face aux pathogènes via des mécanismes liés à l'immunité sociale (Hughes *et al.*, 2002; Cremer *et al.*, 2007). Chez les singes par exemple, l'épouillage social permet d'enlever certains parasites comme les puces, les poux ou les tiques qui peuvent être vecteur de maladies (Nunn *et al.*, 2006). Très étudié chez les insectes eusociaux, notamment les fourmis, de récentes études ont démontré que le réseau de contacts entre les différents individus de la colonie permettait une sorte de vaccination (« immune priming » en anglais) généralisée à tous les individus de la colonie tout en limitant l'infection par l'agent pathogène. Ainsi, lors d'une deuxième infection par ce même pathogène, les fourmis avaient de meilleurs chances de survie (Konrad *et al.*, 2012, 2018)(Konrad et al., 2012 ; Konrad et al., 2018).

Dans ce cas de figure, la vie de famille aurait pu être favorisée en présence de pathogènes via les bénéfices de l'immunité sociale (Meunier, 2015). A l'heure actuelle, le rôle des pathogènes et de l'immunité sociale dans l'évolution des formes de vie eusociale est bien étudié, mais il reste cependant peu connu dans les formes ancestrales de vie de groupe. **Dans le chapitre 4 de cette thèse nous avons décidé de faire une revue de la littérature sur l'immunité sociale dans toutes les formes de vie sociale afin de comprendre son rôle et celui des pathogènes dans l'évolution de la vie de famille.**

Interactions avec le microbiote intestinal

Tous les animaux arborent une communauté microbienne plus ou moins complexe dans leur système digestif, aussi appelée microbiote intestinal (Engel et Moran 2013; Stilling et al. 2014). Bien connu chez les termites chez qui l'impact du microbiote entretient une symbiose nutritionnelle mutualiste(Cleveland, 1923; Brune & Dietrich, 2015), cet impact reçoit de plus en plus d'attention. En effet, de nombreuses études démontrent que les microorganismes présents dans le tube digestif des animaux peuvent impacter de nombreux traits d'histoire de vie comme l'immunité et la reproduction, ils pourraient même influencer l'expression de certains comportements et notamment des comportements sociaux. En effet, chez la blatte grégaire *Blaberus discoidalis* comme chez la fourmi coupe feuille *Acromyrmex echinatior*, il semblerait que la communauté microbienne intestinale joue un rôle sur la socialité des individus et qu'une altération du microbiote engendre l'expression de comportements sociaux anormaux (Wada-Katsumata *et al.*, 2015; Teseo *et al.*, 2019). L'influence importante du microbiote intestinal sur les comportements sociaux a conduit à l'élaboration d'une théorie selon laquelle les microorganismes présents dans le système digestif favoriseraient les interactions sociales coopératives permettant ainsi d'augmenter leur efficacité de transmission d'un individu à un autre (Troyer, 1984; Nalepa *et al.*, 2001; Lombardo, 2008; Lewin-Epstein *et al.*, 2017). Chez les insectes eusociaux par exemple, il est maintenant connu que les interactions sociales telles que le partage de nourriture par trophallaxie ou coprophagie permet la transmission totale ou partielle du microbiote intestinal au sein des membres de la colonie (Nalepa, 1984; Powell *et al.*, 2014). Cependant, et en dépit du fort potentiel du microbiote intestinal dans l'évolution de la socialité, très peu d'études se sont intéressées à son impact sur les soins parentaux (Lewin-Epstein *et al.*, 2017; Gurevich *et al.*, 2020). **Nous nous sommes intéressés au rôle du microbiote intestinal sur l'expression des soins maternels envers les œufs et envers les juvéniles chez le forficule européen dans le chapitre 5 de cette thèse.**

Interactions avec des substances chimiques anthropiques

Depuis la révolution industrielle, de nombreux scientifiques, dont Paul Crutzen et Eugene Stoermer (2000), ont désigné l'époque géologique dans laquelle nous vivons comme celle de l'Antropocène, autrement dit « l'ère de l'humain » (Crutzen & Stoermer, 2000). Bien que ce concept d'Anthropocène puisse faire débat, l'influence de l'activité humaine sur les autres espèces animales est considérable et pourrait jouer un rôle dans les interactions sociales (Le Tallec *et al.*, 2015). Une des activités principales de l'Homme étant l'agriculture, l'utilisation massive de molécules chimiques telles que les pesticides est largement répandue à travers le monde dans le but de lutter contre les arthropodes nuisibles et d'optimiser les rendements agricoles. Or, ces substances chimiques dispersées dans l'environnement peuvent aussi avoir des conséquences non négligeables (même à des doses sublétales) sur la physiologie et les comportements d'espèces non ciblées (Desneux *et al.*, 2007; Arya *et al.*, 2019). Au sein des insectes, l'exposition à l'azadirachtine (un limonoïde) par exemple, perturbe la recherche d'un hôte de ponte chez la guêpe parasitoïde *Hyposoter didymator* (Schneider *et al.*, 2004), tandis que la deltaméthrine (un pyréthrinoïde) altère la mémoire et la recherche de nourriture chez l'abeille domestique *Apis mellifera* (Ramirez-Romero *et al.*, 2005). Alors que de plus en plus de preuves s'accumulent sur la multitude des effets de l'exposition à des pesticides sur le comportement de plusieurs espèces animales (Müller, 2018; Arya *et al.*, 2019; Parkinson *et al.*, 2020), leur impact sur l'expression des soins maternels et sur la vie de famille reste très peu étudié voire inexploré chez les insectes. Or, montrer que les pesticides affectent l'expression des soins parentaux et modulent les coûts et les bénéfices des interactions sociales au sein de la vie de famille pourrait être d'une importance capitale dans notre utilisation future des pesticides dans l'agriculture. **Aussi nous nous sommes interrogés sur l'effet de la deltaméthrine (un pesticide très utilisé en agriculture) dans l'expression des soins maternels chez le forficule européen dans une dernière étude.**

IV- Problématique de la thèse

Ce travail de thèse a pour but d'étudier l'évolution de la vie de famille à travers les interactions sociales et l'écologie chez un insecte précociale, le forficule européen. Nous l'avons organisé en deux parties basées sur un travail de recherche valorisé en six publications scientifiques.

Dans la **première partie** de cette thèse nous nous sommes intéressés aux paramètres ayant favorisé les soins maternels chez une espèce précociale, le forficule européen (**Chapitre 1 et 2**) et nous nous sommes interrogés sur l'existence d'autres aspects des interactions mères-juvéniles peuvent exister chez cette espèce et ainsi avoir un rôle important – bien que jusqu'ici négligé – dans l'émergence et le maintien de la vie de famille dans la nature (**chapitre 3**).

Dans la **deuxième partie** de cette thèse, nous avons voulu savoir si certains facteurs écologiques tels que les pathogènes (**Chapitre 4**), le microbiote intestinal (**Chapitre 5**) et les pesticides (**Chapitre 6**) ont pu ou pourront, par leur effet sur ces interactions sociales mère-descendants, favoriser ou à l'inverse limiter les bénéfices de ces interactions et donc modifier l'évolution de la vie de famille.

Encadré 1

Le modèle biologique de cette thèse

Présentation de l'espèce

Le forficule européen *Forficula auricularia* L., plus communément appelé pince- ou perce-oreille, est un insecte dermaptère appartenant à la famille des *Forficulidae* (Linné) qui compte à l'heure actuelle environ 500 espèces. Largement répandu en France, en Europe, au nord de l'Afrique du Nord et à l'ouest de l'Asie d'où il est natif, le forficule européen se retrouve à l'heure actuelle, partout dans le monde (Lamb & Wellington, 1975; Albouy & Caussanel, 1990; Guillet *et al.*, 2000; Haas, 2018).

Cet insecte a une activité principalement nocturne. Pendant la journée, les individus, lucifuges, se réfugient dans les failles du sol, entre les écorces des arbres ou encore sous les pots de fleurs et les pierres. La nuit, les individus partent à la recherche de nourriture (Lamb & Wellington, 1975; Costa, 2006). Omnivores et possédant des pièces buccales de type broyeur, les forficules peuvent se nourrir d'une grande variété d'aliments tels que des fruits, du pollen, des plantes mais aussi d'autres insectes comme les pucerons ou les psylles, ce qui en fait des auxiliaires de culture notamment dans les vergers (Dib *et al.*, 2010, 2011) et les houblonnières (Madge & Buxton, 1976).

Au niveau morphologique, le forficule européen adulte mesure entre 12 à 15mm de long. Insecte de couleur brune, il est facilement reconnaissable grâce aux cerques qu'il arbore à l'extrémité de son abdomen(Langston & Powell, 1975). Cet appendice servant de défense contre les petits prédateurs (araignées, pseudo scorpion) et contre ses congénères permet aussi de différencier les mâles des femelles et sert d'ornement de cour (Moore & Wilson, 1993 ; Walker & Fell, 2001). Les cerques des mâles ont une forme de crochets et peuvent être de taille extrêmement variable entre les mâles (Figure 5a) tandis que celles des femelles sont de taille moins variable et ont plutôt une forme allongée en ciseaux (Moore & Wilson, 1993 ; Figure 5b).

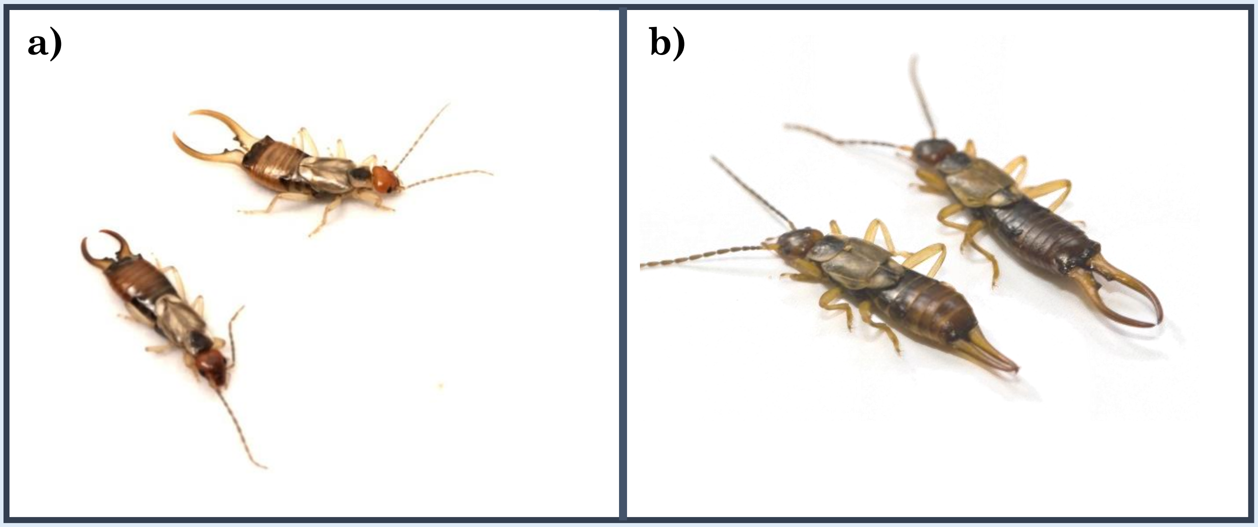

Figure 5: Représentation de la taille variable des cerques entre les mâles chez le forficule (photo : S. Van Meyel) a) et du dimorphisme sexuel présent chez cette espèce b). La femelle est à gauche et le mâle à droite de l'image b (Photo : J. Meunier)

Cycle de vie

La durée de vie du forficule européen est en moyenne d'un an et demi pour les femelles et d'un an pour les mâles. Cependant cette durée de vie peut varier en fonction de la population étudiée (Tourneur & Meunier, 2020). C'est une espèce univoltine, c'est-à-dire qu'une seule génération est produite par an à travers plusieurs pontes (Moerkens *et al.*, 2010). Son cycle de vie commence au début du printemps par l'émergence des adultes (Figure 6). Ces jeunes adultes, grégaires, produisent des phéromones d'agrégation pour se regrouper et s'accoupler durant la saison estivale (Sauphanor, 1992). Les femelles s'accouplent avec plusieurs mâles, les spermatozoïdes des différents partenaires sont ainsi accumulés dans une spermathèque et serviront à la fécondation de l'ensemble des pontes produites par la femelle (Sandrin *et al.*, 2015). A la fin de l'automne, les femelles creusent un nid dans le sol et s'isolent pour pondre leurs œufs et éviter le cannibalisme par leurs congénères (Figure 6). La taille moyenne de la ponte est d'environ 50 œufs mais varie fortement entre les femelles (Ratz *et al.*, 2016; Tourneur & Meunier, 2020).

Cycle de vie (suite)

La durée d’incubation des œufs varie entre 50 et 60 jours pendant l’hiver (Figure 6). Les femelles arrêtent alors de s’alimenter et prodiguent diverses formes de soins à leurs œufs (voir la section sur les soins maternel). Les premiers œufs éclosent au milieu de l’hiver tandis que les derniers arrivent à terme au tout début du printemps. Le forficule européen est une espèce hétérométabole : les juvéniles qui émergent des œufs ressemblent morphologiquement aux adultes en dehors de leurs cerques encore immatures, ce qui empêche l’identification du sexe des individus. Dès leur éclosion, ces juvéniles sont mobiles et capables de rechercher leur propre nourriture de façon autonome. Bien que la présence de la mère ne soit pas requise pour la survie des juvéniles, la mère et les juvéniles restent ensemble et forment une unité familiale pendant une quinzaine de jours (Figure 6) durant laquelle la mère va notamment les protéger contre les prédateurs et partager de la nourriture (voir section sur les soins maternels) (Lamb, 1976; Costa, 2006; Boos *et al.*, 2014). A la fin de la vie familiale, les juvéniles se dispersent et terminent leur développement en devenant des adultes en capacité de se reproduire (Figure 6). Les mères, quant à elles, ont pour certaines la possibilité de réaliser une deuxième voire troisième ponte (Meunier *et al.*, 2012 ; Figure 6). Les températures étant alors plus élevées, les œufs de la deuxième ponte se développent plus rapidement et les juvéniles émergent au bout de deux semaines. Ils deviendront ensuite des adultes avant l’été de la même année (Ratz *et al.*, 2016). Le développement des juvéniles en adultes s’effectue à travers quatre stades larvaires (Figure 6). Le premier et le deuxième stade larvaire s’effectuent dans le nid, environ 8 jours puis 15 jours après l’éclosion des œufs. Le troisième et le quatrième stade larvaire ont lieu environ 25 jours et 45 jours respectivement après l’éclosion des œufs, en dehors du nid. Le stade adulte est atteint après une dernière mue entre 70 et 80 jours après l’éclosion des œufs. Les femelles atteignent l’âge adulte généralement plus rapidement que les mâles (Van Meyel et Meunier ; données non publiées ; Figure 6).

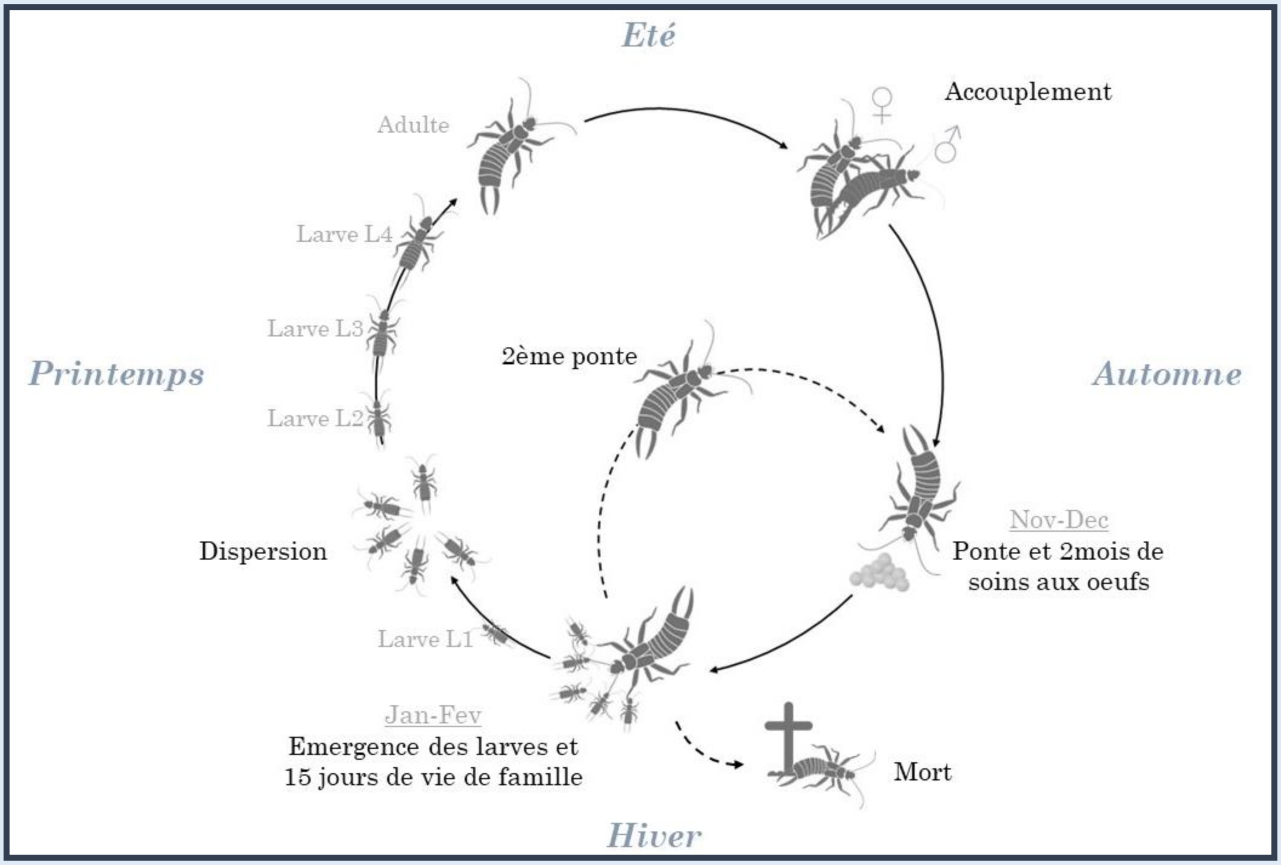

Figure 6: Cycle de vie du forficule européen *Forficula auricularia* en condition naturelle. Adaptation d'après Meunier 2017. A noter qu'une mue intermédiaire appelé L0 vient d'être décrite chez cette espèce. Cette mue s'effectue au moment de l'éclosion de l'œuf (Tourneur *et al.*, 2020 *The Canadian Entomologist, in press*)

Soins maternels

Soins pré-éclosion

Le forficule européen est une espèce remarquable par la complexité et la durée de ces soins parentaux (Lamb, 1976). Les femelles vont dans un premier temps construire un nid avant la ponte de leurs œufs (Figure 7a). Ce nid est généralement creusé dans le sol et permet aux femelles de s'isoler de leurs congénères. Une fois les œufs pondus, elles les nettoient régulièrement et tout au long du développement, tout en appliquant à l'aide de leurs mandibules et de leur salive des produits antifongiques et antimicrobiens (Boos *et al.*, 2014); Figure 7d). En réponse aux variations de température et d'humidité, les femelles déplacent les œufs dans le nid afin d'optimiser leur développement (Lamb, 1976). Les œufs sont toujours rassemblés ensemble afin de former un monticule (Figure 7b). Si jamais des œufs se retrouvent à l'écart, les femelles les recherchent activement grâce à leurs antennes pour les localiser et les transporter vers le reste de la couvée. Les femelles protègent aussi leurs œufs contre des prédateurs (araignées, pseudo-scorpion) ou contre des congénères (en particulier les mâles qui cannibalisent les œufs) à l'aide de leurs cerques. Pendant toute la durée d'incubation, les femelles ne quittent pas leur nid et cessent de se nourrir (Lamb, 1976).

Soins post-éclosion

Après l'éclosion des œufs et bien que les juvéniles soient autonomes et capables de fourrager par eux-mêmes, les femelles restent et présentent des soins envers leurs nymphes (Figure 7c). Elles les protègent d'éventuels prédateurs (Mas & Kölliker, 2011) et partagent aussi de la nourriture par deux méthodes distinctes : l'approvisionnement avec de la nourriture intacte (fruits, pucerons, etc.) ou l'échange de nourriture prédigéré grâce à un transfert par la bouche (trophallaxies stomodéales) ou par l'anus (trophallaxies proctodéales) (Lamb, 1976; Staerkle & Kölliker, 2008; Mas & Kölliker, 2011; Meunier *et al.*, 2012).

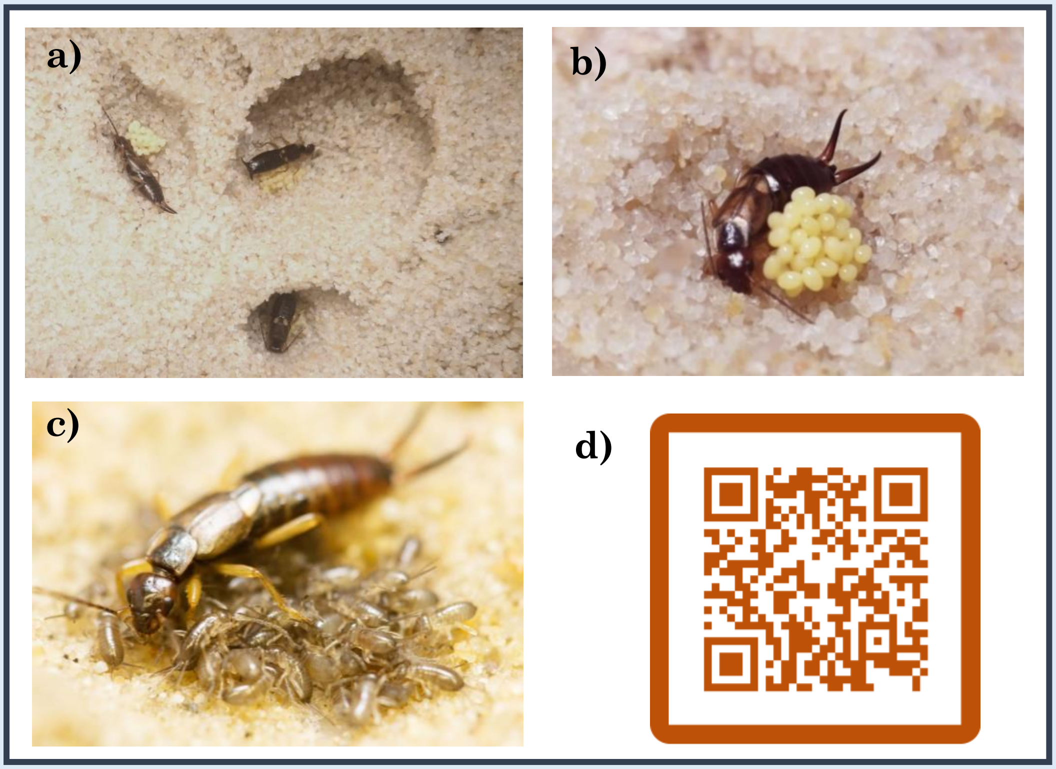

Figure 7: Illustrations des soins maternel envers les œufs et les nymphes chez le forficule européen *Forficula auricularia*. a) Construction du nid (photo : S. Van Meyel), b) défense des œufs (photo : S. Van Meyel) et c) des nymphes contre les prédateurs (photo : J. Meunier) et d) nettoyage des œufs dans la vidéo en lien QRcode (vidéo : S. Van Meyel)

PARTIE I:

Interactions mère-descendants dans l'évolution de la vie de famille

CHAPITRE 1

Discrimination de parentèle pendant les soins maternels chez le forficule européen

Résumé de l’étude

La sélection de parentèle est considérée comme un paramètre central dans l'évolution de la vie de famille. En effet, à cause des coûts importants liés aux soins parentaux, la théorie d’Hamilton (1964) prévoit généralement que les parents rejettent les petits étrangers pour s'assurer que leur investissement dans les soins soit dirigé vers leurs propres descendants. Le fait d’adapter un comportement en fonction du lien de parenté qu’un individu entretient avec un autre individu est appelé « discrimination de la parentèle ». Largement étudiée chez les espèces altriciales où les soins parentaux sont obligatoires, la sélection pour la discrimination de la parentèle est élevée lorsque les soins parentaux sont longs et énergétiquement couteux. A l’inverse, la sélection pour la discrimination de la parentèle est relativement faible lorsque les espèces ont développé des stratégies qui limitent la probabilité de rencontrer des œufs ou des petits étrangers. Dans cette étude, nous avons exploré ces pressions de sélection chez le forficule européen, un insecte où les soins aux œufs peuvent être relativement coûteux pour les femelles. Nos résultats montrent globalement que les femelles forficules n'éliminent pas les œufs étrangers et qu'elles fournissent même un niveau de soins et un investissement équivalent entre les œufs étrangers et leurs propres œufs. Par conséquent, les deux types d’œuf ont une durée de développement et un succès d'éclosion similaires et donnent des juvéniles de qualité comparable. En plus de ces résultats, notre étude révèle aussi que s'occuper des œufs (quelle que soit leur origine) réduit la perte de poids des femelles pendant cette longue période, ce qui pourrait s’expliquer par du cannibalisme des œufs de la part des femelles. En somme, nos résultats soulignent la difficulté de prédire l'apparition de la discrimination de la parentèle dans l’évolution des soins parentaux et démontrent qu’il est nécessaire d'élargir nos connaissances sur les réels bénéfices de ce comportement pour les parents.

Love them all: mothers provide care to foreign eggs in the European earwig *Forficula auricularia*

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ABSTRACT

The rejection of foreign individuals is considered a central parameter in the evolution of social life. Within family units, parents are typically thought to reject foreign offspring to ensure that their investment into care is directed towards their own descendants. Whereas selection for such kin bias is expected to be high when parental care is extended and involves numerous and energetically costly behaviours, it can be reduced when the acceptance of foreigners provide subsequent benefits to offspring and when alternative parental strategies limit the risk of clutch parasitism. In this study, we investigated the outcome of these conflicting selection pressures in the European earwig. Our results overall demonstrate that mothers do not eliminate foreign eggs, provide the same level of care to both foreign and own eggs (egg grooming, egg defense and maternal return) and pay the same costs of care in terms of weight loss and immunity when tending each type of eggs. We also show that foreign and own eggs exhibit similar development time, hatching success and lead to comparable juvenile quality. Interestingly, our results reveal that tending eggs (of any origin) reduces mothers' weight loss during this long period, possibly due to egg cannibalism. Hence, these findings emphasize the difficulty to predict the occurrence of kin bias, and stress the need to broaden our knowledge on the net benefits of egg rejection for parents to better understand the general importance of kin bias in the evolution of pre-hatching parental care.

INTRODUCTION

The capability of an individual to reject unrelated conspecifics is often considered a hallmark of the evolution of cooperation and group living (Tang-Martinez, 2001; Penn & Frommen, 2010). This is because such form of kin bias can enhance the direct and/or indirect fitness of donor individuals by allowing them to direct costly cooperative behaviors to recipient individuals sharing (at least) some of their genes (Hamilton, 1964). Examples of kin bias can be found across a large number of species and contexts, ranging from colony defense in Belding's ground squirrels and eusocial insects (Sherman, 1977; Polizzi & Forschler, 1999; Duffy *et al.*, 2002; Meunier *et al.*, 2011), over matrilineal kinship in hierarchies of Japanese monkeys (Kawai, 1958; Kawamura, 1958), to cooperative courtship in the wild turkey (Krakauer, 2005).

Parental care is one of the main contexts in which kin bias is expected to evolve. Parental care is a taxonomically widespread phenomenon, and involves a broad diversity of processes, such as the defense of eggs and juveniles against predators and pathogens, as well as the provisioning of juveniles with food (Royle *et al.*, 2012; Kramer & Meunier, 2019). While these processes typically enhance offspring development and likelihood to reach adulthood (see Royle et al. 2012 for review), parental care come with significant costs for parents: Investing into care can increase parents' energetic expenditure and risk of predation, both of which ultimately reduce parental investment into future reproduction (Trivers, 1972; Alonso-Alvarez & Velando, 2012). Evolving the capability to reject foreign offspring may thus help parents optimizing the fitness returns of their investment into care by ensuring that it is directed at their own offspring (Hamilton, 1964).

Several parameters, however, may inhibit the evolution of kin bias in family living species (Penn & Frommen, 2010). For instance, kin bias typically comes with an important risk of error for parents (rejecting their own offspring), so that it is expected to evolve only when parents have a high likelihood to encounter foreign offspring in their nest, e.g. due to heterospecific or conspecific clutch parasitism (Tallamy, 2005). Kin selection may also inhibit kin bias and promote the acceptance of foreign offspring when clutch parasitism is primarily done by related conspecifics, e.g. in populations where individuals exhibit low dispersal capabilities (Hatchwell & Komdeur, 2000). Selection for kin bias may also be relaxed if the net costs of care are low for parents, e.g. if care mostly involves passive processes such as thermal protection (Klug & Bonsall, 2014). Finally, a relaxed selection for kin bias may occur if the net costs of care for parents are outweighed by direct benefits of tending larger clutches, e.g. if it reduces the risk of predation of their own offspring through a dilution effect (Krause *et al.*, 2002). Investigating the presence (or absence) of kin bias in family living species may thus offer a unique opportunity to shed light on the effects of the above parameters on the expression of parental care, and more generally, on their importance in the evolution of complex family systems (Hatchwell & Komdeur, 2000; Samuk & Avilés, 2013).

Whereas females of the European earwig *Forficula auricularia* provide extended and complex forms of care to their eggs (Lamb, 1976), it remains unclear whether mothers accept foreign eggs and/or adjust their level of care to egg origin. In this insect, each female constructs a burrow during late autumn, in which they take 1 to 3 days to produce a first clutch of about 40 eggs (Boos *et al.*, 2014; Koch & Meunier, 2014; Ratz *et al.*, 2016; Diehl & Meunier, 2018). Females remain with their eggs for the subsequent 1-2 months, during which they provide multiple forms of egg care. This includes, for instance, the removal of fungal spores from the egg shell, the application of chemical protection against desiccation through egg grooming, the relocation of the clutch under stressful conditions, and a fierce protection against predators (Boos *et al.*, 2014; Koch & Meunier, 2014; Diehl & Meunier, 2018). Egg care is likely to be costly for females, as they typically stop their foraging activity between egg laying and hatching (Kölliker, 2007) and thus only have severely limited resources to spend on both their own survival and egg care for several months. Whereas the presence of conspecific clutch parasitism is unknown in this species, several parameters suggest that it can be present. First, populations have high densities (Moerkens *et al.*, 2009) and exhibit great inter-individual variation in the timing of egg production, with late females laying eggs more than 1 month after early ones (Meunier *et al.*, 2012; Ratz *et al.*, 2016). Tending mothers are thus likely to encounter conspecific females looking for a burrow to lay their eggs (and possibly to parasitize). Moreover, females retain the capability to produce a replacement clutch after a few days if they lose their eggs (J Meunier, pers. obs.), which could be used to replace foreign eggs with their own ones if the occasion arose (e.g. if females eliminate all eggs of their parasitized clutch). Whether this capability evolved as a strategy to combat conspecific clutch parasitism is, however, unknown.

In this study, we investigated whether *F. auricularia* mothers discriminate against foreign conspecific eggs. In particular, we tested whether females eliminate foreign eggs or alter the level of care they provide to these eggs, whether this potential alteration reduces the cost of care for mothers, and whether it reduces the quantity and/or quality of the resulting offspring. We set up four experimental treatments in which either mothers tended their own eggs, mothers tended foreign eggs, mothers had no eggs to tend, or eggs had no tending mother. We then took four measurements over the entire period of egg development. First, we measured the levels of three important forms of egg care: egg grooming, clutch defense and maternal return (Diehl & Meunier, 2018). To shed light on the potential costs of tending foreign eggs for mothers, we then measured changes of maternal weight between egg laying and hatching (a proxy of maternal investment into egg care (Koch and Meunier 2014)), and changes in the basal immunity of mothers (a costly physiological trait that often trades-off with investment into care (Reavey *et al.*, 2014b)). Finally, we investigated the potential costs to offspring of being tended by a foreign mother by measuring egg development time, hatching success and the weight of emerging juveniles. If *F. auricularia* mothers exhibit kin bias, we predict that (i) females are more likely to abandon or kill their clutch when tending foreign compared to their own eggs, and/or that (ii) females alter their level of care when tending foreign eggs. Reducing the amount of care toward foreign eggs might allow females to save energy (for future reproduction) while limiting the costs of discrimination errors. If egg care comes with physiological costs, we also predict (iii) a higher weight loss and (iv) a higher change in the levels of basal immunity from egg laying to hatching in mothers tending their own compared to foreign eggs – both values being higher when compared to mothers tending no eggs. If low investment in egg care incur costs to offspring, we predict that (v) eggs reared by a foreign mother exhibit a slower development, are less likely to hatch and that the resulting offspring are lighter compared to offspring from eggs reared by their own mothers – the three measurements reflecting an overall higher quality when compared to eggs with no tending mother.

MATERIALS AND METHODS

Experimental setup

We tested whether *F. auricularia* females discriminate against foreign eggs using a total of 85 families. The females used in this study were collected in June 2017 in Pont-de-Ruan, France, and maintained under standard laboratory conditions until egg production (Meunier *et al.*, 2012). Mothers and eggs were manipulated five days after egg laying to allow females freely interacting with their own eggs during this period of time and by doing so, to allow females to form a template that would allow them to discriminate between their own eggs and foreign (Penn & Frommen, 2010). At that time, we set up the four following experimental treatments: (i) mothers tending their own eggs (“own eggs treatment”, n = 28), (ii) mothers tending foreign eggs (“foreign eggs treatment”, n = 28), (iii) mothers tending no eggs (“no eggs treatment”, n = 29) and (iv) clutches of eggs without any tending mother (“no mother treatment”, n = 29; eggs taken from the no eggs treatment). The two last treatments were conducted to test whether the costs of tending foreign eggs are higher than the costs of tending no eggs for a female, and whether the costs of receiving care from a foreign mother are lower than receiving no care for eggs. During our manipulations, all eggs were counted and females received foreign clutches of the same size as their own clutches, i.e. clutches were not trimmed or mixed (mean difference between the number of eggs produced and received ± SE = 0.01 ± 0.02). Manipulations were done by first isolating mothers assigned to different treatments (i.e. females from own eggs, foreign eggs and no eggs treatments) and their full clutch of eggs in two small Petri dishes (diameter 5 cm), then transferring the eggs in the Petri dish of the corresponding mother (own eggs or foreign eggs treatments) and finally returning the recipient mother to its original Petri dish. Females and eggs from every treatment were treated the same way, except for the no mother treatment, where eggs were transferred to a new Petri dish. Note that mean clutch sizes were similar across the four treatments (own eggs treatment: mean clutch size ± SE = 36.93 ± 0.93; foreign eggs treatment = 36.68 ± 0.56 and no eggs/no mother treatments = 37.03 ± 0.83; LM, $F_{2,82}$= 0.054, p = 0.948). Moreover, we did not observe any female egg laying (and thus replacement clutches) after setting up of the four treatments.

Measurements

After the setup of each experimental family, we measured the expression of three forms of maternal care: the time spent on egg grooming, the level of clutch defense and the delay of maternal return, which were all measured using standard protocols (Thesing *et al.*, 2015; Diehl & Meunier, 2018). Egg grooming was measured one day after the setup of experimental families (i.e. five days after egg laying) by isolating mothers for 30 minutes, then returning them to their Petri dish at a distance of 5 cm from the eggs and recording their behaviors for the subsequent 15 minutes on camera (SONY© Handycam HDR-CX700 camera). Movies were analyzed using the software BORIS v4.0.3 (Friard & Gamba, 2016) and the total duration of egg grooming was defined as the total amount of time each female spent on cleaning eggs with their mandibles (Boos *et al.*, 2014). Clutch defense, which reflects females' willingness to protect their eggs from predator attacks (Thesing *et al.*, 2015), was measured five days after the setup of experimental families (i.e. 9 days after egg laying). This measurement was performed by poking females on the pronotum with a glass capillary in a standardized manner (one poke per second). We started poking when females were motionless on the brood (or just motionless for "no eggs" treatment). The number of pokes required until the female moved more than one body length away from its initial position was then recorded. Finally, maternal return represents the delay after which females return to their clutch after being chased away by a simulated predator attack (called clutch abandonment in Thesing *et al.*, 2015). It was measured by recording the time the female took to return to its clutch after the end of the clutch defense measurement. We decided to fix the time of maternal return at 20 minutes. Beyond this delay we indicated that female take more than 20 min to return to its clutch. Note that only one female (from the own eggs treatment) did not return to its clutch in less than 20 minutes after being chased away. All these measurements were conducted on females from own eggs and foreign eggs treatments, they were performed under red light due to the nocturnal nature of earwigs and researchers were blind regarding the treatments. Clutch defense was also measured in females from the no eggs treatment to test the importance of egg presence on the number of pokes before withdrawal, and thus to confirm that the measured behavior indeed reflects a form of egg care.

The costs of maternal investment into egg care in terms of weight loss and immunity changes were measured by recording maternal fresh weight and basal immunity both three days after egg laying and one day after egg hatching. The fresh weight of each female was measured to the nearest 0.001 g using a microbalance (OHAUS© Discovery DV215CD). To measure basal immunity, each female was first anesthetized with CO_2 for 30 seconds. We then extracted 1 µL of hemolymph using a glass capillary and diluted it in 25 µL of cold sodium cacodylate/$CaCl_2$ buffer (0.01 M Na-Cac, 0.005 M CaCl2; pH 6.5). The concentration of hemocytes was measured immediately after hemolymph extraction by transferring 10 µL of the diluted hemolymph on a Neubauer chamber and counting using an optical microscope (magnification ×400). Note that the mean hemocyte numbers per female were similar at the setup of the three treatments (own eggs treatment: mean hemocyte numbers ± SE = 86.78 ± 8.39; foreign eggs treatment = 98.37 ± 7.96; no eggs treatment = 99.44 ± 9.06; LM, $F_{2,78}$= 0.65, p = 0.524). When eggs did not hatch (or were absent due to the treatment), the second measurements were done 50 days after egg laying, which is similar to the mean number of days between egg laying and egg hatching in the other females (mean duration of egg development ± SE = 50.7 ± 0.13). All these measurements were done blindly regarding the treatments and conducted in females from the own eggs, foreign eggs, and no eggs treatments.

Finally, egg development time was defined as the number of days between egg laying and hatching. Conversely, hatching success was measured by dividing the number of juveniles present one day after egg hatching by the number of eggs counted during experimental setup. Note that all eggs from a clutch typically hatch within a day (Koch & Meunier, 2014). The mean weight of the resulting juveniles was measured to the nearest 0.001 g by weighing a group of up to 10 juveniles per clutch with a microbalance (OHAUS© Discovery DV215CD). These measurements were conducted in clutches from the own eggs, foreign eggs, and no mother treatments following a blind protocol.

Statistical analysis

Egg grooming and clutch defense were analysed using a general linear model (LM) and a generalized linear model (GLM) with Poisson error distribution, respectively. In each model either the number of pokes or the total duration of egg grooming were used as the response variable, whereas the treatment (own eggs, foreign eggs, or no eggs) were used as the explanatory factor. The delay of maternal return was tested using a Cox proportional hazard regression model allowing for censored data to account for females that did not return to their eggs at the end of the observation time (see data in the results). Note that this analysis assumes that females' delays to return have hazard functions that are proportional over time for the different treatment. Females' changes in basal immunity and fresh weight were analysed using a GLM and a LM, respectively. In these models, the relative change in hemocyte number (or fresh weight) between egg laying and hatching was entered as the response variable, while the treatment (own eggs, foreign eggs, or no eggs) was used as an explanatory variable. These relative changes were obtained by subtracting the number of hemocytes (or the females' fresh weight) at egg laying from the number of hemocytes (or the females' fresh weight) at hatching, and then dividing the result by the number of hemocytes (or the females' fresh weight) at egg laying. Positive values therefore indicate that females gained hemocytes or fresh weight during the period of egg care, and vice versa. The egg development time and mean weight of juveniles were tested using two additional LM, in which the treatment (own mother, foreign mother or no mother) was entered as the response variable. Finally, the hatching success was analysed using a GLM with a quasibinomial error distribution to correct for overdispersion. The hatching success was entered as a response variable using the *cbind* function in R (number of nymphs at hatching, number of egg received), whereas the treatment (own mother, foreign mother, or no mother) was used as a factor in the model.

When required, pairwise comparisons between treatments were conducted using the estimated marginal means (in the LMs and GLMs) and the *survdiff* function (in the Cox models) and corrected for multiple testing using Tukey methods. To fulfill homoscedasticity and normal distribution of model residuals, the number of pokes and mean nymph weight were log-transformed. All statistical analyses were performed with the software R v3.4.3 (http://www.r-project.org/) loaded with the packages *car* (Fox *et al.*, 2011), *survival* (Therneau, 2015) and *emmeans* (Lenth, 2018)

RESULTS

Whether mothers tended their own or foreign eggs did not affect the expression of egg grooming, clutch defense, and maternal return (Table 1.1a, Fig 1.1). Moreover, tending either their own or foreign eggs did not shape how much maternal weight and hemocyte number changed over the period of egg care (Table 1.1b, Fig 1.2). Similarly, the two types of experimental families did not differ in terms of egg development time, hatching success, and nymphs' fresh weight at hatching (Table 1.2, Fig 1.3).

As expected, our measurement of clutch defense reflected a form of egg care: the number of pokes required until mothers move away from their initial position was overall smaller when mothers tended eggs compared to no eggs (Table 1.1a, Fig 1.1b). Somewhat surprisingly, however, the proportion of fresh weight lost by mothers during the period of care was larger in absence compared to presence of eggs (Table 1.1b, Fig 1.2a). By contrast, the presence or absence of eggs did not shape maternal variation in hemocyte number (Table 1.1b, Fig 1.2b). Finally, the presence or absence of a tending mother had no effect on egg development time and nymph weight at hatching, even if the absence of a mother dramatically reduced the proportion of successful egg hatching (Table 1.2, Fig 1.2).

Table 1.1: Effect of treatment (own eggs, foreign eggs, or no eggs) on the three measured forms of maternal egg care and on the relative changes in hemocyte number and maternal fresh weight. Significant p-values are in bold.

	Treatment			Post-hoc tests (Tukey)		
	Model	Statistical values	P-values	Related vs Unrelated	Related vs No eggs	Unrelated vs No eggs
a) Levels of maternal care						
Clutch defense	GLM	LR $\chi^2(2) = 8.69$	**0.0130**	0.9904	**0.0248**	**0.0352**
Maternal return	Cox	$\chi^2(1) = 1.06$	0.3043	-	-	-
Egg grooming	LM	F(1,54) = 0.24	0.8784	-	-	-
b) Effects of clutch attendance on mothers						
Hemocyte number	GLM	LR $\chi^2(2) = 0.48$	0.7881	-	-	-
Fresh weight	LM	F(2,72) = 8.31	**0.0006**	0.9803	**0.0014**	**0.0042**

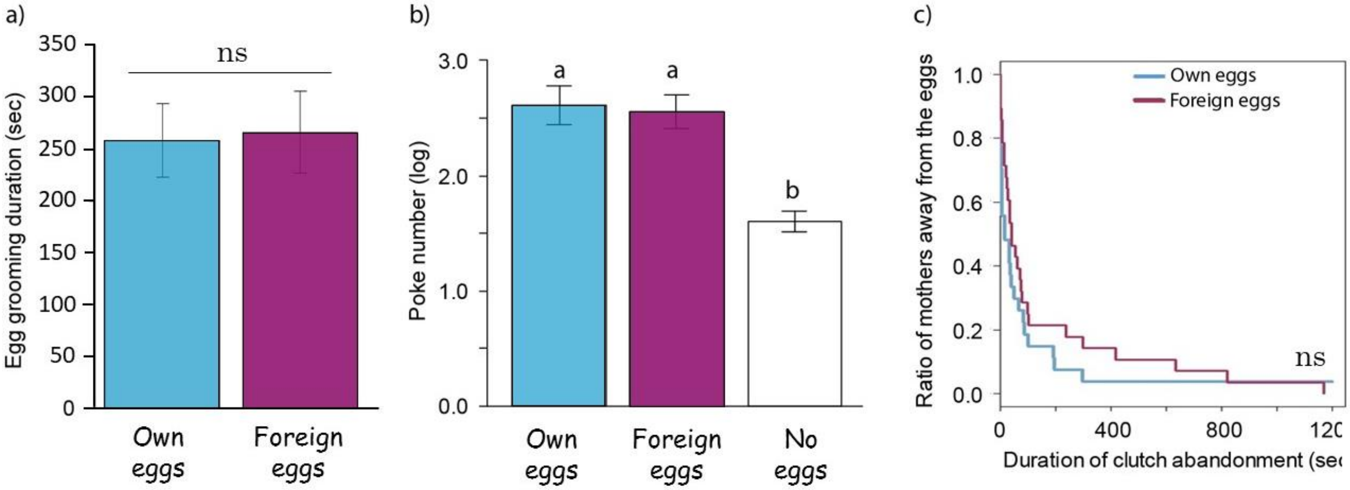

Figure 1.1: Effect of treatment on females' egg care. (a) Egg grooming, which is the sum of the times spent by female to clean the eggs. (b) Egg defense against a simulated predator attack, which represents the number of pokes required to deter mothers from tending their clutch of eggs and (c) Delay of maternal return, which indicates how long mothers remain away from their eggs after poking. Different letters correspond to p-values < 0.0001, ns = not significant. Values are means ± s.e.m. The number of replicates is indicated at the bottom of each bar or between brackets.

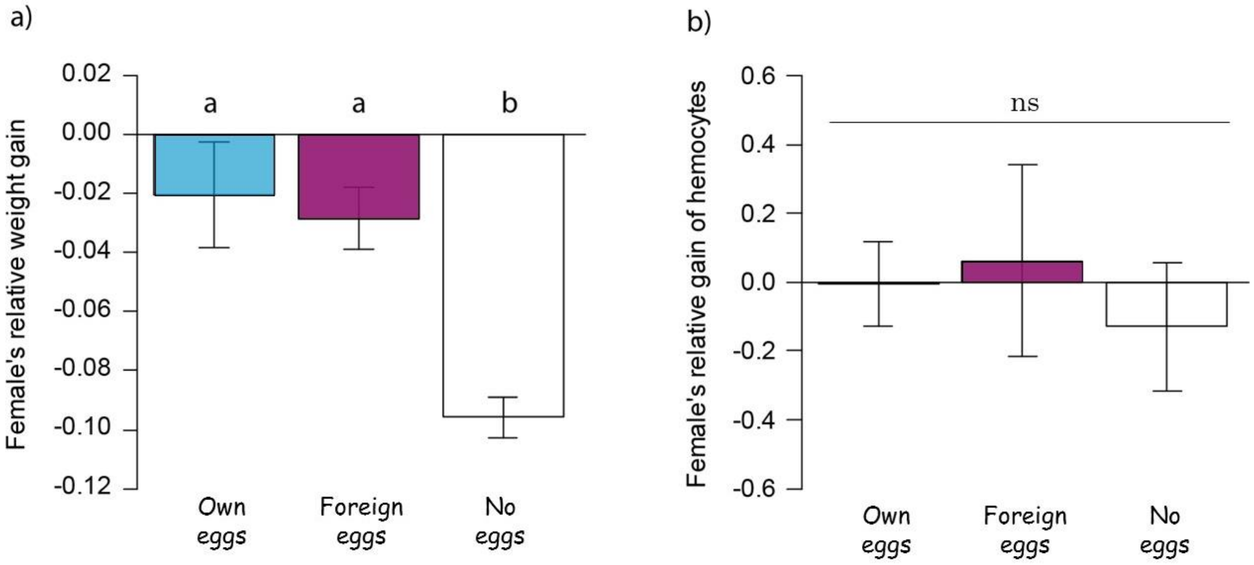

Figure 1.2: Effect of treatment on females' body condition reflecting their investment on egg care. (a) Female's relative differences in weight between egg laying and egg hatching, negative values show a weight loss. (b) Female's relative gain of hemocytes between the egg laying and the egg hatching. Different letters correspond to p-values < 0.01, ns = not significant. Values are means ± s.e.m. The number of replicates is indicated at the bottom of each bar.

Table 1.2: Effect of treatment (own eggs, foreign eggs, or no eggs) on egg development, hatching success and nymph weight at hatching. Significant p-values are in bold.

	Treatment			Post-hoc tests (Tukey)		
	Model	Statistical values	P-values	Related vs Unrelated	Related vs No mother	Unrelated vs No mother
Egg development time	LM	F(2,50) = 0.41	0.6647	-	-	-
Hatching success	GLM	LR χ^2 (2) = 43.16	< **0.0001**	0.7125	< **0.0001**	< **0.0001**
Nymph weight at hatching	LM	F(2,50) = 0.04	0.9654	-	-	-

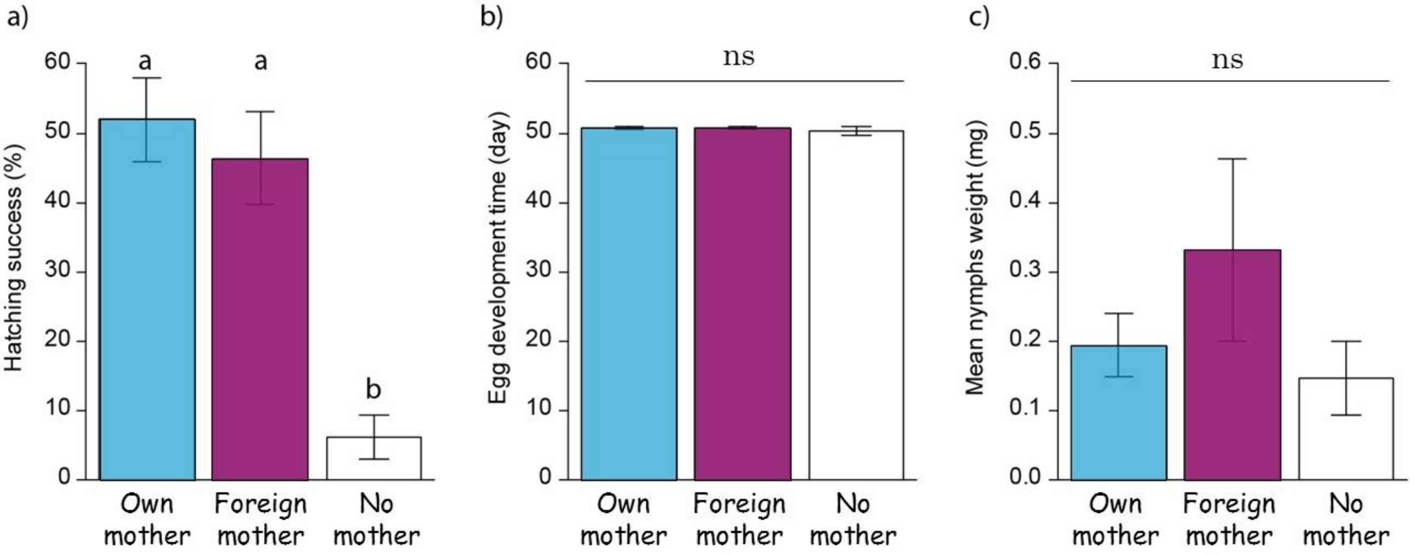

Figure 1.3: Effect of treatment on egg quality in terms of (a) hatching success, (b) duration of egg development and (c) mean of nymph weight at hatching. Different letters correspond to p-values < 0.0001, ns = not significant. Values are means ± s.e.m. The number of replicates is indicated at the bottom of each bar.

DISCUSSION

Theory predicts that the net costs of parental care may compel tending parents to reject foreign eggs, for instance by eliminating these eggs and/or reducing the amount of care they provide to foreign eggs (Hamilton, 1964). In this study, we showed that mothers of the European earwig do neither. The presence of either their own or foreign eggs entailed no change in the three measured forms of maternal care, and no change in two physiological costs often associated with maternal care, i.e. weight loss and altered basal immunity. Somewhat surprisingly, females lost more weight in absence compared to presence of (any type of) eggs, whereas egg presence had no effect on females' changes in basal immunity. The experimental treatment to which these mothers were subjected (i.e own versus foreign eggs) also had no effect on egg development time, egg hatching success and on the weight of the resulting juveniles. Finally, the absence of a mother dramatically reduced egg hatching success, whereas for the few surviving eggs, it had no effect on development time and juvenile weight.

Our data first show that earwig mothers do not eliminate foreign eggs and that they provide extensive forms of care to foreign conspecific eggs just as they do to their own eggs. This is in line with results obtained in other subsocial arthropods, such as the hemiptera *Tectocoris diophthalmus* (Giffney & Kemp, 2016), the wolf spider *Pardosa milvina* (Culley *et al.*, 2010) and many eusocial Hymenoptera (Bourke, 1994; Strassmann *et al.*, 2000). It also supports results from several cross-fostering studies in *F. auricularia* showing that eggs reared by foreign mothers can exhibit a high hatching success (Meunier & Kölliker, 2012a; Boos *et al.*, 2014, 2015; Thesing *et al.*, 2015). Here, our finding suggests that (i) eggs do not possess cues (e.g. chemicals on egg surface) that are specific to their own mother and can be used by females to express kin bias or that (ii) there is no or limited selection pressures promoting the rejection of foreign eggs. The first hypothesis is unlikely to explain our results. Earwig mothers are known to immediately and regularly apply a bouquet of cuticular hydrocarbons on the surface of their eggs (Boos *et al.*, 2014), the composition of this bouquet is family specific (Wong *et al.*, 2014b) and it has been proposed that this bouquet mediates kin discrimination between juveniles (Dobler & Kölliker, 2010). The second hypothesis is more likely to explain our results. First, that is because conspecific clutch parasitism could be absent in this species, as no study ever reported (or actually investigated) its occurrence in *F. auricularia*. This would suggest that even if females may compete for limited nest availability (due to the combination of high population densities and inter-individual variation in the timing of egg production), it is not necessarily associated with parasitic behaviours during egg laying. Second, earwig females may have evolved alternative strategies to prevent clutch parasitism. For instance, some females close their nest during the period of egg care and most females remain close to their eggs until they hatch, which may have evolved to limit the risk of intrusion by conspecifics (Meunier *et al.*, 2012; Diehl & Meunier, 2018). Thirdly, kin selection may actually promote the adoption of conspecific foreign eggs. That is because *F. auricularia* individuals are likely to live in populations with high genetic relatedness (due to low dispersal capabilities (Moerkens *et al.*, 2010)), so that females might be related to most foreign conspecific eggs and could thus increase their inclusive fitness by tending them. Finally, the acceptance of foreign eggs by a mother could be selected to provide nutritional benefits to its own juveniles later on, as these latter selectively cannibalise foreign juveniles during earwig family life (Kölliker & Vancassel, 2007; Dobler & Kölliker, 2010). Future studies should be conducted to disentangle which of these four parameters lead to the absence of the reported kin bias in our model species.

Independent of the absence of kin bias, our results confirm that maternal presence is crucial to maximize hatching success in earwigs (Boos *et al.*, 2014). Across species and taxa, the presence of mothers with eggs often mitigates the costs of external stressors acting during egg development, such as predation (Swennen *et al.*, 1993; Machado & Oliveira, 2002; Requena *et al.*, 2009; Miller *et al.*, 2011), pathogen infection (Grindstaff *et al.*, 2003; Herzner & Strohm, 2007; Kudo *et al.*, 2011; Trumbo & Balshine, 2012; Boos *et al.*, 2014), desiccation (Aubret *et al.*, 2005; Poo & Bickford, 2013), and other environmental changes (Green & McCormick, 2004; Smiseth *et al.*, 2012). Given the standard laboratory conditions used in the present study, our findings suggest that maternal presence buffers the otherwise lethal effects of small variation in the nesting environment, e.g. humidity and/or the development of non-pathogenic microbes such as mold (see also Boos *et al.*, 2014). Nevertheless, our results also reveal that maternal presence does not change the intrinsic quality of the eggs – as defined by a faster development and the production of heavier juveniles (Körner *et al.*, 2016, 2018). This suggests that the natural variation in egg quality reported between *F. auricularia* clutches (Diehl & Meunier, 2018) does not reflect alternative strategies consisting in increasing (or reducing) the level of pre-hatching care to compensate for low (or to benefit from high) investment into egg quality, respectively.

Although *F. auricularia* mothers stop their foraging activity during the period of egg care (Lamb, 1976), our data surprisingly show that mothers lost three times less weight when they tended (any type of) eggs as compared to no eggs at all. This difference in weight loss could be due either (i) to a stress-triggered excess of metabolic activity when mothers lost their full clutch of eggs, or (ii) to the lack of opportunity for isolated mothers to cannibalise their own eggs. The first hypothesis is unlikely to explain our results: we found no effect of egg presence/absence on the basal immunity of females, a physiological trait that is typically shaped by stress in animals (Adamo, 2012). By contrast, filial egg cannibalism might be more likely to explain our results. This phenomenon has been reported in several earwig species, including *F. auricularia* (Miller & Zink, 2012; Koch & Meunier, 2014). Filial egg cannibalism is generally considered as an adaptive process if it either (i) targets damaged, parasitized or diseased eggs, (ii) reduces the future level of sibling competition for limited resources, (iii) allows parents to re-allocate their resources into future reproduction after a sudden deterioration in their environmental conditions or (iv) if it targets unfertilized trophic eggs and is used as a regular food source to parents when other food sources are scarce (Bartlett, 1987; Crespi, 1992;

Lindström & Sargent, 1997; Thomas & Manica, 2003; Lourdais *et al.*, 2005; Klug *et al.*, 2006; Miller & Zink, 2012; Zeng *et al.*, 2017). This fourth hypothesis could operate in earwigs, as mothers constantly remain in their nest over the period of egg care (Kölliker, 2007) which largely occurs during the period when food sources are typically very limited (Lamb, 1976). To determine whether egg cannibalism is the main driver of our results and to disentangle its underlying processes, further research will investigate the effects of physical damage and pathogen infection on egg cannibalism, test whether earwig females produce unfertilized trophic eggs and explore the link between changes in environmental conditions and egg cannibalism.

To conclude, our study shows that *F. auricularia* mothers do not reject foreign conspecific eggs. This emphasizes that prolonged and extensive periods of maternal egg care, together with the absence of foraging activity and the capability to produce a replacement clutch do not necessarily select for maternal adjustment of egg care in function of eggs origin. Moreover, our results stress the importance of other parameters such as the risk of clutch parasitism, the development of alternative strategies to fight against clutch parasitism and the potential benefits of adopting additional eggs, in the selection for a broad tolerance toward foreign eggs. Hence, improving our knowledge on these parameters and their associated net benefits (or costs) for parents will be of central importance to get a better understanding of the role of kin bias in the evolution of parental care across species and taxa.

CHAPITRE 2

Implication du cannibalisme filial partiel des œufs dans l’évolution des soins maternels chez le forficule européen

Résumé de l’étude

Chez de nombreuses espèces animales, les mères peuvent manger une partie de leur ponte. Bien que ce comportement puisse paraître contre-intuitif pendant les soins parentaux, il peut avoir différents avantages. Le cannibalisme filial partiel des œufs peut en effet limiter la compétition entre les juvéniles à l’éclosion, permettre d’enlever les œufs infectés ou parasités qui pourraient infecter le reste de la couvée et aussi permettre à la mère de réallouer des ressources énergétiques dans une reproduction future. Bien que l’expression de ce comportement ait de profondes répercussions sur la fitness des parents et des descendants, les liens entre l’expression de ce comportement et l’évolution des soins parentaux sont peu connus. Dans cette étude nous avons notamment voulu savoir si, d’une part, ce comportement est un simple sous-produit lié à un faible investissement de la femelle dans ses soins aux œufs et si, d’autre part, ce comportement est une stratégie déterminée avant ou après la production des œufs. Nos résultats révèlent d’abord que le cannibalisme des œufs procure des bénéfices directs aux femelles forficule : les femelles qui ont consommé une partie de leurs œufs ont eu, par la suite, un meilleur investissement dans leur seconde ponte. Nous avons ensuite montré que ce cannibalisme filial partiel était indépendant du niveau d'investissement de la mère dans deux formes importantes de soins aux œufs. Cela suggère que des pressions de sélection différentes ont agi, d’un côté, sur l’expression des soins parentaux et, de l’autre côté, sur l’expression du cannibalisme filial partiel des œufs. Enfin, il semblerait que le cannibalisme d’une partie de la ponte soit une stratégie déterminée après la ponte des œufs. Dans l'ensemble, nos résultats apportent un éclairage nouveau sur notre compréhension de l'émergence des soins parentaux, car ils suggèrent que les bénéfices directs liés au cannibalisme partiel des œufs pour les femelles pourraient avoir favorisé le fait que ces dernières restent auprès de leurs œufs et ne les abandonnent pas.

Filial egg cannibalism in the European earwig: its determinants and implications in the evolution of maternal egg care

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ABSTRACT

In many animal species, mothers eat some of their own eggs. While this filial egg cannibalism can have profound impacts on both parental and offspring fitness, it remains unclear whether this behaviour is a simple by-product of a generally low maternal investment in egg care and whether it is determined before or after egg production. Here, we addressed these questions in the European earwig, *Forficula auricularia,* an insect in which females vary in their level of investment in egg care and in their expression of egg cannibalism. Using a cross-fostering experiment, we investigated the benefits of egg cannibalism for mothers, the potential association between egg cannibalism and egg care, and whether egg cannibalism is a maternal strategy that is determined before or after egg laying. Our results first revealed that egg cannibalism provided direct benefits to mothers, as the females that consumed some of their eggs increased their investment in future reproduction. We then showed that egg cannibalism was independent of the level of maternal investment in two important forms of egg care (egg defence and the time taken to return after a simulated predator attack). This overall suggests that access to egg cannibalism may select for mothers to remain with their eggs, independent of the potential benefits of care for the eggs. Finally, we demonstrated that the number of eggs received, but not the number of eggs produced by foster mothers, determined the level of egg cannibalism. This highlights that egg cannibalism is determined after egg laying and is not the outcome of anticipatory maternal effects. Overall, these findings provide novel insights into our understanding of the early evolution of parental care, as they suggest that the direct benefits of filial egg cannibalism for mothers could have promoted egg attendance from an ancestral egg abandonment state.

INTRODUCTION

Parents can employ multiple strategies to maximize their own and/or their descendants' fitness after egg production (Smiseth *et al.*, 2012). Most of these strategies are energetically costly for parents and consist of providing care to protect their eggs against predators, pathogens and harsh environmental conditions (Clutton-Brock, 1991; Royle *et al.*, 2012). However, parents of several animal species (Elgar & Crespi, 1992) adopt strategies that are energetically beneficial for them and costly for some of their eggs, such as reducing the clutch size through egg consumption (Manica, 2002; Klug & Bonsall, 2007). This so-called partial filial egg cannibalism is often viewed as an adaptive strategy because it can provide some benefits to mothers. For instance, egg consumption can allow mothers to limit the level of posthatching sibling competition and to remove unviable and infected eggs that could otherwise favour the spread of microbial pathogens to the rest of the clutch (Bartlett, 1987; Creighton, 2005). Similarly, filial egg cannibalism may allow females to reallocate resources to future reproduction and/or a higher quality of care to current clutches (Sargent, 1992; Rohwer, 2002).

While partial filial egg cannibalism has a profound impact on both parental and offspring fitness (Manica, 2002; Klug & Bonsall, 2007), the link between this behaviour and the general parental investment in egg care, as well as the proximate drivers of this behaviour, remains unclear. Demonstrating that egg cannibalism and egg care are independent would first suggest that egg cannibalism is not a simple by-product of a low or high investment of mothers in care. This would indicate that the sole benefits of egg cannibalism (for instance in terms of future reproduction) could select for mothers to remain with their eggs when these do not require care to survive, a scenario that probably prevailed in the early evolution of egg brooding (Royle *et al.*, 2012; Furness & Capellini, 2019). Moreover, demonstrating that partial filial egg cannibalism is a maternal strategy that is determined before and/or after egg laying may provide key information on the selection pressures operating on the evolution of egg cannibalism and on how this phenomenon can ultimately (and somewhat counterintuitively) improve maternal fitness. In particular, a strategy determined before egg laying may allow mothers to account for future access to food resources (i.e. eggs) during times where either egg care or other reproductive or seasonal limitations prohibit foraging. This strategy could operate, for instance, in species where females are under a physiological constraint enforcing the development of (even unfertilized) eggs in their reproductive tract, a phenomenon found in numerous species such as the terrestrial isopod *Armadillidium pelagicum* (Hamaied *et al.*, 2004), the fruit fly *Drosophila mangabeirai* (Murdy & Carson, 1959) and the European earwig, *Forficula auricularia* (S. Van Meyel & J. Meunier, personal observation). On the other hand, a strategy determined after egg laying could allow parents to optimize their energetic resources in unpredictable environments (Sargent, 1992; Rohwer, 2002) and should thus reflect a process not primarily determined by the maternal genetic background and/or by anticipatory maternal effects.

In this study, we investigated whether filial egg cannibalism (1) provides benefits to mothers in the European earwig, (2) depends on the level of maternal investment in egg care and (3) is determined before and/or after egg production. In this insect species, females produce up to two clutches in their lifetime without remating (Meunier et al., 2012; Ratz, Kramer, Veuille, & Meunier, 2016; Tourneur & Meunier, 2020). Earwig mothers typically tend their first clutch of eggs for several weeks in winter (Lamb, 1976; Kölliker, 2007), during which they stop foraging and provide multiple forms of care (Kölliker, 2007; Boos *et al.*, 2014; Diehl & Meunier, 2018). Hence, during the period of egg care, earwig females have no food source other than their own eggs. Recent studies have shown that earwig females can consume some of their eggs during this period (Koch & Meunier, 2014) and that mothers with large clutches are more likely to express this behaviour than those with small clutches (Koch & Meunier, 2014). The underlying drivers of this link, however, are unknown.

Here, we cross-fostered clutches of different sizes and then measured the occurrence and level of filial egg cannibalism by the foster mothers (based on their weight gain), as well as their investment in egg care and second-clutch production. If egg consumption benefits mothers, we predicted that a second clutch would be both more likely to be produced and larger in cannibal than noncannibal mothers, i.e. in females that gained weight compared to those that lost weight during the period of egg care. If filial egg cannibalism is independent of egg care, we predicted no association between the level of egg care expressed by a female and its weight gain during this period. Finally, if filial egg cannibalism is determined before (or after) egg laying, we predicted that the number of eggs produced (or the number received) by a foster mother would drive the known positive association between clutch size and likelihood of egg cannibalism (Miller & Zink, 2012; Koch & Meunier, 2014).

METHODS

Experimental design and measurements

The 74 earwig females used in this study were collected in June 2017 at Pont-de-Ruan (France) and were then maintained under standard laboratory conditions until egg laying (Meunier *et al.*, 2012). Because females need up to 3 days to produce their full clutch of eggs (Koch & Meunier, 2014), we counted the eggs produced by each female 3 days after egg laying. One day later, we randomly exchanged the full clutch of each mother with the first clutch of another, unrelated mother. This cross-fostering was possible because earwig mothers do not discriminate against foreign eggs (Van Meyel, Devers, & Meunier, 2019). Overall, this process led to 74 experimental families in which each mother originally produced from 12 to 53 eggs and then received a clutch that was from 70% smaller to 95% larger than its original clutch (see distribution in supplementary material Fig. S2.1). There was no association between the number of eggs produced and those received by the foster mothers (Spearman correlation: rho = -0.102, S = 7 4388, P = 0.389). These experimental families were then maintained at 10 °C in constant darkness to mimic winter conditions and allow egg development (Meunier *et al.*, 2012). They were not given any food source between egg production and egg hatching, as females typically stop foraging during this period (Kölliker, 2007).

Five days after the cross-fostering, we used standard protocols to measure maternal investment in two important forms of egg care (Thesing *et al.*, 2015; Diehl & Meunier, 2018; Van Meyel *et al.*, 2019). We first measured clutch defence, which reflects females' willingness to protect their eggs from predator attacks, by standardly poking females on the pronotum with a glass capillary and then recording the number of pokes required until they moved more than 1 body length away from the clutch. High values of clutch defence (poke number) therefore show high maternal investment in egg care and vice versa. We then measured the delay after which females returned to their clutch after being chased away by a simulated predator attack (henceforth, 'delay of maternal return') by recording the time it took each female to return to its clutch after the end of the clutch defence measurement. Long delays of maternal return indicate low maternal investment in egg care and vice versa. Overall, females showing high levels of clutch defence tended to have shorter delays of maternal return, but this was not significant (Spearman correlation test: rho = -0.222, S = 7 6012, P = 0.061).

One day after egg hatching, families were transferred to standard summer conditions (Meunier *et al.*, 2012) and received an ad libitum amount of standard food twice a week (see detailed food composition in Meunier et al., 2012). Fourteen days later, juveniles were removed from their mothers to mimic natural family dispersion (Lamb, 1975; Meunier *et al.*, 2012), while mothers were maintained in constant darkness to allow second-clutch production (Meunier *et al.*, 2012). When these mothers produced a second clutch, we counted the eggs 3 days after egg laying.

The occurrence and levels of filial egg cannibalism were measured using two proxies based on female weight gain. We used female weight gain instead of simply counting the eggs that did not hatch, because multiple factors independent of filial egg cannibalism can shape hatching success in earwigs. For instance, failures in egg developmental processes result in eggs that either dry out and disappear (the most frequent) or remain unhatched, and clutches suffer from recurrent egg cannibalism by newly hatched siblings (Koch & Meunier, 2014; Miller & Zink, 2012). Our first proxy of egg cannibalism was the absolute weight gained by mothers between egg laying and egg hatching. This proxy provides quantitative information on egg consumption (continuous variable), as earwig mothers do not have access to any food source between egg laying and hatching and thus the absolute weight gained by a mother during this period necessarily (and at least partly) reflects the number of eggs it eats (Koch & Meunier, 2014; Miller & Zink, 2012). The limit of this proxy, however, is that maternal idiosyncrasies may also affect female weight during this period and could thus blur our quantitative interpretation of egg cannibalism. To confirm that this limit does not alter the robustness of our conclusions, we also used a qualitative proxy of egg cannibalism (categorical variable). This proxy defined 'cannibal mothers' as the females that gained weight between egg laying and egg hatching (N = 19 mothers; see Results) and 'noncannibal mothers' as the females that lost weight during this period (N = 55). The benefit of this second proxy is that 'cannibal mothers' may only include females that ate eggs, whereas its limit is that 'noncannibal mothers' may also include a few females that did consume a small part of their clutch (but the weight gained through this consumption was lower than the weight lost for other reasons). Given the complementarity of these two proxies, we believe that their combined use comprehensively and conservatively characterizes filial egg cannibalism and thus allowed us to explore its costs and benefits, as well as its determinants in earwigs. Female weight gain during the period of egg care was calculated by subtracting the mother's fresh weight measured 3 days after egg laying from the mother's fresh weight measured 1 day after egg hatching. All weights were measured to the nearest 0.01 mg using a microbalance (OHAUS Discovery DV215CD)

Statistical analyses

The potential costs and benefits of egg cannibalism for mothers were analysed using a series of two generalized linear models (GLM) fitted with binomial error distributions, and three general linear models (LM). In these models, the response variable was either the hatching success (GLM), the likelihood of producing a second clutch (GLM), the number of second-clutch eggs produced (LM), the level of clutch defence (LM) or the delay of maternal return (LM). All these models included three explanatory variables, which were the weight gained by foster mothers during the period of egg care (our quantitative proxy of filial egg cannibalism), the number of eggs they produced in their first clutch and the interaction between these two factors. These models finally included one covariable, the weight of the foster mothers at egg production, which was used to account for female body condition in the potential costs and benefits of egg cannibalism. To confirm that our results did not depend on the selected proxy of filial egg cannibalism (and its associated limits), we then reran the exact same series of five statistical models but used cannibal/noncannibal mothers as a categorical explanatory variable instead of the weight gained by foster mothers.

We then tested whether filial egg cannibalism depends on the number of eggs produced and/or received by the females using two statistical models, in which the response variable was either the quantitative proxy (i.e. female absolute weight gain, LM) or the qualitative proxy (i.e. cannibal versus noncannibal mothers, GLM) of filial egg cannibalism. In these models, the explanatory variables were the number of first-clutch eggs produced by the foster females, the number of first-clutch eggs they received and the interaction between these two variables. Note that a significant interaction between these two factors would suggest that filial egg cannibalism depends on whether mothers received more or fewer eggs than they had laid. In these models, we also entered the weight of foster mothers at egg production as a covariable.

All these models were conducted with the software R v3.6.0 (R Core Team, 2017). Each model was checked for homoscedasticity and normality of residuals and, when required, variables were transformed to fulfil model assumptions. In particular, the weights gained by mothers during the period of egg care were log+0.01 transformed, the level of clutch defence was log transformed and the delay of maternal return was log+1 transformed. These transformations were also used in the figures. When applicable, the hatching success was entered through the cbind function in R, in which we used the number of nymphs at hatching on the one hand, and the difference between the number of eggs received and the number of newly hatched nymphs on the other.

Ethical Note

We used a total of 74 individuals in this study. No animal ethics approval was required. All individuals were handled with care.

RESULTS

Overall, variation in female weight during the period of egg care ranged from a loss of 7.84 mg (13.5% of its initial weight) to a gain of 22.14 mg (48.6% of its initial weight), with a total of 19 (24.7%) females that gained weight and were thus the most likely to have performed filial egg consumption (hereafter called ‘cannibal mothers’ in contrast to the 55 'noncannibal mothers’). Among the 74 tested females, 53 (71.6%) produced a second clutch, which contained 5 - 43 eggs (mean ± SE = 22.42 ± 1.38).

Female weight gain between egg production and egg hatching (our quantitative proxy of filial egg cannibalism) was overall negatively correlated with egg hatching success (model estimate ± SE = -18.90 ± 3.88; Table 2.1, Fig. 2.1a). By contrast, it was positively correlated with both the likelihood of producing a second clutch (model estimate ± SE = 1.47 ± 0.67; Table 2.1, Fig. 2.1b) and, when present, with the number of second-clutch eggs they produced (model estimate ± SE = 7.37 ± 2.14; Table 2.1, Fig. 2.1c). The use of the qualitative proxy of egg cannibalism provided the same results. Cannibal mothers had a lower egg hatching success (Table 2.1, Fig. 2.1d), but a higher likelihood of producing a second clutch (Table 2.1, Fig. 2.1e) and a larger number of second-clutch eggs (Table 2.1, Fig. 2.1f) compared to noncannibal mothers. Note that the negative associations between egg hatching success and filial egg cannibalism (found with both proxies, Fig. 2.1a, d) remain significant when females with a hatching success of zero are removed from the data sets (Table S2.1). Independent of the level of filial egg cannibalism and of the proxy we used, the egg hatching success was negatively correlated with the weight of females at egg laying (model estimate ± SE = -85.01 ± 31.95; Table 2.1).

Table 2.1: Effects of female body mass at egg laying, egg cannibalism and egg production on hatching success, likelihood of female producing a second clutch, the number of second-clutch eggs, clutch defence and delay of maternal return after predator attack

	Female body mass		Filial egg cannibalism (FEC)		No. of first-clutch eggs (NE)		FEC * NE	
	LR/*F*	*P*	LR/*F*	*P*	LR/*F*	*P*	LR/*F*	*P*
Female weight gain used as a proxy of egg cannibalism								
Hatching success	$LR_1=7.51$	**0.006**	$LR_1=43.60$	**<0.0001**	$LR_1=1.26$	0.262	$LR_1=0.08$	0.782
Likelihood of second-clutch production	$LR_1=0.25$	0.615	$LR_1=7.05$	**0.008**	$LR_1=10.18$	**0.001**	$LR_1=3.53$	0.060
No. of second-clutch eggs	$F_{1,69}=0.12$	0.730	$F_{1,69}=13.89$	**0.0004**	$F_{1,69}=15.1$	**0.0002**	$F_{1,69}=0.32$	0.573
Clutch defence	$F_{1,69}=2.92$	0.092	$F_{1,69}=0.09$	0.770	$F_{1,69}=0.39$	0.533	$F_{1,69}=1.92$	0.171
Delay of maternal return	$F_{1,67}=0.06$	0.802	$F_{1,67}=0.13$	0.716	$F_{1,67}=0.71$	0.402	$F_{1,67}=1.40$	0.241
Category of female cannibalism used as a proxy of egg cannibalism								
Hatching success	$LR_1=4.64$	**0.031**	$LR_1=37.19$	**<0.0001**	$LR_1=3.08$	0.079	$LR_1=0.04$	0.844
Likelihood of second-clutch production	$LR_1=0.01$	0.936	$LR_1=11.58$	**0.001**	$LR_1=14.58$	**0.0001**	$LR_1=1.64$	0.200
No. of second-clutch eggs	$F_{1,69}=0.05$	0.832	$F_{1,69}=16.24$	**0.0001**	$F_{1,69}=21.67$	**<0.0001**	$F_{1,69}=0.33$	0.565
Clutch defence	$F_{1,69}=3.81$	0.055	$F_{1,69}<0.01$	0.976	$F_{1,69}=0.46$	0.502	$F_{1,69}=1.39$	0.242
Delay of maternal return	$F_{1,67}=0.22$	0.642	$F_{1,67}<0.01$	0.998	$F_{1,67}=0.62$	0.433	$F_{1,67}=0.52$	0.472

In these models, filial egg cannibalism was entered either as a continuous variable in the form of female weight gain or as a categorical variable in the form of cannibal versus non cannibal mothers. LR: likelihood ratio. Significant *P* values are in bold.

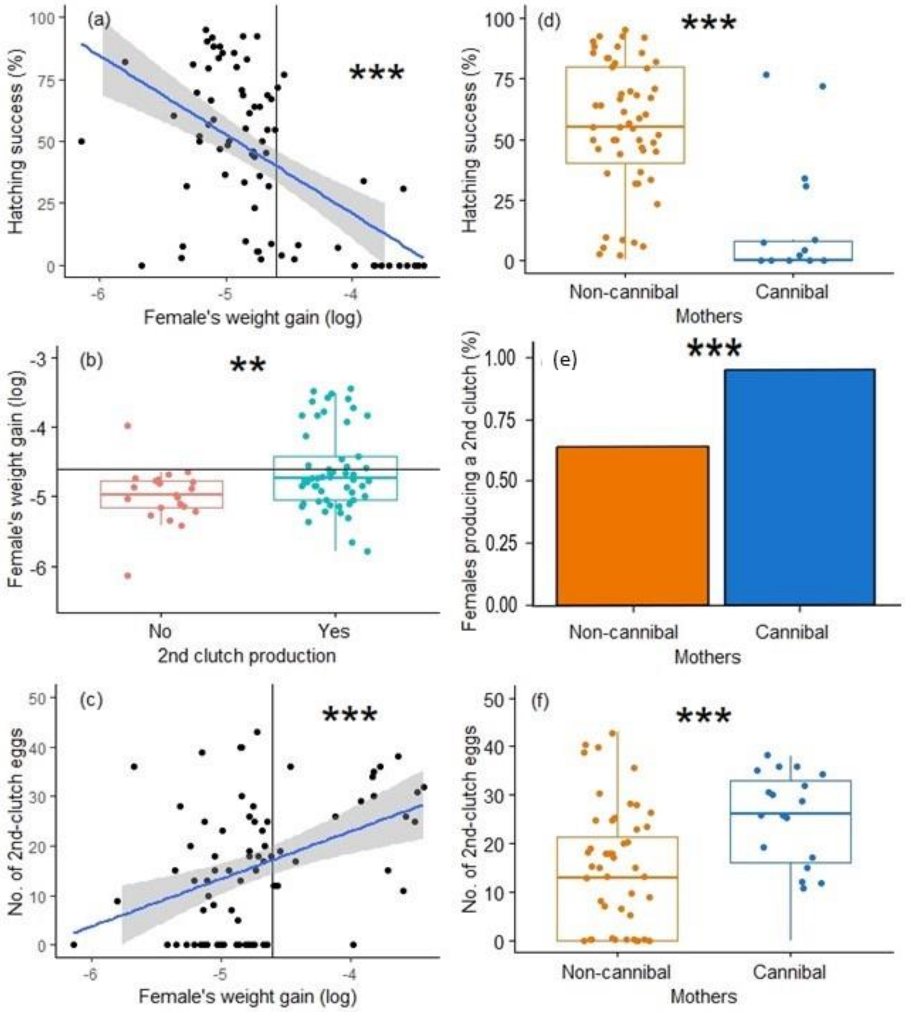

Figure 2.1: Associations between filial egg cannibalism and (a, d) egg hatching success, (b, e) likelihood of producing a second clutch and (c, f) number of second-clutch eggs produced. (a, b, c) Quantitative proxy of filial egg cannibalism (i.e. female weight gain). (d, e, f) Qualitative proxy of egg cannibalism (cannibal versus noncannibal mothers). (a, c) Grey zones represent the standard error of each regression line. Vertical lines show the value corresponding to a null female weight gain, i.e. log(0.01). (b, d, f) Box plots depict median (bold bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. $^{**}P < 0.01$; $^{***}P < 0.001$.

The range in level of clutch defence expressed by the tested mothers was 2 - 100 pokes (Fig. 2.2a; mean ± SE = 21.14 ± 2.06), while that in the delay of maternal return was 0 - 1200 s (Fig. 2.2b; mean ± SE = 121.9 ± 32.3). These two forms of maternal care were independent of filial egg cannibalism (with both quantitative and qualitative proxies; Table 2.1, Fig. 2.2a, b, c, d), as well as of their initial body weight and production of first-clutch eggs (Table 2.1).

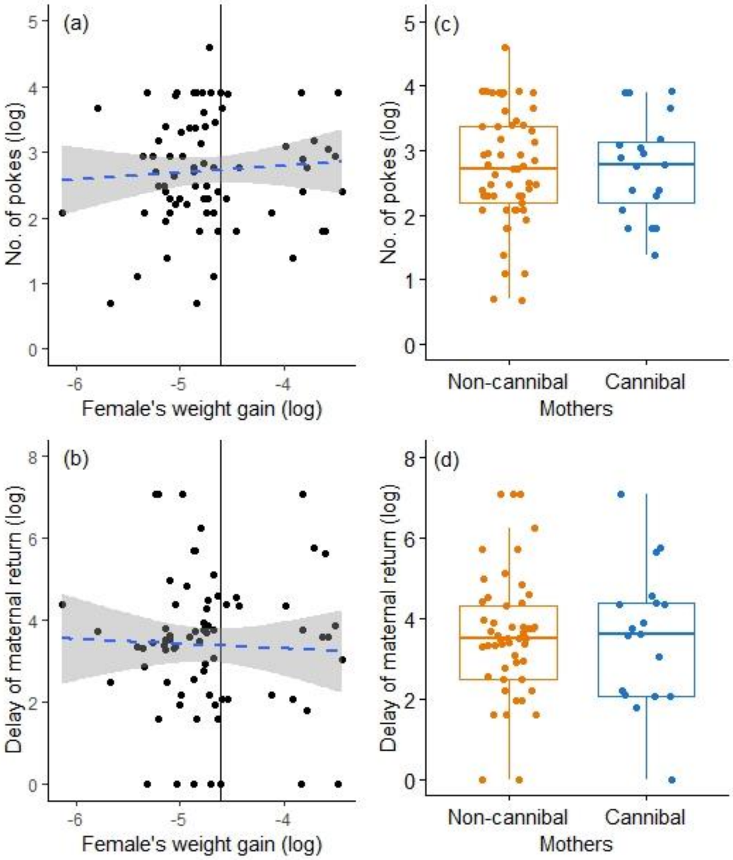

Figure 2.2: Association between filial egg cannibalism and two forms of maternal care: (a, c) clutch defence and (b, d) delay of maternal return (the delay after which females returned to their clutch after being chased away by a simulated predator attack, i.e. poking with a capillary). (a, b) Quantitative proxy of filial egg cannibalism (i.e. female weight gain). (c, d) Qualitative proxy of egg cannibalism (cannibal versus noncannibal mothers). (a, b) Grey zones represent the standard error of each regression line. Vertical lines show the value corresponding to a null female weight gain, i.e. log(0.01). (c, d) Box plots depict median (bold bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values.

Female weight gain was positively associated with the number of eggs they received ($F_{1,69}$ = 6.77, P = 0.011; Fig. 2.3a). However, it was independent of the number of eggs they produced ($F_{1,69}$ = 1.29, P = 0.259; Fig. 2.3b), the interaction between the number of eggs they received and produced ($F_{1,69}$ = 0.10, P = 0.752) and their initial weight at egg laying ($F_{1,69}$ = 0.83, P = 0.367). The use of the qualitative proxy of egg cannibalism provided comparable results: females defined as 'cannibal mothers' received more eggs than non cannibal females (LR_1=4.55, P = 0.033; Fig. 2.3c), whereas they produced the same number of eggs (LR_1=0.15, P = 0.703; Fig. 2.3d) and showed no difference in initial body weight (LR_1<0.01, P = 0.982).

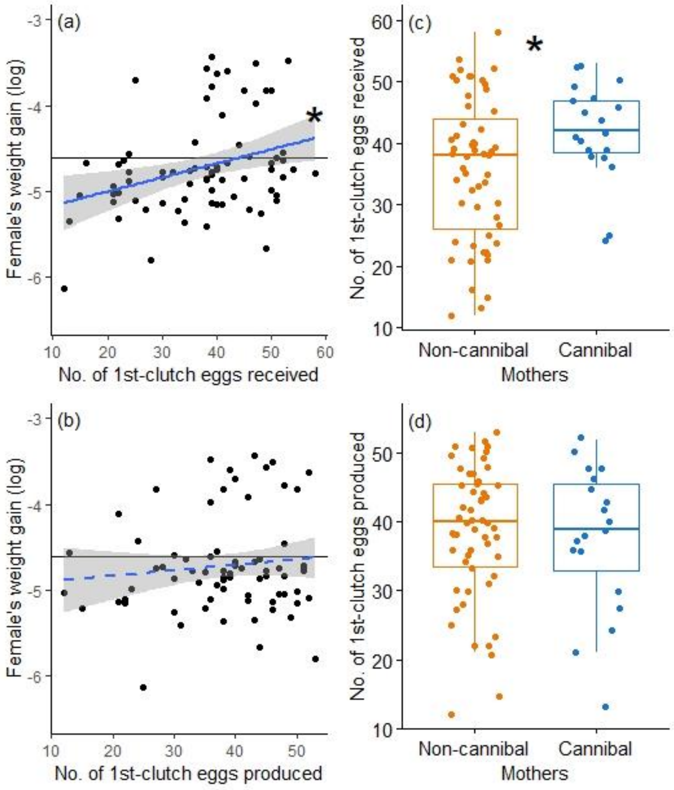

Figure 2.3: Association between filial egg cannibalism and the number of eggs either (a, c) received or (b, d) produced by the foster mothers. (a, b) Quantitative proxy of filial egg cannibalism (i.e. female weight gain). (c, d) Qualitative proxy of egg cannibalism (cannibal versus noncannibal mothers). (a, b) Grey zones represent the standard error of each regression line. Vertical lines show the value corresponding to a null female weight gain, i.e. log(0.01). (c, d) Box plots depict median (bold bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. *P < 0.05.

DISCUSSION

Shedding light on the nature and determinants of filial egg cannibalism is of central importance to better understand how and why this surprising phenomenon has emerged in nature and to reveal its potential link to the evolution of parental care. Using a cross-fostering experiment in the European earwig, we demonstrated that filial egg consumption, measured through maternal weight gain, was (1) associated with an increase in maternal investment in future egg production, (2) independent of how much mothers invested in two classic forms of egg care and (3) determined by the number of eggs tended, and not by the eggs produced, by the mother. Interestingly, our results also showed a negative association between egg hatching success and female body weight at egg laying, and confirmed both the absence of a trade-off between first- and second-clutch production in this species and the coexistence of females exhibiting either high or low overall investment in egg production (Meunier *et al.*, 2012; Ratz *et al.*, 2016).

We first found that filial egg cannibalism was positively associated with both the likelihood of producing and the size of a second (terminal) clutch. These associations suggest that mothers expressing egg cannibalism have either a better intrinsic quality than mothers that do not or that egg consumption allows mothers to increase their investment in future reproduction. The first hypothesis is unlikely to explain our results, as we showed that female weight at egg laying did not differ between mothers that did or did not eat eggs. Conversely, the second hypothesis is in line with results from studies conducted in other animal species (Manica, 2002; Klug & Bonsall, 2007). These results show that egg cannibalism can allow parents to reallocate resources from a current reproductive effort to a future one when current environmental conditions become harsh and render their present eggs unlikely to develop and survive (Sargent, 1992; Manica, 2002). In our experiment, however, earwig mothers experienced neither harsh nor changing environments, so that the benefits of reallocating resources from current to future reproduction were low (if any) and thus unlikely to be the main driver of this phenomenon. One hypothesis to explain why earwig mothers consumed some of their eggs is that clutch size reduction limits the risks of competition between future juveniles (Mock & Parker, 1998) and that its associated direct benefits for mothers (increasing nutrient intake) are simple by-products. In line with this explanation, previous studies reported that earwig family life is shaped by fierce competitive interactions between siblings (Dobler & Kölliker, 2010, 2011) and that mothers reduce the number of newly hatched juveniles under specific conditions (Kramer et al., 2017; Kramer & Meunier, 2016; Kramer, Thesing, & Meunier, 2015). The high likelihood of egg cannibalism when clutches are large (data in the present study; Koch & Meunier, 2014) could thus reflect an efficient maternal strategy to limit the subsequent level of sibling competition, as reported in the burying beetle, *Nicrophorus tomentosus* (Trumbo, 1990). Further work exploring the costs and benefits of egg cannibalism for offspring fitness is nevertheless required to confirm this hypothesis.

The fact that cannibal mothers were more likely both to produce a second clutch and to produce more second-clutch eggs does not necessarily imply that they had a higher fitness due to a larger net productivity (i.e. total number of uneaten eggs produced) or to producing better-quality offspring. Given that the two types of mothers produced a comparable number of first-clutch eggs, the net productivity of cannibal mothers can be larger than that of noncannibal mothers only if their production of second-clutch eggs outweighs their consumption of first-clutch eggs. Our results, however, show that the 19 females classified as 'cannibal mothers' produced 25.78 ± 2.17 second-clutch eggs, whereas 36.21 ± 2.79 of their first-clutch eggs did not hatch (Welch t test: $t_{33.48} = 2.96$, $P = 0.006$). Even if the number of unhatched eggs does not include cannibalized eggs (see Methods), these values suggest that cannibal mothers produced only as many, or fewer, second-clutch eggs as the number of first-clutch eggs they ate, and thus that their net productivity was comparable to or lower than that of noncannibal ones. Similarly, previous studies showed that the quality of earwig juveniles (estimated from their weight, developmental speed and survival rate) is comparable between first and second clutches (Meunier et al., 2012; Ratz et al., 2016; Tourneur & Meunier, 2020), suggesting that a preferential investment in the production of one of these two clutches does not necessarily reflect a preferential investment in high- or low-quality offspring. Overall, the absence of a clear fitness benefit of filial egg cannibalism for mothers in terms of net productivity and/or high-quality offspring may explain why few mothers perform this behaviour in the European earwig (see also Koch & Meunier, 2014).

Our results also demonstrate that earwigs' filial egg cannibalism was independent of two important forms of maternal egg care: clutch defence and the delay of maternal return. The level of maternal investment in egg care can show broad interindividual variation in nature, which often leads to important variation in the development and survival of the resulting eggs and offspring (Westneat *et al.*, 2011; Van Dijk *et al.*, 2012; Williams & Fowler, 2015). This link is known in the European earwig, where females' investment in both pre- and posthatching care varies greatly, which has major consequences for offspring in terms of, for instance, egg development time or weight at hatching (Kölliker, 2007; Kölliker & Vancassel, 2007; Boos *et al.*, 2014; Van Meyel *et al.*, 2019). Here, we have shown that low maternal investment in egg care does not necessarily come with high levels of egg cannibalism (and vice versa). This reveals that egg cannibalism is not on a continuum from poor to high maternal investment in egg care and instead reflects a specific phenomenon that could evolve under different selection pressures than those driving the evolution of egg care. This opens the scope for future research exploring these selection pressures and studying their roles in the early emergence of brood care from the ancestral nonbrood care state (Wong *et al.*, 2013; Furness & Capellini, 2019).

The use of a cross-fostering experimental design finally allowed us to demonstrate that the known positive link between clutch size and filial egg cannibalism (Koch & Meunier, 2014) is driven by the number of eggs that are tended by the mothers and not by the number of eggs they have laid or by differences between the number of eggs laid and received. An effect of the number of eggs produced could have been expected in species in which females both incur physiological constraints forcing them to lay their eggs and experience long and planned periods of starvation, two parameters present in the European earwig (Lamb, 1976). Assuming that the resulting costs of egg production would be very limited in these species compared to the benefits of ensuring access to future food resources, these mothers would benefit directly from producing a number of eggs tightly associated with their future needs. Our results, however, do not support this scenario in earwigs. Instead, they suggest that the costs of egg production are typically larger than the benefits of egg consumption and thus that egg cannibalism is a behaviour determined after egg laying.

On a more general level, our findings highlight that the presence of egg care can be entangled with the presence of other behaviours that directly or indirectly improve maternal investment in future reproduction at the cost of (some) current egg survival. The benefits of egg cannibalism for mothers could be important regarding the evolution of egg brooding from a nonegg-brooding state, because access to partial egg cannibalism may (at least partly) select for mothers to remain with their eggs (i.e. egg brooding). Enhancing egg development and survival may thus not be the sole evolutionary drivers of the emergence and consolidation of egg brooding. This alternative (but non-mutually exclusive) evolutionary scenario could be particularly well suited for species in which mothers have long life spans and limited access to food after egg laying, as in the European earwig in which females live for 1.5 years and stop their foraging activities during the period of egg care (Kölliker, 2007; Lamb, 1976; Tourneur & Meunier, 2020). Moreover, the cost of maternal presence for the survival of some eggs reported in this study is in line with that for some earwig juveniles (when food resources are limited) reported in previous studies (Kramer et al., 2017; Meunier & Kölliker, 2012). Altogether, these results demonstrate that the presence of a mother with its descendants (eggs or juveniles) is not necessarily associated with net benefits for the latter (although the benefits of egg cannibalism for the surviving offspring have yet to be explored), which questions the central roles of parental care and its benefits for offspring in current theories on the early evolution of family life (Kramer et al., 2017; Kramer & Meunier, 2018; Meunier & Kölliker, 2012).

SUPPLEMENTARY MATERIAL

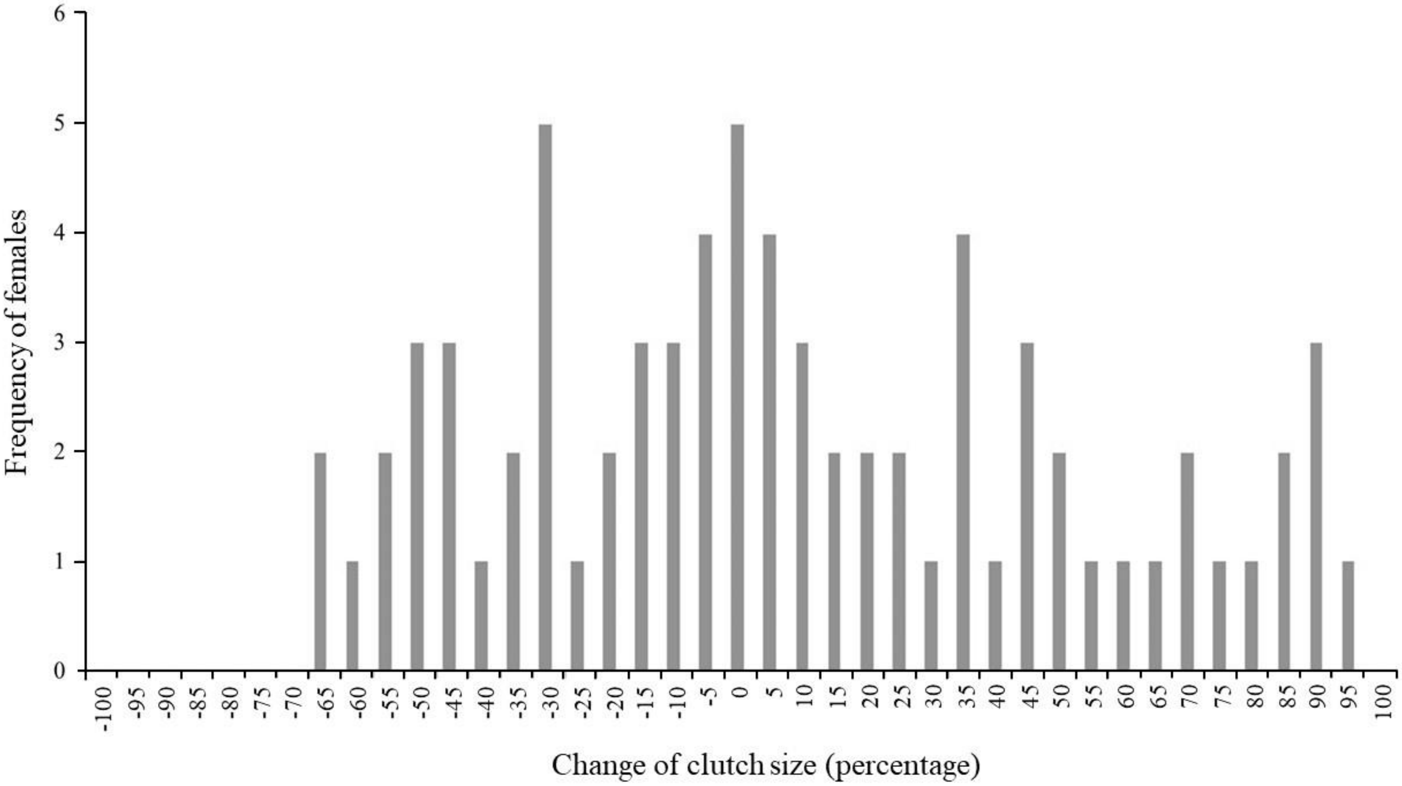

Figure S2.1: Number of females per change in clutch size [(number of eggs received - number of eggs produced)/number of eggs produced] when eggs were cross-fostered between females.

Table S2.1: Effects of female weight at egg laying, egg cannibalism and egg production on hatching success using a data set without females that had a hatching success of zero

	Female weight gain used as a proxy of egg cannibalism		Category of female cannibalism used as a proxy of egg cannibalism	
	LR_1	*P*	LR_1	*P*
Initial female weight	5.63	**0.018**	4.44	0.351
Filial egg cannibalism (FEC)	11.24	**0.001**	7.01	**0.008**
No. of first-clutch eggs (NE)	0.64	0.425	1.27	0.260
FEC * NE	0.06	0.807	1.44	0.231

In these models, filial egg cannibalism was entered either as a continuous variable in the form of female weight gain or as a categorical variable in the form of cannibal versus non cannibal mothers. LR: likelihood ratio. Significant *P* values are in

CHAPITRE 3

Les juvéniles peuvent-ils apporter une assistance nutritionnelle à leur mère chez le forficule européen ?

Résumé de l’étude

On a depuis longtemps considéré que la vie de famille menait à de multiples conflits impliquant les parents et/ou les descendants ainsi qu’à une seule forme d’interaction coopérative par le biais des soins parentaux. Cependant, des études récentes suggèrent que d'autres interactions sociales peuvent se produire au cours de la vie familiale et jouer un rôle clé dans son émergence, comme les comportements coopératifs des jeunes envers leurs parents. Dans cette étude, nous avons testé si ce type d’interaction existait chez le forficule européen et si les juvéniles aidaient leurs mères par le biais de la coprophagie lorsque celle-ci était privée de nourriture. Pour cela, nous avons mis en place quatre types de familles expérimentales dans lesquelles nous avons manipulé les conditions nutritionnelles des mères et de leurs juvéniles. Nous avons ensuite mesuré l’effet du manque de nourriture sur la consommation de fèces par les mères et sur la production de fèces par les juvéniles. Nos résultats confirment tout d'abord le fait que les mères consomment les fèces de leurs juvéniles. De plus, cette consommation est plus probable en présence de fèces produites par des juvéniles nourris que par des juvéniles privés de nourriture. La quantité de fèces consommés, quant à elle, est plus importante chez les mères privées de nourriture que chez les mères nourries. Cependant, nous avons constaté que les juvéniles nourris n'augmentaient pas leur production de fèces lorsque leurs mères étaient privées de nourriture. Par conséquent, les juvéniles chez le forficule européen ne semblent pas fournir d'assistance nutritionnelle de façon proactive à leur mère. Dans l'ensemble, ces résultats appellent à des travaux futurs explorant les avantages de l'allocoprophagie chez les espèces précociales. Ils soulignent qu'une meilleure compréhension des moteurs évolutifs de l'évolution de la vie familiale nécessite des recherches plus approfondies sur toutes ces formes négligées d'interactions sociales qui se produisent dans les unités familiales.

Do juveniles provide nutritional assistance to food-deprived mothers in the European earwig?

Sophie Van Meyel, Séverine Devers and Joël Meunier

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Van Meyel S., Devers S. and Meunier J. Do juveniles provide nutritional assistance to food-deprived mothers in the European earwig *Forficula auricularia*. Under review in *Behavioral Ecology and Sociobiology*.

ABSTRACT

Family interactions have long been thought to reflect either cooperation through parental care, or competition through the multiple conflicts involving parents and/or offspring. However, recent works suggest that other social interactions can occur during family life and be of central importance in its early evolution, such as cooperative behaviours of juveniles towards their parents. In this study, we tested whether this behaviour occurs and whether juveniles help their food-deprived mothers in the European earwig *Forficula auricularia.* In this family-living insect, juveniles exhibit early foraging capabilities and share food with their siblings through coprophagy. We set up four types of experimental families in which we manipulated the nutritional needs of mothers and their juveniles, and then measured the effects on mothers' consumption of juvenile feces, and juveniles' feces production. Our results first show that mothers indeed consumed the feces of their juveniles. This consumption was more likely in the presence of feces from fed compared to food-deprived juveniles, and the number of feces pellets eaten was larger in food-deprived compared to fed mothers. However, we found that the fed juveniles did not increase their production of feces when their mothers were food-deprived. Hence, earwig juveniles do not provide active nutritional assistance to their mothers via allocoprophagy. Overall, these findings call for future works exploring the benefits of allo-coprophagy in precocial insect families and emphasize that better understanding the early evolutionary drivers of family life requires further explorations of all the neglected forms of social interactions occurring in family units.

INTRODUCTION

The social interactions occurring during family life have long been thought to reflect either cooperation in the form of parental care or competition in the forms of sexual and parent-offspring conflicts. Parental care takes a great diversity of forms across taxa and species and typically provides essential benefits to offspring by improving their protection against predators, pathogens and starvation when juveniles have poor foraging capabilities (Alonso-Alvarez & Velando, 2012; Balshine, 2012; Klug & Bonsall, 2014; Machado & Trumbo, 2018). Conversely, three main conflicts occur between family members. One of them occurs among parents, as they are expected to compete with each other to reduce their investment into parental care at the other parent's expense (Trivers, 1972; Lessells, 2012; Meunier & Kölliker, 2012a; Smiseth, 2019). The two other conflicts are between offspring and either their siblings, as offspring are expected to compete with each other to selfishly monopolize parental resources (Mock & Parker, 1997; Roulin & Dreiss, 2012) or their parents, as offspring are selected to demand more parental investment than the parents are selected to provide (Trivers, 1974; Kilner & Hinde, 2012; Kölliker *et al.*, 2015).

This traditional vision of disequilibrium between a single form of cooperation (parental care) and the many forms of competition (sexual conflict, sibling rivalry and parent-offspring conflict) has long fostered the idea that parental care is the main (if not sole) source of benefits for family members and thus that it likely is the main promoter of the emergence and maintenance of all forms of family life in nature (Kramer & Meunier, 2019; Socias-Martínez & Kappeler, 2019). However, a growing number of studies questions this idea. In particular, they emphasize that other neglected forms of cooperation possibly occur between family members and that their associated benefits might be particularly crucial when the benefits of having a tending mother are limited for offspring, a scenario that likely prevailed during the early evolutionary transition from solitary to family life (Kramer & Meunier, 2019). For instance, juveniles of several vertebrate and arthropod species show forms of cooperation instead of exclusively competing with each other during family life. This is the case in the barn owls *Tyto alba* where offspring express allopreening and food sharing towards their siblings (Marti, 1989; Roulin et al., 2016), in the domestic rabbit *Oryctolagus cuniculus* where newborn siblings cooperate by sharing the best thermal positions within the nest (Bautista *et al.*, 2008) and in the European earwig *Forficula auricularia* L. where juveniles share food through allo-coprophagy and proctodeal trophallaxis (mouth-to-anus contacts; Falk et al. 2014). Hence, these findings call for an update of our general understanding of the nature of social interactions occurring between family members and for studies exploring their neglected forms (Kramer & Meunier, 2019).

Among the neglected forms of social interactions possibly occurring between family members (Roulin & Dreiss, 2012; Falk *et al.*, 2014; Kramer *et al.*, 2015; Magneville *et al.*, 2018; Rebar *et al.*, 2020), the presence of cooperative behaviours from juveniles toward their parents remains poorly explored. Yet, this cooperation could be particularly important to explain the early evolution and maintenance of precocial family life, an ancestral form of family where juveniles exhibit early foraging capabilities and can develop and survive in absence of parental care (Falk *et al.*, 2014; Kramer & Meunier, 2019). In these families, offspring assistance to parents in the form of food provisioning could provide significant benefits to all family members, as it may help parents to access a broad diversity of food resources provided by their large number of foraging offspring or any food source when their mobility is limited (e.g. due to poor condition), while it may provide direct benefits to juveniles by maintaining parents in the nest to display efficient defences against predators and pathogens (Alonso-Alvarez & Velando, 2012; Balshine, 2012; Klug & Bonsall, 2014; Machado & Trumbo, 2018). Interestingly, the nutritional assistance of adult offspring towards parents is a common phenomenon in species with highly derived forms of family life such as in eusocial insects (which includes ants, termites and some bees and wasps; Rubenstein & Abbot, 2017). In these so-called ‘extended’ families (Kramer & Meunier, 2019), parents (queens and sometimes kings) are almost exclusively fed by their worker offspring (which can be juveniles in termites societies; Eggleton, 2011). Whether nutritional assistance of juveniles toward parents occurs in precocial species remains, however, unknown.

In this study, we investigated whether juveniles provide nutritional assistance to their mothers in the precocial European earwig *Forficula auricularia* clade B (González-Miguéns et al., 2020; Wirth et al., 1998), a species where juveniles both exhibit early foraging capabilities and are relatively independent of parental care. Earwig mothers remain with their clutch of eggs overwinter and then with the emerged juveniles (called nymphs) during the first two weeks following egg hatching (Lamb, 1976; Kölliker, 2007; Van Meyel *et al.*, 2019; Tourneur & Meunier, 2020). During this post-hatching family life, mothers provide multiple forms of care to their nymphs, including fierce protections against predators, grooming behaviours and food provisioning through regurgitation (Staerkle & Kölliker, 2008; Mas & Kölliker, 2011; Ratz *et al.*, 2016). The level of care expressed by earwig mothers often depends on the nutritional needs of both nymphs and mothers, which are typically assessed by all family members via individual chemical signatures (Mas *et al.*, 2009, 2011; Wong *et al.*, 2014a). Although maternal care is costly for earwig mothers, e.g. it delays their production of a second clutch (Kölliker, 2007), it is also facultative: earwig nymphs are quickly capable of foraging for themselves and return to their nest after their foraging trips (Wong & Kölliker, 2012), they survive and develop well in the absence of mothers (Kölliker, 2007; Thesing *et al.*, 2015; Kramer *et al.*, 2017) and they can share food with their siblings through the production and consumption of feces pellets (allo-coprophagy; Falk et al. 2014). Here, we tested whether 1) earwig mothers can get food from their juveniles through the consumption of their feces, 2) mothers adapt their consumption to their own nutritional needs and/or the nutritional condition of the feces-producing juveniles (which may, for instance, shape the nutritional quality of the feces) and finally whether 3) juveniles actively adapt their production of feces (e.g. via a modified foraging activity and/or food intake) to the nutritional needs of their mother. Note that we did not investigate whether earwig nymphs provide food to their mothers through stomodeal trophallaxis (i.e. mouth-to-mouth contacts) because the frequency of this behaviour is low (Mas & Kölliker, 2011) and previous works showed that it does not mediate food transfer between nymphs during family life (Falk *et al.*, 2014).

We set up four types of experimental families in which we provided food either to the mothers, the juveniles, both mothers and juveniles, or none of them, for eight days (half the period of post-hatching family life) - isolated earwig nymphs are known to survive up to 15 days without food access (Körner *et al.*, 2016). We then counted the number of feces pellets produced by juveniles on day eight and the occurrence and level of maternal consumption of these nymphal feces pellets on day nine. If nymphs share food with their mother via allo-coprophagy (just like they do with their siblings; Falk et al. 2014), we predicted that this same mechanism occurs between offspring and mothers, with mothers consuming at least some feces pellets produced by their juveniles. If the level of allo-coprophagy is driven by the nutritional needs of the mother, we predicted feces consumption to be higher in food-deprived compared to non-food deprived mothers. Finally, if this coprophagy is an active form of nutritional assistance from juveniles to food-deprived mothers, we predicted that juveniles produced more feces (e.g. due to a higher foraging activity and food intake) when they were tended by a food-deprived compared to a non-food deprived mother.

MATERIALS AND METHODS

Experimental setup

The experiment involved 95 earwig families produced by 95 *F. auricularia* females field-sampled in June 2018 in Pont-de-Ruan (France) and then maintained under standard laboratory conditions until egg-laying (Nov-Dec 2019) and then egg hatching (Jan-Feb 2019; details of the standard rearing conditions are provided in Meunier et al., 2012). One day after egg hatching, each clutch was randomly assigned to one of four treatments consisting of the provisioning of food to either i) the mother (*N*=24), ii) the nymphs (*N*=24), iii) both the mother and the nymphs (*N*=23) or iv) none of them (*N*=24). To this end, the number of nymphs per family was first standardized to 25 (initial mean ± SE number of nymphs per clutch = 34.52 ± 0.57; nymphs were never mixed between clutches) and the resulting experimental family transferred in large Petri dishes (10 cm diameter) lined with moistened sand and maintained under 20°C and 12h:12h light: dark cycle. The manipulation of food provisioning was done by temporarily separating mothers and nymphs in two small Petri dishes (5 cm diameter) lined with moistened sand, then providing them with an *ad libitum* amount of food or no food (depending on the treatment) for two hours, and finally returning mothers and their nymphs to their original large Petri dishes. Food provisioning was manipulated two, four, six and eight days after egg hatching. Mothers were fed with a standard food preparation (mostly containing pollen, carrots, and cat food; see detailed food composition in Meunier et al., 2012) on each of these days. By contrast, nymphs were fed with the standard food preparation on days two and four, whereas they were fed with green-coloured pollen pellets on day six and eight (Kramer et al., 2015). The consumption of green pollen allowed the subsequent production of green feces pellets by juveniles, which facilitated both their counting by the experimenter and the discrimination between nymph and (when present) mother produced feces (Falk *et al.*, 2014). Immediately after our last manipulation of food provisioning on day eight, 15 nymphs per family were isolated in a new container to allow feces production. Fourteen hours later, we removed the nymphs, counted the number of feces pellets in the container (without touching them as they are very fragile and cannot be removed and/or moved) and then transferred each mother to the container with the feces of their own nymphs. Two hours later, we counted the number of remaining feces pellets in the container. The very few feces pellets produced by mothers during these two hours (when present) were typically non-green and larger than the ones produced by nymphs and were thus excluded from this counting. To minimize observer bias, the feces counting was done blindly regarding the treatment applied to the mothers and their nymphs. For feces production (day 8) and feces consumption (day 9), family members were transferred to new Petri dishes

(10 cm diameter) lined with a piece of white, moistened filter paper (Macherey-Nagel GmbH & Co. KG, Düren, Germany) to facilitate the counting of green feces pellets.

We defined the number of feces pellets eaten by a mother as the number of green feces pellets present in the container before minus after maternal introduction. Somewhat surprisingly, this number was negative in 4 of the 95 replicates (4.2%). A likely explanation for these values is that even if mothers typically produce feces pellets that are non-green and larger compared to the feces of their nymphs, a few mothers might have produced some ‘small’ indistinguishable feces pellets during the experiment and this production happened to be larger than the number of nymph’s feces pellets they have possibly consumed in these four replicates. Among these four females, two belonged to the treatment where both females and nymphs had no food access (our calculation led to -1 and -1 eaten feces pellets, respectively), one to the treatment where both females and nymphs had food access (-2 eaten feces pellets) and one to the treatment where only the nymphs had food access (-1 eaten feces pellets). For consistency, we removed these four values in all our statistical analyses. Nevertheless, either changing these values to zero, removing or maintaining them in our analyses do not qualitatively change the results (see details in Table S3.1). Note that only a few replicates contained the typical ‘large’ non-green maternal feces pellets, which suggests that maternal feces production was overall very limited during the 2h of our experiment and thus that the potential limit of the method discussed above is unlikely to affect our general conclusions.

Statistical analysis

We analyzed the effects of nymphs’ and mothers’ food status on the production of feces pellets using a linear general model (LM), in which the number of feces pellets produced by the fifteen nymphs was entered as a response variable, while nymphs’ food status, mothers’ food status and the interaction between these two factors were entered as explanatory factors. Because a large number of mothers did not consume any fecal material produced by their nymphs (see results and Figure S1), this data was analysed using two successive approaches. We first analyzed the effects of nymphs' and mothers' food status on the consumption of at least one nymphs' feces pellet by a mother using a generalized linear model (GLM) with binomial error distribution. In this model, whether a mother was or was not coprophagous (1 or 0, respectively) was entered as a response variable, while nymphs' food status, mothers' food status and the interaction between these two factors were entered as explanatory factors. Second, we analyzed the effects of nymphs' and mothers' food status on the level of feces consumption by coprophagous mothers using two additional LM, in which the proportion and the absolute number of feces pellets eaten by a coprophagous mother were used as a response variable, while nymphs' food status, mothers' food status and the interaction between these two factors were entered as explanatory factors. To fulfil model assumptions, we log-transformed both the number of feces pellets produced by nymphs and the absolute number of feces pellets eaten by mothers. When required, we conducted pairwise comparisons between treatments using estimated marginal means of the LMs and GLM, and Tukey-adjusted *P*-values. All these models were conducted with R v4.0.2 (R Core Team, 2017) loaded with the packages *car* and *emmeans*.

RESULTS

Overall, the groups of nymphs produced from 2 to 194 feces pellets (mean ± SE = 48.32 ± 4.97; Figure S3.1A), which corresponds to an average of 3.2 feces pellets per nymph per 14h. This production was shaped by an interaction between nymphs' and mothers' food access ($F_{1,91}$ =7.57, $P = 0.007$; Figure 3.1; see table S3.1 for full model description). Contrary to our predictions, however, this interaction reveals that the fed nymphs produced more feces when they were tended by fed compared to food-deprived mothers (pairwise comparison: $t_{91} = -3.37$, $P = 0.006$) and that this effect was absent in the food-deprived nymphs (pairwise comparison: $t_{91} = 0.50$, $P = 0.959$).

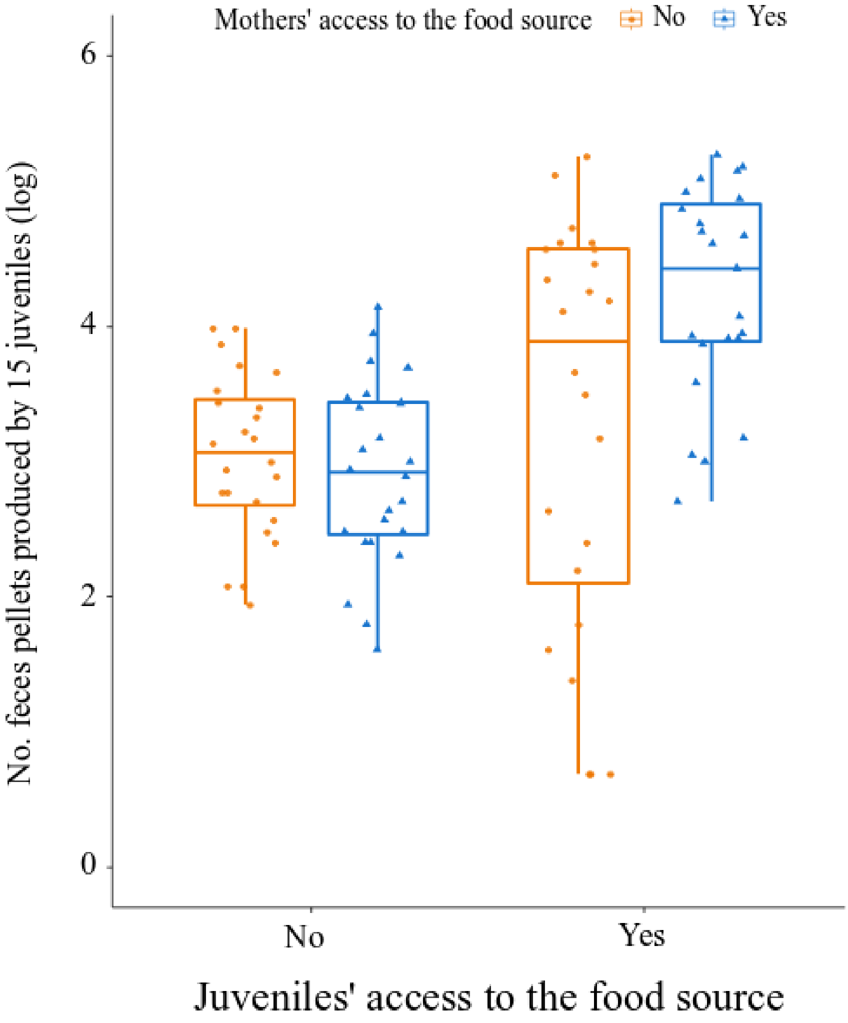

Figure 3.1: Effects of juveniles' or mothers' access to food during family life on the number of feces pellets produced by fifteen juveniles over 14h. Boxplots depict median and interquartile range, with whiskers extending to 1.5 times the interquartile range and dots representing experimental values.

The consumption of at least one nymphs' feces pellet by the mother was present in 46 of the 91 (50.5%) trials. This maternal coprophagy was overall more frequent when the feces were produced by fed compared to food-deprived nymphs (Figure 3.2A; Likelihood Ratio $\chi^2_1 = 5.99$, $P = 0.014$), whereas it was independent of mothers' food access (LR $\chi^2_1 = 0.39$, $P = 0.532$; Figure 3.2A) and an interaction between mothers' and nymphs' food access ($\chi^2_1 = 1.51$, $P = 0.220$).

In the 46 coprophagous mothers described above, the absolute number of nymphs' feces pellets eaten by each female varied from 1 to 125 (mean ± SE = 12.98 ± 3.28; Figure S3.1B). These numbers correspond to proportions ranging from 1 to 75 % (mean ± SE = 20.05 ± 2.80 %) of the total number of nymphs' feces pellets available during the tests, i.e. coprophagous mothers never consumed all the feces pellets produced by their nymphs. The proportion of nymph's feces pellets eaten by coprophagous mothers was shaped by an interaction between nymphs' and mothers' food access (Figure 3.2B; $F_{1,42} = 4.54$, $P = 0.039$; see table S3.1 for full model description). In the presence of feces produced by fed nymphs, the proportion of feces eaten was larger in food-deprived compared to fed mothers (Figure 3.2B; pairwise comparison: $t_{42} = 3.87$, $P = 0.002$). This effect was, however, not detected in the presence of feces produced by starved nymphs (Figure 3.2B; pairwise comparison: $t_{42} = 0.29$, $P = 0.992$). Interestingly, the absolute number of feces eaten by coprophagous mothers was driven by the same interaction between nymphs' and mothers' food access (Figure 3.2C), even if this interaction was just above the significance level ($F_{1,42} = 3.87$, $P = 0.056$). This last statistical model also showed that the absolute number of nymphs' feces pellets eaten by a mother was overall higher when the feces were produced by fed compared to food-deprived nymphs (Figure 3.2; $F_{1,42} = 12.28$, $P = 0.001$) and independent of mothers' food access ($F_{1,42} = 1.57$, $P = 0.217$).

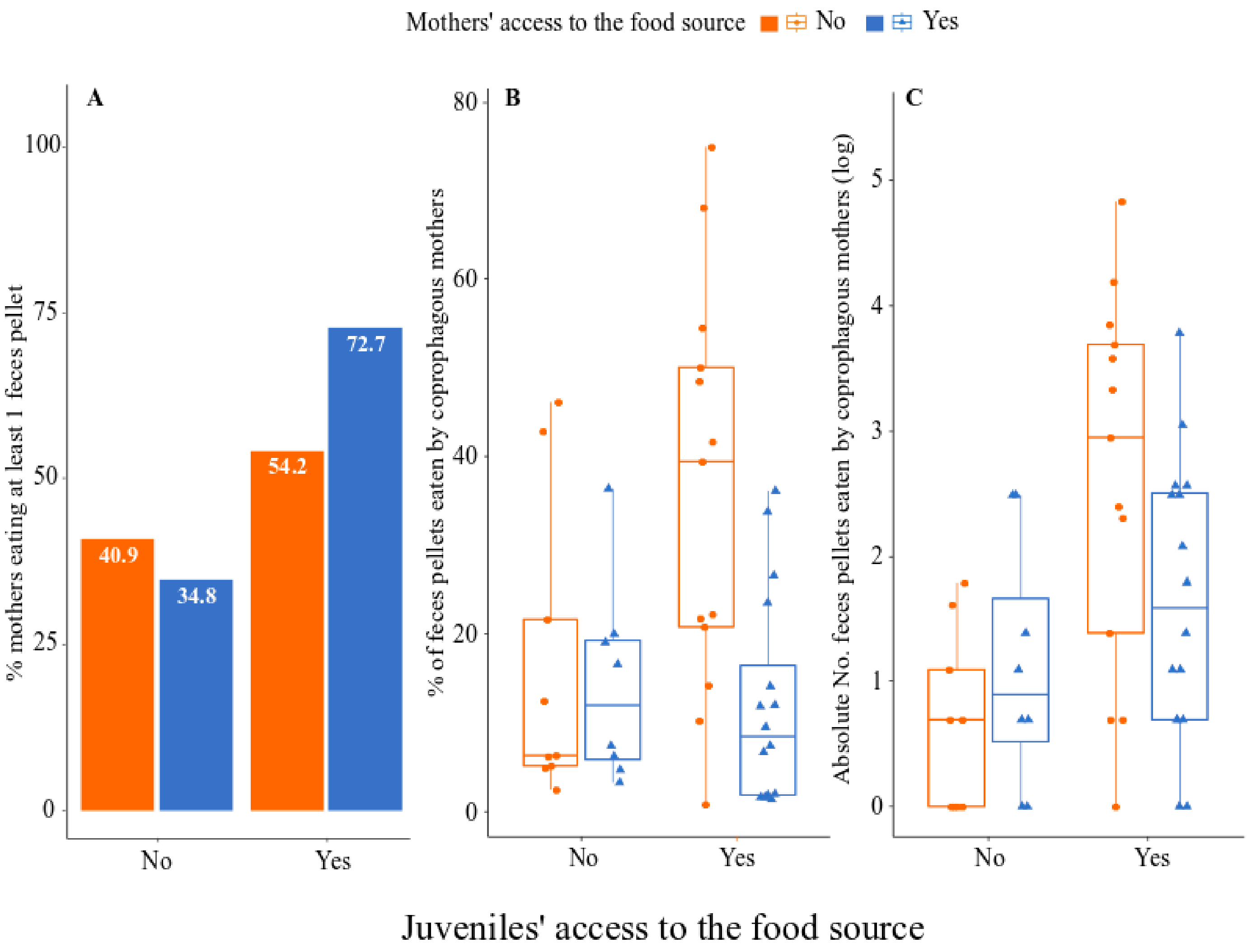

Figure 3.2: Effect of juveniles' or mothers' access to food during family life on the (A) likelihood of mothers to eat at least one nymphs' feces pellet, (B) proportion of nymphs' feces pellet eaten by a coprophagous mother and on the (C) absolute number of nymphs' feces pellet eaten by a coprophagous mother over 2h. Boxplots depict median and interquartile range, with whiskers extending to 1.5 times the interquartile range and dots representing experimental values.

DISCUSSION

Whereas a great diversity of social interactions may occur between family members (Roulin & Dreiss, 2012; Falk *et al.*, 2014; Kramer *et al.*, 2015; Magneville *et al.*, 2018; Rebar *et al.*, 2020), decades of research on this topic have often neglected the occurrence and impact of several types of family interactions (Kramer & Meunier, 2019). Cooperative behaviours from juveniles toward parents are one of these neglected interactions, whereas it could both operate and be a significant source of benefits for both parents and offspring in species with a precocial family system (Kramer & Meunier, 2019). In this study, we addressed this gap in knowledge by testing whether juveniles can assist their food-deprived mothers via food provisioning (allocoprophagy) in the precocial European earwig *F. auricularia*. Our results first show that about 50% of the tested earwig mothers indeed consumed the feces produced by their juveniles during family life. The likelihood that a mother becomes coprophagous was higher in the presence of feces produced by fed compared to food-deprived nymphs. Similarly, we showed that food-deprived coprophagous mothers exhibited a higher level of feces consumption only when the feces were produced by fed compared to food-deprived nymphs. These findings are overall in line with the presence of a higher amount of poorly digested nutrients in the feces of fed compared to food-deprived nymphs (Körner *et al.*, 2016). Despite the consumption of nymphs' feces pellet by some mothers, we finally showed that the production of nymphs' feces was not positively linked with the nutritional needs of the mother, and instead that the fed nymphs produced more feces when they were tended by fed compared to food-deprived mothers.

Our results first show that about 50% of the tested earwig mothers actively consumed the feces of their juveniles during family life. The consumption of feces produced by conspecifics is a well-documented phenomenon in group-living animals where it can serve to clean the nesting area or provide other direct benefits to recipients (Weiss, 2006). In insects, for instance, this consumption can help to complement normal diet by obtaining additional nutrients such as in the mosquito

Culiseta bergrothi (Nilsson, 1983), to acquire mutualistic micro-organisms that aid in the digestion of specific food sources such as in many wood-feeding insects (Mirabito & Rosengaus, 2016), or to simply improve resistance against starvation such as in the German cockroach *Blattella germanica* (Kopanic *et al.*, 2001). In earwigs, this consumption is unlikely to reflect a maternal behaviour of nest cleaning, as nests' walls and grounds are typically covered with mothers and nymphs feces (Körner *et al.*, 2016) and these feces exhibit antimicrobial properties preventing the development of mould and other potential pathogens (Diehl *et al.*, 2015). By contrast, improving resistance against starvation may (at least partly) explain this maternal behaviour, as we found that feces consumption was overall more likely to occur when mothers had access to good-quality feces (i.e. feces produced by well-fed nymphs), was higher in starved compared to non-starved earwig mothers and a previous study showed that allo-coprophagy allows earwig nymphs to survive longer in absence of any other food source (Körner *et al.*, 2016). Given that resistance against starvation is but one possible benefits of coprophagy, our results nevertheless call for future researches investigating other potential benefits for earwig mothers, such as access to specific nutrients, mutualists symbionts and/or immune components (Diehl et al. 2015).

Contrary to our predictions, the data revealed that the fed nymphs did not increase their production of feces when their mothers were food-deprived, but instead that they increased this production when their mothers were fed. Assuming that all the feces pellets produced by fed nymphs are of equivalent quality and size (Falk *et al.*, 2014; Kramer *et al.*, 2015), this result does not provide support to our prediction of active nutritional assistance of offspring toward their starved mothers in *F. auricularia*. This was surprising, as earwig nymphs can assess the nutritional needs of their mothers (via chemical signals; Wong et al., 2014), and their precocious mobility grants them the capability to adapt their food intake to the condition of the family members they interact with (Wong & Kölliker, 2012; Kramer *et al.*, 2015; Kramer & Meunier, 2016b). Moreover, previous studies suggest that earwig nymphs can modify their production of feces to help siblings during family interactions (Falk *et al.*, 2014; Kramer *et al.*, 2015; Kramer & Meunier, 2016b). Hence, our present results emphasize that increasing feces production to help starved family members may be a form of cooperation that is expressed only between siblings in this species (Falk *et al.*, 2014; Kramer *et al.*, 2015; Kramer & Meunier, 2016b) and thus, that the selection pressures favouring the evolution of cooperative behaviours such as allo-coprophagy could be age-specific in precocial families.

Whereas half of the tested mothers expressed allo-coprophagy, the other half did not consume any nymphs' feces pellets. This split is particularly striking in experimental families where starved mothers only had access to feces produced by their fed nymphs. Such an amount of variation in the expression of maternal allo-coprophagy reveals that selection could operate on this behaviour, but that it has not in the tested context – i.e. when mothers have no other food source available. One potential reason is that the foraging success of earwig mothers and their nymphs rarely mismatch under natural conditions, e.g. because it relies on the same environment surrounding the same nest (Albouy & Caussanel, 1990). Such a rare mismatch may have reduced opportunities for selection to operate on this specific form of offspring assistance and lead to the lack of a systematic expression of allo-coprophagy in starved mothers. Hence, our data shed lights on the limits possibly imposed by family life in the evolvability of certain social interactions and suggests that the evolution of juveniles' assistance towards starved mothers necessitate that juveniles have frequent access to better/more food resources compared to their parents. This scenario could operate when parents terminally invest in their clutch of eggs, are poorly mobiles and/or present peculiarities (e.g. in terms of size or buccal appendices) preventing them from access to resources exclusively available to juveniles.

Finally, we showed that nymphs tended by a fed mother produced more feces when these nymphs had access to food compared to when they had no access to food, whereas nymphs tended by a starved mother produced a comparable number of feces pellets when they had access to food compared to when they had no access to food.In addition to reflecting a pattern opposite to our predictions, these findings suggest that the fed mothers provided food to nymphs that were already well-fed and thus likely to be of good-condition (causing an increase in feces production by the fed nymphs), but not to nymphs that were starved and thus of poor-condition. This finding is in line with previous works showing that earwig mothers increase their investment into care (i.e. foraging activity, allocation of food resources and grooming behaviours) when they are exposed to chemical cues extracted from well-fed compared to poorly-fed juveniles (Mas *et al.*, 2009; Mas & Kölliker, 2011). It is generally expected that parents preferentially feed offspring of higher reproductive value to maximize their fitness return on parental investment (Haig, 1990). Given that post-hatching maternal care is costly in earwigs (Kölliker, 2007), our results support this general prediction.

To conclude, our experiments revealed that earwig mothers can consume the feces of their juveniles during family life that this consumption (at least) partly depends on their own nutritional needs, but that juveniles do not adapt their feces production to the nutritional needs of their mothers. These findings overall indicate that juveniles are unlikely to provide active nutritional assistance to their food-deprived mothers in the European earwig. Nevertheless, they call for future studies exploring as to why mothers eat the feces of their juveniles across the different treatments. More generally, they also call for studies disentangling whether this behaviour is a mere product of selection for immediate food access with no further implication in the early evolution of family life, or whether it may have such an implication by selecting for the maintenance of mothers in the vicinity of their foraging juveniles and thus, by ultimately promoting the emergence and consolidation of family living from a solitary state. By testing for the first time the hypothesis positing that juveniles may provide help to their parents during family life (Kramer & Meunier, 2019), the present study paves the way for future works aimed at disentangling the diversity of social interactions that shape precocial family life and evaluating their impacts in its early evolution (Kramer & Meunier, 2019).

SUPPLEMENTARY MATERIAL

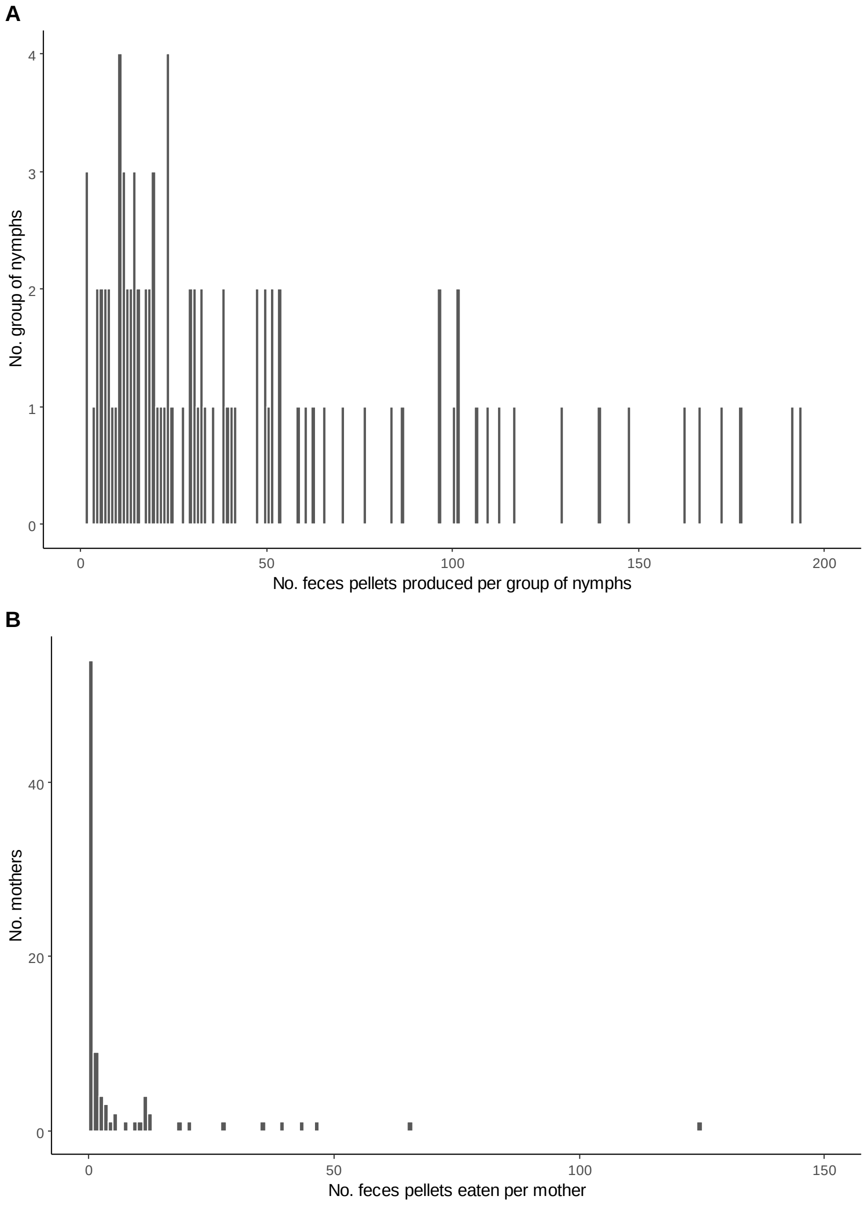

Figure S3.1: Overall distribution of the (A) number of feces pellets produced per group of nymphs and (B) number of nymphs' feces pellets eaten per mother in the corresponding experiments.

Table S3.1: Effects of juveniles' or mothers' access to a food source during family life on the likelihood of maternal coprophagy using data sets where the negative values of feces consumption were either (a) removed or (b) changed in zero. The results are qualitatively similar between the three data sets. Significant p-values are in bold. LR = Likelihood Ratio.

	(a) Negative values removed (main text)		(b) Negative values changed in zero	
	LR χ^2_1	P	LR χ^2_1	P
Juveniles	5.99	**0.014**	6.70	**0.010**
Mothers	0.39	0.532	0.31	0.577
Juveniles : Mothers	1.50	0.220	0.97	0.326

PARTIE II:

Les facteurs écologiques dans l'évolution de la vie de famille

CHAPITRE 4

Immunité sociale : pourquoi il est important d'étudier son évolution au travers de toutes les formes sociales

Résumé de l'étude

Au cours de son cycle de vie, chaque animal interagit avec un grand nombre d'agents pathogènes présents dans son environnement. Ces pathogènes peuvent impacter de façon drastique la survie et la fitness de l'animal. Les systèmes de défenses contre les pathogènes ont donc un rôle majeur pour tous les animaux. Bien que l'on sache depuis longtemps que ces défenses reposent sur des processus individuels tels que le système immunitaire, des études récentes ont souligné l'importance des défenses liées aux comportements sociaux pour les organismes vivant en groupe. Ces systèmes de défense, appelés immunité sociale, ont été principalement étudiés chez les insectes eusociaux tels que les abeilles, les termites et les fourmis. L'immunité social comprend, entre autres, le nettoyage mutuel et la gestion des déchets. Cependant, ces dernières années, un nombre croissant d'étude ont proposé une exploration plus large de l'immunité sociale chez les espèces non eusociales. Dans cette revue, nous résumons les raisons de ces recherches et examinons-en quoi élargir notre vision de l'immunité sociale peut apporter des éclairages majeurs dans notre compréhension du rôle des pathogènes dans l'évolution de la vie sociale. Dans un premier temps, nous présentons le cadre conceptuel originel de l'immunité sociale développée chez les insectes eusociaux et nous mettons en lumière son importance dans les systèmes sociaux hautement dérivés. Dans un second temps, nous remettons en cause trois grands aspects que ce cadre originel a pu favoriser et nous montrons en quoi ils ont rendu nécessaire le passage à une définition plus large de l'immunité sociale. Enfin, et parce que toute définition (même plus large) a besoin de limites, nous présentons trois critères permettant de distinguer ce qui est une forme d'immunité sociale de ce qui ne l'est pas. Dans l'ensemble, nous soutenons que l'étude de l'immunité sociale à travers tous les systèmes sociaux n'apporte pas seulement une nouvelle vision de la façon dont les pathogènes affectent et ont pu affecter l'évolution de l'eusocialité, mais aussi sur leur rôle dans l'émergence et le maintien de la vie sociale à partir d'un état solitaire comme l'évolution de la vie de famille chez les espèces précociales.

Social immunity: why we should study its nature, evolution and functions across all social systems

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ABSTRACT

Mounting defences against pathogens is a necessity for all animals. Although these defences have long been known to rely on individual processes such as the immune system, recent studies have emphasized the importance of social defences for group-living hosts. These defences, called social immunity, have been mostly studied in eusocial insects such as bees, termites and ants, and include, for instance, mutual cleaning and waste management. Over the last few years, however, a growing number of works called for a broader exploration of social immunity in non-eusocial species. In this review, we summarize the rationales of this call and examine why it may provide major insights into our current understanding of the role of pathogens in social evolution. We start by presenting the original conceptual framework of social immunity developed in eusocial insects and shed light on its importance in highly derived social systems. We then clarify three major misconceptions possibly fostered by this original framework and demonstrate why they made necessary the shift towards a broader definition of social immunity. Because a broader definition still needs boundaries, we finally present three criteria to discriminate what is a form of social immunity, from what is not. Overall, we argue that studying social immunity across social systems does not only provide novel insights into how pathogens affect the evolution of eusociality, but also of the emergence and maintenance of social life from a solitary state. Moreover, this broader approach offers new scopes to disentangle the common and specific anti-pathogen defences developed by eusocial and non-eusocial hosts, and to better understand the dependent and independent evolutionary drivers of social and individual immunity.

INTRODUCTION

During its life cycle, every animal encounters large numbers of pathogens such as viruses, protozoans, bacteria, helminths and fungi (Lu & St. Leger, 2016). Pathogen infections often have dramatic consequences in a host, ranging from premature death to the modification of a broad set of fitness-related physiological, morphological and behavioural traits (Siva-jothy *et al.*, 2005). To limit the costs of pathogen infection, hosts have thus developed a multitude of defences encompassed in the term individual immunity (Siva-jothy *et al.*, 2005; De Roode & Lefèvre, 2012; Schmid-Hempel, 2014). In insects, these defences typically rely on physiological changes limiting pathogen development into the host body (i.e. immune system) (Siva-jothy *et al.*, 2005; Hillyer, 2016) and on behavioural processes reducing the risk of pathogen exposure and infection, for instance, by prophylactively or therapeutically consuming food sources with anti-pathogenic properties, a process called self-medication (De Roode & Lefèvre, 2012).

Over the last decades, a growing number of studies has revealed that protection against pathogens may not only rely on the defences exhibited by the host itself, but also on defences generated by its surrounding relatives (Cremer *et al.*, 2007; Cotter & Kilner, 2010a; Meunier, 2015). Textbook examples of this *social immunity* typically come from eusocial insects such as bees, ants and termites (Figure 1) (Schmid-Hempel, 1998; Cremer *et al.*, 2007; Wilson-Rich *et al.*, 2009; Meunier, 2015). One of these examples is allo-grooming, a behaviour frequently reported in eusocial insects, during which workers groom each other to remove the pathogens present on the cuticle (Reber *et al.*, 2011). Another example encompasses sanitary behaviours, during which workers remove food waste and/or cadavers from their colony to prevent the development of microbial pathogens, as found in many bees, ants and termites (Visscher, 1983; Zeh *et al.*, 1999; Hart *et al.*, 2002; Ulyshen & Shelton, 2012). Social immunity can also be illustrated by social isolation, during which infected individuals leave their colony (Heinze & Walter, 2010; Rueppell *et al.*, 2010) or reduce contacts to the brood (Ugelvig & Cremer, 2007; Bos *et al.*, 2012) to limit the transfer of pathogens to colony members. Finally, ant and termite workers frequently use self-produced secretions to sanitize the nest walls and/or the brood (Yek & Mueller, 2011; Baracchi *et al.*, 2012; López-Uribe *et al.*, 2017), which is also a common form of social immunity (for an exhaustive list of all the classical forms of social immunity, please refer to (Cremer *et al.*, 2007; Meunier, 2015)).

The discovery of social immunity rapidly led to major advances in our understanding of why and how eusocial insects are efficiently protected against pathogens (Schmid-Hempel, 1998; Cremer *et al.*, 2007; Cremer & Sixt, 2009). It also gave rise to two evolutionary scenarios on the role of social immunity in the evolution of group living. The first scenario posited that social immunity is a phenomenon that has secondarily derived from eusocial systems and thus only plays a role in the consolidation of complex, permanent and obligatory forms of group living exhibiting reproductive division of labour (thereafter called the *eusocial framework*) (Cremer *et al.*, 2007, 2018; Schmid-Hempel, 2017). The other (more recent) scenario postulates that social immunity is an ancestral phenomenon that can be found in many forms of group living and thus, that social immunity also plays a key role in the early emergence and maintenance of group living from a solitary state (thereafter called the *group-living framework*) (Cotter & Kilner, 2010a; Meunier, 2015).

In this study, we review recent empirical data across eusocial and non-eusocial (i.e. group living species that do not exhibit a eusocial organisation) insects to emphasize why it is now time to study the nature, evolution and functions of social immunity across all social systems. Specifically, we first present the origin and implications of the eusocial framework in our current understanding of anti-pathogen defences in eusocial insects. We then discuss the rationales of the recent call for a switch from a eusocial to a group living framework by shedding light on three major misconceptions that can be fostered by the eusocial framework. In a final part, we stress that understanding social immunity requires boundaries in its definition and thus propose a newly defined group-living framework detailing three criteria that could allow discriminating what is a form of social immunity, from what is not. Overall, we argue that expanding the number of studies on social immunity in a broad taxonomical spectrum of non-eusocial species would provide novel major insights into our general understanding of the common and specific solutions developed by each type of social host to counteract infections and thus, into the role of pathogens in social evolution.

The eusocial framework of social immunity

The eusocial framework of social immunity emerged at the beginning of the 21th century as the result of works conducted by researchers investigating how eusocial insects limit the inherently high risks of pathogen exposure and transmission between colony members (Schmid-Hempel, 1998; Naug & Camazine, 2002; Cremer *et al.*, 2007; Wilson-Rich *et al.*, 2009). The central idea of this framework is that social immunity mimics the individual immunity of multicellular organisms when the unit of selection has shifted from the individual to the colony (Cremer & Sixt, 2009; Masri & Cremer, 2014). In other words, social immunity has "evolved in convergence with individual immunity to protect the entire reproductive entity (i.e. the superorganism, (Kennedy *et al.*, 2017)) and maximize its fitness" [25]. Three examples typically illustrate this parallel between personal and social immunity in eusocial insects. First, wood ants, honeybees and stingless bees collect and incorporate plant resin with antimicrobial properties into their nests to limit the development of microbial pathogens (Christe *et al.*, 2003; Duangphakdee *et al.*, 2009; Simone *et al.*, 2009), a process mimicking individuals' self-medication process to fight an infection (De Roode *et al.*, 2013). Second, honeybee workers can fan their wings simultaneously to increase the temperature of their hive and thereby eliminate heat-sensitive pathogens (Starks *et al.*, 2000), a process mimicking the fever exhibited by a body to fight an infection. Finally, workers of the ant *Lasius neglectus* administer antimicrobial poison inside infected cocoons to prevent pathogen replication and establishment within the colony, just like the individual immune system targets and eliminates infected cells from host body (Pull *et al.*, 2018).

The accumulation of results supporting the parallel between individual and social immunity in eusocial insects rapidly led to the adoption of the eusocial framework by researchers interested in collective defences against pathogens. This adoption then fostered the claim that social immunity is “necessary and essential to eusocial systems” [25] and thus, that social immunity should be considered as a major and unique social parameter once eusociality has emerged [6,9,24,25].

The limit of the eusocial framework

One pillar of the original eusocial framework is thus that all collective defences against pathogens employed by individuals living in non-eusocial groups are not social immunity, but instead reflect non-derived defences such as communal disease defences and parental care [24,25]. This boundary between eusocial and non-eusocial species rapidly became a major issue in deciphering the common and/or separate evolutionary pathways of collective defences against pathogens across group living species (Cotter & Kilner, 2010a; Meunier, 2015). Moreover, this restriction to eusocial systems opened scope for several important misconceptions concerning the link between social immunity and social evolution. For instance, it might suggest that 1) reproductive division of labour is essential to allow the evolution of social immunity, 2) the presence of social immunity should lower investments into individual immunity in eusocial species and finally, that 3) social immunity does not have counterparts in non-eusocial species (Schmid-Hempel, 1998, 2017; Naug & Camazine, 2002; Cremer *et al.*, 2007, 2018; Wilson-Rich *et al.*, 2009). In the following part, we clarify these three misconceptions using recent empirical findings and demonstrate why they call for considering social immunity as a broader phenomenon that is not exclusive to eusocial species (Cotter & Kilner, 2010a; Meunier, 2015).

On the importance of reproductive division of labour

One misconceptions possibly fostered by the eusocial framework is that the direct fitness costs of performing social immunity are so high for a donor individual that they should prevent the evolution of social immunity in groups where the donors' fitness relies on their own reproduction. In other words, the net benefits of performing social immunity should only be present in groups where donor individuals forego personal reproduction, i.e. in eusocial species with reproductive division of labour [25]. The first issue with this prediction is that it neglects that some forms of social immunity are not only unlikely to provide significant fitness costs to donors (e.g. the use of self-produced secretion to sanitize the nest, the removal of fresh corpses from the nest (Cremer *et al.*, 2007)), but may also provide direct benefits to donor individuals. These direct benefits have been recently revealed in allogrooming, a textbook example of social immunity (Cremer *et al.*, 2007; Meunier, 2015). This behaviour has long been thought to be exclusively costly for donor individuals, because it increases their risk of being exposed to the pathogens present on the recipient individuals. In an elegant study conducted in the ant *Lasius neglectus*, however, Konrad *et al* (Konrad *et al.*, 2012) demonstrated that allogrooming provides direct benefits to both recipients and donors, as it allows donors to prime their own immune system and thus boost their defences against future pathogen exposure. Interestingly, a follow-up study recently showed that these direct benefits are pathogen-specific in that workers immune-primed with one type of pathogen preferentially direct their future allogrooming behaviours toward individuals infected with the same compared to a different pathogen (Konrad *et al.*, 2018).

The second issue with this prediction is that it overlooks the central role of kin selection in the evolution of some extreme forms of social immunity and neglects the fact that kin selection also operates in groups of individuals undergoing personal reproduction (Kramer & Meunier, 2016a). This central role can be illustrated by the self-exclusion of infected workers (figure 4.1), another textbook example of social immunity reported in ants and bees (Heinze & Walter, 2010; Rueppell *et al.*, 2010). This behaviour reflects that infected workers leave their nest to die alone and thereby limit the infection of their colony members. The evolution of such a behaviour typically relies on kin selection, as it becomes adaptive only if it allows the genes of the sacrificed individual to be passed on to the next generation by one or more of the saved group members, i.e. only if the benefactor and the beneficiaries are genetically related. Based on the same reasoning, such a sacrificial behaviour could *in principle* evolve in subsocial (family) groups if the self-exclusion of infected offspring significantly improves the reproduction of their related siblings. Whether such sacrifices occur in non-eusocial species is, however, unexplored so far. Overall, social immunity is thus not necessarily associated with net fitness costs for donor individuals and it is therefore possible for social immunity to evolve in group-living species without reproductive division of labour.

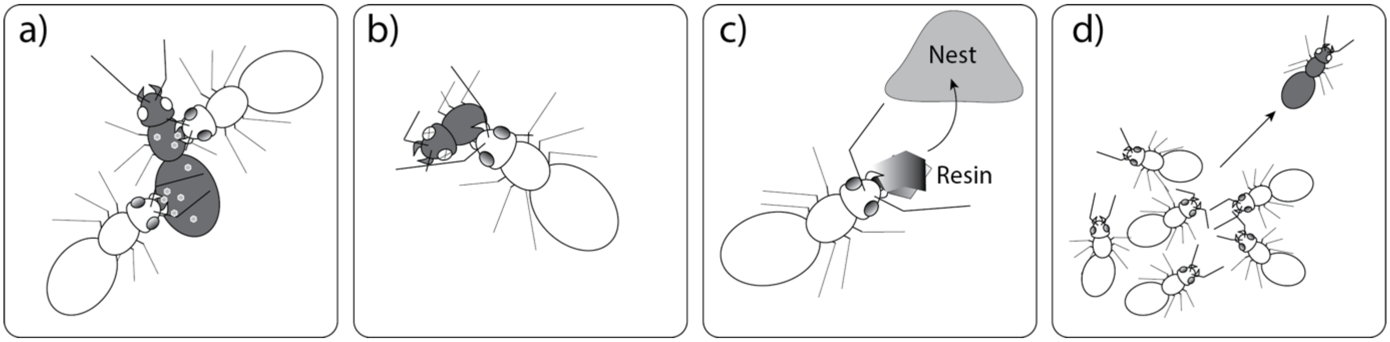

Figure 4.1: Four classical examples of social immunity that can be found in ant colonies. (a) Two workers groom an infected nestmate (grey) to remove external pathogens. (b) A worker carries a corpse (grey) away from the nest. (c) A worker collects a piece of resin with antimicrobial properties and brings it back to its nest. (d) An infected worker (grey) isolates itself from the group to limit the risk of pathogen spread. References can be found in (Cremer *et al.*, 2007; Meunier, 2015).

On the relaxed selection on individual immunity in eusocial species

A second misconception possibly fostered by the eusocial framework is that the emergence of social immunity should relax selection on individual immunity and thus, that individual immunity should be less efficient and/or involve a lower number of genes in eusocial compared to non-eusocial species (Evans *et al.*, 2006; Weinstock *et al.*, 2006). The interest of this prediction resided in the fact that it was relatively easy to test empirically. Unfortunately, the results were at odds with this prediction.

On one hand, physiological studies showed that antimicrobial peptides (a component of individual immunity) are more effective in eusocial compared to solitary sister species in bees (Stow *et al.*, 2007) and trips (Turnbull *et al.*, 2011). On the other hand, the recent accumulation of genomic studies comparing insects with different levels of social organization reports no general association between eusociality and the number and/or expression of immune-related genes across ants, bees and termites (reviewed in (Otani *et al.*, 2016)). Hence, shifting the unit of selection from individual to superorganism may not affect the selection pressures exerted on individual immunity (Barribeau *et al.*, 2015). Interestingly, this apparent absence of a general link between individual and social immunities suggests that the emergence of social immunity does not reduce, but instead complements individual defences against pathogens.

On the absence of social immunity in non-eusocial species

A third misconception possibly fostered by the eusocial framework is that social immunity is exclusive to eusocial species. A recent study, however, revealed that 11 of the 30 anti-pathogen defences found in eusocial insects and classically considered as forms of social immunity (Cremer *et al.*, 2007) can also be found in non-eusocial insects (Meunier, 2015) (see also (Cotter & Kilner, 2010a)). For instance, the use of self-produced components with antimicrobial properties as colony material is not only present in ants and termites (Christe *et al.*, 2003; Chouvenc *et al.*, 2013), but has been reported in nests of the wood cockroach *Cryptocercus punctulatus*, the European earwig *Forficula auricularia* and the Burying beetle *Nicrophorus vespilloides* (Cotter & Kilner, 2010b; Rosengaus *et al.*, 2013; Diehl *et al.*, 2015). Sanitary behaviours consisting in the removal of waste and feces material from the colony can also be found in several non-eusocial species with high nest fidelity (reviewed in (Weiss, 2006)), such as the subsocial cricket *Anurogryllus muticus* (West & Alexander, 1963). Finally, allogrooming is a behaviour frequently observed in arthropod species where parents remain with juveniles after egg hatching (e.g. (Thiel, 1999; Mas & Kölliker, 2011)), even if its role against pathogen infection needs to be further explored.

Whereas (at least) some forms of social immunity can be present in non-eusocial insects, it is also important to stress that (at least) some forms of social immunity are not present in all eusocial insects. For instance, queens of the pharaoh ant *Monomorium pharaonic* and the wood ant *Formica paralugubris* surprisingly prefer habitats contaminated with a pathogenic fungus to establish their colony (Brütsch *et al.*, 2014; Pontieri *et al.*, 2014), whereas the avoidance of contaminated areas is classically considered as a form of social immunity in eusocial insects (Cremer *et al.*, 2007). Similarly, experimental exposure to pathogen spores did not trigger higher levels of allogrooming between workers in the ants *Formica selysi* and *Myrmica rubra* (Reber *et al.*, 2011; Leclerc & Detrain, 2016), and co-founding queens of the ant *Lasius niger* perform only very little allogrooming and did not exhibit a better resistance against pathogens when compared to solitary queens (Brütsch *et al.*, 2017). The claim derived from the eusocial framework and stating that social immunity is “necessary and essential to protect the entire reproductive entity and maximize its fitness” [25] should therefore be taken with caution. Arguably, social immunity encompasses a great diversity of forms (Cremer *et al.*, 2007; Meunier, 2015), so that the absence of evidence for one form of social immunity should not be considered as an evidence for the absence of any form of social immunity. Nevertheless, the above findings warn us on the risk to over-interpreting the expression of certain behaviours as social immune responses on the sole basis that they are present in a eusocial species.

What is and what is not a form of social immunity?

The shift from a eusocial to a group-living framework has recently generated some confusion on the boundaries of social immunity, which in turn blurred our general view of its nature, evolution and function across species. Here, we clear up this confusion by proposing a newly defined group-living framework detailing three criteria that can be used to determine whether a given defence is a form of social immunity. First, this defence should help recipient individuals to reduce their risks of infection by pathogens, which refers to anything that can produce a disease such as viruses, bacteria, protozoa, prion, fungus and helminths. This encompasses all the potential steps of an infection, which include direct contact to a pathogen, penetration, development and replication of pathogens into the recipients' body and ultimately infection-derived death of the host (Cremer *et al.*, 2007; Meunier, 2015). The second criterion is that donors and at least some of the recipients should belong both to the same species and to the same social group. This excludes, for instance, all behaviours and collective processes during which individuals from one species provide anti-pathogen defences to individuals from another species, as commonly reported in the context of symbiosis and *cleaning symbiosis* in cleaner fishes (Hopkins *et al.*, 2017). Finally, the third criterion is that the defence should be "at least partly" selected for the anti-pathogen benefits it provides to the recipients. This stresses that social immunity is a target of selection and cannot be a simple by-product of individual immunity. This criterion excludes all individual defences that are either passively enhanced by group living (e.g. herd immunity (Babayan & Schneider, 2012)), selfishly driven by the nearby presence of conspecific individuals (e.g. density-dependent prophylaxis (Wilson & Cotter, 2009)) or that only happens to limit the risk of infection of solitary individuals encountered during a life cycle, such as during mating and/or competitive events. This third criterion also clarifies the rationale to separate the nomenclature between individual and social immunity.

Overall, these three criteria can be fulfilled 1) when group living is permanent, obligatory, temporary and/or facultative and 2) in a broad range of species ranging from insects and arachnids, over birds and fishes, to mammals and social microbes (Cotter & Kilner, 2010a; Meunier, 2015). Importantly, this absence of a dichotomy between eusocial and non-eusocial systems emphasizes that similar selection pressures are likely to have driven the evolution of comparable forms of social immunity across group living species. For instance, the evolution of the spread of feces with antimicrobial properties on nest walls by eusocial workers in termites (Chouvenc *et al.*, 2013) is very likely to have evolved under the same selection pressures that the ones selecting for the spread of feces with antimicrobial properties on nest walls by juveniles in family units of burying beetles and earwigs (Reavey *et* *al.*, 2014a; Diehl *et al.*, 2015). To summarize, social immunity can be defined as “any collective or personal mechanism that has emerged and/or is maintained at least partly due to the anti-pathogen defence it provides to other homospecific group members”, which is an edited definition of social immunity previously formulated by Meunier (Meunier, 2015).

CONCLUSION

In this review, we emphasized that individuals living either in facultative/temporary groups or in obligatory/permanent colonies can all perform defences against pathogens that may not only help themselves, but also their group members. The presence of these defences in such a large diversity of social systems recently made necessary the shift from a eusocial to a broad conceptual framework of social immunity [6–8,24,25]. This shift has generated novel works using the term ‘social immunity’ in a few subsocial insects such as the European earwig (e.g. (Boos *et al.*, 2014; Diehl *et al.*, 2015; Kohlmeier *et al.*, 2016)) and the burying beetle (e.g. (Reavey *et al.*, 2014a, 2015; Duarte *et al.*, 2016)). Here, we claim that it is crucial to expand these first works to a taxonomically broader number of non-eusocial species. The resulting studies would first allow us to disentangle whether the selection pressures favouring the emergence of social immunity have either secondarily evolved to limit the inherently high risk of pathogen exposure in species with an obligatory and permanent social life (i.e. some forms of social immunity derive from eusociality), or whether they remained constant after the evolutionary shift from solitary to group living (i.e. social immunity is an ancestral process) (Cotter & Kilner, 2010a; Meunier, 2015). Interestingly, it would also allow testing an alternative evolutionary scenario positing that the general risk of pathogen exposure for a solitary individual could have selected for the emergence of group living in order to obtain an additional line of defence such as social immunity (Biedermann & Rohlfs, 2017). Second, a taxonomically broader number of studies on social immunity would allow us exploring the potential trade-off between social and individual immunity across group-living species (Cotter *et al.*, 2010) and thus shed light on the dependent or independent evolutionary drivers of these two lines of anti-pathogen defences across animals. For instance, it would allow us to address questions such as whether certain types of pathogens are more likely to apply selection pressure onto individual instead of social immunity, or whether these two lines of defences necessarily trade-off across social systems (Cotter *et al.*, 2010). Finally, non-eusocial species could offer experimental opportunities that are not available in eusocial species and thus allow exploration of novel factors possibly underlying the expression of social immunity. For instance, a recent study in the European earwig allowed to demonstrate that the recent (but not prolonged!) social isolation of group-living adults induces a stress that specifically lowers their resistance against pathogens, whereas comparing the effects of pathogens on necessarily-newly isolated and non-isolated individuals is often used to test for the occurrence of social immunity in eusocial insects (Kohlmeier *et al.*, 2016). Overall, adopting the group living framework thus opens new perspectives to explore and better understand the common and specific solutions developed by each type of social host to counteract infections and thus, to improve our general understanding of the role of pathogens in the evolution of all forms of social life. Given the comparatively large amount of works on social immunity in eusocial insects, it is now time to further explore social immunity in a larger and taxonomically broader number of non-eusocial species.

CHAPITRE 5

L'altération du microbiote intestinal par la rifampicine n'influence pas les soins maternels chez le forficule européen.

Résumé de l’étude

Les microorganismes présents dans le microbiote intestinal sont connus pour influencer de nombreux traits d’histoire de vie de leur hôte comme les fonctions physiologiques et reproductives et même l’expression de certains comportements sociaux. Cependant, leur effet sur l'expression des soins parentaux reste jusqu’à maintenant inexploré. Cela est surprenant car les soins parentaux sont très répandus dans la nature et sont considérés comme un pilier central dans l'évolution de la vie de famille et de la vie sociale en général. Dans cette étude nous avons testé si l'altération du microbiote intestinal à l’aide d’un antibiotique à spectre large (la rifampicine) pouvait perturber l'expression des soins maternels envers les œufs et envers les juvéniles chez le forficule européen. Nos résultats ont d'abord confirmé que l’antibiotique utilisé altérait bien les communautés microbiennes intestinales des femelles traitées, et ont révélé que les femelles abritaient des microbiotes intestinaux différents avant et après les soins aux œufs. Cependant, contrairement à nos prédictions, l’altération du microbiote intestinal par la rifampicine n'a pas perturbé l'expression des soins maternels envers les œufs et envers les juvéniles. De plus, indépendamment de ces résultats sur les soins maternels, la rifampicine n'a modifié aucun des 18 autres traits d’histoire de vie liés à la physiologie, la reproduction et la longévité des femelles que nous avons mesurés tout au long de leur vie. A noter néanmoins que le traitement à la rifampicine a augmenté la production de fèces par les femelles et a conduit à la production de juvéniles et d'œufs plus légers (sans impacter la survie des juvéniles et la viabilité des œufs). Dans l'ensemble, ces résultats révèlent que l'altération du microbiote intestinal n'affecte pas nécessairement l’expression des comportements sociaux des hôtes dans les différentes formes de vie sociale ni chez toutes les espèces. Cela souligne que tous les animaux n'ont pas développé une co-dépendance avec leur microbiome.

Alteration of gut microbiota with rifampicin does not impair maternal care in the European earwig.

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Van Meyel S., Devers S., Dupont S., Dedeine F. and Meunier J. Alteration of gut microbiota with rifampicin does not impair maternal care in the European earwig.

ABSTRACT

The microbes residing within the gut of an animal host often maximise their own fitness by modifying their host's physiological, reproductive, and behavioural functions. Whereas recent studies suggest that they may also shape host sociality and therefore have critical effects on animal social evolution, the impact of the gut microbiota on maternal care remains unexplored. This is surprising, as this social behaviour is widespread among animals, often determines the fitness of both juveniles and parents, and is essential in the evolution of complex animal societies. Here, we address this gap in knowledge by testing whether life-long alterations of the gut microbiota with rifampicin - a broad-spectrum antibiotic – impair the expression of pre- and post-hatching maternal care in the European earwig, an insect exhibiting extensive forms of maternal care towards eggs and juveniles. Our results first confirm that rifampicin altered the mothers' gut microbial communities and revealed that the composition of the gut microbiota differs before and after egg care. Contrary to our predictions, however, the rifampicin-induced alterations of the gut microbiota did not modify the expression of pre- or post-hatching care. Independent of maternal care, rifampicin increased the females' feces production and resulted in lighter eggs and juveniles. By contrast, rifampicin altered none of the other 23 physiological, reproductive and longevity traits measured over the females' lifetime. Overall, these findings reveal that altering the gut microbiota does not necessarily affect host sociality. More generally, our results emphasize that not all animals have evolved a co-dependence with their microbiota.

INTRODUCTION

Almost all animals harbour a gut microbiota, i.e. a community of microorganisms residing within the gut of the host (Kolodny *et al.*, 2020). Some of these gut microbes have long been known for their pathogenic effects in the hosts (Engel & Moran, 2013) and others for the benefits they provide to the hosts in terms of nutritional mutualism (Cleveland, 1923). Over the last decades, however, a growing number of works has been revealing that the effects of gut microbes are much more diverse than previously thought and shape numerous physiological, reproductive, and behavioural functions of the host (Moran *et al.*, 2019). In the fruit fly *Drosophila melanogaster*, for instance, the gut microbiota is associated with hormone signalling, metabolism and ageing (Broderick & Lemaitre, 2012). Gut microbes can also shape the hosts' immunocompetence and resistance against pesticides and toxic plant metabolites (Itoh *et al.*, 2018), such as in the mosquito *Anopheles stephensi* (Wei *et al.*, 2017), the bean bug *Riptortus pedestri* (Kikuchi *et al.*, 2012) and the wasp *Nasonia vitripennis* (Wang *et al.*, 2020). Similarly, the gut microbiota is a key parameter in host reproduction and mating incompatibilities, as found in the fruit fly *Bactrocera minax* (Andongma *et al.*, 2018), in the terrestrial isopod *Armadillidium vulgare* (Fortin *et al.*, 2019), and the parasitic wasp *Asobara tabida* (Dedeine *et al.*, 2001). Finally, gut microbes shape the expression of numerous host behaviours, such as nutritional preference and kin recognition in *D. melanogaster* (Lizé *et al.*, 2014; Wong *et al.*, 2017), offspring activity in the stinkbug *Megacopta punctissima* (Hosokawa *et al.*, 2008), and different behavioural tasks in honeybees (Jones *et al.*, 2018).

In addition to these multiple effects, recent studies suggest that gut microbiota may also play a critical role in the sociality of their hosts by shaping the expression and nature of social interactions among group members. For instance, individuals with an altered gut microbiota exhibited deficient sociability and increased social avoidance in family-living rats (Desbonnet *et al.*, 2014; Buffington *et al.*, 2016), as well as showed higher levels of aggressiveness toward conspecifics in colonies of the leaf-cutting ant *Acromyrmex echinator* (Teseo *et al.*, 2019). Similarly, experimental alterations of gut microbial communities rendered hosts less attractive to conspecifics both in the gregarious German cockroach *Blattela germanica* (Wada-Katsumata *et al.*, 2015) and in the swarm-living desert locust *Schistocerca gregaria* (Dillon *et al.*, 2000). Most of these social alterations were reverted when individuals received transplants of their original gut microbiota (Desbonnet *et al.*, 2014; Wada-Katsumata *et al.*, 2015; Buffington *et al.*, 2016; Teseo *et al.*, 2019), suggesting that certain microbes and/or the gut community as a whole were responsible for the sociality of their host and thus, more generally, supporting the hypothesis that gut microbes could have a key role in the evolution of their hosts' social life.

Despite these apparent links between the hosts' gut microbial communities and their social behaviours, the role of gut microbes on the expression of parental care remains experimentally unexplored. This is surprising, because this form of social behaviour is present in a large and taxonomically diverse number of animal species (Royle *et al.*, 2012), has considerable effects on the fitness of both juveniles and parents (Klug & Bonsall, 2014) and because shedding light on this link may provide crucial information on the role of gut microbes in the early evolution of complex animal societies (Kramer & Meunier, 2019). On one hand, gut microbes may indirectly drive parental care, because parents are expected to adjust their level of care to their own condition (Klug *et al.*, 2012) and altered gut microbial communities can lower these conditions in multiple ways (see above). On the other hand, gut microbes could serve as a direct promoter of parental care because, by enforcing the expression of care in adult hosts, parental gut microbes could maximize their chances to reach novel hosts that are both young and offer limited scope for microbial competition with resident gut microbes (Stilling *et al.*, 2014a; Biedermann & Rohlfs, 2017; Lewin-Epstein *et al.*, 2017; Figueiredo & Kramer, 2020; Gurevich *et al.*, 2020; Nalepa, 2020)(but see (Johnson & Foster, 2018)). The transfer of gut microbes through parental care has been reported in several insect species, such as the stinkbug *Parastrachia japonensis* (Hosokawa *et al.*, 2012), the Japanese common plataspid stinkbug *Megacopta punctatissima* (Fukatsu & Hosokawa, 2002) and the wood cockroach *Cryptocercus punctulatus* (Nalepa, 1984, 1990). However, whether the gut microbiota drives the expression of parental care remains untested.

In this study, we address this gap in knowledge by investigating whether gut microbiota alteration with rifampicin - a broad-spectrum antibiotic - impaired the expression of pre- and post-hatching maternal care in the European earwig *Forficula auricularia* L, clade B (González-Miguéns *et al.*, 2020). In this omnivorous insect, mothers tend their clutch of eggs over winter. During this period, mothers stop their foraging activity to provide extensive maternal egg care in the forms of protection against desiccation, predators and pathogens (Lamb, 1976; Van Meyel *et al.*, 2019). Upon egg hatching, mothers continue tending their brood of newly emerged juveniles (called nymphs) for two weeks, during which they provide post-hatching care in the forms of fierce protections against predators, grooming behaviours, and food provisioning through regurgitation (Staerkle & Kölliker, 2008; Mas & Kölliker, 2011). Interestingly, pre-hatching care allows mothers to transfer microbes exhibiting antifungal properties to their eggshell in the maritime earwig *Anisolabis maritima* (Greer *et al.*, 2020), a behaviour that could also occur in the European earwig (Boos *et al.*, 2014). In *F. auricularia*, pre-hatching maternal care is necessary to ensure egg development and hatching (Boos *et al.*, 2014), whereas post-hatching maternal care is facultative for the development and survival of nymphs (Kölliker, 2007) - even if its absence can have long-term negative effects on the resulting adults (Thesing *et al.*, 2015; Vogelweith *et al.*, 2017). Here, we altered the gut microbiota of *F. auricularia* females by feeding them with rifampicin during their entire adult lifetime (about 14 months) and measured whether and how this treatment affected gut microbial communities, maternal care, and other life-history traits. Specifically, we first determined how the antibiotherapy alters the diversity and the structure of the gut bacterial community of females at two periods of their life-cycle (before oviposition and at egg hatching) by sequencing 16S rRNA gene (V3-V4 region) amplicons. We then tested the effects of rifampicin on the expression of four pre- and two post-hatching forms of maternal care. Finally, to disentangle whether the potential link between gut microbiota alteration and the level of maternal care is direct and/or indirect, we investigated the effects of rifampicin on 32 other traits measured throughout the females' lifetime and reflecting their general physiological state, reproductive success and longevity, as well as their juveniles' development, sex-ratio and survival.

MATERIALS AND METHODS

Insect rearing and rifampicin treatment

The experiment involved a total of 296 *Forficula auricularia* males and females. These were the first laboratory-born descendants of 74 females field-sampled in Pont-de-Ruan, France, in 2017 and then maintained under standard laboratory conditions (Meunier *et al.*, 2012). For each of these 74 families, 2 males and 2 females were isolated at adult emergence and immediately fed with a standard food mixed with either 10 µL of rifampicin (1 male and 1 female per family; Sigma-Aldrich, PHR1806; 0.2 mg/ml) or 10 µL of water (1 male and 1 female per family). The standard food contained agar, carrots, pollen, and cat and bird dry food (Meunier *et al.*, 2012). Two weeks later, 148 mating pairs were set up (1 female and 1 male from the same family and same treatment). The use of sibling pairs allowed us limiting the risk of cytoplasmic incompatibility due to inter-familial microbiome variability, and there are only limited signs of inbreeding depression in this species (Meunier & Kölliker, 2013). They continued to receive the same rifampicin- and water-treatment for about two months. At that time, females were isolated to mimic natural dispersal for oviposition (Meunier *et al.*, 2012). From oviposition to egg hatching, four forms of egg care were measured (details below). During that time, females were not provided with food (and thus not treated with rifampicin), as mothers typically stop foraging during the period of egg care (Kölliker, 2007). One day after egg hatching, each family (a mother with its newly hatched juveniles) was provided with either rifampicin or water to follow up on the pre-oviposition treatment. Three forms of maternal care towards juveniles were measured during the following 14 days (details below), which corresponds to the natural duration of family life (Meunier *et al.*, 2012). At the end of these 14 days, families were split to allow newly isolated mothers to produce a 2nd clutch and permit groups of nymphs to continue their development. The mothers and groups of nymphs continued to receive the same treatment (rifampicin or water) until the end of the experiment, i.e. until the mother died and nymphs reached adulthood. Throughout the experiment, the treatments were renewed twice a week (except during egg care). All isolated adults, groups of nymphs, and families were maintained in Petri dishes (diameter 9 cm) lined with moistened sand. More details on the experimental setup in the supplementary material.

Rifampicin is considered one of the most potent and broad-spectrum antibiotics due to its high-affinity binding to the RNAP β subunit, which causes the inhibition of the bacterial DNA-dependent RNA polymerase RNAP by directly blocking the RNA elongation path (Campbell *et al.*, 2001). It is also commonly used to experimentally alter gut microbial communities in insects (e.g. (Chouaia *et al.*, 2012; Shan *et al.*, 2016; Rosas *et al.*, 2018)). The high dose of rifampicin used in this study (about 10 times higher than the dose generally used in smaller insect species (Chouaia *et al.*, 2012; Shan *et al.*, 2016)) was chosen to ensure gut microbial alteration and because it did not trigger an excess of mortality in the German cockroach (Rosas *et al.*, 2018), an insect that is about the size of the European earwig.

Effects of rifampicin on the gut microbiota

To determine whether and how rifampicin treatment altered the earwigs' gut microbial communities, we extracted the gut of 10 females per treatment (n total = 20) on the day we observed the first oviposition (i.e. about 2 months after being fed with or without rifampicin), and 10 rifampicin- and 8 water-treated females one day after egg hatching (i.e. about 1 month later). For gut extraction, we first anaesthetized each female for 2 min at -20°C and then dissected them in a watch glass with sterilized double Q water. All dissections and manipulations were conducted on a sterilized bench, under a Bunsen burner's sterility area and using sterile material. Whole individual guts were extracted, placed in 100 µl of T1 extraction buffer (Nucleo-Spin Tissue, Macherey-Nagel), and stored at −80°C until DNA extraction. Protocols for DNA extractions and bioinformatic pipelines are detailed in the supplementary material.

Measurements of pre- and post-hatching maternal care

We measured the effects of rifampicin on four classical forms of earwig maternal egg care: egg grooming, egg defence, delay of maternal return and egg searching exploration rate (Diehl & Meunier, 2018; Van Meyel *et al.*, 2019). Egg grooming, which is used by earwig females to deposit chemical substances on the eggs and to clean eggshell from dirt and fungi (Boos *et al.*, 2014), was measured 15 days after egg production. We first isolated each mother for 30 min, then returned them to their Petri dish and gently deposited them at a distance of 5 cm from their egg clutch, and finally recorded their behaviours for the subsequent 15 minutes on camera (SONY© Handycam HDR-CX700 camera). The resulting movies were analysed using the software BORIS v4.0.3 (Friard & Gamba, 2016) and the total duration of egg grooming was defined as the total amount of time each female spent on cleaning eggs with their mandibles (Boos *et al.*, 2014). Clutch defence, which reflects the females' willingness to protect their eggs from a predator attack (Thesing *et al.*, 2015), was measured 16 days after egg production. We standardly poked each female on the pronotum with a glass capillary (one poke per second) and then recorded the number of pokes required until the female moved more than one body length away from the clutch. The delay of maternal return after clutch abandonment (Van Meyel *et al.*, 2019), which represents the delay after which females return to their clutch after being chased away by a simulated predator attack (Thesing *et al.*, 2015), was measured by recording the time the female took to return to its clutch just after the end of the clutch defence measurement. Finally, the egg searching exploration rate, which represents the level of exploration of a novel area by a mother looking for her eggs, was measured 21 days after egg production. We deposited each female at the centre of a square arena (W: 9 cm; L: 9 cm; H: 0.5 cm) covered by a glass sheet and video-tracked their activity during the following 35 min. The video was done under infra-red light, while the individual video tracking and calculation of exploration rate were conducted using the software ToxTrac v2.83 (Rodriguez *et al.*, 2018).

We then measured the effects of rifampicin on two classical forms of post-hatching maternal care: the defence of and search for juveniles (Thesing *et al.*, 2015; Diehl & Meunier, 2018). These two forms of care were measured 10 days and 12 days after egg hatching, respectively, following the above-detailed protocols for egg defence and egg searching activity. All the measurements of pre- and post-hatching maternal care were conducted in the afternoon and under a red light as earwigs are nocturnal. These measurements were conducted blindly regarding the treatments (rifampicin versus control). The number of replicates for each of our measurements ranged from 21 to 56 (details in Tables 5.1 and S5.1a,b).

Measurements of the 24 other life-history traits in mothers and offspring

We tested the effects of rifampicin on 7 proxies of female physiology, 16 proxies of female reproduction and on female longevity using standard protocols (Meunier *et al.*, 2012; Körner *et al.*, 2016). We measured the females' physiology through their feces production (reflecting their digestive/foraging activity) and weight gains between two life stages. Feces production was measured two months after the beginning of the treatments. Females were isolated in a new Petri Dish for 24 hours, after which we counted the number of feces pellets present on the ground. The weight gains of each female were measured between the days of adult emergence and oviposition, and between the days of oviposition and egg hatching. Reproductive success was measured in the 1st and 2nd clutches (if any), by counting the number of eggs produced, the number of days between oviposition and egg hatching (egg development time), and by measuring the mean egg weight at oviposition, the egg hatching rate, and the mean offspring weight at egg hatching. We also counted the number of days between adult emergence and oviposition (delay until oviposition), between the females' isolation after family life and 2nd clutch oviposition (delay until 2nd clutch production), and between adult emergence and death (female longevity). We finally assessed the females' likelihood to produce a 2nd clutch (1 or 0) and the females' reproductive allocation between the two clutches (reproductive strategy (Meunier *et al.*, 2012)). This allocation was defined as the number of 2nd clutch eggs divided by the total number of eggs produced by a female.

Because mothers and groups of nymphs continued to receive their treatment after the end of family life, we also tested the effects of rifampicin on juvenile developmental time between each developmental instar, on the survival rates from egg hatching until both the end of family life and adulthood, and on the sex ratio of the resulting adults. Juvenile developmental time was defined as the differences between the moulting date in a given instar (or hatching date) and the moulting date of its subsequent instar at the family-level, i.e. focusing on the first individual moulting in the clutch. Every weighing was done to the nearest 0.01 mg using a microbalance (OHAUS© Discovery DV215CD). Sample sizes for each measurement are detailed in Tables 5.1 and S5.1a,b. More details on the methods are provided in the supplementary material.

Statistical analyses

Analyses of the α and β-diversity indices.

The structure, composition and diversity of the microbial communities were analysed using PHYLOSEQ R package (McMurdie & Holmes, 2013) implemented in the FROGSSTAT Phyloseq tools (Bernard *et al.*, 2017). Diversity within the gut microbial communities (alpha-diversity) was assessed using two richness indices which estimate the number of species in the microbiome with correction for subsampling (Chao1; ACE), and three metrics that aim to measure diversity by accounting for evenness or homogeneity (Shannon, Simpson, Inverse Simpson, and

Fisher) (Jost, 2007). Diversity between the gut microbial communities (beta-diversity) was assessed using two non-phylogenetically informed (Bray Curtis dissimilarity; Jaccard indice) and two phylogenetically informed (uniFrac; Weighed uniFrac) measures of community similarity. The metrics were analysed individually using either a General Linear Model for α-diversity, or a Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA) for β-diversity. In these models, the values (or distance matrix for β-diversity) of each index were entered as a response variable, while the treatment (rifampicin or water), the sampling stage of the female (before oviposition or at egg hatching) and the interaction between them were used as fixed factors. When required, a post-hoc analysis was conducted by splitting the data set according to the sampling stage and then conducting PERMANOVA on each of the resulting subsets.

Analyses of the life-history traits.

Although the presented experimental design was originally paired, i.e. 2 females per family were distributed among the two treatments, the 38 life-history traits were often measured in only one of the paired individuals (detailed sample sizes in Tables 5.1 and S5.1a,b). This was mostly due to time constraints, and because some females died during the 18-months course of this experiment. These overall led to critical reductions in the number of replicates that could be involved in a paired statistical approach (details in Table S5.2a,b). We, therefore, analysed the effects of rifampicin on the 32 measurements using a series of 31 non-paired exact Mann Whitney U tests and 1 Pearson's Chi-squared test (for the likelihood to produce a 2nd clutch), in which we compared the values of all the available replicates fed with rifampicin to the values of all the available replicates fed with water. Note that the results do not qualitatively change when we use paired analyses with the associated smallest sample sizes (results presented in Table S5.2a,b). To correct for the inflated risk of Type I errors due to multiple testing, all p-values were adjusted using the

False Discovery Rate (FDR) method (Benjamini & Hochberg, 1995). All these analyses were conducted using the software R v4.0.2 (www.r-project.org).

RESULTS

Description of the earwig gut microbiota

A total of 1636435 sequenced reads of the 16S rRNA V3-V4 region were obtained from the 38 female earwig gut samples. After sequence processing, this number went down to 1130241, with 21069 to 35385 sequences per sample (median = 30595.5). The sequences were aggregated and filtered in a total of 161 unique OTUs, which were resolved down to the family or genus level to increase the confidence in the taxonomic assignation. All detailed information on OTUs is given in Table S5.3 on request. More than 99.90% of the sequences were assigned to four bacterial phyla: Proteobacteria (65.94%), Firmicutes (21.12%), Bacteroidota (9.89%) and Actinobacteriota (2.96%) (Figure 5.1). The remaining OTUs were assigned to Bdellovibrionota (0.04%) and Patescibacteria (0.04%).

Comparative analyses of the α and β diversity of the gut microbiota

The gut microbial α-diversity (i.e. species richness) decreased between oviposition and egg hatching when this diversity was measured using Chao1 ($F_{1,34} = 21.63$, $P < 0.0001$), ACE ($F_{1,34} = 24.46$, $P < 0.0001$) and Fisher ($F_{1,34} = 20.85$, $P < 0.0001$; Figure 5.2) indices. This decrease was, however, not significant when α-diversity was measured using Shannon ($F_{1,34} = 3.18$, $P = 0.084$; Figure 5.2) and Simpson ($F_{1,34} = 1.60$, $P = 0.214$) indices. The α-diversity was overall independent of the rifampicin treatment (Chao1: $F_{1,34} = 0.72$, $P = 0.401$; ACE: $F_{1,34} = 0.62$, $P =0.435$; Fisher: $F_{1,34} = 0.59$, $P = 0.447$; Shannon: $F_{1,34} = 1.67$, $P = 0.205$; Simpson: $F_{1,34} = 0.55$, $P = 0.465$; Figure 5.2), and of an interaction between female sampling stage and rifampicin treatment (all $P > 0.525$).

The gut microbiota β-diversity (i.e. species composition) overall changed with female sampling stage and rifampicin treatment. This was the case with the four measured indices of β-diversity: Bray-Curtis (Stage: $F_{1,34} = 5.77$, $P < 0.0001$; Rifampicin: $F_{1,34} = 4.23$, $P < 0.0001$), JACCARD (Stage: $F_{1,34} = 7.76$, $P < 0.0001$; Rifampicin: $F_{1,34} = 2.37$, $P = 0.0036$), unweighted UniFrac (Stage: $F_{1,34} = 6.51$, $P < 0.0001$; Rifampicin: $F_{1,34} = 3.39$, $P = 0.0006$) and weighted UniFrac (Stage: $F_{1,34} = 14.10$, $P < 0.0001$; Rifampicin: $F_{1,34} = 6.42$, $P = 0.0006$). In particular, females before oviposition harboured less Actinobacteriota and Proteobacteria compared to females at egg hatching, while rifampicin females overall harboured less Bacteroidota and more Firmicutes compared to untreated females (Figure 5.1). Interestingly, the interaction between female sampling stage and rifampicin had no effect on the β-diversity measured using all (all $P > 0.117$) but the weighted UniFrac indices ($F_{1,34} = 2.94$, $P = 0.026$). This interaction reflected an effect of rifampicin on the β-diversity before oviposition ($F_{1,34} = 0.17$, $P = 0.018$) but not at egg hatching ($F_{1,34} = 0.97$, $P = 0.356$).

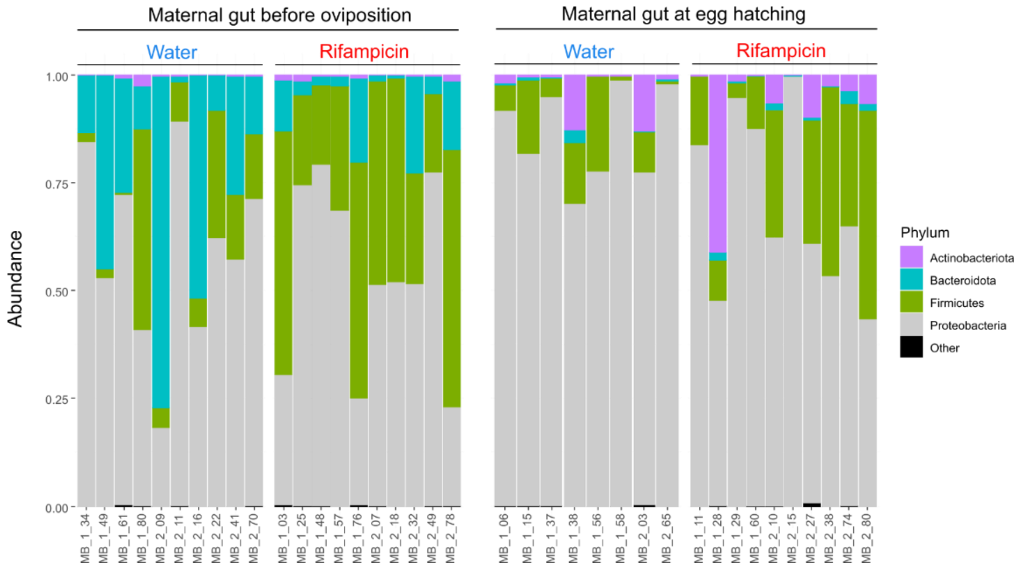

Figure 5.1: Gut microbial composition in females. Guts were sampled either before oviposition or at egg hatching in females treated either with water or rifampicin. The ID of each female is provided on the x-axis. More details in table S5.3 on request.

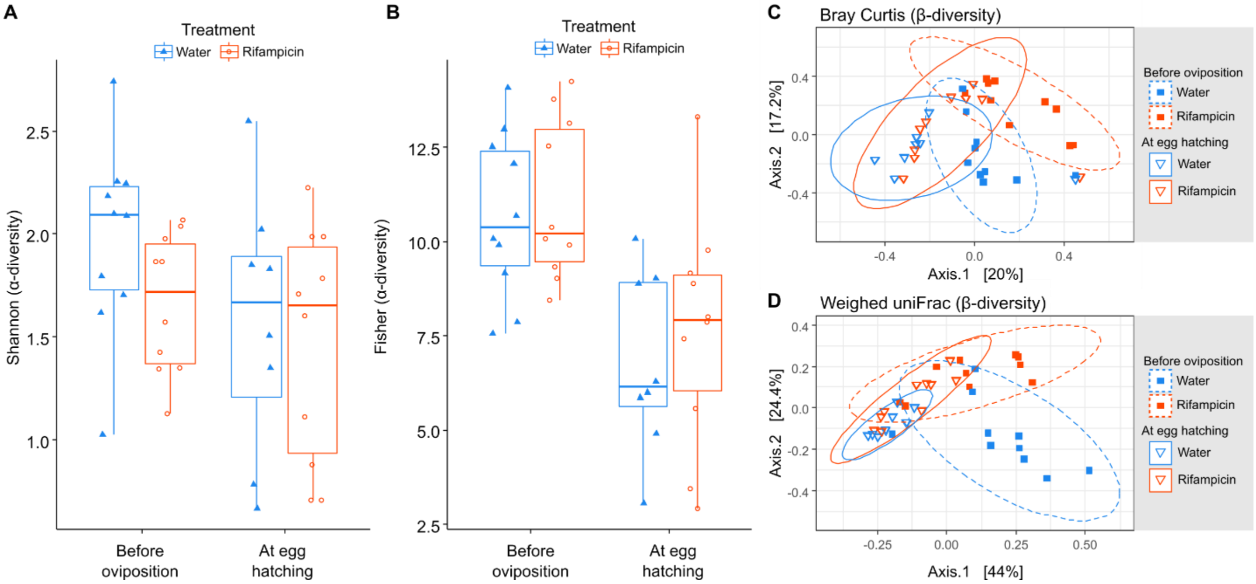

Figure 5.2: Effects of rifampicin and female sampling stage on gut microbial α- and β-diversities. Guts were sampled either before oviposition or at egg hatching in females treated either with water or rifampicin. (A, B) *Alpha*-diversity based on Shannon and Fisher indices (representative of all the tested metrics), respectively. Box plots depict median (middle bar) and interquartile range (box), with whiskers extending to 1.5 times the interquartile range and dots/triangles representing values of each sample. (C, D) *Beta*-diversity based on Bray-Curtis and weighed- uniFrac indices (representative of all the tested metrics). Illustrations report multidimensional scaling (MDS) results, where dots show values and ellipses represent 95% confidence intervals.

Rifampicin and maternal care

We did not detect any effect of rifampicin on the six measured forms of egg and nymph care (Table 5.1). In particular, rifampicin- and control-fed mothers spent the same amount of time in egg grooming (Figure 5.3A), showed the same levels of both egg and juvenile defences against a simulated predator attack (Figures 5.3B and 5.3E), exhibited the same delay to return to their eggs after egg defence (Figure 5.3C) and showed comparable exploration rates when looking for their eggs or their juveniles (Figures 5.3D and 5.3F).

Rifampicin and female's physiology, reproduction, and longevity

The consumption of rifampicin altered 3 of the 23 measured proxies of female physiology, reproduction, and longevity. In particular, females fed with rifampicin produced on average twice as many feces pellets per 24h (Figure 5.4; Table S5.1a), had newly hatched nymphs that were 15% lighter ($W = 1244$, $P = 0.002$, adjusted-$P = 0.025$; Table S5.1b) and laid 2nd clutch eggs that were 7% lighter ($W = 628$, $P = 0.002$, adjusted-$P = 0.025$; Table S5.1b) compared to control females. By contrast, we did not detect any effect of rifampicin on the 20 other traits (Tables 5.1 and S5.1a,b).

Rifampicin and nymphs' development, sex-ratio and survival

We did not detect any effect of rifampicin on the juveniles' developmental speed from hatching to adult and at every step of their development, as well as the sex-ratio of the 1st clutches (Tables 5.1 and S5.1b). Similarly, the survival rate of nymphs throughout family life (i.e. from egg hatching until day fourteen) and from the end of family life until they reached adulthood were not impacted by the consumption of rifampicin (Tables 5.1 and S5.1b).

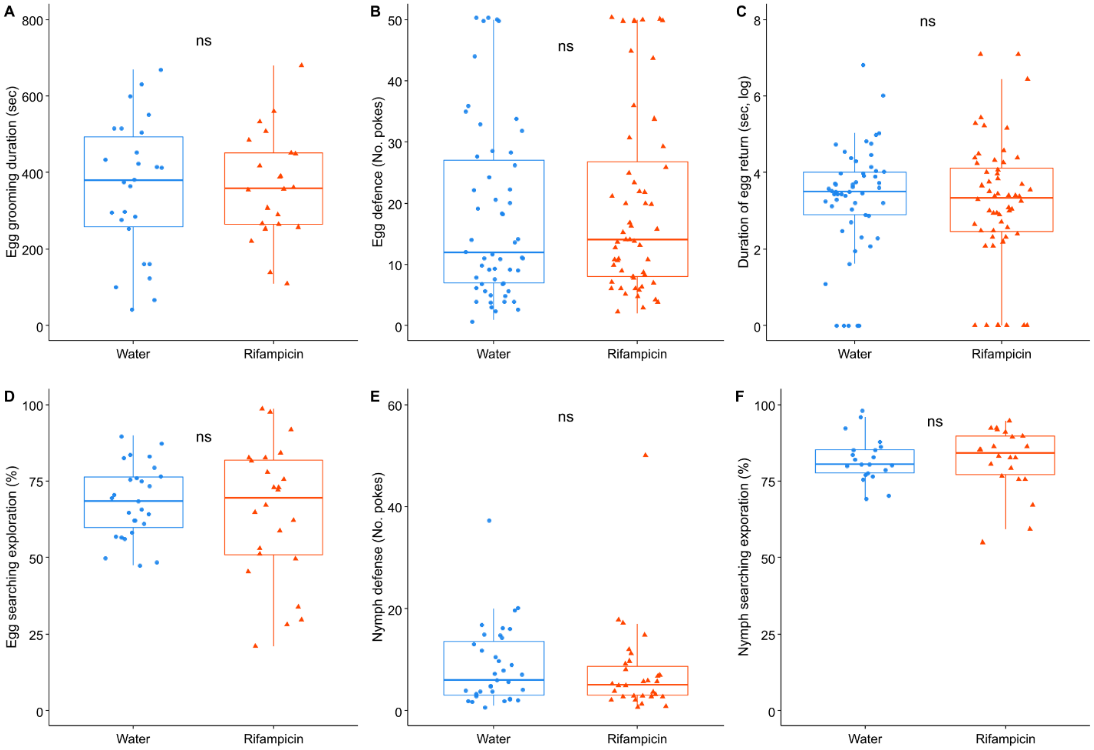

Figure 3 – Effect of rifampicin on maternal care. (A) duration of egg grooming, (B) egg defence against a simulated predator, (C) delay of maternal return after egg defence, (D) egg searching, (E) nymph defence against a simulated predator and (F) nymph searching. Box plots depict median (middle bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. *ns* stands for P < 0.05.

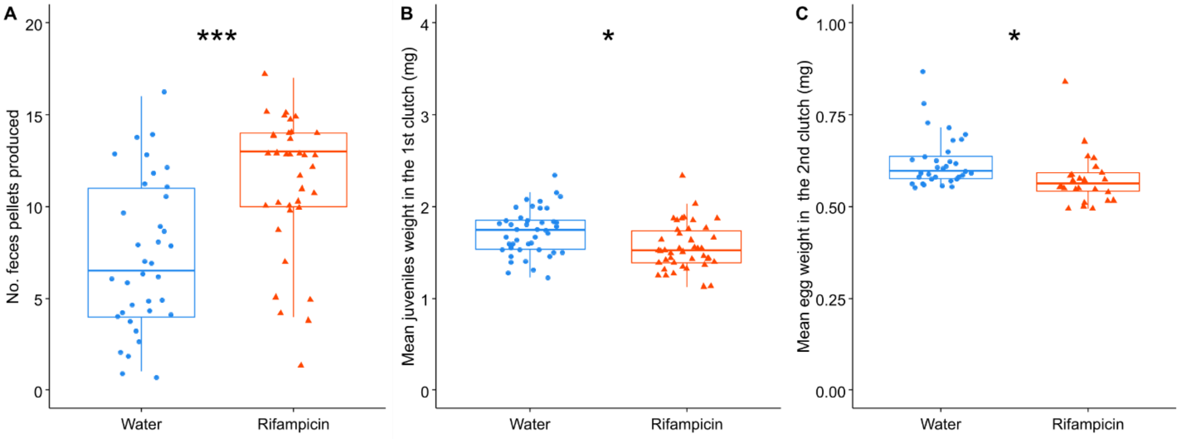

Figure 4 – Effects of rifampicin on (A) females' feces production, (B) mean juveniles weight in the first clutch and (C) mean egg weight in the 2nd clutch. Box plots depict median (middle bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. ***$P < 0.001$ and *$P < 0.05$.

Table 5.1: Effects of rifampicin on a representative selection of 15 of the 36 measured traits reflecting maternal care, female physiology, reproduction and longevity, as well as nymph development and survival. The effects of rifampicin on all 36 traits are presented in table S5.1a,b. P-values significant after correction for multiple comparisons (Adj-P) are in bold. Med = Median; 1Q and 3Q = first and third quartile, respectively. N = sample size.

	Water				**Rifampicin**				**Statistics**		
	Med	1Q	3Q	N	Med	1Q	3Q	N	W	P	Adj-P
Maternal care											
Egg grooming (sec)	379.34	259.20	492.28	26	359.54	264.49	450.18	22	288.5	0.963	0.963
Egg defense	12.00	7.00	27.00	55	14.00	8.00	26.75	56	1397.0	0.398	0.582
Delay maternal return (sec)	32.00	17.00	54.00	55	27.00	10.75	60.50	56	1677.5	0.417	0.582
Egg searching (%)	68.37	59.72	76.28	27	69.55	50.78	81.88	24	339.5	0.775	0.807
Juveniles defense	6.00	3.00	13.50	35	5.00	3.00	8.75	30	592.5	0.377	0.582
Nymph searching (%)	80.61	77.55	85.20	21	84.16	77.17	89.55	22	208.0	0.584	0.716
Female physiology											
Feces production	6.50	4.00	11.00	36	13.00	10.00	14.00	36	303.0	**<0.001**	**<0.001**
Abs. weight gain during egg care (mg)	1.28	-1.28	4.82	52	2.32	0.19	4.48	59	1308.0	0.182	0.407
Female reproduction & longevity											
No. eggs produced in the 1st clutch	55.00	48.50	60.00	59	53.00	43.00	58.75	62	2037.0	0.280	0.560
No. eggs produced in the 2nd clutch	28.00	20.00	33.00	33	23.00	14.00	28.50	27	561.0	0.087	0.383
Total No. nymphs produced	32.50	22.00	52.00	32	21.50	7.50	32.50	28	596.5	0.027	0.203
Female longevity (days)	323.00	293.50	361.00	39	306.00	284.50	343.25	42	994.5	0.098	0.310
Nymph development and survival											
Dvptal time from hatching to adults	81.00	75.50	85.50	35	82.00	77.25	85.75	34	565.5	0.727	0.807
Survival rate from hatching to day 14	84.38	77.50	90.00	35	85.16	75.37	91.37	34	572.0	0.786	0.807
Survival rate from day 14 to adults	42.86	29.86	59.91	35	39.88	27.27	66.67	34	620.5	0.763	0.807

DISCUSSION

Whereas gut microbial communities shape the physiology, reproduction and behaviours of a great diversity of hosts, their importance on parental care – a key behaviour in social evolution (Royle *et al.*, 2012; Klug & Bonsall, 2014; Kramer & Meunier, 2019) - remains poorly explored (Gurevich *et al.*, 2020). In this study, we addressed this gap in knowledge by treating females of the European earwig with rifampicin and measuring the effects on gut microbial communities, maternal care and female physiology, reproduction, and longevity. Our results first reveal that rifampicin altered the composition of the gut microbial community of earwig females and show that this modification diminishes during the period of egg care. Contrary to our predictions, however, the rifampicin-induced alterations of gut microbial communities did not impair the expression of pre- or post-hatching care: rifampicin-treated and control mothers showed similar levels of egg grooming, clutch defences against a predator, maternal return and clutch searching. Independent of maternal care, our results reveal that the consumption of rifampicin increased the females' production of feces pellets, as well as lead to the production of lighter nymphs and lighter 2nd clutch eggs. By contrast, rifampicin affected none of the other 23 physiological, reproductive and longevity traits measured over the females' lifetime.

Our experiment first demonstrates that the ingestion of rifampicin by earwig females induced stage-specific modifications in the species composition (β-diversity) of the gut microbiota but did not shape its species richness (α-diversity). These findings confirm that the earwig gut microbiota harbours both bacterial taxa (and/or genetic variants of certain taxa) that are sensitive and taxa that are resistant to rifampicin, and thus that our treatment successfully altered gut microbial communities (just like in other animal species (Chouaia *et al.*, 2012; Shan *et al.*, 2016; Rosas *et al.*, 2018)). Independent of rifampicin, our data also reveal that both α- and β-diversity changed from pre-oviposition to egg hatching. This stage-specific pattern could be due to the absence of food intake for about four weeks before gut sampling in females at egg hatching compared to before oviposition (Montoya-Ciriaco *et al.*, 2020), and/or due to different rearing temperatures (Sepulveda & Moeller, 2020) and differences in female age (Anderson *et al.*, 2018) between the two life stages. Notwithstanding the drivers of this stage-specific effect, it is important to note that all the tested females were re-treated with rifampicin (or water) after egg hatching so that the resulting alteration of their gut microbiota reported at oviposition likely operated during their entire lifetime and could thus have affected all the traits measured after egg-hatching.

Although gut microbial communities shape the expression of hosts sociality in numerous vertebrate and arthropod species (Dillon *et al.*, 2000; Desbonnet *et al.*, 2014; Wada-Katsumata *et al.*, 2015; Buffington *et al.*, 2016; Teseo *et al.*, 2019), our findings reveal that rifampicin-induced alterations of this community did not affect the expression of pre- and post-hatching maternal care in earwigs. Gut microbes were expected to directly drive the expression of parental care, because enforcing the expression of this social behaviour may allow symbionts to reach new hosts (i.e. offspring) that are typically young (thus offering long-lived habitats), display poor immune defences (thus facilitating bacterial establishment and development (Vogelweith *et al.*, 2017)) and harbour only a few resident microbes (thus limiting the risk of competition within the microbiome (Figueiredo & Kramer, 2020)). This apparent absence of a link between rifampicin and maternal care may first suggest that earwig parental care is shaped by microbes that are non-sensitive to rifampicin. In insects, gut microbial communities do not only encompass a broad diversity of bacteria (among which some are resistant to rifampicin) but also fungi, protists and other microorganisms that could have key roles in hosts biology (Urubschurov & Janczyk, 2011; Arcila & Meunier, 2020; Greer *et al.*, 2020). Even if the previous experimental works linking gut microbiota and host sociality focused on bacteria (Dillon *et al.*, 2000; Desbonnet *et al.*, 2014; Wada-Katsumata *et al.*, 2015; Buffington *et al.*, 2016; Teseo *et al.*, 2019), future studies will be required to confirm that no other members of the gut microbiota shape parental care in our study species. A second hypothesis is that microbial symbionts never developed any specific capabilities to manipulate host sociality, either because adapted strain never occurred within the microbial populations associated with these earwig species (or populations), or because certain antagonistic interactions (e.g. competition) among the members of the microbial community have prevented the emergence of host social manipulation. Any symbiont species (or strain) investing its resources to manipulate host behaviour is indeed expected to be outcompeted within the microbiome by other species or variants that, instead, direct their resources into growth, survival or directly transmission (Johnson & Foster, 2018)(but see for the evolution of paternal care (Gurevich *et al.*, 2020)). Finally, a third hypothesis is that the symbionts' capability to manipulate host sociality may have changed and/or disappeared during host social evolution and consequently vanished in the European earwig. The evolutionary drivers of family life indeed change over time (Kramer & Meunier, 2019) and, while gut microbes may have (at least partly) driven the ancestral evolutionary shift from solitary to family living for the reasons detailed above, the resulting benefits of parental care for the hosts could have consolidated the expression of care and thus reduced the capability of symbionts to control host social behaviours and/or reduced the sensitivity of the hosts to this manipulation. Based on this hypothesis, alterations in gut microbial communities should not shape the expression of parental care once this behaviour is established. In earwigs, pre- and post-hatching maternal care are well established (even if their levels of expression differ between females and the associated benefits appear to be limited for juveniles (Kölliker, 2007; Thesing *et al.*, 2015)), which may thus have limited the maintenance of symbiont control over parental care. Overall, our findings provide the first experimental evidence that alteration of the gut microbiota (with rifampicin) does not directly or indirectly impair the expression of maternal care and thus emphasize the potentially limited role of the gut microbiota in this important social behaviour.

Despite its apparent lack of effects on maternal care, rifampicin altered three female life-history traits related to physiology and reproduction. The first trait is the production of feces pellets, which was twice as high in rifampicin-treated compared to control females. This result was not surprising, as the gut microbiota often plays a key role in nutrient extraction and digestion (Douglas, 2015) and its alteration by antibiotics typically disturbs the host's digestive efficiency and triggers an overproduction of fecal material. The two other traits were the weights of the 2nd clutch juveniles and 2nd clutch eggs, which were lighter in rifampicin compared to control females. Light eggs and newly hatched juveniles are often thought to reflect low offspring quality in insects (Berrigan, 1991). In the present study, however, heavier eggs and newly hatched juveniles did not translate into higher offspring survival and improved development compared to lighter counterparts. On a proximate level, these findings suggest that rifampicin breaks the association between offspring weight and quality, either due to alteration in gut microbial communities and/or antibiotic toxicity. On an ultimate level, these findings stress that rifampicin only has a limited impact on offspring fitness, as least under laboratory conditions.

Rifampicin altered none of the 23 others developmental, physiological, and reproductive and longevity traits measured in earwig mothers and offspring. Whereas these findings contrast with a large amount of literature showing the broad impact of altered gut microbial communities on host biology (Moran *et al.*, 2019), they are in line with a few recent studies showing that antibiotic-induced alteration of gut microbial communities does not affect development and survival of the three Lepidopteran *Danaus chrysippus*, *Ariadne merione* and *Choristoneura fumiferana* (Hammer *et al.*, 2017; Phalnikar *et al.*, 2019; Ravenscraft *et al.*, 2019). Together with these findings, our results thus provide support to the idea that essential microbial symbioses are not universal across insect species (Hammer *et al.*, 2019; Phalnikar *et al.*, 2019). In these three Lepidoptera species, the lack of microbial symbioses has been explained by the fact that they do not depend on specific gut bacteria to derive critical nutrition from their dietary resources (Chaturvedi *et al.*, 2017; Hammer *et al.*, 2017; Phalnikar *et al.*, 2018). This might also be the case in the European earwig because it is omnivorous (Meunier *et al.*, 2012) and thus a partnership with bacteria facilitating the digestion of specific food sources might not have been required during species evolution. Future works are nevertheless required to test whether (or which part of) its gut microbiota is transient.

To conclude, our study reveals that rifampicin consumption alters female gut microbial communities in earwigs, but provides no evidence for a link between this alteration and the expression of parental care, and no evidence for a strong impact of this alteration on earwig physiology, reproduction and survival. Our study also shows that earwig females harbour different gut microbial communities before and after the period of egg care, a result in line with temporal variation in the microbial communities present on eggshells in the maritime earwig (Greer *et al.*, 2020). Overall, these findings provide support to the recent proposal that microbial enforcement of host social interactions is unlikely to evolve (Johnson & Foster, 2018) and to the emerging idea that not all animals have evolved a co-dependence with their microbiome (Hammer *et al.*, 2019; Phalnikar *et al.*, 2019). Nevertheless, shedding light on whether and how a symbiotic community shape hosts biology is a difficult task, mostly due to the number of players possibly involved and the complexity of their potential interactions (Ravenscraft *et al.*, 2019). Hence, our findings pave the way for follow-up studies testing whether other (non-rifampicin sensitive) members of the gut microbial community could shape the expression of parental care in family-living animals and/or drive important fitness parameters of earwig biology.

SUPPLEMENTARY MATERIAL

Rifampicin treatment and insect rearing

The experiment involved a total of 296 *Forficula auricularia* males and females. These were the first laboratory-born descendants of 74 females field-sampled in Pont-de-Ruan, France, in 2017 and then maintained under standard laboratory conditions (detailed in (Meunier *et al.*, 2012)). In brief, the entire experiment consisted of feeding these 296 earwig adults with a standard food mixed with either rifampicin (Sigma-Aldrich, PHR1806) or water from adult emergence until death, and then measuring a set of parameters reflecting the behaviour, physiology, reproduction and longevity of the tested females. Rifampicin is considered one of the most potent and broad-spectrum antibiotics due to its high-affinity binding to the RNAP β subunit, which causes the inhibition of the bacterial DNA-dependent RNA polymerase RNAP by directly blocking the RNA elongation path (Campbell *et al.*, 2001). It is also commonly used to experimentally alter gut microbial communities in insects (e.g. (Chouaia *et al.*, 2012; Shan *et al.*, 2016; Rosas *et al.*, 2018)).

To obtain rifampicin- and control-treated mothers, we first isolated two virgin males and two virgin females per family (n = 74 families for a total of 296 individuals) four days after adult emergence, and then fed them for two weeks with green-coloured pollen pellets mixed with either 10 µL of rifampicin (0.2 mg/ml) or 10 µL of water. This relatively high dose of rifampicin (about 10 times higher than the dose generally used in smaller insect species (Chouaia *et al.*, 2012; Shan *et al.*, 2016)) was chosen to ensure gut microbial alteration and because it did not trigger an excess of mortality in the German cockroach (Rosas *et al.*, 2018), an insect that is about the size of the European earwig. During these two weeks, each individual was isolated in a Petri Dish (diameter 9 cm) grounded with a humid filter paper that was changed twice a week to prevent the consumption of their own feces pellets (deposited on the paper) and, therefore, to limit the risk of self-transplantation of gut microbes through coprophagy. We also renewed the treatment twice a week to maximize rifampicin activity and its potential effect on gut microbes. The use of a green-coloured food allowed us to verify that the tested individuals produced green-coloured feces pellets and therefore accepted the consumption of rifampicin treated food (which was the case for all the tested individuals).

At the end of these two weeks, the resulting 296 individuals were used to set up 148 mating pairs composed of one virgin female and one virgin male from the same family and the same treatment (2 pairs per family – one rifampicin and one control – for a total of 74 pairs per treatment). The use of sibling pairs allowed us limiting the risk of cytoplasmic incompatibility due to inter-familial microbiome variability, and there are only limited signs of inbreeding depression in this species (Meunier & Kölliker, 2013). Each of the resulting 148 mating pair was maintained under standard autumn laboratory conditions until egg production (Meunier *et al.*, 2012). Until that time, each pair continued to receive a standard food source mostly composed of agar, carrots, pollen, and cat and bird dry food (see detailed composition in (Meunier *et al.*, 2012)) mixed with either 10 µL of rifampicin (0.2 mg/ml) or 10 µL of water (depending on the treatment) twice a week.

On the day we observed the first egg-laying across the 148 pairs (i.e. about 2 months after adult emergence), all males were discarded from the experiment both to mimic natural females' isolation for egg-laying and avoid egg cannibalism by males (Meunier *et al.*, 2012). Females were then maintained under standard winter laboratory conditions (Meunier *et al.*, 2012) until egg hatching, during which we measured the expression of four forms of maternal egg care (details below). Females received no food (and thus no treatment) between egg-laying and egg hatching, as mothers typically stop foraging activity during the period of egg care (Kölliker, 2007). One day after egg hatching, each experimental family was maintained under spring laboratory conditions (Meunier *et al.*, 2012) and we then continued the treatments by feeding them with the same standard food source mixed with either 10 µL of rifampicin (0.2 mg/ml) or 10 µL of water. The food and treatment were renewed twice a week. We measured three forms of maternal care towards juveniles during the following 14 days (details below), which corresponds to the period of family life in this species (Meunier *et al.*, 2012). At the end of this period, we isolated the mothers to mimic the natural end of family life and allow both females' second clutch production and further nymph's development. Isolated mothers and groups of nymphs then continued receiving their treatment (rifampicin or water) and were maintained under standard laboratory spring conditions (Meunier *et al.*, 2012), during which we monitored juvenile development until adulthood, as well as mothers' and nymphs' survival.

Protocols of DNA extraction and bioinformatics pipeline for the gut microbiota

Genomic DNA was extracted from guts using Nucleo-Spin Tissue extraction kit, following the manufacturer instructions. Before the lysis step, a sterile stainless-steel bead (5 mm, Qiagen) was placed in each tube and samples were ground using a Tissue-Lyser II (Qiagen) for 30 s at 30 Hz. Two PCR amplifications were performed for each sample in a final volume of 35 µl to amplify a 450-bp portion of the V3–V4 region of the 16S rRNA gene (forward primer: 5′-CTT TCC CTA CAC GAC GCT CTT CCG ATC T**AC GGR AGG CAG CAG**-3′; reverse primer: 5′-GGA GTT CAG ACG TGT GCT CTT CCG ATC T**TA CCA GGG TAT CTA ATC**-3′; the Illumina adapters and primers *per se* appeared in non-bold and bold, respectively). For each PCR (Polymerase Chain Reaction), 3.5 µl of MTP Taq buffer 10× (Merck), 0.7 µl of dNTP mix (10 mM, Merck), 0.88 µl of each primer (20 µM), 0.6 µl of MTP Taq DNA Polymerase (Merck) and 25.45 µl of water (Merck) were mixed with 3 µl of DNA template. PCR amplifications were performed with 1 min at 94°C followed by 30 cycles composed of 1 min at 94°C, 1 min at 65°C and 1 min at 72°C, and a final step at 72°C for 10 min. Fifty microliters of PCR product was then sent to the GeT-PlaGe genomic platform (GenoToul, Toulouse, France), which performed library preparation and 2 × 250 paired-end Illumina Miseq sequencing according to the manufacturer's instructions.

At the GeT-PlaGe genomic platform, the quality of the run was checked using PhiX (Illumina), each pair-end sequence was assigned to its sample and pair-end reads were assembled using Flash software (Magoč & Salzberg, 2011). The sequencing reads were processed following the FROGS standard operation procedure (Escudié *et al.*, 2018). A pre-process tool was used to remove sequences without primers, with not expected lengths, containing ambiguous bases, and to trim the primers. Sequence clustering was carried out with SWARM (Mahé *et al.*, 2014) with a first denoising step (aggregation distance = 1) followed by a second clustering step (aggregation distance = 3). Chimeric sequences were detected and removed with VSEARCH (Rognes *et al.*, 2016), and a filtering tool was used to remove clusters with a read number abundance of less than 0.005 % of all sequences. Sequences were finally classified with SILVA taxonomy version 138 (Quast *et al.*, 2013; Yilmaz *et al.*, 2014) for V3V4 reads.

Details on the measurements of female's physiology, reproduction, and longevity

We tested the effects of rifampicin on 7 proxies of females' physiology, 16 proxies of females' reproduction and females' longevity. We measured females' physiology through their feces production (reflecting their digestive/foraging activity) and weight gains between two life stages. Feces production was measured 2 months after the beginning of the feeding treatment (on the day where males were removed from the mating pairs). Females were isolated in a Petri Dish lined with a moistened filtered paper (Whatman, diameter: 9cm) for 24 hours, after which we carefully counted the number of feces pellets present on the ground (Körner *et al.*, 2016). The absolute and relative weight gains of each female were measured between adult emergence and egg-laying, and between egg-laying and egg hatching. These measurements were taken to the nearest 0.01 mg using a microbalance (OHAUS© Discovery DV215CD) and by weighing each female on the day of adult emergence, three days after egg laying and one day after egg hatching. The relative weight gains between adult emergence and egg-laying, and between egg-laying and egg hatching were calculated by dividing the absolute weight gain of each female during this period by the fresh weight of this female at the beginning of this period.

We measured females' reproductive parameters on the 1st clutch, 2nd clutch and overall, as well as female's longevity. For the first clutch, we measured the delay until egg-laying, the number of eggs produced, egg weight at production, egg development time, egg hatching rate and juveniles' weight at hatching. The delay until egg-laying corresponds to the number of days between the date of adults' emergence (considered as day 0) and the date of egg production. The number of eggs produced was counted three days after oviposition, as earwig females typically take three days to lay their full clutch of eggs (Koch & Meunier, 2014). The mean egg weight at production was measured in a random group of 10 eggs per clutch. Eggs development time was defined as the number of days between egg-laying and hatching. Egg hatching rate was measured by dividing the number of juveniles present one day after egg hatching (all eggs from a clutch typically hatch within a day (Koch & Meunier, 2014)) by the number of eggs counted three days after egg-laying. Finally, nymphs' weight was measured using a random group of 10 nymphs per clutch. Weighing were to the nearest 0.01 mg (OHAUS© Discovery DV215CD). For the second clutch, we measured females' likelihood to produce a second clutch (1 or 0), the delay until second clutch production (if any) defined as the number of days between the date of females' isolation (14 days after egg hatching, i.e. at the end of family life) and the date of egg production. We also measured the number of eggs produced (if any), mean egg weight at production, egg development time (in days), egg hatching rate and juveniles' weight at hatching. All these measurements were conducted in the same way than for the 1st clutch. Finally, the lifetime reproduction of each female was measured by summing the total number of eggs and the total number of nymphs produced in the first and second clutches, respectively. We also determined their reproductive allocation between first and second clutches (females' reproductive strategy (Meunier *et al.*, 2012)), by calculating their relative investment into 2nd clutch production defined as the number of 2nd clutch eggs divided by the total number of eggs produced by a female. Finally, we defined females' longevity as the difference between the date of adult emergence of each female and its date of death. The sample sizes for each measurement are detailed in Tables 5.1 and S5.1a,b.

Details on the measurements of juveniles' development, sex-ratio, and survival

Because mothers and groups of nymphs continued to receive their treatment after the end of family life, we also tested the effects of rifampicin on juveniles' developmental time between each developmental instar, survival rates (until the end of family life and until adulthood), and the sex ratio of the resulting adults. Juveniles' developmental time between each instar was defined as the differences between the moulting date in a given instar (or hatching date) and the moulting date of its subsequent instar at the clutch-level, i.e. focusing on the first individual moulting in the clutch. This method robustly reflects the developmental time of the entire clutch (Gómez & Kölliker, 2013). It provided us with the following five developmental times: from hatching to 2nd instar, from 2nd to 3rd instar, from 3rd to 4th instars, from 4th instar to adulthood and, overall, from hatching to adulthood. Finally, juveniles' survival from hatching to the end of family life (i.e. 14 days later) was calculated by dividing the total number of juveniles alive 14 days after egg hatching by the number of newly hatched juveniles, while juveniles' survival from the end of family life to adulthood was calculated by dividing the total number of newly emerged adults by the number of juveniles alive 14 days after egg hatching. Finally, the clutch sex ratio at adulthood was calculated using the number of resulting adult males and females in each experimental family (it is not possible to disentangle earwig sex before they reach adulthood). All sample sizes are detailed in Tables 5.1 and S5.1a,b.

Table S5.1a: Effects of the consumption of antibiotics (Rifampicin) on 13 different traits which regroup maternal care and female physiology. P-values that remain significant after correction for multiple comparisons (Adjusted P) are in bold.

	Water				**Rifampicin**				**Statistics**		
Traits	Med Water	1Q Water	3Q Water	N Water	Med Rifam	1Q Rifam	3Q Rifam	N Rifam	W	P	Adjusted P
Maternal care											
Egg grooming (sec)	379.34	259.2	492.28	26	359.54	264.49	450.18	22	288.5	0.963	0.963
Egg defense	12	7	27	55	14	8	26.75	56	1397	0.398	0.582
Delay maternal return (sec)	32	17	54	55	27	10.75	60.5	56	1677.5	0.417	0.582
Egg searching exploration (%)	68.37	59.72	76.28	27	69.55	50.78	81.88	24	339.5	0.775	0.807
Juveniles defense	6	3	13.5	35	5	3	8.75	30	592.5	0.377	0.582
Nymph searching exploration (%)	80.61	77.55	85.2	21	84.16	77.17	89.55	22	208	0.584	0.716
Female physiology											
Feces production	6.5	4	11	36	13	10	14	36	303	**<0.001**	**<0.001**
Females absolute weight gain until egg laying (mg)	13.74	7.24	20.14	59	13.29	8.56	18.22	62	1989	0.407	0.582
Females relative weight gain until egg laying	33.52	13.61	46.14	59	26.83	16.46	46.14	62	1937	0.575	0.716
Females absolute weight during egg care (mg)	1.28	-1.28	4.82	52	2.32	0.19	4.48	59	1308	0.182	0.407
Females relative weight during egg care	1.89	-1.82	7.96	52	4.14	0.32	7.61	59	1308	0.182	0.407
Females absolute weight during egg care of the 2nd clutch (mg)	-1.36	-2.55	2.49	21	1.05	0.38	4.61	9	51	0.05	0.215
Females relative weight during egg care of the 2nd clutch	-2.28	-4.11	3.31	21	1.73	0.58	6.91	9	51	0.05	0.215

Table S5.1b: Effects of the consumption of antibiotics (Rifampicin) on 25 different traits which regroup female reproduction and longevity and nymphs' development, sex ratio and survival. P-values that remain significant after correction for multiple comparisons (Adjusted P) are in bold.

	Water				Rifampicin				Statistics		
Traits	Med Water	1Q Water	3Q Water	N Water	Med Rifam	1Q Rifam	3Q Rifam	N Rifam	W	P	Adjusted P
Female reproduction & longevity											
Age at egg production (days)	183	173	197.5	59	183	172	195	62	1901	0.709	0.807
No eggs produced	55	48.5	60	59	53	43	58.75	62	2037	0.28	0.56
Mean egg weight (mg)	0.62	0.58	0.67	59	0.59	0.57	0.64	62	2297.5	0.015	0.142
Egg developmental time (days)	54	53	55	52	55	53.5	55	59	1215	0.051	0.215
Egg hatching rate (%)	54.54	17.39	68.17	52	42	0	69.3	59	1702	0.318	0.575
Mean juveniles weight (mg)	1.75	1.53	1.85	43	1.52	1.39	1.74	42	1244	**0.002**	**0.025**
Delay until 2nd clutch production	17	12	26	24	21	16	23	17	187	0.66	0.784
Likelihood to produce a second clutch	0.59	-	-	56	0.5	-	-	54	1647	0.347	0.582
No eggs produced on the 2nd clutch	28	20	33	33	23	14	28.5	27	561	0.087	0.383
Mean egg weight of the 2nd clutch (mg)	0.6	0.58	0.64	33	0.56	0.54	0.59	26	628	**0.002**	**0.025**
Egg developmental time of the 2nd clutch (days)	15	15	16	21	16	15	16	9	77	0.361	0.582
Egg hatching rate of the 2nd clutch (%)	38.33	0	75.89	32	0	0	33.24	26	545	0.032	0.203
Mean juveniles of the 2nd clutch weight (mg)	1.49	1.38	1.6	21	1.39	1.35	1.48	10	139.5	0.15	0.395
Female's investment on the 2nd clutch	0.33	0.26	0.38	33	0.32	0.21	0.35	26	495.5	0.312	0.575
Overall reproduction in term of egg	84	71	91	33	76.5	57	84.75	26	553.5	0.057	0.217
Overall reproduction in term of nymph	32.5	22	52	32	21.5	7.5	32.5	28	596.5	0.027	0.203
Female longevity (days)	323	293.5	361	39	306	284.5	343.25	42	994.5	0.098	0.31
Nymph development, survival & clutch sex-ratio											
Juveniles developmental time from hatching to 2nd instar	15	14	16,5	35	15	14	17	34	529.5	0.429	0.582
Juveniles developmental time from 2nd to 3rd instar	15	13	16	35	15	14	16	34	539.5	0.505	0.662
Juveniles developmental time from 3rd to 4th instar	17	16	20	35	19,5	17	21	34	444	0.069	0.238
Juveniles developmental time from 4th instar to adults	32	29	37	35	31	25.25	34.75	34	713.5	0.156	0.395
Juveniles developmental time from hatching to 2nd instar	81	75.5	85.5	35	82	77.25	85.75	34	565.5	0.727	0.807
Juveniles survival from hatching to day 14	84.38	77.5	90	35	85.16	75.37	91.37	34	572	0.786	0.807
Juveniles survival from day 14 to adults	42.86	29.86	59.91	35	39.88	27.27	66.67	34	620.5	0.763	0.807
Sex-ratio	0.5	0.33	0.64	35	0.5	0.4	0.69	34	502	0.266	0.56

Table S5.2a: Effects of antibiotics (Rifampicin) on traits reflecting maternal care and females' physiology, reproduction and longevity and nymphs' development, sex ratio and survival based on paired analyses (i.e. analyses involving water and rifampicin treated individuals from the same family). P-values significant after correction for multiple comparisons (Adj-P) are in bold. Median.diff = Median of the paired difference between rifampicin and water treatments; 1Q and 3Q = first and third quartile of the paired difference, respectively. N = number of paired samples.

Traits	Med Diff	1Q	3Q	N	W	P	Adjusted P
Maternal care							
Egg grooming (sec)	-58.47	-170.44	-2.52	7	4.0	0.219	0.845
Egg defense	-3.00	-13.75	6.25	40	300.0	0.312	0.845
Delay maternal return (sec)	3.00	-23.50	32.50	40	432.0	0.772	1.000
Egg searching distance (mm)	-17.76	-19.56	-16.33	9	16.0	0.496	0.873
Juveniles defense	-1.00	-7.00	2.00	13	35.5	0.806	1.000
Nymph searching distance (mm)	0.97	-3.12	4.06	4	5.0	1.000	1.000
Female physiology							
Feces production	-2.50	-7.25	-1.00	20	23.0	0.002	**0.034**
Females absolute weight gain until egg-laying (mg)	0.56	-4.92	8.20	49	713.0	0.323	0.845
Females relative weight gain until egg-laying	3.39	-11.47	19.69	49	679.0	0.515	0.873
Females absolute weight during egg care (mg)	-1.19	-3.86	2.69	41	344.0	0.268	0.845
Females relative weight during egg care	-1.72	-7.09	4.89	41	346.0	0.280	0.845
Females absolute weight during egg care of the 2nd clutch (mg)	-3.02	-5.12	-0.93	2	1.0	1.000	1.000
Females relative weight during egg care of the 2nd clutch	-5.20	-8.44	-1.97	2	1.0	1.000	1.000

Table S5.2b: Effects of antibiotics (Rifampicin) on traits reflecting maternal care and females' physiology, reproduction and longevity and nymphs' development, sex ratio and survival based on paired analyses (i.e. analyses involving water and rifampicin treated individuals from the same family). P-values significant after correction for multiple comparisons (Adj-P) are in bold. Median.diff = Median of the paired difference between rifampicin and water treatments; 1Q and 3Q = first and third quartile of the paired difference, respectively. N = number of paired samples.

Traits	Med Diff	1Q	3Q	N	W	P	Adjusted P
Female reproduction & longevity							
Age at egg production (days)	2.00	-12.00	13.00	49	622.5	0.923	1.000
No eggs produced	1.00	-7.00	8.00	49	639.0	0.432	0.873
Mean egg weight (mg)	0.04	-0.02	0.06	49	884.5	0.002	**0.034**
Egg developmental time (days)	0.00	-1.00	1.00	41	219.5	0.275	0.845
Egg hatching rate (%)	8.54	-29.13	48.52	41	435.0	0.539	0.873
Mean juveniles weight (mg)	0.22	0.07	0.39	22	202.0	0.013	0.147
Delay until 2nd clutch production	-0.50	-3.00	5.25	4	3.0	1.000	1.000
No eggs produced on the 2nd clutch	2.00	-6.00	12.00	13	52.5	0.310	0.845
Mean egg weight of the 2nd clutch (mg)	0.01	0.01	0.08	13	64.5	0.197	0.845
Egg developmental time of the 2nd clutch (days)	0.50	-0.25	1.25	2	2.0	1.000	1.000
Egg hatching rate of the 2nd clutch (%)	0.00	-14.29	50.00	13	35.0	0.492	0.873
Mean juveniles weight of the 2nd clutch (mg)	0.42	0.41	0.43	2	3.0	0.500	0.873
Female longevity (days)	18.50	-25.75	56.25	22	147.0	0.284	0.845
Nymph development, survival & clutch sex-ratio							
Juveniles developmental time from hatching to 2nd instar	0.00	-1.00	1.00	13	16.0	0.844	1.000
Juveniles developmental time from 2nd to 3rd instar	1.00	-1.00	2.00	13	40.0	0.946	1.000
Juveniles developmental time from 3rd to 4th instar	1.00	-3.00	5.00	13	44.5	0.685	1.000
Juveniles developmental time from 4th instar to adults	2.00	-2.00	5.00	13	60.5	0.315	0.845
Juveniles developmental time from hatching to 2nd instar	1.00	-2.00	7.00	13	49.0	0.460	0.873
Juveniles survival from hatching to day 14	2.39	-3.62	16.23	13	51.0	0.735	1.000
Juveniles survival from day 14 to adults	-3.15	-36.36	11.90	13	38.0	0.635	0.981
Sex-ratio	0.00	-0.22	0.10	13	24.0	0.465	0.873

CHAPITRE 6

L'expression des soins aux œufs est perturbée par une exposition sublétale à la deltaméthrine chez le forficule européen

Résumé de l’étude

L’utilisation accrue des pesticides en agriculture entraîne généralement une exposition à des doses létales et sublétales d’un grand nombre d’insectes non-ciblés. Alors que les recherches sur ces effets sublétaux se sont principalement concentrées sur les paramètres reproductifs et physiologiques, un nombre croissant d'études met en lumière la grande diversité des effets sublétaux d’une exposition aux pesticides sur le comportement des insectes. Cependant, ces effets sur les soins maternels chez les insectes restent à l’heure actuelle inconnus. Dans cette étude, nous avons testé si l'exposition à plusieurs doses sublétales de deltaméthrine (un insecticide de la famille des pyréthrinoïdes) pouvait modifier l'expression des soins maternels envers les œufs chez les femelles forficule. Nos résultats révèlent tout d'abord que l'exposition à la deltaméthrine altère l'expression de trois formes de soins maternels sur six. Plus précisément, cette exposition réduit la propension des mères à rassembler leurs œufs, prolonge le temps d’abandon de la couvée par la femelle après une attaque de prédateur (simulée) et enfin, réduit la durée du nettoyage des œufs. Il est important de noter que ces effets sublétaux sur les soins maternels ne reflètent pas une baisse d’activité de la part des femelles exposées à la deltaméthrine car ces mêmes femelles ont augmenté leur temps d’auto toilettage et n’ont eu aucune perturbation dans leur mobilité et leur taux d’exploration. Enfin, nous avons constaté que les effets négatifs de la deltaméthrine sur les soins maternels ne modifiaient pas le développement intrinsèque des œufs ni le taux d'éclosion et le poids des juvéniles. Dans l'ensemble, nos résultats révèlent qu'une exposition sublétale à un pesticide comme la deltaméthrine peut altérer l’expression des soins maternels chez un insecte auxiliaire de culture et non ciblé. Notre étude appelle donc à l'intégration de ce type de mesure dans les essais d'application des pesticides.

Sublethal exposure to deltamethrin impairs maternal egg care in the European earwig Forficula auricularia

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ABSTRACT

The application of pesticides typically leads to lethal and sublethal exposure of non-target insects. Whereas our current understanding of these sublethal effects typically focuses on reproductive and physiological parameters, recent works emphasize that sublethal effects on behaviors such as maternal care could be of major importance in non-target species. However, it remained unknown whether these sublethal effects occur in insects. Here, we tested if exposure to sublethal doses of deltamethrin - a pyrethroid insecticide commonly used in crops - alters the expression of maternal egg care in females of the European earwig *Forficula auricularia*, a predator insect and pest control. Our results first reveal that deltamethrin exposure impaired the expression of three forms of maternal egg care: It decreased the likelihood of mothers to gather their otherwise scattered clutch of eggs, increased the time during which the female abandoned the clutch after a predator attack and reduced egg grooming duration. These sublethal effects did not reflect a lower activity of deltamethrin-exposed females, as these females increased their expression of self-grooming, and deltamethrin exposure did not affect females' exploration and mobility. Finally, we found that the negative effects of deltamethrin on egg care did not modify egg development, hatching rate and juvenile weight, possibly due to the transient effects of deltamethrin on maternal behaviors. Overall, our results reveal that sublethal exposure to a pesticide may diminish maternal egg care in a natural pest control and call for the integration of this measurement in assays on pesticides application.

INTRODUCTION

The widespread and increased applications of pesticides inherently lead to the exposure of a great number of non-target insects (Aktar *et al.*, 2009; Guedes *et al.*, 2016). Among these insects, some can be of key agricultural, economic and ecological importance, as they often offer long-term sustainable pest control solution by consuming herbivore invertebrates and plant pathogen vectors, provide pollination services facilitating propagation and fruit production for many plants, and represent keystone species maintaining ecosystem balances. Understanding how pesticides affect the survival, physiology and behavior of these beneficial insects is therefore critical to safely and effectively use these compounds.

Pyrethroids are one of the most common class of pesticide used in agrosystems (Davies *et al.*, 2012; Liao *et al.*, 2018) and includes allethrin, bifenthrin, cyhalothrin, cypermethrin, permethrin and deltamethrin. Deltamethrin in particular is a type II synthetic pyrethroid which is well known to affect the sodium channel and trigger a broad release of neurotransmitters at the synapse (Enan & Matsumura, 1992). Due to its low toxicity for vertebrates, high efficiency against insects, and low persistence in soil (Dietz *et al.*, 2009; Li *et al.*, 2019), it is applied in many crops worldwide (e.g. apples, pears, peaches, sorghum, pineapple, coffee, and eucalyptus), as well as used in the public health field to control mosquitoes, flies, cockroaches, and ticks (Davies *et al.*, 2012; Liao *et al.*, 2018). In addition to its well-known lethal effects (e.g. Gutiérrez *et al.* 2017; Shaw *et al.* 2019; Vander Pan *et al.* 2019), sublethal concentrations of deltamethrin can entail major physiological damages in a multitude of target and non-target insects (Cutler, 2013; Müller, 2018). For instance, exposure to sublethal doses of deltamethrin reduces females' fecundity in honeybees, parasitoid wasps and cockroaches (Lee *et al.*, 1998; Dai *et al.*, 2010; Teder & Knapp, 2019), impairs larval development in honeybees and the wasp *Trichogramma achaeae* (Oliveira *et al.*, 2018; Yang *et al.*, 2020) and inhibits molting processes in the stable fly *Stomoxys calcitrans* (Reissert-Oppermann *et al.*, 2019).

Exposure to sublethal concentrations of deltamethrin may also indirectly and directly alter crucial behaviors in insects. Indirect alterations often take the form of an avoidance of deltamethrin contaminated areas by non-exposed individuals. This is the case, for instance, in the maize weevil *Sitophilus zeamais* where individuals modify their walking activity in presence of grains exposed to deltamethrin (Vélez *et al.*, 2019), and in the mosquito *Anopheles sinensis*, where adults show longer flying time and more frequent takeoffs when maintained close to deltamethrin-treated bed nets (He *et al.*, 2019). On the other hand, direct behavioral alterations after deltamethrin exposure have been reported in few insects, such as in Trichogramma species where it disrupted the discrimination of sex pheromones (Delpuech *et al.*, 2012), in the cotton leafworm *Spodoptera littoralis*, where it had an effect on peripheral olfactory system and sexual behavior (Lalouette *et al.*, 2016), and in the backswimmer *Buenoa tarsalis*, where it disrupted swimming activity (Gutiérrez et al., 2017). In the honeybee *Apis mellifera*, it also impaired olfaction and learning capabilities (Decourtye *et al.*, 2004), altered homing flight (Vandame *et al.*, 1995), disturbed orientation and dancing communication (Thompson, 2003; Zhang *et al.*, 2020), and modified foraging activity and memory (Ramirez-Romero *et al.*, 2005).

Whereas a growing number of studies shed light on the broad diversity of sublethal effects associated with pesticide exposure on insect behaviors (Haynes, 1988; Mazzi & Dorn, 2012; Müller, 2018; Parkinson *et al.*, 2020), their impacts – and the impact of deltamethrin - on maternal care remain unexplored in insects. This is surprising, as maternal care is a common phenomenon in insects and forms the backbone of key insect societies such as in many bees (Wong *et al.*, 2013; Klug & Bonsall, 2014; Machado & Trumbo, 2018; Kramer & Meunier, 2019). Moreover, sublethal effects of pesticides on maternal care have been recently suggested to be of central importance because the resulting alterations in offspring could shape the long-term efficiency of pesticide use, and the maintenance and population dynamics of non-target organisms (Cummings *et al.*, 2010; Fong-Mcmaster *et al.*, 2020). To date, however, these sublethal effects on maternal care were investigated in only a few studies conducted in rats and mice (Punzo, 2003; De Castro *et al.*, 2007; Stürtz *et al.*, 2008; Venerosi *et al.*, 2009; Udo *et al.*, 2014; Keller *et al.*, 2019). These studies showed, for example, that mothers fed with lindane (an organochlorine pesticide) exhibit a lack of retrieval behavior and a consequential litter loss (Matsuura *et al.*, 2005), that mothers exposed to a glyphosate-based herbicide (an organophosphorus pesticide) increased maternal licking behaviors (Dechartres *et al.*, 2019) or that mothers fed with methoxychlor (an organochloride pesticide) reduced the amount of time spent in nursing (Palanza *et al.*, 2002). Whether and how sublethal effects of pesticide exposure (such as deltamethrin) alter maternal care in non-mammalian species remains unknown.

The European Earwig *Forficula auricularia* L. is a common Dermapteran in many agro-ecosystems worldwide and is well-known to exhibit multiple forms of maternal care (Lamb, 1976; Albouy & Caussanel, 1990; Orpet *et al.*, 2019). It is considered an effective generalist predators of several pests such as aphids, leafrollers, and psyllids in pip-fruit orchards (Sauphanor & Sureau, 1993; Dib *et al.*, 2010, 2011; Moerkens *et al.*, 2011; Lordan *et al.*, 2015; Orpet *et al.*, 2019) and data are available on the lethal and physiological effects (or absence of effects) of a number of pesticides commonly applied in crops and vineyards (French-Constant & Vickerman, 1985; Colvin & Cranshaw, 2010; Malagnoux *et al.*, 2014; Le Navenant *et al.*, 2019; Orpet *et al.*, 2019). Individuals of this non-target species (it is sometimes considered as a pest in North America; Colvin and Cranshaw, 2010) are nocturnal and may therefore encounter pesticides only in the form of residuals through direct contacts with (dried) treated surfaces (leaf and soil; Orpet et al., 2019). Although many pesticides used in orchards are toxic to this species (Fountain & Harris, 2015; Malagnoux *et al.*, 2015b), direct exposures to normal application rates of deltamethrin have no impact on their survival and predator activities (Malagnoux *et al.*, 2015a). The effects of deltamethrin on earwig maternal egg care remain unknown. Earwig females produce a first and second (if any) clutch of eggs in late autumn and early spring, respectively, with which they remain in a nest for several weeks to provide extensive egg care (Meunier *et al.*, 2012; Van Meyel *et al.*, 2019). This egg care is considered essential for egg development and survival, as it allows the removal of external pathogens and mold from the egg shell, the deposition of chemical compounds (hydrocarbons) on the eggs to limit the risk of desiccation, and the expression of fierce maternal behaviors to deter predators (Buxton & Madge, 1974; Boos *et al.*, 2014; Diehl & Meunier, 2018).

In this study, we exposed females to different concentrations of deltamethrin and subsequently measured the expression of six forms of egg care, three forms of non-egg care behaviors, and measured the resulting effects on egg hatching date, hatching rate and juveniles' fresh weight at egg hatching. We also investigated the sublethal effects of deltamethrin on females' allocation between self- and egg-grooming, two key behaviors that can trade-off and typically allow insects to respectively clean their cuticle and egg shell from pathogens and dirt of different natures (Boos *et al.*, 2014; Weiß *et al.*, 2014). To confirm that the reported sublethal effects of deltamethrin do not reflect population-specific expression of egg care (Ratz *et al.*, 2016; Tourneur & Meunier, 2020), we tested females from two distant populations.

MATERIAL AND METHODS

Earwig sampling and maintenance

Our study involved a total of 120 *Forficula auricularia* females that were field-sampled in pip fruit orchards under Integrated Pest Management (IPM) during the first week of July 2019 in Pont-de-Ruan, France (n = 71) and Joué-les-tours, France (n = 49). These females were then maintained under standard laboratory conditions until egg-laying. In brief, earwig adults were caught with wood traps previously placed on tree branches for one week. On the day of field-sampling, males and females were transferred in large plastic containers to allow uncontrolled mating (Sandrin *et* *al.*, 2015). All the containers were then maintained at 18°C under a 12:12h Light:Dark cycle. Four months later, each female was isolated in a Petri dish (diameter 9 cm) to mimic natural dispersion and encourage egg production (Körner *et al.*, 2018). The Petri dish was grounded with humid sand and maintained at 10°C under constant darkness to trigger females' nest construction (Koch & Meunier, 2014). Each female was provided with an *ad libitum* amount of an artificial diet mainly consisting of pollen, cat food, carrots and agar (see details in Kramer et al., 2015). These last rearing conditions were maintained until egg hatching. Each Petri dish was checked once a week to record the week of egg laying. Because females cease feeding between egg-laying and egg hatching (Kölliker, 2007), we removed the food present in each Petri dish at egg-laying.

Deltamethrin exposure

Sixteen days after egg laying (i.e. half egg development time; Ratz *et al.* 2016), each mother was randomly exposed to a deltamethrin or a control (absolute ethanol) solution using a protocol adapted from Malagnoux *et al.* (2015a) and mimicking commercial sprayers (Sauphanor *et al.*, 1992). We used four (sublethal) concentrations of deltamethrin (68.750, 13.750, 6.875 or 3.438 ng/cm^2) obtained by diluting the active deltamethrin molecule (SIGMA #45423) in pure analytical grade (>99.5% absolute) ethanol. These concentrations were respectively 4 times larger, and 1.25, 2.5 and 5 times smaller than the normal application rate (NAR) allowed in French apple orchards that is 0.75 g/hl active ingredient equivalent to 17 ng/cm^2 (Malagnoux *et al.*, 2015a). Maternal exposures were conducted by directly applying 88 µL of one of the four deltamethrin solutions or an absolute ethanol solution (control) on the surface of a Petri dish (diameter 3.5 cm). The Petri dish was then immediately and gently rotated to fully cover its ground and walls with the pesticide or control solution. These solutions were allowed to evaporate for 30 min under an extractor hood. Each mother was subsequently removed from its clutch of eggs, transferred to this Petri dish and allowed to walk freely on its ground contaminated with deltamethrin (or evaporated ethanol for the control). Four hours later, each mother was returned to her clutch of eggs in the original Petri dish and we subsequently conducted egg care measurements. Overall, this process allowed us to obtain 120 exposed mothers, of which 24 mothers exposed to deltamethrin at a level of 68.750 ng/cm^2 (14 and 10 from Pont-de-Ruan and Joué-les-tours, respectively), 24 mothers exposed at a level of 13.750 ng/cm^2 (14 and 10, respectively), 24 mothers exposed at a level of 6.875 ng/cm^2 (15 and 9, respectively), 24 mothers exposed at a level of 3.438 ng/cm^2 (14 and 10, respectively) and 24 mothers exposed to (evaporated) ethanol only (14 and 10, respectively). During exposure, mothers were maintained under a constant artificial (white) light to stimulate their walking activity (the European earwig is a lucifugous species) and thus maximise contacts with molecules deposited on the Petri dish. The tested mothers were randomly distributed among the five treatments, so that there was no association between the number of tended eggs and deltamethrin concentration (Linear model, $F_{4,155} = 0.07$, $P = 0.992$).

Sublethal doses of pesticides are typically defined as doses inducing no apparent mortality in the tested population, but potentially causing physiological and/or behavioral effects on individuals that survive the pesticides exposure (Desneux *et al.*, 2007; Müller, 2018). In our study, we therefore defined the four doses of deltamethrin as sublethal on the basis of previous works showing that deltamethrin doses corresponding to normal application rates (NAR) in French apple orchards do not induce mortality in *F. auricularia* adults (Malagnoux *et al.*, 2015a), and on our current results showing that there was no excess of mortality with the four tested doses (see results).

Behavioural and reproductive measurements

The 120 mothers exposed to deltamethrin or evaporated ethanol (control) were then used to measure the expression of six forms of egg care (egg gathering, egg defence against a predator, delay of maternal return after clutch abandonment, egg grooming, egg displacement and egg antennation), three female behaviours that are not associated with egg care (see below), females' allocation between self- and egg-grooming, as well as to record females' reproductive outcome in terms of date of egg hatching, egg hatching rate and larval weight at egg hatching. For egg gathering, we carefully scattered the clutch of each female in its original Petri dish during the 4h maternal exposure (see above), then transfered each mother to its scattered clutch and, 17h later, recorded whether each mother gathered (or not) all the eggs, i.e. whether all eggs were within one egg distance from each other. The time spent by mothers on egg grooming, egg displacement and egg antennation was measured just after egg gathering measurements and involved all the tested mothers, i.e. includes the few mothers that did not gather their eggs after 17h (see results). We first isolated each mother for 15 min to increase their future motivation to interact with the eggs (Van Meyel *et al.*, 2019). We then put back mothers in their Petri dish at a distance of 5 cm from the eggs and finally video recorded their behaviours for the next 20 min (SONY© Handycam HDR-CX700 camera). The total durations of egg grooming, egg displacement and egg antennation were respectively defined as the total amount of time each female spent on cleaning eggs with their mandibles, displacing eggs within or away from the clutch, and antennating at least one egg without mandibular contacts (Boos *et al.*, 2014). These three measurements are classical forms of maternal care and reflect the amount of time earwig mothers allocate to the cleaning or application of chemicals (hydrocarbons) on the eggs, the moving of eggs to safer area and/or to the re-organisation of clutch structure and to the touching of the eggs before triggering other care behaviours, respectively (Van Meyel *et al.*, 2019). Movies were analysed using the software BORIS v4.0.3 (Friard & Gamba, 2016). The measurements of egg defence (Thesing *et al.*, 2015), which reflects females' willingness to protect their eggs from a predator attack, were conducted just after the above-detailed movies recordings. We standardly poked females on the pronotum with a glass capillary (1 poke per second) and recorded the number of pokes required until the female moved more than 1 body length away from its initial position. Finally, the delay of maternal return after clutch abandonment (Van Meyel *et al.*, 2019) was measured by recording the time the female took to return to its clutch just after the end of the egg defence measurement. At the end of these behavioral measurements, all females were returned with their clutch of eggs and then maintained under standard laboratory conditions. All the behavioural measurements (here and below) and video analyses were done under red light (earwigs are nocturnal) and blind regarding the type of female exposure.

The effects of deltamethrin exposure on three non-care behaviours (self-grooming, exploration and inactivity) and females' allocation between self- and egg-grooming were measured on the 20 min movies used to measure egg care behaviors (see above). The total duration of females' self-grooming, which is a behavior during which individuals typically clean off dirt, remove external parasites and apply chemical protections on their cuticle (Weiß *et al.*, 2014), was defined as the total amount of time each female spent on cleaning itself with its mandibles. Females' exploration, which is a behavior reflecting the general mobility of an individual, was defined as the total amount of time each female spent on being mobile neither interacting with the eggs nor expressing self-grooming. Females' inactivity was defined as the total amount of time each mother did not show any movement or behaviour directed at themselves or the eggs during the 20 min video recordings. These three behaviours were analysed following the same protocol than for maternal egg care. Finally, females' allocation between self- and egg-grooming, which may reflect a selfish versus cooperative maternal strategy, was calculated for each female by dividing the total amount of time spend in egg-grooming by the total amount of time spend in both egg-grooming and self-grooming.

All females and eggs used in the behavioural measurements detailed above were then maintained under standard conditions during the 40 days following female exposure. They were checked daily to record the date of death (if any) and thus confirm our use of sublethal doses of deltamethrin, as well as to record the date of egg hatching and, one day later, to count and weight the resulting larval. For the weighing, a group of 10 juveniles (called nymphs) was randomly sampled per clutch (or all of them if less than 10 were available), weighed to the nearest 0.01 mg using a microbalance (OHAUS© Discovery DV215CD) and these measurements then used to calculate mean nymph weight.

Statistical analyses

We used three generalised linear models (GLM) fitted with binomial error distribution to analyse egg gathering (1 or 0), hatching rate and females' allocation between self- and egg-grooming, while we used a series of nine GLM fitted with Poisson error distribution corrected for over-dispersion to analyse egg defence, delay of maternal return, egg grooming, egg displacement, egg antennation, self-grooming, exploration, inactivity and mean nymph weight. In each of these models, females' exposure (68.750, 13.750, 6.875, 3.438 ng/cm^2 or evaporated ethanol), females' population of origin (Pont-de-Ruan or Joué-les-tours) and the interaction between these two factors were entered as categorical factors, while the number of eggs produced by a female was used as a covariable to control for its possible impact on the level of egg care. Egg hatching rate and females' allocation between self- and egg-grooming were entered in the models using the *cbind* function in R (number of juveniles at hatching, number of unhatched eggs; and time spend in egg grooming, time spend in self grooming, respectively). We also analyzed whether mothers exposed to each tested dose of deltamethrin allocated the same amount of time between egg- and self-grooming using one sample t-tests, in which we compared females' allocation between self- and egg-grooming in each treatment to the value 0.5. For egg gathering, pairwise comparisons between deltamethrin treatments were conducted using a series of fisher exact tests and the p-values were corrected for multiple testing using the mean false discovery rate method (Benjamini & Hochberg, 1995; Kramer *et al.*, 2015). For the delay of maternal return, egg grooming and self-grooming, pairwise comparisons between deltamethrin treatments were conducted using the estimated marginal means and the p-values were corrected for multiple testing using Tukey methods. All models were checked for homoscedasticity and normal distribution of model residuals. All statistical analyses were performed with the software R v3.6.3 (http://www.r- project.org/) loaded with the packages *car* and *emmeans*.

RESULTS

Out of the 120 females involved in this study, only 11 (9.2%) died within the 40 days following experimental exposures. These deaths were independent of deltamethrin exposure (Fisher's exact test, $p = 0.949$), as 3 (12.5%) females died after exposure to the 68.750 ng/cm^2 solution of deltamethrin, 3 (12.5%) after exposure to the 13.750 ng/cm^2 solution, 2 (8.3%) after exposure to the 6.875 ng/cm^2 solution, 2 (8.3%) females after exposure to the 3.438 ng/cm^2 solution and 1 (4.2%) female after exposure to evaporated ethanol (control) solution.

There were contrasting dose-dependent effects of deltamethrin on maternal care in terms of likelihood of egg gathering, total time spent egg grooming and delay of maternal return after clutch abandonment (Figure 6.1; Table 6.1). First, the likelihood of females to gather their eggs within the 17h post-exposure period was significantly lower when they were exposed to the highest quantity of deltamethrin (45.9% of the females at 68.750ng/cm^2) compared to the control (100% of the control females) or any others doses of deltamethrin (Tables 6.1 & 6.2 , Figure 6.1A). This likelihood was not different among the three lowest doses of deltamethrin and the control (Table 6.2). Second, the total duration of egg grooming was about 3.5 times shorter when females were exposed to the highest quantity of deltamethrin (51.4 sec of the females at 68.750ng/cm^2) compared to the control (177.9 sec) or any other doses of deltamethrin (Tables 6.1 & 6.2, Figure 6.1B). This duration was also not different among the three lowest doses of deltamethrin and the control (Table 6.2). Finally, the delay of maternal return after clutch abandonment was about 9 times longer when females were exposed to the highest dose of deltamethrin (194.5 sec of the females at 68.750ng/cm^2) compared to the control (21.5 sec), a delay also present for all the other tested quantities of deltamethrin (Tables 6.1 & 6.2, Figure 6.1B). The delay of maternal return was comparable among the four tested doses of deltamethrin (Table

6.2). Contrary to these effects, there was no impact of deltamethrin on the total amount of time spend in egg displacement and egg antennation, nor on the level of egg defense against a simulated predator attack (Figures 6.1D, 6.1E and 6.1F; Table 6.1).

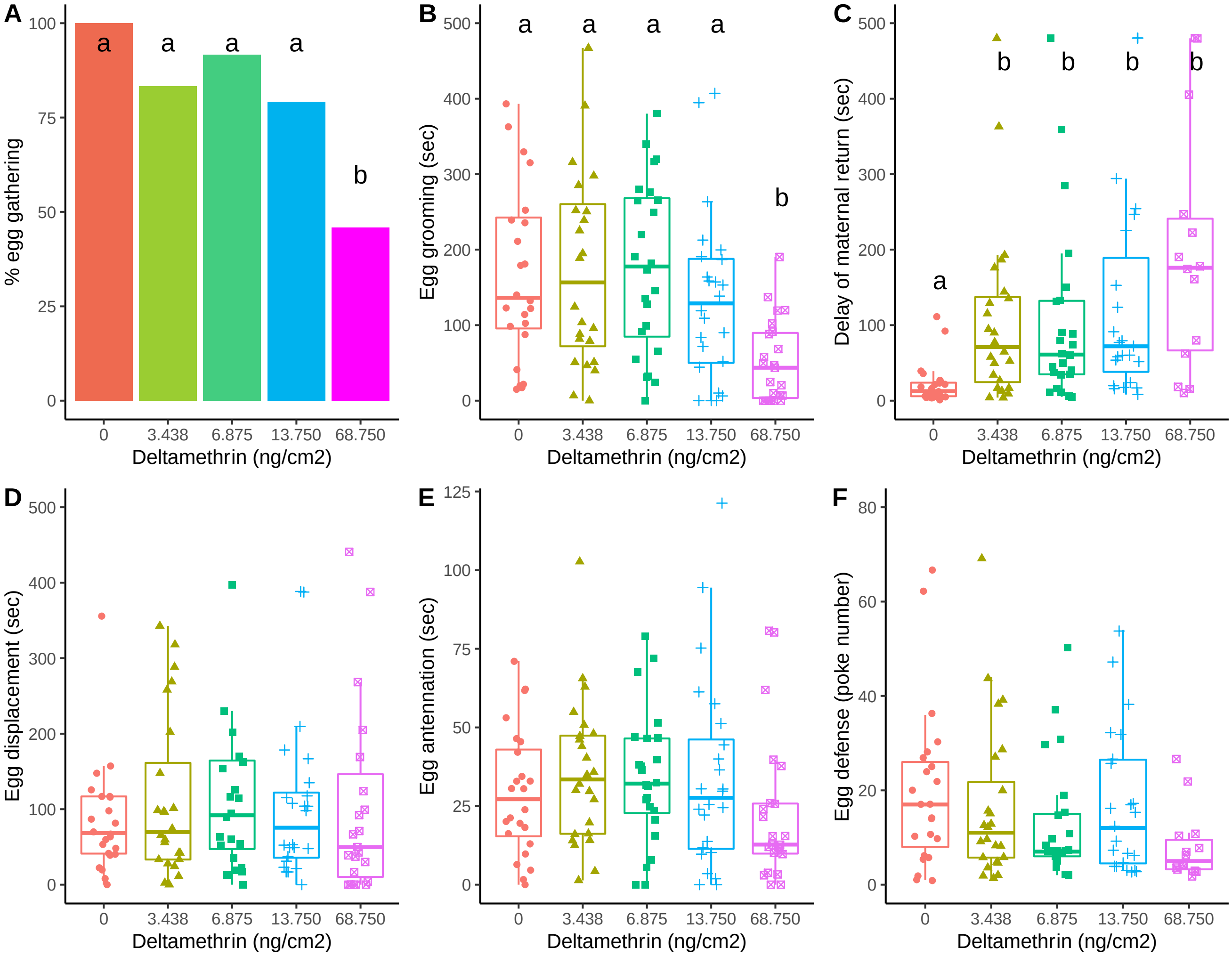

Figure 6.1: Effects of deltamethrin on maternal egg care in the forms of A) egg gathering, B) egg grooming, C) delay of maternal return after clutch abandonment, D) egg displacement, E) egg antennation and F) egg defence against a predator. Boxplots depict median (bold bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. For pairwise comparisons, different letters correspond to *P* <0.05. The absence of letter on a figure indicates an absence of significant difference between all the tested doses.

Dose-dependent effects of deltamethrin were present in self-grooming behavior, but not on the time spent in exploration and inactivity (Tables 6.1 & 6.2, Figure 6.2). In particular, the total duration of self-grooming was about 2.4 times longer when females were exposed to the highest quantity of deltamethrin (270.2 sec of the females at 68.750ng/cm^2) compared to the control (111.6 sec) and the two lowest doses of deltamethrin (Tables 6.1 & 6.2, Figure 6.2A). Females exposed to the second highest quantity of deltamethrin (13.750 ng/cm^2) spent 197.4 sec in self-grooming on average, and intermediate value that was not different from the other tested doses of deltamethrin and the control (Tables 6.1 & 6.2; Figure 6.2A).

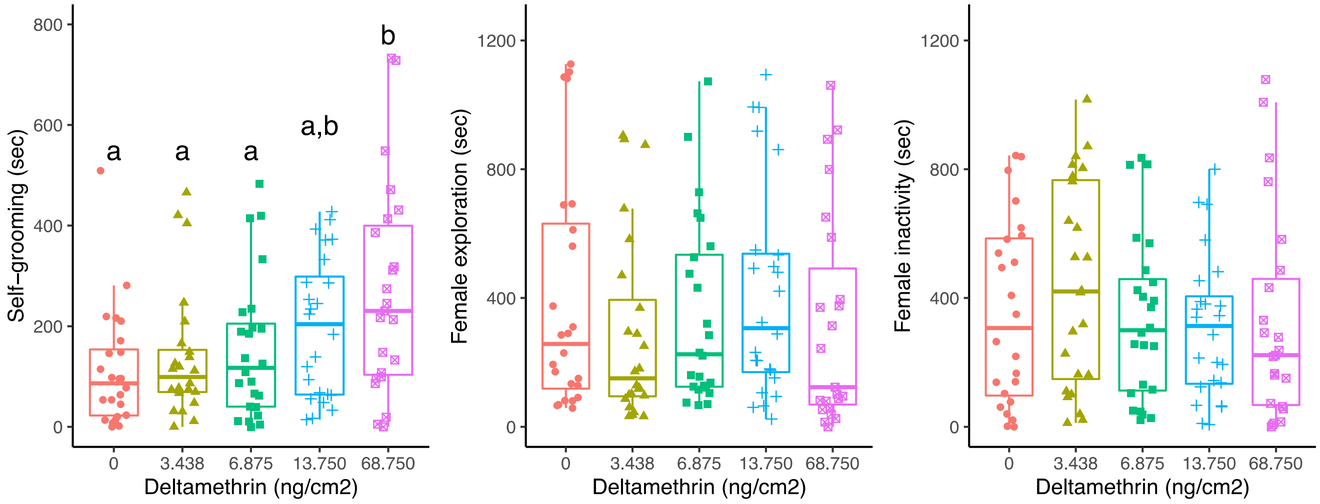

Figure 6.2: Effects of deltamethrin on the total durations of females' A) self-grooming, B) exploration and C) inactivity. Boxplots depict median (bold bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. For pairwise comparisons, different letters correspond to $P < 0.05$. The absence of letter on a figure indicates an absence of significant differences between all the tested doses.

Dose-dependent effects of deltamethrin also shaped the presence and outcome of females' allocation between self- and egg-grooming (Figure 6.3, Table 6.1). Of the total amount of time spend in grooming behaviors (i.e. egg- plus self-grooming), control females allocated on average 64.2% of their grooming time to their eggs, whereas females exposed to the two highest doses of deltamethrin (68.750 and 13.750 ng/cm^2) allocated a 4 and 1.7 times smaller proportion of time (Tables 6.1 & 6.2, Figure 6.3). Females exposed to the two smallest doses of deltamethrin exhibited a proportion in egg grooming that was intermediate between the control and highest doses of deltamethrin (Table 6.2; Figure 6.3). Interestingly, females exposed to control allocated a larger proportion of their total grooming time to their eggs (one sample t-test, t = 2.94, df = 23, P = 0.007; Figure 6.3), females exposed to the smallest (8.750 ng/cm^2) and second smallest (3.438 ng/cm^2) quantities of deltamethrin shared their total grooming time between the eggs and themselves (t = 1.14, df = 23, P = 0.266 and t = 1.26, df = 23, P = 0.220, respectively), while females exposed to the highest (68.750 ng/cm^2) and second highest (13.750 ng/cm^2) doses of deltamethrin allocated a larger proportion of their total grooming time to themselves (t = -10.59, df = 21, P < 0.0001 and t = -2.15, df = 23, P = 0.043, respectively).

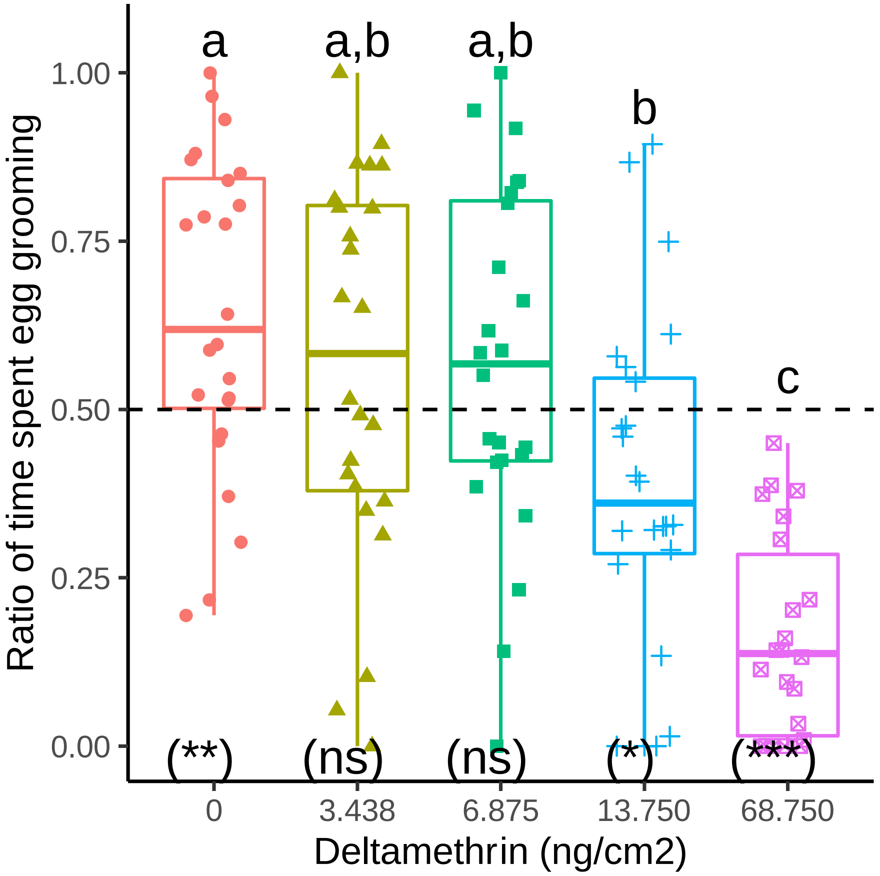

Figure 6.3: Effects of deltamethrin on female's allocation between egg- and self-grooming. A ratio of time spent on egg grooming of 0.5 indicates an absence of the trade-off, while ratios between 0.5 and 1 indicates a trade-off in favour of egg grooming and between 0 and 0.5 a trade-off in favour of self-grooming. Values between brackets (bottom) compare each column to 0.5 (using one sample t-tests), i.e. tests whether females from each category did express the same amount of egg- and self-grooming. *** stands for $P < 0.001$; ** for $P < 0.01$, * for $P < 0.05$ and *ns* for $P > 0.05$. For pairwise comparisons, different letters correspond to $P < 0.05$.

There were no effects of deltamethrin exposure on egg hatching date, egg hatching rate and on the mean weight of the resulting juveniles (Figure 6.4; Table 6.1). Interestingly, the eggs from Joué-les-tours overall hatched 8.30 ± 0.03 (mean ± SE) days after exposure, a value that was shorter than the 10.05 ± 0.04 days of the eggs from Pont-de-Ruan (Table 6.1). Females from Joué-les-tours also spent 419.9 ± 41.6 (mean ± SE) sec in inactivity, which was longer than the 306.1 ± 32.0 sec of females from Pont-de-Ruan (Table 6.1). Nevertheless, the population had no other effect (neither a main effect nor in interaction with deltamethrin exposure) on the six measured forms of egg care, on females' allocation between self- and egg-grooming, on the two other non-care behaviors, and on the two other measurements of reproductive outcome (Table 6.1). Similarly, egg number was negatively associated with the likelihood of egg clustering 17h post-exposure (model estimate ± SE = -0.37 ± 0.019; Table 6.1) and with the mean juvenile's weight at egg hatching (model estimate ± SE = -0.0052 ± 0.0019; Table 6.1), while it was positively associated with maternal allocation in egg- compared to self-grooming (model estimate ± SE = 0.03 ± 0.01; Table 6.1). Egg number had no effect on the ten other measured traits (Table 6.1).

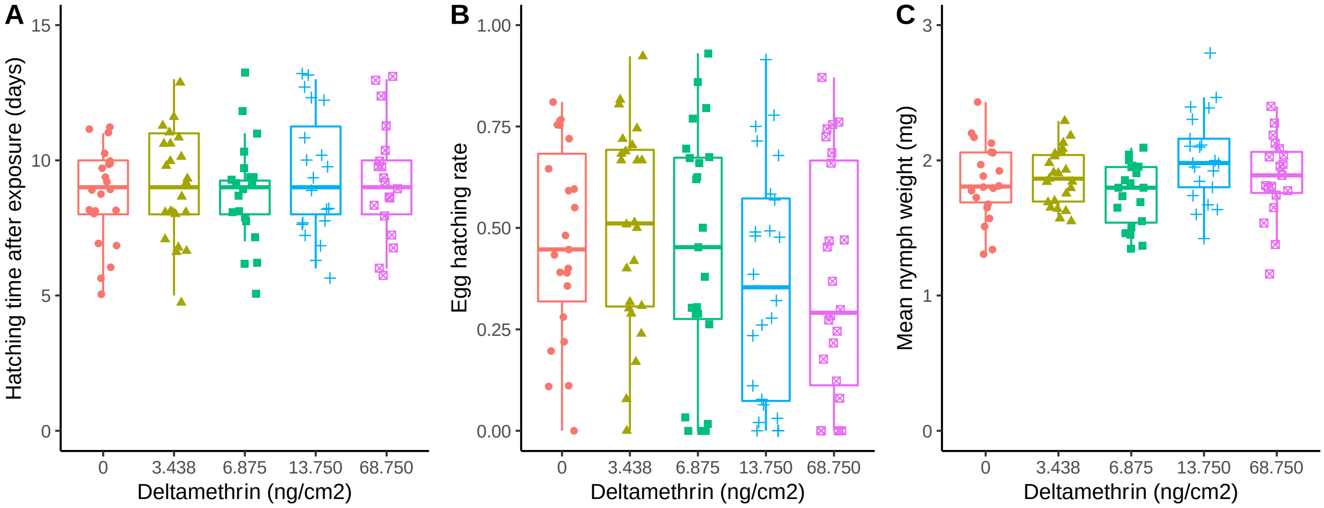

Figure 6.4: Effects of deltamethrin on reproductive outcomes in term of A) hatching time after exposure, B) egg hatching rate and C) mean nymph weight at egg hatching. Boxplots depict median (bold bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. For the three measurements, there was no significant differences between the tested doses.

Table 6.1: Effect of exposure to different concentrations of deltamethrin, females' population and egg number on the expression of six forms of egg care, three female behaviors not associated with egg care, the trade-off between self- and egg-grooming, and on three proxies of females' reproductive outcome. Statistical values from generalized and general linear models conducted on each measurement (one model per line; see methods). Significant p-values are in bold. $LR\chi^2$ stands for Likelihood Ratio χ^2.

	Exposure			Population			Egg number			Exposure : Population		
	F / $LR\chi^2$	df	P	F / $LR\chi^2$	df	P	F / $LR\chi^2$	df	P	F / $LR\chi^2$	df	P
Egg care behaviors												
Egg gathering	28.48	4	**<0.0001**	1.03	1	0.310	6.45	1	**0.011**	9.18	4	0.057
Egg grooming	27.64	4	**<0.0001**	0.21	1	0.643	3.18	1	0.075	2.49	4	0.647
Maternal return	29.62	4	**<0.0001**	2.06	1	0.151	0.08	1	0.774	3.43	4	0.489
Egg displacement	1.08	4	0.897	2.37	1	0.124	4.06	1	0.044	1.68	4	0.794
Egg antennation	5.17	4	0.270	0.54	1	0.462	0.62	1	0.432	2.99	4	0.560
Egg defense	7.32	4	0.120	1.44	1	0.230	0.73	1	0.392	1.37	4	0.849
Non-care behaviours												
Self-grooming	15.81	4	**0.003**	2.35	1	0.125	1.96	1	0.162	0.79	4	0.939
Exploration	2.50	4	0.644	0.34	1	0.561	0.25	1	0.619	2.13	4	0.712
Inactivity	3.17	4	0.530	4.5	1	**0.034**	0.02	1	0.886	5.71	4	0.222
Allocation self/egg grooming	71.87	4	**<0.0001**	0.28	1	0.594	9.3	1	**0.002**	2.47	4	0.649
Reproductive outcome												
Hatching date	0.73	4	0.571	27.22	1	**<0.0001**	2.92	1	0.091	0.87	4	0.488
Hatching rate	4.61	4	0.329	0.22	1	0.638	0.19	1	0.663	2.34	4	0.674
Nymph weight	2.38	4	0.057	1.50	1	0.223	7.24	1	**0.008**	1.65	4	0.168

Table 6.2: Pairwise comparisons among deltamethrin treatments (C1 = 68.750 ng/cm^2, C2 = 13.750 ng/cm^2, C3 = 6.875 ng/cm^2, C4 = 3.438 ng/cm^2) and control (evaporated ethanol). The values represent the estimated marginal means of the statistical models on egg gathering, egg grooming, delay of maternal return, self-grooming and allocation self/egg grooming (see methods). Significant p-values are in bold.

			Egg gathering	Egg grooming			Delay of maternal return			Self-grooming			Allocation self/egg grooming		
			P	Est.	SE	P	Est.	SE	P	Est.	SE	P	Est.	SE	P
C1	-	C2	**0.036**	-1.01	0.33	**0.019**	0.58	0.34	0.431	0.31	0.24	0.196	-1.28	0.32	**0.001**
C1	-	C3	**0.001**	-1.23	0.32	**0.001**	0.57	0.31	0.357	0.52	0.25	**0.036**	-1.82	0.32	**<0.001**
C1	-	C4	**0.015**	-1.33	0.32	**<0.001**	0.65	0.32	0.263	0.64	0.25	**0.012**	-1.98	0.32	**<0.001**
C1	-	Control	**<0.001**	-1.27	0.32	**0.001**	2.14	0.51	**<0.001**	0.82	0.27	**0.002**	-2.10	0.32	**<0.001**
C2	-	C3	0.416	-0.22	0.22	0.859	-0.01	0.33	1.000	0.21	0.26	0.427	-0.54	0.26	0.251
C2	-	C4	1.000	-0.32	0.22	0.587	0.07	0.34	1.000	0.33	0.27	0.220	-0.70	0.26	0.062
C2	-	Control	0.050	-0.26	0.22	0.751	1.56	0.52	**0.023**	0.52	0.29	0.072	-0.82	0.27	**0.021**
C3	-	C4	0.666	-0.10	0.21	0.990	0.08	0.31	0.999	0.12	0.28	0.657	-0.16	0.26	0.970
C3	-	Control	0.489	-0.04	0.21	1.000	1.57	0.50	**0.015**	0.31	0.29	0.299	-0.28	0.27	0.829
C4	-	Control	0.109	0.05	0.20	0.999	1.49	0.51	**0.029**	0.18	0.30	0.547	-0.12	0.27	0.992

DISCUSSION

Whereas exposure to sublethal doses of pesticides is well known to shape a broad diversity of insect behaviors (Haynes, 1988; Mazzi & Dorn, 2012; Müller, 2018; Parkinson *et al.*, 2020), their effects on maternal egg care remained surprisingly unknown. In this study, we addressed this gap in knowledge by testing whether exposures to different concentrations of deltamethrin (a commonly used pesticide in agriculture and public health fields (Davies *et al.*, 2012; Liao *et al.*, 2018; Li *et al.*, 2019) altered the expression of maternal egg care in the European earwig. Our results first revealed that sublethal exposure to deltamethrin indeed impaired earwig maternal egg care by reducing their propensity to gather eggs, shortening the time spent in egg grooming and prolonging the time spent away from the clutch after a predator attack. These effects were unlikely to result from a general lower activity of deltamethrin exposed mothers, as we also found that this exposure increased the time spend by mothers in self-grooming, while it had no effects on egg displacement, egg antennation, females exploration and inactivity, as well as on the number of pokes received before escaping from a simulated predator attack. Interestingly, deltamethrin exposure modified females allocation between self- and egg-grooming: mothers exposed to the control solution exhibited a higher allocation of time in egg- compared to self-grooming, whereas exposure to the two smallest concentrations induced mothers to equally share their time between egg- and self-grooming, and exposure to the two highest concentrations of deltamethrin induced mothers to favor self- over egg-grooming. Finally, our results confirm that the low concentrations of deltamethrin used in our study did not trigger a higher mortality in the exposed mothers (and are thus sublethal doses), as well as show that it did not impair their reproductive outcomes in terms of egg hatching date, egg hatching rate and juveniles' weight at hatching. All these findings were independent of the population of origin of the tested females.

It has been recently proposed that studying sublethal effects of pesticides on maternal care could be of central importance to improve our general use and regulation of pesticides (Cummings *et al.*, 2010; Keller *et al.*, 2019; Fong-Mcmaster *et al.*, 2020). To date, however, only few studies in rodents did investigate the occurrence and nature of these sublethal effects (Cummings *et al.*, 2010; Keller *et al.*, 2019; Fong-Mcmaster *et al.*, 2020). Our results in earwigs thus confirm that sublethal effects of pesticides can impair the expression of maternal care in a broader number of animal species and, importantly, reveal for the first time that these effects can apply both before juveniles' emergence (i.e. toward eggs) and in non-mammalian species. Given that maternal egg care is taxonomically widespread in insects (Wong *et al.*, 2013; Klug & Bonsall, 2014; Machado & Trumbo, 2018), our results emphasize that this currently neglected sublethal effects could have a broad impact on the target and non-target insects present in crops, and therefore call for a better integration of this measurement in future assays on sublethal effects.

The effects of sublethal exposures to pesticides on insect behaviors can take multiple forms ranging from, for instance, increased locomotor activity in the predatory beetle *Platynus assimilis* (Tooming *et al.*, 2014), over altered calling behaviors in tortricid moth pests (Navarro-Roldán *et al.*, 2017), to impaired mating behaviors in the predatory stink bug *Podisus nigrispinus* (Oliveira *et al.*, 2012). The sublethal effects of deltamethrin on insect behaviors have been well studied in honeybees, where deltamethrin exposure typically impairs orientation and memory, increases stimulus sensitivity and general excitement, and triggers disordered movements (Thompson, 2003; Decourtye *et al.*, 2004; Ramirez-Romero *et al.*, 2005; Zhang *et al.*, 2020). The sublethal effects reported on earwig behaviors only partly support these modes of action. On one hand, disordered movements, overexcitement and impaired orientation may explain why earwig females exposed to deltamethrin were less likely to gather their eggs and then spent less time in egg grooming. On the other hand, these modes of action fail to explain why deltamethrin exposure increased the expression of self-grooming and did not affect the level of reaction to a stimulus (poking number in egg defense) and the time spent in exploration and inactivity. This apparent discrepancy may suggest specificity in the mode of action of deltamethrin on maternal care and/or earwig behaviors. In *Spodoptera littoralis,* it has been shown that deltamethrin can disrupt peripheral olfactory system and sexual behaviors (Lalouette *et al.*, 2016). It might thus be interesting to study whether deltamethrin affects the receptivity of earwig mothers to recognition signals emitted by the eggs, even if we do not observe any hormetic effect on their maternal behaviors (contrary to the study on *S. podoptera littoralis*).

Studying sublethal effects of pesticides on maternal care may provide novel tools to unravel the neurobiological, physiological and/or molecular pathways regulating maternal behaviors in animals (Keller *et al.*, 2019). This could be particularly decisive in insects, where these pathways remain poorly understood (Trumbo, 2019; Bridges, 2020; Körner *et al.*, 2020; Wu *et al.*, 2020). The principal molecular mode of action of deltamethrin in insects is the prolonged opening of the sodium channel, which results in membrane depolarization of neurons, repetitive discharges and synaptic disturbances leading to hyperexcitation of the nervous system (Narahashi *et al.*, 1992). How these effects can modulate specific behaviors is still unclear. A recent study suggests that the impaired honeybee waggle dance observed after exposure to deltamethrin is associated with a deregulation of the signaling pathway of neurotransmitter dopamine, which in turn impairs learning and memory (Zhang *et al.*, 2020). Further studies should test whether deltamethrin can alter maternal care behaviors by disrupting neurotransmitter systems and more generally, decipher which molecular mode of action could lead to this behavioral effect.

Although maternal egg care is considered essential to ensure egg development and hatching in the European earwig (Buxton & Madge, 1974; Boos *et al.*, 2014; Van Meyel *et al.*, 2019), our results surprisingly showed that the sublethal effects of deltamethrin on egg care did not translate into a delayed hatching date, a lower hatching rate nor into the production of lighter larvae at hatching. These findings first suggest that earwig mothers did not transfer the deltamethrin molecules present on their body to their eggs during egg care and/or that the quantities of transferred molecules are not toxic after the eggs have reached half of their development time. The first hypothesis is unlikely to explain our results, as frequent and tight contacts between individuals often facilitate the horizontal transfer of pesticides from exposed to non-exposed insects, a process reported in cockroaches, termites and ants (Rust & Saran, 2006; Choe & Rust, 2008; Buczkowski & Schal, 2009). On the other hand, earwig mothers regularly clean their eggs during the period of egg care (Boos *et al.*, 2014), a behavior that might help them removing the few molecules possibly transferred on the egg shell. Determining whether this horizontal transfer is possible with deltamethrin and occurs during egg care will require future works and measurements of deltamethrin on the eggs.

The absence of effects of maternal exposure to deltamethrin on the egg fate may also suggest that the observed sublethal effects on maternal care are transient and return to a normal level a few days after maternal exposure (a behavior that was not recorded in the present experiment) and/or that impaired maternal care occurring after the eggs have reached half of their development time is not detrimental in absence of natural constraints such as pathogens and predators. In insects, the duration of sublethal effects of pesticides on insect behaviors are difficult to predict, as they strongly depend on the type and dose of pesticide, mode of pesticide exposure, species, and measured behavior (Haynes, 1988; Mazzi & Dorn, 2012; Müller, 2018; Parkinson *et al.*, 2020). Our findings therefore pave the way for follow-up studies disentangling among the formulated hypotheses and investigating the long-lasting effects of deltamethrin on the physiology and behaviors of earwig mothers, as well as the short- and long-term effects of deltamethrin exposure on the impact of impaired egg care under natural conditions. These studies should also investigate the effects of (more natural) repeated maternal exposures to deltamethrin, as earwig females are likely to repeatedly encounter deltamethrin when exploring treated areas (deltamethrin residues can be found on plants and soil several weeks after application; Dietz et al., 2009; Mukherjee et al., 2015).

On a more general level, our results highlight the benefits and novel opportunities provided by the use of non-model organisms (such as the European earwig) to study the impacts of pesticides and reveal novel markers of fitness reduction. Developing a comprehensive understanding of the environmental impacts of pesticides typically requires us to consider species' idiosyncrasies, the diversity of fitness-relevant parameters, and the multiple routes of contamination. These three factors, however, cannot be necessarily taken into account when focusing on a small number of model organisms. For instance, the effects of pyrethroids have been recently investigated in insect aquatic models (Gutiérrez *et al.*, 2017), because this ecosystem is the receptacle of numerous routes of contaminations. Beyond tests of toxicity, assays on sublethal effects on specific behaviors such as swimming and position within the water body column have been developed, as they are relevant with regard to the consequences of these behaviors in terms of food seeking and predator avoidance (Gutiérrez *et al.*, 2017). Concerning terrestrial insect species, pollinating bees are the model organisms that have attracted most attention in the literature, particularly regarding the sublethal effects of deltamethrin on insect behaviors (Thompson, 2003; Decourtye *et al.*, 2005; Ramirez-Romero *et al.*, 2005; Desneux *et al.*, 2007; Lalouette *et al.*, 2016). However, the sublethal effects of deltamethrin on maternal egg care remained unexplored in these studies (possibly due to difficulties in its measurements), overall fostering the neglect of this key behavior (Wong *et al.*, 2013; Klug & Bonsall, 2014; Machado & Trumbo, 2018) in the insect literature.

To conclude, our results demonstrate that sublethal exposure to deltamethrin, a pesticide commonly applied in a broad diversity of crops worldwide, alters the expression of maternal egg care in the European earwig, a pest control insect present on almost every continent (Wirth *et al.*, 1998; Quarrell *et al.*, 2018; Tourneur & Meunier, 2020). These findings provide important insights in our general understanding of the diversity of sublethal effects possibly occurring among insects and highlight the benefits and novel opportunities provided by the use of non-model organisms to study these effects. They also shed light on novel potential markers of fitness reduction associated with pesticide exposure, and thus open new research avenues to allow safe and effective use of these compounds in crops and orchards.

DISCUSSION GÉNÉRALE

« La science n’est jamais qu’une succession de questions conduisant à d’autres questions. »

Terry Pratchett

Comprendre l'évolution de la vie de famille est depuis longtemps un défi en biologie évolutive et de nombreuses études se sont penchées sur les mécanismes ayant favorisé l'apparition des soins parentaux jusqu'alors considérés comme le pilier de la vie de famille(Clutton-Brock, 1991; Stearns, 1992; Schrader *et al.*, 2015; Duarte *et al.*, 2016; Jarrett *et al.*, 2017). Au cours de cette thèse, nous avons étudié l'évolution de la vie de famille à un stade ancestral en explorant les interactions mère-descendants et les facteurs écologiques qui ont pu ou pourront moduler ces interactions chez une espèce précociale : le forficule européen. Après une présentation succincte des principaux résultats obtenus durant cette thèse, je discute en détails certains de leurs éléments qui me semblent pertinents pour améliorer notre compréhension de l'évolution de la vie de famille chez cette espèce et plus généralement dans la nature.

Le premier objectif de ce travail de thèse était d'étudier l'interaction mère-descendants chez une espèce où la vie de famille est facultative : le forficule européen. Le but était de mieux comprendre l'émergence de la vie de famille chez cette espèce. Ainsi, dans les deux premiers chapitres, nous nous sommes intéressés à l'expression des soins maternels. Nous avons voulu savoir, d'une part, si la discrimination de parenté (un paramètre clé dans les soins parentaux) était présente chez notre espèce (**Chapitre 1**) et, d'autre part, si les modalités du cannibalisme filial partiel des œufs pouvaient jouer un rôle dans l'évolution des soins maternels chez le forficule européen (**Chapitre 2**). Dans une troisième étude (**Chapitre 3**), nous nous sommes demandés si une interaction coopérative favorisant l'émergence de la vie familiale à un stade ancestral pouvait exister entre les juvéniles et leur mère. Plus particulièrement, nous avons voulu savoir si les juvéniles pouvaient venir en aide à leur mère via l'allo-coprophagie. Nos résultats ont montré que les femelles du forficule européen ne discriminent pas les œufs étrangers de leurs propres œufs à travers leur investissement maternel dans les soins (**Chapitre 1**). De plus, nous avons démontré que, chez notre espèce, le cannibalisme filial partiel des œufs n'est pas un comportement lié aux soins maternels et que différentes pressions de sélection ont pu favoriser l'expression de ce comportement (**Chapitre 2**). Enfin, nos résultats suggèrent que même si les femelles consomment les fèces de leurs juvéniles et peuvent en tirer des bénéfices, les juvéniles ne coopèrent pas de façon proactive avec leur mère via la production de fèces (**Chapitre 3**).

Le deuxième objectif de cette thèse était de savoir si des facteurs écologiques tels que les pathogènes (**Chapitre 4**), le microbiote intestinal (**Chapitre 5**) ou l'exposition à un pesticide (**Chapitre 6**) pouvaient moduler l'expression des soins maternels chez le forficule européen et pourraient donc jouer un rôle dans l'évolution des soins parentaux chez les espèces précociales. Notre revue sur l'immunité sociale (**Chapitre 4**) souligne les limites de son étude quasiment exclusive chez les espèces eusociales, ce qui nous invite à reconsidérer les pathogènes comme pouvant être une pression de sélection dans toutes formes de socialité et dans l'émergence de la vie de famille. Dans le **chapitre 5**, nos résultats montrent que l'altération du microbiote par la rifampicine (un antibiotique à spectre large) n'induit pas de modification dans l'expression des soins maternels. Enfin, nous avons démontré qu'une exposition à une dose sublétale de deltaméthrine (un pesticide pyréthrinoïde) altère l'expression des soins maternels envers les œufs chez les femelles forficule sans pour autant perturber leur activité générale (**Chapitre 6**).

I- Emergence des soins aux œufs chez le forficule européen

1- Mécanistique des soins maternels envers les œufs

Les soins parentaux sont généralement coûteux pour les parents (hausse du risque de prédation, perte d'énergie, etc.) (Trivers, 1972; Alonso-Alvarez & Velando, 2012). La théorie d'Hamilton sur la sélection de parentèle (1964) prédit que ces coûts peuvent favoriser la discrimination de parentèle et ainsi contraindre les parents à rejeter les œufs étrangers (en les éliminant et/ou en réduisant la quantité de soins) afin que les soins ne s'adressent qu'à leurs propres descendants (Hamilton, 1964). Contrairement à cette prédiction, les résultats du **chapitre 1** ont démontré que les femelles ne rejettent pas les œufs d'une autre femelle et, qu'en conséquence, les femelles en présence de leurs œufs et les femelles en présence d'œufs étrangers investissent de la même manière dans les soins maternels. Pour expliquer ces résultats, une hypothèse serait que les femelles sont dans la capacité de reconnaitre leurs œufs à l'aide de composés chimiques mais que différentes pressions de sélection ont pu favoriser l'acceptation de ces œufs (Penn & Frommen, 2010).

Le fait que les femelles soient dans la capacité de reconnaitre leurs propres œufs est une hypothèse plausible chez notre espèce. En effet, les juvéniles forficules sont capables de reconnaitre leurs frères et sœurs de juvéniles étrangers (Kölliker & Vancassel, 2007; Dobler & Kölliker, 2010; Falk *et al.*, 2014). Cela suggère que les récepteurs permettant la reconnaissance des apparentés sont présents chez le forficule européen dès l'éclosion et donc que les femelles adultes seraient dans la capacité physiologique de reconnaitre leurs œufs si ces derniers possédaient une signature chimique propre. En ce qui concerne ce point, les femelles forficules nettoient régulièrement la surface de leurs œufs pour les protéger du développement de champignon (Boos *et al.*, 2014). De plus, il a été montré dans cette même étude, qu'en plus de nettoyer les œufs, les femelles déposent des hydrocarbures sur la surface des œufs (Boos *et al.*, 2014). Parallèlement, des études réalisées chez la punaise à dentelle d'aubergine *Gargaphia solani* suggèrent que, chez cette espèce, des composés chimiques sont déposés sur les œufs et permettraient la discrimination de parenté par les femelles (Monaco *et al.*, 1998; López-Sepulcre & Kokko, 2002). De futures études pourraient donc explorer le profil chimique des œufs de différentes femelles chez le forficule européen afin de déterminer si une signature chimique maternelle existe à la surface des œufs. La chromatographie en phase gazeuse couplée à une spectrométrie de masse pourrait être utilisée, cette technique d'analyse étant couramment effectuée pour déterminer le profil chimique cuticulaire et la signature coloniale chez les fourmis et les termites par exemple (Haverty *et al.*, 1988; Liu *et al.*, 2001; Lucas *et al.*, 2004). Dans de futures études, il serait cependant intéressant de savoir si les profils chimiques des hydrocarbures déposés sur les œufs sont seulement spécifiques à l'espèce (Boos *et al.*, 2015) ou s'il y a aussi une signature individuelle permettant la reconnaissance de la parentèle envers les œufs.

Même si les femelles sont dans la capacité de reconnaître les œufs qui ne sont pas les leurs, il semblerait que, chez notre espèce, différentes pressions de sélection aient pu favoriser l'acceptation des œufs étrangers par les femelles. Nous proposons que la sélection de parentèle agit ici comme pression de sélection. En effet, le forficule européen a une faible capacité de dispersion, ce qui sous-entend que les femelles proches géographiquement sont susceptibles d'être apparentées génétiquement (Moerkens *et al.*, 2010). Selon la même théorie d'Hamilton sur la sélection de parentèle, le fait de s'occuper d'œufs étrangers mais apparentés pourrait être un avantage pour la fitness inclusive des femelles et favoriserait le fait que les femelles acceptent de s'occuper d'œufs d'autres femelles (Hamilton, 1964). Cette hypothèse semble cohérente avec l'écologie de notre espèce ainsi qu'avec d'autres études réalisées chez plusieurs espèces d'insectes (voir la revue de Tallamy 2005). Dans ces études, les femelles acceptent et s'occupent des œufs de leurs congénères. De plus, la sélection de la parentèle aurait favorisé le comportement de parasitisme de ponte (« egg dumping » en anglais) où les femelles vont préférentiellement parasité les couvée de femelles apparentées génétiquement (Monaco *et al.*, 1998; Loeb *et al.*, 2000; Tallamy, 2005). Pour explorer davantage cette hypothèse, une étude de la structure génétique des populations du forficule pourrait être réalisée afin de savoir si les femelles dont les nids sont proches géographiquement (ce qui favoriserait le parasitisme de ponte) sont aussi proche génétiquement (ce qui favoriserait l'acceptation d'œufs apparentés via la sélection de parentèle).

Enfin, les études réalisées sur le parasitisme de ponte chez les différentes espèces de punaise et de membracinae (Tallamy, 2005) soulèvent le fait que l'acceptation d'œufs étrangers pendant les soins maternels pourrait être favorisée lorsque les coûts des soins aux œufs sont relativement faibles. En effet, chez les différentes espèces d'insectes où le parasitisme de ponte est présent, le seul comportement de soin exprimé par les femelles est la défense des œufs face aux prédateurs. Or, l'expression de ce soin n'est pas plus couteuse si la femelle reçoit des œufs d'autres femelles en plus des siens (Hardin & Tallamy, 1992). Contrairement à ces espèces, chez le forficule européen, en plus de la défense des œufs, les femelles déplacent les œufs dans le nid en fonction de la température et de l'humidité et les nettoient en déposant par la même occasion des composés antifongiques (Lamb, 1976; Boos *et al.*, 2014). Dans l'ensemble, ces éléments soulèvent la question des coûts réels de l'expression des soins aux œufs chez notre modèle d'étude.

Les soins envers les œufs sont-ils réellement couteux pour les femelles ?

Dans la majorité des travaux menés sur l'évolution des soins parentaux, ces soins sont considérés comme couteux pour les parents (Clutton-Brock, 1991; Royle *et al.*, 2012). Ces coûts peuvent être issus de contraintes écologiques comme un risque accru à la prédation (Schradin & Anzenberger, 2001; Cox & Calsbeek, 2010) ou au parasitisme (Nordling *et al.*, 1998), ou de contraintes physiologique liées au compromis d'allocation des ressources entre les soins parentaux et d'autres fonctions métaboliques comme l'immunité (Pike *et al.*, 2007; Tyndale *et al.*, 2008). Dans la majorité des cas, ces coûts altèrent la survie des parents et/ou leur investissement dans une future reproduction (Alonso-Alvarez & Velando, 2012). Chez le forficule, il est attendu que les soins maternels envers les œufs soient particulièrement couteux pour les femelles étant donné que ces soins sont relativement longs (2 mois) et qu'ils ont lieu pendant l'hiver, période durant laquelle les femelles arrêtent de se nourrir (Kölliker, 2007). Cependant les résultats obtenus dans le **chapitre 1** de cette thèse nous suggèrent que, chez notre espèce, les soins aux œufs pourraient être peu voire non couteux pour les femelles contrairement à ce qui est prédit par la littérature. En effet, nos résultats ne montrent pas de coûts physiologique liés aux soins aux œufs (perte de poids et immunité basale) et le fait que les femelles acceptent les œufs étrangers renforce cette hypothèse. Ces résultats ouvrent la porte à de futures études qui pourraient étudier les coûts réels des soins aux œufs chez le forficule européen en augmentant de façon artificielle la taille des pontes des femelles, comme cela a été réalisé chez de nombreuses espèces d'oiseaux (Reid, 1987; Sanz, 1997; De Heij *et al.*, 2006), et en mesurant les coûts physiologiques et non-physiologiques pour les femelles. S'il s'avérait que les soins aux œufs sont peu couteux, il serait intéressant d'étudier les mécanismes qui permettent aux femelles de réduire les coûts des soins maternels.

Dans la littérature, un des mécanismes possibles permettant de réduire les coûts liés aux soins maternels est la baisse de l'activité physiologique pendant la durée des soins. Ce mode d'économie d'énergie peut notamment être observé chez les espèces qui arrêtent de se nourrir pendant la période de soins maternels. Chez l'araignée loup *Pardosa saltans*, les mères arrêtent leur activité de chasse pendant les soins envers leur cocon (Ruhland, 2016). Pendant cette période, une baisse de leur activité locomotrice peut aussi être observé, ce qui leur permet d'économiser de l'énergie en stockant les lipides qui seront alors utilisé pour la période de soins (Ruhland, 2016; Ruhland *et al.*, 2016). Cette hypothèse est probable chez le forficule européen, car pendant les soins aux œufs, les femelles restent dans le nid et ont ainsi peu d'activité locomotrice. Il serait cependant intéressant d'étudier d'un point de vue physiologique : comment les femelles économisent-elles leur énergie et comment limitent-elles les coûts des soins maternels. Une façon de procéder pourrait être de doser les lipides stockés dans le corps gras chez les femelles avant la ponte des œufs, puis pendant la période de soins à différentes étapes du développement des œufs en fonction du nombre d'œufs (Benowitz *et al.*, 2017).

Comment les soins maternels sont-ils déterminés chez le forficule européen et plus généralement chez les insectes ?

L'expression des soins maternels chez les animaux est assez bien documentée, en particulier chez les vertébrés (Lévy *et al.*, 2004; Rilling & Young, 2014; Angelier *et al.*, 2016). Chez les mammifères, deux mécanismes sont en synergie dans l'apparition et le maintien des soins maternels. D'une part, les signaux envoyés par les jeunes (olfactifs, sonores, tactiles) stimulent l'expression des soins maternels (Corona & Lévy, 2015) et, d'autre part, l'action d'hormones (plus particulièrement l'œstradiol et l'ocytocine) libérées lors de l'expulsion du fœtus déclenchent l'expression du comportement maternel (Kendrick & Keverne, 1991; Poindron *et al.*, 2007). A l'inverse, les mécanismes comportementaux physiologiques et neurobiologiques dans l'expression des comportements maternels chez les arthropodes semblent beaucoup moins connus (Trumbo, 2019; Bridges, 2020; Yang *et al.*, 2020). Cependant, quelques études donnent des pistes de réflexion. En effet, il semblerait que des mécanismes similaires à ceux opérant chez les vertébrés pourraient être présents. Chez l'araignée loup *Pardosa saltans*, l'expression des soins maternels implique des signaux émis par les juvéniles et une réponse physiologique hormonale de la part de la femelle (Ruhland, 2016; Ruhland *et al.*, 2019). Chez les insectes, la revue de Mas et Kölliker (2008) suggère aussi la présence de signaux chimiques produits par les descendants afin de solliciter l'expression des soins maternel, ainsi que l'implication des hormones juvéniles (Trumbo, 2002; Mas & Kölliker, 2008). Chez le forficule européen, on sait déjà que la présence des jeunes et leur état nutritionnel induisent l'expression de soins maternels via une communication chimique (Mas *et al.*, 2009). De plus, il semblerait que les hormones juvéniles (JH) jouent bien un rôle dans l'expression des soins (Rankin *et al.*, 1997). Il serait cependant intéressant d'étudier si les mêmes mécanismes opèrent lors des soins aux œufs et en particulier si les œufs communiquent avec leur mère via des molécules chimiques. Les résultats obtenus dans le **chapitre 6** de cette thèse suggèrent l'importance de l'olfaction pendant les soins aux œufs chez les femelles forficules. En effet, nos résultats montrent que l'exposition à une dose sublétale de deltaméthrine perturbe l'expression de deux formes de soins maternels sans pour autant affecter l'activité générale de la femelle (auto-nettoyage, activité locomotrice). Or, il a été démontré que la deltaméthrine pouvait, par son mode d'action, agir sur le canal sodique et sur l'homéostasie des ions calcium au sein des cellules, perturbant ainsi l'olfaction des insectes comme l'abeille *Apis mellifera* (Decourtye *et al.*, 2005). Nos résultats suggèrent donc qu'une dose sublétale de deltaméthrine pourrait interférer dans la communication chimique entre les œufs et la femelle ce qui induirait une perturbation dans l'expression des soins maternels. Cependant, nous n'avons pas exploré en détail l'impact de la deltaméthrine sur les systèmes de neurotransmission des signaux olfactifs chez notre espèce et des études complémentaires sont nécessaire afin de mieux comprendre les mécanismes neurobiologiques de l'expression des soins maternels envers les œufs chez le forficule européen.

En dehors de l'aspect mécanistique des soins maternels envers les œufs, des stratégies comportementales ont aussi pu être sélectionnées pour limiter les coûts liés aux soins aux œufs. Une de ces stratégies est le cannibalisme filial que nous avons étudié dans le **chapitre 2** de cette thèse.

2-Le rôle du cannibalisme filial partiel des œufs pendant les soins maternels

Bénéfices du cannibalisme filial partiel des œufs pour les femelles

Le cannibalisme filial pendant les soins parentaux n'est pas un comportement rare dans la nature (Polis, 1981; Elgar & Crespi, 1992). Il se retrouve chez les mammifères (Klemme *et al.*, 2006), les oiseaux (Gilbert *et al.*, 2005), les arthropodes (Anthony, 2003; Miller & Zink, 2012) et les poissons (Manica, 2002; Thomas & Manica, 2003). L'expression du cannibalisme filial d'une partie des œufs est généralement adaptative et l'hypothèse qui a reçu le plus d'intérêt est que le cannibalisme filial d'une partie des œufs permet une réallocation des ressources énergétiques. En consommant une partie de leurs œufs, les parents récupèrent un apport énergétique qui est à nouveau alloué aux soins envers les œufs restant ou aux futurs descendant (Sargent, 1992; Manica, 2002; Rohwer, 2002). Ainsi, l'expression du cannibalisme filial partiel des œufs permettrait aux parents en mauvaise condition physiologique de limiter les coûts liés aux soins parentaux (Hoelzer, 1992;

Marconato *et al.*, 1993; Kraak, 1996; Kvarnemo *et al.*, 1998; Manica, 2004). Chez le sergent-major à queue en ciseaux *Abudefduf sexfasciatus* et le demoiselle de Cortez *Stegastes rectifraenum*, il a en effet été démontré que les mâles adaptent leur taux de cannibalism en fonction de la disponibilité en nourriture (Hoelzer, 1992; Manica, 2004). Cette hypothèse pourrait être d'autant plus probable chez les espèces ayant une durée de vie longue, pouvant produire plusieurs pontes, et dont l'accès à la nourriture est limité. Ce qui est le cas du forficule européen (Lamb, 1976; Kölliker, 2007; Tourneur & Meunier, 2020). Les résultats obtenus dans le **chapitre 2** favorisent en partie cette hypothèse. En effet notre étude montre que les femelles considérées comme cannibales ont eu un meilleur investissement dans leur seconde ponte, et donc ont été plus susceptibles de produire une deuxième couvée avec un plus grand nombre d'œufs. Cependant, il est intéressant de noter que, contrairement aux prédictions exprimées dans la littérature, le taux de cannibalisme chez les femelles forficule n'est ni lié à leur investissement dans les soins aux œufs ni à leur condition physiologique (poids avant la ponte). Cela suggère que d'autre pressions de sélection ont pu agir sur l'expression du cannibalisme filial pendant les soins maternels chez le forficule européen. Il serait donc intéressant de tester d'autres hypothèses pour expliquer le maintien de ce comportement chez notre espèce.

Parmi les facteurs pouvant moduler l'expression du cannibalisme filial partiel des œufs chez le forficule européen, la présence de pathogènes et les conditions environnementales pourraient être des facteurs particulièrement intéressants à étudier. Concernant la présence de pathogène dans l'expression du cannibalisme envers les œufs, il a en effet été montré chez de nombreuses espèces de poissons, par exemple le poisson dard *Etheostoma squamiceps* et le gobie tacheté *Pomatoschistus microps*, que les mâles consomment préférentiellement les œufs infectés par des microorganismes pathogènes présents dans l'eau (Lehtonen & Kvarnemo, 2015; Bandoli, 2016; Vallon *et al.*, 2016). Cette stratégie permet notamment aux mâles de limiter l'infection à seulement quelques œufs et d'éviter que toute la ponte soit infectée (Lehtonen & Kvarnemo, 2015; Bandoli, 2016). Cette explication pourrait être intéressante à étudier chez notre espèce. En effet, nous savons que chez le forficule, les femelles évitent le développement de champignon pathogènes en nettoyant les œufs (Boos *et al.*, 2014). Si quelques œufs sont déjà infestés, il se pourrait donc que les femelles les mangent préférentiellement pour éviter une contamination des autres œufs. Enfin, il serait aussi pertinent d'étudier l'impact des conditions environnementales, comme le climat, sur l'expression du cannibalisme chez le forficule. En effet, une étude récente chez les forficules du Canada suggère que le taux de cannibalisme filial partiel des œufs par les femelles serait plus important lorsque les automnes sont chauds, ce qui implique une éclosion des œufs au milieu de l'hiver alors que les ressources alimentaires sont limitées (Tourneur & Meunier, 2020). Etudier le taux de cannibalisme en fonction des variations de température et d'humidité pourrait donc être une piste intéressante chez notre modèle.

Possible rôle du cannibalisme filial partiel des œufs dans l'évolution des soins maternels

Outre nos résultats sur le mécanisme du cannibalisme filial partiel des œufs et les pressions de sélection qui auraient pu le favoriser chez le forficule européen, le fait que le cannibalisme filial partiel des œufs apporte des bénéfices directs aux femelles soulève l'idée que ce comportement aurait pu favoriser la présence des femelles auprès de leurs œufs, leur donnant un certain avantage dans leur reproduction future. Ces bénéfices auraient ainsi, très bien pu initier l'émergence des soins maternels chez le forficule européen indépendamment du fait d'augmenter la fitness des femelles via les soins aux œufs. Ce scénario évolutif est particulièrement probable chez le forficule européen, due au fait que les femelles peuvent produire plusieurs couvées et que les soins aux œufs ont lieu pendant l'hiver, période pendant laquelle la disponibilité en nourriture dans l'environnement est relativement faible (Lamb, 1976; Kölliker, 2007; Tourneur & Meunier, 2020). Cependant, il est important de noter que ce scénario évolutif n'est valable que si les femelles consomment les œufs en plusieurs fois et de façon régulière pendant le développement des œufs. En effet, si les femelles consomment une partie des œufs à un seul moment, par exemple juste après la ponte, l'accès au cannibalisme n'aurait pas favorisé la persistance des femelles auprès de leurs œufs. Je propose donc d'étudier l'expression du comportement de cannibalisme des œufs par les femelles au cours du temps.

II- Emergence de la vie de famille chez le forficule européen

1- Evolution des soins maternels envers les juvéniles

Le microbiote intestinal peut-il jouer un rôle dans l'émergence des soins envers les juvéniles ?

Les microorganismes présents dans le microbiote intestinal sont connus pour influencer l'expression de certains comportements sociaux. Ainsi, chez les souris *Mus musculus*, les individus avec un microbiote intestinal altéré ont une sociabilité déficiente (Desbonnet *et al.*, 2014; Buffington *et al.*, 2016). De façon similaire, l'altération du microbiote intestinal chez la fourmi coupe-feuille *Acromyrmex echinator*, conduit à des niveaux plus élevés d'agressivité envers les congénères (Teseo *et al.*, 2019). Pour expliquer le lien entre le microbiote intestinal et l'expression des comportements sociaux, de récentes études ont émis l'hypothèse que les microorganismes pourraient favoriser les interactions sociales coopératives en augmentant leurs taux de transmission (Troyer, 1984; Nalepa *et al.*, 2001; Lombardo, 2008; Lewin-Epstein *et al.*, 2017). Le transfert de microorganismes intestinaux entre les membres de la colonie via les comportements sociaux est notamment bien étudié chez les insectes eusociaux comme les termites ou les abeilles (Nalepa, 1994; Powell *et al.*, 2014). Dans cette thèse, nous avons émis l'hypothèse que le microbiote intestinal pouvait aussi moduler l'expression des soins maternels chez le forficule européen et aurait ainsi favorisé l'émergence de la vie de famille chez cette espèce. Contrairement à cette prédiction, les résultats du **chapitre 5** ont démontré que l'altération du microbiote intestinal par la rifampicine ne perturbe pas l'expression des soins maternels envers les œufs et envers les juvéniles. Ces résultats suggèrent que le microbiote intestinal n'a pas de rôle dans l'expression des soins maternels chez le forficule européen. Cette hypothèse est probable car le lien entre microbiote est comportement sociaux n'est pas universel dans le règne animal. Ainsi chez plusieurs espèces de lépidoptères, il a été montré que l'altération du microbiote n'induisait aucun changement dans la survie et l'expression des comportements des larves (Ravenscraft *et al.*, 2019).

Néanmoins, dans notre étude, nous n'avons utilisé qu'un seul antibiotique, la rifampicine. Hors, même si c'est un antibiotique à spectre large qui cible une grande partie des bactéries présentes dans le microbiote intestinale des insectes (Campbell *et al.*, 2001; Chouaia *et al.*, 2012; Shan *et al.*, 2016; Rosas *et al.*, 2018), nous ne pouvons pas exclure l'hypothèse que les microorganismes qui pourraient agir sur l'expression des soins maternels ne sont pas sensibles à cet antibiotique, tels que des protistes ou des champignons, aussi présents dans le système digestif des insectes (Urubschurov & Janczyk, 2011 ; Arcila & Meunier, 2020 ; Greer et al., 2020). Cette hypothèse est probable chez le forficule, en effet, il s'avère que l'altération du microbiote intestinal à l'aide d'un cocktail d'antibiotique conduit à une altération de la socialité chez les adultes (données non publiées). Aussi d'autres études futures seront nécessaires pour confirmer qu'aucun autre membre du microbiote intestinal ne façonne les soins parentaux chez le forficule européen.

2-Interaction coopérative des juvéniles envers leur mère

La coopération entre les descendants et leur parents et une interaction qui est présente et largement étudiée chez les espèces communales et eusociales. Chez ces espèces les jeunes adultes, aident leurs parents pour diverses tâches comme la protection du territoire, la recherche de nourriture ou encore les soins aux jeunes (Hailman *et al.*, 1994; Clutton-Brock *et al.*, 1998). Dans les colonies de fourmis, les reines sont d'ailleurs presque exclusivement nourris par leur descendante ouvrière (Rubenstein & Abbot, 2017). L'étude de cette interaction coopérative des juvéniles envers leur mère chez le forficule européen effectuée dans le **chapitre 3** révèle cependant, que les juvéniles ne coopèrent pas de façon pro-active avec leurs mères. En effet, nos résultats montrent que les juvéniles n'adaptent pas leurs production de fèces pour venir en aide à leurs mère lorsque celle-ci est en manque de nourriture alors qu'ils effectuent exactement ce même type de coopération avec leurs frères et sœurs (Körner *et al.*, 2016). Nos résultats pourraient donc suggérer que les pressions de sélections qui ont favorisé la coopération des juvéniles envers leurs parents n'opèrent que chez les formes de vie sociales plus complexes. Il serait donc intéressant d'explorer d'avantage l'interaction coopérative entre les juvéniles et leurs parents dans les différentes formes de vie sociale pour savoir quels sont les mécanismes évolutifs qui les ont favorisés et quel est le rôle de cette interaction dans l'évolution de la vie de famille.

Outre le fait que les juvéniles ne coopèrent pas de façon pro-active avec leur mère chez le forficule européen, nos résultats montrent tout de même que les femelles ont des bénéfices directs à rester avec leurs descendants via la consommation de leurs fèces. Nous allons donc maintenant discuter plus en détails des fonctions que pourrait avoir l'allo-coprophagie chez notre espèce.

Les bénéfices potentiels liés à l'allo-coprophagie

L'allo-coprophagie n'est pas un comportement rare dans le règne animal. On peut notamment le retrouver chez les mammifères, les oiseaux et les insectes (Crowell-Davus & Houpt, 1985; Watarai *et al.*, 2018; Kobayashi *et al.*, 2019). Chez ces espèces, l'allo-coprophagie peut avoir différentes fonctions adaptatives. En effet chez les jeunes rats *Rattus rattus* comme chez la blatte germanique *Blattella germanica* la consommation des fèces sert au transfert de nutriments (Galef, 1979; Kopanic *et al.*, 2001). Chez les lagopèdes alpins *Lagopus muta japonica*, comme chez de nombreuses espèces de termites, l'allo-coprophagie permet le transfert de bactérie essentielles pour la digestion (Mirabito & Rosengaus, 2016; Kobayashi *et al.*, 2019). Enfin, l'allo-coprophagie peut servir au transfert d'hormones comme chez les rats taupe nu *Heterocephalus glaber* (Watarai *et al.*, 2018) ou de composés immunitaires comme chez le cheval *Equus caballus* (Crowell-Davus & Houpt, 1985). Les résultats obtenus dans le **chapitre 3,** ainsi que dans une précédente étude réalisée aussi chez le forficule européen (Körner *et al.*, 2016), démontrent que les juvéniles et les mères consomment des fèces produites par les membres de la famille. Les juvéniles consomment des fèces produites par leurs frères et sœur et par leurs mères (Körner *et al.*, 2016) et les mères consomment les fèces produites par leurs juvéniles.

Une première hypothèse est que, comme chez les blattes germaniques (Kopanic *et al.*, 2001), l'allo-coprophagie chez le forficule européen pourrait servir au transfert de nutriment et permettre ainsi au coprophage de résister au manque de nourriture chez le forficule. En effet, nos résultats montrent que les femelles mangent plus de fèces quand elles ont été privé de nourriture et qu'elles ont une préférence pour les fèces issues des juvéniles nourris (c-à-d de meilleure qualité). Une future étude pourrait consister à tester les quantités de nutriments encore présents dans les fèces après la digestion.

En plus, des bénéfices liés à l'accès aux nutriments, il semble que l'allo-coprophagie chez le forficule ait aussi une autre fonction. En effet, nous avons également montré dans le **chapitre 3** que même si les femelles étaient suffisamment nourris, elles consommaient tout de même les fèces de leurs juvéniles. De la même manière, les juvéniles consomment les fèces de leurs congénères même si ils ont accès à d'autres formes de nourriture (Körner *et al.*, 2016).

La seconde fonction de l'allo-coprophagie chez notre modèle est que, comme chez le cheval, ce comportement servirait au transfert de molécules impliquées dans l'immunité (Crowell-Davus & Houpt, 1985). Cette hypothèse est probable chez le forficule étant donné que de récentes études ont démontrés que les fèces produites par les juvéniles ont des propriétés antimicrobiennes (Diehl *et al.*, 2015) . Cependant, ce dernier aspect nécessite d'être testé dans de futures études. Pour cela, je propose d'effectuer une expériences dans laquelle des femelles auraient accès ou non aux fèces de leurs juvéniles puis des mesures d'immunité basale et de résistance aux pathogènes pourraient être effectuées sur ces deux types de femelles.

En dehors des bénéfices directs de l'allo-coprophagie pour les femelles forficule, il semblerait aussi que les mères pourrait aussi profiter de comportements liés à l'immunité sociale.

Les bénéfices potentiels liés à l'immunité sociale

La présence d'agents pathogènes dans l'environnement est connue pour jouer un rôle clé dans l'expression des soins parentaux. En effet, les parents limitent souvent le risque d'infection de leurs descendants via le transfert d'agents antimicrobiens par l'alimentation, le nettoyage ou par l'utilisation de matériaux antimicrobiens comme les plantes aromatiques ou la résine (Mennerat *et al.*, 2009). Chez le forficule européen, des études récentes montrent, qu'en présence de pathogènes, les femelles adaptent l'expression de leurs soins envers leurs œufs, ce qui les protègent de l'infection par un champignon entomopathogène ou des moisissures (Boos *et al.*, 2014; Diehl & Meunier, 2018). En plus de ce rôle sur les soins parentaux, la présence de pathogènes peut aussi moduler l'expression d'autres comportements liés à l'immunité sociale au sein de la vie de famille. Par exemple chez les fourmis et les abeilles, les ouvrières pratiquent entre elles le nettoyage réciproque (« allo-grooming » en anglais) (Cremer *et al.*, 2007), ces comportements participent au mécanisme d'immunité sociale largement décrit chez les espèces eusociales. Chez notre modèle, ce genre de comportements entre descendants s'observe aussi. En effet, les juvéniles produisent des fèces ayant des capacités antimicrobiennes, ce qui protège ainsi le nid d'infection par des agents pathogènes et des champignons (Diehl *et al.*, 2015). De ce point de vue, les mères présentes dans le nid pourraient avoir des bénéfices directes à rester avec leurs descendants, notamment dans la lutte contre les pathogènes.

Il serait maintenant intéressant de tester si d'autres mécanismes de l'immunité sociale présents chez les espèces eusociales se retrouvent au sein de la vie de famille chez les espèces subsociales précociales. Je pense notamment au phénomène de vaccination (« immune priming » en anglais). En effet, chez les fourmis, l'organisation sociale dans la colonie et les interactions entre les individus permettent la transmission d'une petite quantité de pathogène entre les individus (Konrad *et al.*, 2012), cette dose non mortelle permet aux fourmis de se « vacciner » et d'avoir une meilleure réponse immunitaire lors d'une deuxième infection (Konrad *et al.*, 2012, 2018). Il serait donc intéressant de savoir si, chez le forficule, un système similaire de vaccination existe au sein de la famille et si les mères peuvent en bénéficier.

Dans notre revue de la littérature (**chapitre 4**) sur le rôle de l'immunité sociale dans l'évolution de la vie sociale nous y soulignons que les pathogènes ont pu agir en tant que pression de sélection dans toutes formes de socialité et qu'ils auraient bien pu favoriser l'émergence de la vie de famille de la même façon que les formes de vie sociale plus complexe (Cremer et al., 2007 ; Meunier, 2015).

IV- Conclusion

Dans son ensemble, cette thèse apporte une vision nouvelle des bénéfices et des coûts liés aux interactions entre la mère et ses descendants, ainsi que du rôle de facteurs écologiques sous-estimés dans l'évolution de la vie de famille chez un insecte précocial. Nous avons notamment mis en lumière que les bénéfices directs liés au cannibalisme filial partiel des œufs avaient pu favoriser en partie la présence des femelles auprès de leur couvée à un stade ancestral, et que l'amélioration de la survie des œufs n'était pas le seul mécanisme évolutif permettant l'émergence des soins maternels envers les œufs. De plus, à partir de nos résultats, nous proposons un nouveau scénario de l'évolution de la vie de famille basé sur le parasitisme social effectué par la mère sur ses descendants. Les femelles auraient ainsi des avantages directs à rester auprès de leurs juvéniles comme un accès à une protection contre les pathogènes ou l'accès aux bénéfices de l'allo-coprophagie. De manière générale, les études menées durant cette thèse invitent à repenser le rôle des soins parentaux dans l'évolution de la vie de famille au sein des formes ancestrales de vie sociale et apportent de nouvelles perspectives quant aux scénarios ayant favorisés l'émergence et le maintien de la vie de famille chez le forficule européen et plus généralement chez les insectes. En conclusion, il semble crucial d'ouvrir davantage la recherche sur l'évolution de la vie sociale aux espèces précociales car elles peuvent nous permettre de mieux comprendre les prémices de l'évolution de la socialité.

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« Rappelez-vous de regarder les étoiles et non pas vos pieds. »

Stephen Hawking

Sophie VAN MEYEL

ÉVOLUTION DE LA VIE DE FAMILLE CHEZ UN INSECTE PRÉCOCIAL :

Importance des interactions sociales et de l'écologie

Résumé

Alors que la vie de famille est un phénomène omniprésent dans la nature, son évolution et notamment son émergence et son maintien au cours du temps restent encore des phénomènes peu compris. Dans cette thèse, nous nous sommes intéressée à ces phénomènes en étudiant les interactions mère-descendants et les facteurs écologiques pouvant moduler le rapport coûts/bénéfices de la vie de famille chez le forficule européen. Nos résultats proposent d'abord de reconsidérer l'importance de paramètres souvent jugés centraux dans l'évolution des soins maternels, que ce soit à travers la discrimination de parentèle et le cannibalisme des œufs. Ils montrent aussi que les mères peuvent tirer des bénéfices à rester avec leurs juvéniles, même si ces derniers ne prodiguent pas activement de soins envers leurs parents. Enfin, nous avons mis en évidence l'importance d'explorer l'impact de facteurs écologiques comme les pathogènes, le microbiote intestinal et les pesticides sur les interactions familiales. Dans son ensemble, cette thèse propose une vision nouvelle de l'évolution de la vie de famille chez les insectes.

Mots-clés : Vie de famille, Soins maternels, Espèce précociale, Facteurs écologiques, Dermaptère.

Résumé en anglais

Whereas family life is a common phenomenon in nature, its evolution and especially its emergence and maintenance over time are still little understood. In this thesis, we addressed this gap in knowledge by studying mother-offspring interactions and the ecological factors possibly shaping the cost/benefit ratio of family life in the European earwig. Our results first propose to reconsider the importance of parameters often considered central in the evolution of maternal care, which are kin discrimination and egg cannibalism. They also reveal that mothers can get direct benefits from staying with their juveniles, even if the latter do not actively care for their parents. Finally, our results highlight the importance of studying the impact of ecological factors such as pathogens, gut microbiota and pesticides on family interactions. Overall, this thesis provides novel insights in our general understanding of the evolution of family life in insects.

Key-words: Family life, Maternal care, Precocial species, Ecological factors, Dermaptera.