La diversité de taille des ouvrières au sein des colonies de fourmis : intérêts du groupe ou des individus ?

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La diversité de taille des ouvrières au sein des colonies de fourmis : intérêts du groupe ou des individus ?

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Sommaire

INTRODUCTION

1. De la vie solitaire à l'eusocialité : les apports de la vie en groupe

La transition sociale

Le passage de la vie solitaire à la vie en groupe est considéré comme l'une des transitions majeures de l'évolution (Bourke, 2011; Szathmáry & Smith, 1995). L'évolution a engendré un panel de mode de vie possible : des individus purement solitaires, aux rassemblements incoordonné ou saisonnier dus à une caractéristique de l'environnement (par exemple l'agrégation durant l'hiver chez les coccinelles ; Durieux et al., 2012), aux groupements cordonnés simples tels que les phénomènes de migration chez les oiseaux (Ramenofsky & Wingfield, 2007), en passant par la vie en groupe à l'instar de nombreuses espèces de primates (Markham, Gesquiere, Alberts, & Altmann, 2015) jusqu'à des sociétés dites vraies comme les insectes sociaux (Wilson, 1971).

La transition vers la socialité réside dans les soins parentaux. Alors que le mode de vie solitaire n'implique pas ou très peu d'interactions avec les parents (souvent réduites à la surveillance des jeunes ou à l'approvisionnement en nourriture), l'extension des soins parentaux à plus large échelle temporelle et de manière plus régulière mène à la subsocialité. Cette subsocialité, qui peut être autrement assimilée à une vie familiale (Kramer & Meunier, 2019), accroit les interactions entre parents et progénitures. Chez le forficule (*Forficula auricularia*), les mères défendent et approvisionnent en nourriture leurs progénitures pendant plusieurs semaines après l'éclosion (Costa, 2006). Ce groupement d'individus entraine par conséquent des interactions entre progénitures, que ce soit de nature compétitive (cannibalisme ; Dobler & Kölliker, 2010) ou coopérative (partage de nourriture via les fèces ; Falk, Wong, Kölliker, & Meunier, 2014). D'ailleurs, les interactions sociales entre les progénitures ont potentiellement un rôle crucial dans l'évolution de la vie en groupe (Körner, Diehl, & Meunier, 2016), bien que cela ne soit que marginalement étudié pour le moment (Kramer & Meunier, 2019). La coopération entre progénitures et l'aide de celles-ci dans la vie de groupe pourraient respectivement compléter les bénéfices de la vie en groupe et contrebalancer certains couts liés aux soins parentaux (Kramer & Meunier, 2019).

Les espèces sociales se divisent en plusieurs catégories, maintes fois renommées et redéfinies (encore récemment : Dew, Tierney, & Schwarz, 2016; Richards, 2019). Les espèces communales ont des membres d'une même génération vivant ensemble. Lorsque les individus du groupe sont engagés dans des soins coopératifs aux jeunes, les espèces sont qualifiées de quasisociales ou semisociales (Crespi & Yanega, 1995; Michener, 1969). Une division du travail peut parfois avoir lieu. Enfin, le stade le plus avancé connu de vie sociale est l'eusocialité. Les membres forment un groupe permanent, caractérisé par trois composantes majeures : une division du travail reproductif, un soin coopératif aux jeunes et le chevauchement d'individus issus de la même génération. L'eusocialité peut être à nouveau découplée en deux catégories. La première est dite primitivement ou facultativement eusociale lorsqu'il n'y a pas de différences morphologiques entre les individus reproducteurs et non-reproducteurs, à l'inverse la société est qualifiée de hautement ou obligatoirement eusociale (notion de super-organisme) lorsque c'est le cas (Crespi & Yanega, 1995; Michener, 1969). Bien que les insectes représentent la majorité des espèces eusociales (termites : Thorne (1997), coléoptères : Kent & Simpson (1992), fourmis : Wilson (1971), pucerons : Itô (1989), thrips : Crespi (1992)), deux exemples sont trouvés chez les crustacées (crevettes : Duffy (1996)) et les mammifères (rats taupes nus : Jarvis (1981)).

Les différents degrés de socialité cités ici sont résumés dans la figure 1 en prenant pour exemple la classe des insectes (issue de Schultner, Oettler, & Helanterä (2017)).

Les bénéfices de la vie en groupe …

Les bénéfices de la vie en groupe peuvent être directs et/ou indirects. Dans le premier cas, ils concernent l'amélioration de la survie et de la reproduction de l'individu focal. Dans le second cas, ils sont liés à la reproduction et la survie d'individus apparentés. Ces deux types de bénéfices sont regroupés sous le terme de *fitness* inclusive ou *fitness* globale (Bourke, 2011; West, Griffin, & Gardner, 2007). Les individus tirent ainsi des bénéfices en *fitness* dans l'augmentation de leur productivité et de leur survie mais aussi des individus qui leur sont apparentés (Bourke, 2014).

De manière générale, la vie en groupe procure de nombreux bénéfices directs : une meilleure protection contre les prédateurs (Unglaub, Ruch, Herberstein, & Schneider, 2013), une plus grande efficacité dans la recherche de nourriture (fourragement social : Galef & Giraldeau (2001) - Stander, 1992; Vanthournout et al., 2016), un succès reproducteur accru et un taux de survie plus élevé (Krause & Ruxton, 2002; Silk, 2007). Pour donner des exemples d'avantages de la vie en groupe sur un taxon particulier, les araignées sociales coopèrent pour la construction d'un nid commun servant de refuge protecteur contre les prédateurs mais également à moduler les perturbations environnementales (Henschel, Ward, & Lubin, 1992). La survie des individus est liée positivement à la taille du nid et du groupe (Bilde et al., 2007). La vie de groupe chez ces araignées sociales permet également de partager l'entretien et le nettoyage des toiles tissées, mais aussi d'élever coopérativement les jeunes et de capturer des proies plus volumineuses (Yip, Powers, & Avilés, 2008). Pour citer quelques exemples plus généraux, les regroupements chez les espèces endothermes permettent de minimiser les pertes de chaleur et ainsi de réduire les dépenses énergétiques (Gilbert et al., 2010). Chez les ectothermes, ces regroupements permettent de réduire à la fois les pertes en eau (Broly, Devigne, Deneubourg, & Devigne, 2014) et les dépenses métaboliques (Tojo, Nagase, & Filippi, 2005). Les comportements d'allo-grooming chez les primates permettent d'éliminer les saletés et ectoparasites (Russell & Phelps, 2013). Chez les oiseaux, vivre avec un grand nombre de congénères facilite l'émergence et la diffusion de comportements innovants (dans la recherche alimentaire par exemple : Ashton, Thornton, & Ridley, 2019). Enfin, la vie sociale regroupe des individus différant aussi bien comportementalement, morphologiquement, physiologiquement et génétiquement et cette diversité peut apporter des bénéfices au groupe (Dornhaus, Powell, & Bengston, 2012).

La part des bénéfices directs et indirects peut fluctuer entre les espèces, notamment chez les insectes sociaux où les bénéfices indirects jouent un rôle crucial dans le fonctionnement des sociétés. La construction commune de nids élaborés apporte un premier filtre aux perturbations de l'environnement externe (Jones & Oldroyd, 2006; Theraulaz, Bonabeau, & Deneubourg, 1998). La vie en colonie, regroupant parfois des milliers d'individus, entraine un second filtre possible et une forte résilience aux contraintes extérieures (Straub, Williams, Pettis, Fries, & Neumann, 2015). Plusieurs études ont trouvé une relation positive entre la taille des colonies et la résistance à des perturbations telles qu'une exposition à des pesticides (Crall, de Bivort, Dey, & Versypt, 2019), une infection à des parasites (Scharf, Modlmeier, Beros, & Foitzik, 2012), une température stressante (Molet, Péronnet, Couette, Canovas, & Doums, 2017) ou la saisonnalité (Kaspari & Vargo, 1995). Chez les sociétés d'insectes, les bénéfices indirects jouent un rôle plus important dans le maintien de la coopération, comparé aux espèces moins sociales (figure 1). En effet, la société repose sur une division du travail, articulée autour de castes définies morphologiquement ou comportementalement, menant ainsi à un scindement entre la caste reproductive (une reine unique la plupart du temps) et la caste ouvrière qui assure le reste des tâches ergonomiques (soins aux couvains, construction du nid, recherches de nourriture, etc ; Wilson, 1971). Ainsi, généralement, la caste ouvrière ne se reproduit jamais et tire seulement des bénéfices indirects via l'unique reproductrice de la colonie. La coopération et l'altruisme des ouvrières résultent de leur lien de parenté avec leur mère, la reine, selon la théorie d'Hamilton (Hamilton, 1964). Cette théorie, aussi appelée la sélection de parentèle, indique que l'altruisme est sélectionné dans le cas où le coût du donneur (l'ouvrière) à ne pas se reproduire est inférieur aux bénéfices associés à la valeur sélective (ou *fitness*) directe du receveur (la reine) pondéré par le coefficient d'apparentement liant le donneur et le receveur. Avec la théorie d'Hamilton (1964), la valeur sélective des individus ne réside plus seulement dans les opportunités directes de reproduction (*fitness* directe), mais aussi dans les gains issus de la reproduction des individus apparentés (*fitness* indirecte).

La vie en groupe génère ainsi de nombreux avantages. En revanche, les interactions au sein du groupe peuvent être majoritairement conflictuelles (Krause & Ruxton, 2002) et les bénéfices peuvent être fortement contrebalancés par les coûts liés à cette vie sociale. Ces coûts doivent rester toutefois inférieurs aux bénéfices pour la vie en groupe persiste (Vanthournout et al., 2016).

… mais non sans coûts et conflits potentiels

Le fait de vivre en groupe peut par conséquent entrainer une compétition pour les ressources lorsque celles-ci sont limitantes (Koenig, 2002), mais aussi pour l'accès à la reproduction (Huchard & Cowlishaw, 2011; Vigilant et al., 2015). La vie en communauté et ses interactions sous-jacentes augmentent les risques d'infections par des pathogènes ou des parasites (Loehle, 1995). Pour reprendre l'exemple des araignées sociales, la vie en groupe est caractérisée par un système d'accouplement consanguin, entrainant une diversité génétique réduite, aussi bien au niveau de la population que de l'espèce (Settepani, Bechsgaard, & Bilde, 2014). Cela peut aboutir à des effets génétiques délétères (Charlesworth & Willis, 2009) et fortement diminuer les capacités d'adaptation face aux changements environnementaux et aux pathogènes (Bijlsma & Loeschcke, 2012).

L'apparentement entre les individus eusociaux peut être également une source de conflit au sein des sociétés. En effet, comme mentionné précédemment, la coopération au sein des colonies d'insectes réside principalement dans les gains en bénéfices indirects liés à la relation de parenté avec ses congénères. Toutefois, cet apparentement n’est parfois pas suffisant pour garantir la coopération (Ratnieks, Foster, & Wenseleers, 2006) et peut varier au sein des sociétés. Par exemple, chez les hyménoptères, les reines et les ouvrières sont issues d’œufs fécondés, et donc diploïdes, alors que les mâles sont issus d’œufs non-fécondés et haploïdes (Wilson, 1971). Cette détermination du sexe entraine des asymétries d’apparentement génétique entre les reines, ouvrières et males, générant des conflits entre ces différentes castes (Bourke & Ratnieks, 1999; Ratnieks et al., 2006). L’intensité des conflits peut varier en fonction de la structure sociale des colonies, à savoir s’il y a une unique reine ou plusieurs, si celle(s)-ci est (sont) fécondée(s) par un ou plusieurs mâles. La polygynie (colonie à plusieurs reines) et la polyandrie (reine fécondée par plusieurs mâles) contribuent à réduire l’apparentement et ainsi à accroitre les sources de conflits (Ratnieks et al., 2006; Wenseleers, Ratnieks, & Billen, 2003). Ratnieks et al. (2006) ont résumé les grands conflits opérant dans les sociétés d’insectes, dont l’un des principaux résulte dans la détermination des castes. Les larves femelles en développement sont totipotentes et sont plus étroitement liées à leur propre potentielle progéniture qu’à celles des autres femelles (Bourke & Ratnieks, 1999). Ainsi, la théorie prédit que les individus ont plus d’intérêts à devenir reproducteurs (des reines) plutôt que des ouvrières afin d’obtenir une plus grande *fitness* inclusive (Bourke & Ratnieks, 1999; Wenseleers & Ratnieks, 2004; Wenseleers et al., 2003). La résolution des conflits trouve sa source dans la coercition (comportements émis afin de prévenir les actes égoïstes ou de réduire les bénéfices à les faire) et dans les contraintes (détermination génétique ou environnementale via le nourrissage par exemple) issues de la reine ou des ouvrières (Wenseleers, Hart, & Ratnieks, 2004), en complément de l’appariement génétique. Tous ces conflits peuvent être fortement couteux pour les individus et la société, la résolution de ces conflits est donc un élément central dans l’évolution sociale (Bourke, 2014; Ratnieks et al., 2006).

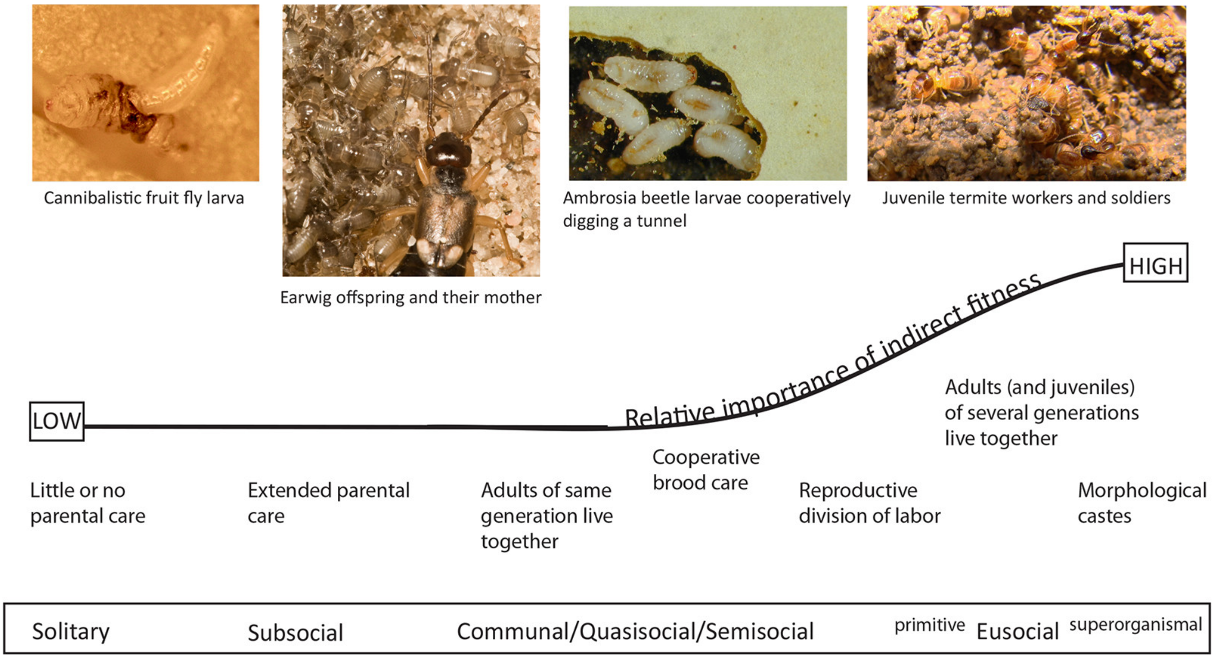

Figure 1 : Les différents modes de vie des insectes, et l'importance relative des bénéfices indirects en *fitness* (issue de Schultner et al., 2017).

2. Déterminisme de la caste et de la taille chez les insectes sociaux

Étant l'un des conflits majeurs au sein des sociétés d'insectes, la détermination des castes a été largement étudiée jusqu'à présent (Anderson, Linksvayer, & Smith, 2008; Schwander, Lo, Beekman, Oldroyd, & Keller, 2010). Il s'agit d'un phénomène très important car il détermine le rôle des individus (reproducteur ou subordonné) dès l'issue du stade larvaire chez les hyménoptères (cycle de vie holométabole). La différentiation des castes implique deux composantes : soit génétique soit environnementale. La part de chaque composante varie fortement entre espèces, et bien souvent il s'agit d'un continuum entre ces deux paramètres plutôt qu'un effet strict d'une composante (Schwander et al., 2010). La caste et la taille sont fortement corrélées, les reines sont généralement plus grandes que les ouvrières, bien que ce soit l'inverse chez quelques espèces de fourmis notamment chez les reines ergatoïdes non ailées (par exemple (Molet, Fisher, Ito, & Peeters, 2009; Molet, Peeters, & Fisher, 2007). La détermination de la taille intervient par conséquent également dès le stade larvaire et est un trait d'histoire de vie fondamental influençant la reproduction et la survie des organismes (Wills, Powell, Rivera, & Suarez, 2018). La taille affecte tous les aspects d'un individu, incluant son métabolisme, sa locomotion, sa longévité, son alimentation et sa thermorégulation (Peters, 1986). A nouveau cela implique des composantes génétiques et environnementales (Wills et al., 2018), c'est pourquoi je vais détailler les facteurs impliqués conjointement pour la détermination des castes et de la taille chez les insectes sociaux.

Facteurs intrinsèques

Les facteurs intrinsèques comprennent les facteurs internes aux œufs et aux larves, se résumant principalement aux facteurs génétiques et développementaux. Par exemple, chez la fourmi *Vollenhovia emeryi*, la génétique a un rôle quasi-exclusif dans la détermination de la caste, les reines sont homozygotes et les ouvrières hétérozygotes (Ohkawara, Nakayama, Satoh, Trindl, & Heinze, 2006). Le même processus est retrouvé chez les fourmis du genre *Pogonomyrmex*, où les ouvrières sont issues d'une hybridation de deux espèces cryptiques et les reines issues d'une lignée pure (Helms Cahan et al., 2002; Julian, Fewell, Gadau, Johnson, & Larrabee, 2002; Volny & Gordon, 2002). Chez la fourmi *Wasmannia auropunctata*, les ouvrières sont produites par reproduction sexuée tandis que les gynes sont des clones de la reine (Fournier et al., 2005).

L'influence génétique est retrouvée chez la taille des individus produits. La souche paternelle influence à la fois la taille des gynes produites mais aussi celles des ouvrières (Kovacs, Hoffman, Marriner, Rekau, & Goodisman, 2010; Smith, Anderson, Tillberg, Gadau, & Suarez, 2008) chez *P. badius*. Chez la fourmi *Acromyrmex echinatior*, les individus de différentes lignées paternelles diffèrent dans leur propension à se développer en grande (*major*) ou petite (*minor*) ouvrières (Hughes, Sumner, Van Borm, & Boomsma, 2003). Chez *Eciton burchellii*, plus les lignées paternelles augmentent, plus les ouvrières sont grandes (Jaffé, Kronauer, Kraus, Boomsma, & Moritz, 2007). En plus de la polyandrie, la polygynie affecte la distribution de taille des ouvrières. Chez *Formica selysi*, les colonies polygynes produisent des ouvrières plus petites et moins polymorphes que les colonies monogynes (Schwander, Rosset, & Chapuisat, 2005). Chez cette espèce, les gynes produites sont également plus grandes chez les colonies monogynes (Meunier & Chapuisat, 2009).

Outre le facteur génétique, on retrouve également les effets épigénétiques. Chez *Pogonomyrmex*, la production de nouvelles reines peut dépendre d'effets maternels, plus précisément de l'état physiologique (âge et hibernation subie) de la reproductrice mais aussi du niveau d'ecdystéroïdes fournis dans les œufs à la ponte (Schwander et al., 2008 - voir aussi

Libbrecht et al., 2013). Chez la fourmi *Temnothorax curvispinosus*, les effets maternels, entre autres, jouent également un rôle dans la détermination des castes (Linksvayer, 2006). Chez l'abeille *Apis mellifera*, un effet maternel est retrouvé dans la taille des gynes produites (Wei et al., 2019). Chez cette même espèce, la méthylation de l'ADN joue un rôle dans la détermination de la caste (Chen et al., 2020; Kucharski, Maleszka, Foret, & Maleszka, 2008) ; tandis que chez la fourmi *Camponotus floridanus*, elle peut générer de la diversité de taille continue en régulant quantitativement la transcription d'un gène (Alvarado, Rajakumar, Abouheif, & Szyf, 2015). Enfin, l'hormone juvénile peut jouer à la fois un rôle dans la détermination de caste et dans la taille des ouvrières. Des changements durant le développement (souvent induits par l'environnement externe, par exemple la saison chez *Pheidole*) peuvent mener à différents phénotypes (Lillico-Ouachour & Abouheif, 2017; Rajakumar et al., 2012).

Facteurs externes

L'environnement de développement a longtemps été considéré comme le facteur principal de la détermination des castes et de la taille (Michener, 1974; Wheeler, 1986), avant l'avènement des recherches en génétique. De ce fait, elles ont été largement étudiées et sont relativement bien connues (Brian, 1979, 1980; Hölldobler & Wilson, 1990; Wheeler, 1986, 1991).

La nourriture est une composante majeure de la détermination environnementale, tant au niveau qualitatif que quantitatif. Chez l'abeille *A. mellifera*, le nourrissage via une substance hautement nutritive, la gelée royale, induit un développement en reine (Allsopp, Calis, & Boot, 2003; Haydak, 1943; Kamakura, 2011). Chez la fourmi *C. tartessica*, une nourriture plus protéinée induit un développement en individus reproducteurs (Amor et al., 2016). Des études ont mis en exergue un rôle de la quantité de nourriture reçue pour la différenciation en reines par exemple chez *A. mellifera* (Slater, Yocum, & Bowsher, 2020) et la guêpe *Polistes metricus* (Judd, Teal, Hernandez, Choudhury, & Hunt, 2015). La taille du corps des ouvrières fluctue en fonction de la nourriture reçue chez de nombreux taxons d'insectes sociaux (Couvillon & Dornhaus, 2009; Linksvayer et al., 2011; Roulston & Cane, 2002; Segers, Menezes, Vollet-neto, Lambert, & Grüter, 2015; Slater et al., 2020; Smith et al., 2008; Smith & Suarez, 2010).

La température est connue pour affecter la différenciation de la caste. Chez la fourmi *Myrmica rubra*, une forte température conduit à une production orientée vers la caste ouvrière (Brian, 1973). Une température chaude stressante chez *T. nylanderi* réduit la taille des ouvrières produites (Molet et al., 2017) et la variation de taille augmente chez *Bombus impatiens* (Kelemen & Dornhaus, 2018). Elle peut affecter la survie du couvain, une forte température est corrélée négativement à la survie des œufs alors qu'à l'inverse les larves sont négativement affectées par une température basse (Abril, Oliveras, & Gómez, 2010). L'hibernation vécue par une larve influence fortement son devenir en reine (Brian, 1963, 1973). La saisonnalité impacte la taille des ouvrières produites également. Chez la fourmi *Veromessor pergandei*, de grandes ouvrières sont produites lorsque la nourriture (les graines) est en accès à la belle saison (Rissing, 1987).

La reine est un facteur important dans la production de nouvelles gynes dans la colonie (Brian, 1980; Wheeler, 1986), via des messages chimiques appelés phéromones qui agissent soit directement sur le développement des larves soit influencent le comportement des ouvrières (Keller & Nonacs, 1993). La seule présence de la reine entraine une production quatre fois plus orientée envers des ouvrières chez la fourmi *M. rubra* (Brian, 1963) et inhibe la production de nouvelles gynes chez le bourdon *B. terrestris* (Shpigler et al., 2013). Chez les fourmis *Pogonomyrmex*, la reine doit avoir subi une phase d'hibernation avant de produire des gynes (Schwander et al., 2008). L'âge de la reine influence sa propension à produire des gynes, plus elle vieillit plus elle en produit (Schwander et al., 2008). Comme vu précédemment, le nombre de reines dans la colonie influence la détermination et la taille des individus produits (Meunier & Chapuisat, 2009; Schwander et al., 2005). Chez la fourmi *Solenopsis invicta*, la diversité de taille des ouvrières est plus importante, les reines sont plus grandes et plus fécondes chez les colonies monogynes que chez les colonies polygynes (Goodisman, Mack, Pearse, & Ross, 1999; Goodisman & Ross, 1996; Keller & Ross, 1993, 1999). Enfin, la présence de la reine affecte également la taille des individus produits chez *B. terrestris*, les ouvrières produites sont plus grandes en l'absence de reine (Shpigler et al., 2013).

Feedbacks entre les facteurs intrinsèques, facteurs écologiques et environnement social

L'effet de la reine rejoint un effet social plus global, appelé environnement social. Cet environnement social a été défini par Wills et al. (2018) comme étant tous les facteurs qui sont directement liés aux traits coloniaux et à son ontogénie dans son ensemble : l'âge de la colonie, l'effectif, la structure sociale (une ou plusieurs reines), la distribution de la caste ouvrière (phénotypes des ouvrières) et l'influence du contrôle social sur le développement de la colonie.

A cela nous pourrions ajouter la présence et la distribution du couvain, tant il joue un rôle crucial dans la vie sociale chez les hyménoptères, que ce soit dans la coopération ou les conflits (Schultner et al., 2017) mais aussi dans les individus produits (Warner, Kovaka, & Linksvayer, 2016). Linksvayer propose la notion de « sociogénome » quant à lui, afin de démontrer l'importance de l'environnement social dans le développement des individus et de la colonie (Linksvayer et al., 2011). En effet, les phénotypes produits dans la colonie résultent à la fois du génotype des individus focaux (les larves), mais aussi du génotype de la reine et des ouvrières qui élèvent ces larves ainsi que leurs comportements associés, notamment dans les soins (Linksvayer, 2006, 2007; Linksvayer, Fondrk, & Page Jr., 2009). Le comportement de ces mêmes ouvrières peut également être modulé par la reine comme mentionné ci-dessus (Keller & Nonacs, 1993). Plus généralement, l'environnement social joue un rôle crucial de rétro-contrôle entre les facteurs externes et le développement de la colonie. La société peut moduler ainsi l'environnement de développement des larves, en régulant la nourriture apportée, le microclimat au sein du nid et les phéromones émises pouvant moduler le développement (Linksvayer, 2006; Linksvayer & Wade, 2005). Chez *B. impatiens* et *Tetragonisca angustula*, la position des larves dans le nid affecte leur quantité de nourriture reçue et engendre une variation de taille des ouvrières (Couvillon & Dornhaus, 2009; Segers et al., 2015). A l'inverse, les larves peuvent jouer un rôle actif dans la prise de nourriture en quémandant directement de nouvelles ressources chez les fourmis (Creemers, Billen, & Gobin, 2003; Kaptein, Billen, & Gobin, 2005). Le ratio ouvrières/larves est une composante majeure de l'environnement social, il affecte la survie et le temps de développement du couvain mais aussi la taille des ouvrières émergentes ensuite chez la fourmi *F. selysi* (Purcell, Brütsch, & Chapuisat, 2012).

L'effectif des colonies d'insectes sociaux influence les phénotypes produits, la taille moyenne et la diversité de taille augmentent souvent en fonction de l'effectif (Clémencet & Doums, 2007; Ferguson-Gow, Sumner, Bourke, & Jones, 2014; Porter & Tschinkel, 1985b; Shpigler et al., 2013; Tschinkel, 1988, 1993, 1998 - mais voir Couvillon, Jandt, Duong, & Dornhaus, 2010; Dornhaus et al., 2012; Fjerdingstad & Crozier, 2006). Les petites ouvrières, moins coûteuses à produire, sont produites en grand nombre au début de la fondation alors que les grandes ouvrières sont produites une fois la colonie établie, parfois au bout de plusieurs années (Billick & Carter, 2007; Tschinkel, 1988). Chez certaines espèces, des ouvrières « naines » (*nanitic*) sont même parfois retrouvées au début de la colonie (Hölldobler & Wilson, 1990; Porter & Tschinkel, 1986; Tschinkel, 1988).

Figure 2 : exemple de phragmose chez *Cephalotes varians* (©Alex Wild)

La distribution des castes au sein des colonies régule également le développement des larves. Chez *Pheidole*, la présence de la caste soldat inhibe la production de nouveaux soldats (Wheeler, 1986; Wheeler & Nijhout, 1984). Cette distribution peut à l'inverse être impactée par l'environnement externe. Par exemple, les colonies peuvent produire des soldats en réponse à une compétition intraspécifique chez *P. pallidula* (Passera, Roncin, Kaufmann, & Keller, 1996). De même, la distribution de la taille des ouvrières peut être influencée par les risques de prédation (Powell & Clark, 2004) ou de compétition pour les ressources (Davidson, 1978). Chez différentes espèces de fourmis, certains soldats présentent un morphotype adapté pour boucher l'entrée du nid (appelé phragmose, figure 2) et ainsi protéger le reste de la colonie des intrus (Hasegawa, 1993; Powell, Price, & Kronauer, 2020).

Enfin, la vie en société permet de moduler les effets des perturbations externes (Crall et al., 2019; Kaspari & Vargo, 1995; Molet et al., 2017; Scharf et al., 2012; Straub et al., 2015) mais aussi de diluer les coûts potentiels à avoir des phénotypes divergents, qui ne sont pas forcément adaptatifs (Molet, Wheeler, & Peeters, 2012). La présence d'un individu morphologiquement atypique et son coût lié pour assurer sa viabilité sont dilués parmi les membres de la colonie. Ainsi, les sociétés d'insectes peuvent diminuer les pressions opérant sur les individus, que ce soit les pressions externes ou bien les pressions internes liées au développement (Londe et al., 2015; Molet et al., 2012). La vie en société pourrait ainsi autoriser une certaine fluctuation de la taille sans que celle-ci soit forcément bénéfique à la colonie (Colin, Doums, Péronnet, & Molet, 2017; Hunt et al., 2011).

En résumé, la détermination des castes et de la taille réside dans deux composantes principales : la génétique et l'environnement externe. L'environnement social joue une part importante dans les phénotypes en modulant l'environnement de développement (température, nourriture) en fonction des contraintes écologiques (compétition, habitat) et des besoins coloniaux (allocation des ressources). L'effet de l'environnement social est peut-être encore sous-estimé. De récentes études mettent en évidence la transmission d'hormones juvéniles (régulant le développement et la métamorphose chez les insectes) via la trophallaxie (LeBoeuf,

2017) entre adultes et larves, ainsi les adultes pourraient avoir un contrôle encore plus accru sur les phénotypes produits chez certaines espèces comme la fourmi *Camponotus floridanus* (LeBoeuf et al., 2018, 2016). L'ensemble des facteurs agissant sur la détermination de la taille des ouvrières est résumé dans la figure 3, issue de Wills et al (2018).

Ainsi, la diversité de taille des ouvrières au sein des colonies de fourmis, sur laquelle nous allons nous focaliser dans cette thèse, résulte fortement de l'environnement social dans lequel se développent les larves et donc des intérêts de la colonie. Toutefois, des intérêts individuels dans la taille peuvent persister notamment via des comportements égoïstes tentant de déjouer le contrôle social durant le développement.

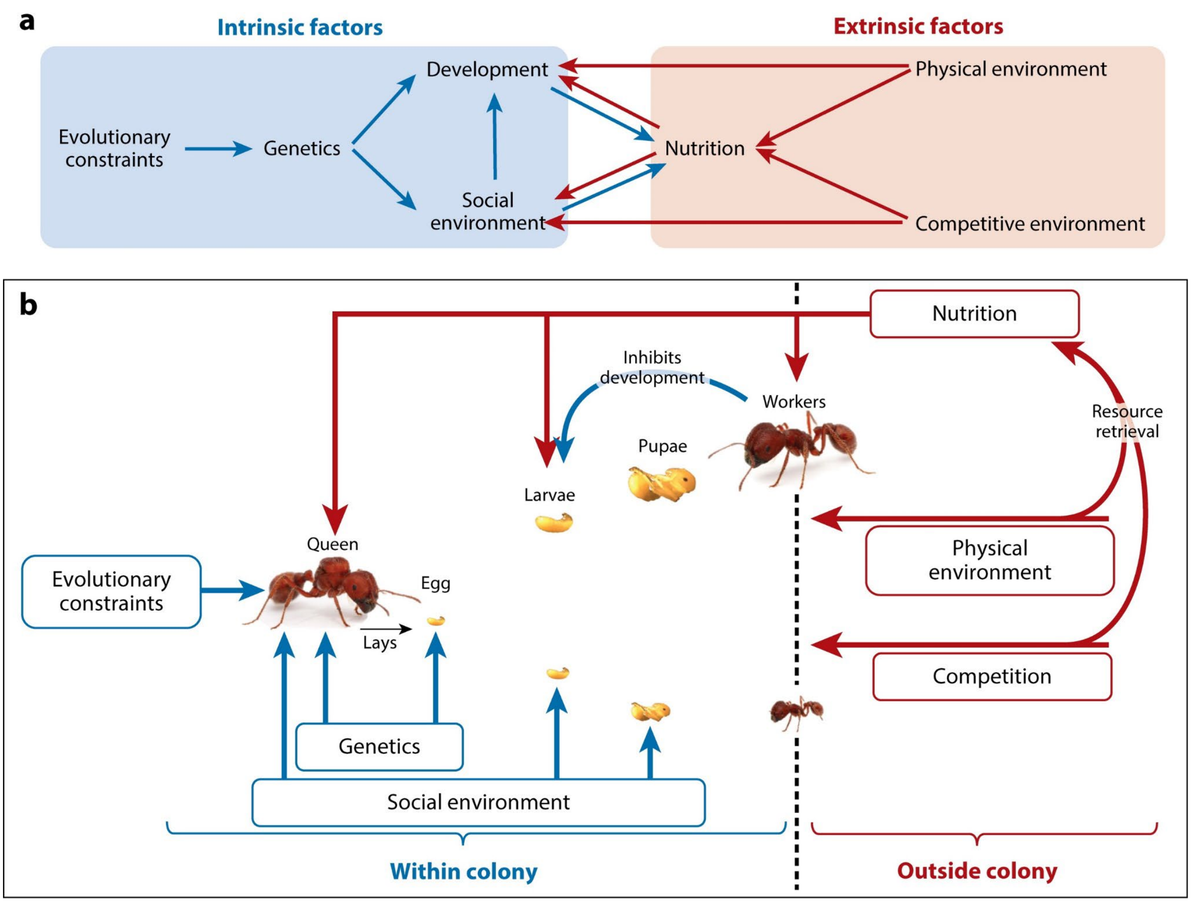

Figure 3 : (a) Facteurs influençant la distribution de taille de la caste ouvrière. La génétique et l'environnement externe jouent un rôle important, le tout étant régulé par l'environnement social. (b) Tous les facteurs peuvent interagir, aux différents stades de vie (des œufs aux ouvrières), et ainsi moduler les phénotypes produits (issue de Wills et al., 2018).

3. Rôle de la taille des ouvrières chez les insectes sociaux

La diversité au sein des colonies

Outre pour l'aspect individuel déjà énoncé (Peters, 1986; Wills et al., 2018), la taille présente un rôle phare dans l'organisation des sociétés d'insectes. En effet, la division du travail est souvent régie selon la morphologie des individus, on parle alors de polyéthisme de caste (Hölldobler & Wilson, 1990; Oster & Wilson, 1978; Wilson, 1971). Ce polyéthisme de caste est supposé améliorer l'efficacité des tâches réalisées et ainsi augmenter la *fitness* des sociétés (Frumhoff & Ward, 1992; Hölldobler & Wilson, 1990; Oster & Wilson, 1978; Wheeler, 1986). La taille va donc déterminer comment les individus interagissent avec les paramètres abiotiques et biotiques de leur environnement. La taille est souvent sujette à une forte sélection, sa variation peut répondre à des contraintes évolutives mais aussi à des compromis (Stearns, 1992; Wills et al., 2018). Par exemple, les sociétés peuvent avoir à choisir entre le nombre et la taille des individus produits (Oster & Wilson, 1978; Smith & Fretwell, 1974; Wheeler, 1991). En plus de la sélection à l'échelle du groupe, la taille peut également résulter d'une sélection à l'échelle de l'individu. En effet, bien qu'elles n'aient généralement pas la capacité de se reproduire, les ouvrières ont gardé la capacité de pondre des œufs haploïdes pour produire des mâles (Giehr et al., 2020). Cette capacité à produire des mâles, à savoir la fertilité, est fortement influencée par la taille du corps des individus chez de nombreuses espèces (Clémencet, Rome, Fédérici, & Doums, 2008; Dietemann, Hölldobler, & Peeters, 2002; Frumhoff & Ward, 1992; Gobin & Ito, 2003; Modlmeier, Pamminger, Foitzik, & Scharf, 2012; Smith, Schoenick, Anderson, Gadau, & Suarez, 2007). La taille et sa diversité pourraient ainsi être affectées simultanément entre les facteurs agissant à l'échelle coloniale (facteurs écologiques et compromis), mais aussi sur les facteurs affectant les opportunités reproductives des ouvrières (Fjerdingstad & Crozier, 2006).

La variation de taille, aussi appelée le polymorphisme, chez les ouvrières peut être discrète ou continue (figure 4). Lorsque la variation est discrète, elle implique la présence de différentes sous-castes distinctes : soldats (parfois appelés *major*) et ouvrières (parfois appelés *minor*). Chez certaines espèces on peut retrouver des supersoldats (figure 4 A et C). Lorsque la variation est continue, il n'y a plus de caste soldats dans la colonie, seulement des ouvrières à proprement dites. Un des sujets centraux sur la taille et sa diversité chez les insectes a été d'étudier sa relation avec la division du travail, notamment si la distribution de taille améliore effectivement la division des tâches à l'intérieur des colonies (Hölldobler & Wilson, 1990; Oster & Wilson, 1978). Beshers & Traniello (1994) ont proposé que la diversité de taille pourrait ne pas être forcément liée à la division du travail mais plutôt à la survie et à la croissance coloniale. Toutefois, cette variation de taille est relative. En effet, chez les fourmis qui représentent le groupe d'insectes sociaux le plus diverse morphologiquement et où la plus grande diversité au sein des colonies peut être observée (Wheeler, 1991), seulement 13% des espèces de fourmis (approximativement 16% des genres) présentent une variation considérable de taille et de forme (Fjerdingstad & Crozier, 2006; Hölldobler & Wilson, 1990; Oster & Wilson, 1978; Wills et al., 2018). La plupart des espèces de fourmis sont même parfois considérées comme monomorphique à cause de leur diversité limitée (voir par exemple : Wills et al., 2018). Fjerdingstad & Crozier (2006) ont comparé la diversité de taille des ouvrières chez 35 espèces de fourmis à travers le monde. Ils ont montré que la plupart des espèces avaient une diversité modérée ou faible, ayant un coefficient de variation inférieur à 0.2, incluant même les espèces ayant un fort succès écologique (voir aussi la variation de taille chez *Formica* : Tawdros, West, & Purcell, 2020). Toutefois, cette variation, bien que limitée, est bien présente et mérite d'être davantage prise en compte et étudiée. Dans cette thèse nous allons nous focaliser sur le groupe des fourmis car elles présentent un large panel de diversité morphologique (Londe et al., 2015; Molet et al., 2012; Wheeler, 1991), et le rôle de la taille chez les espèces à diversité modérée/limitée reste largement non résolu.

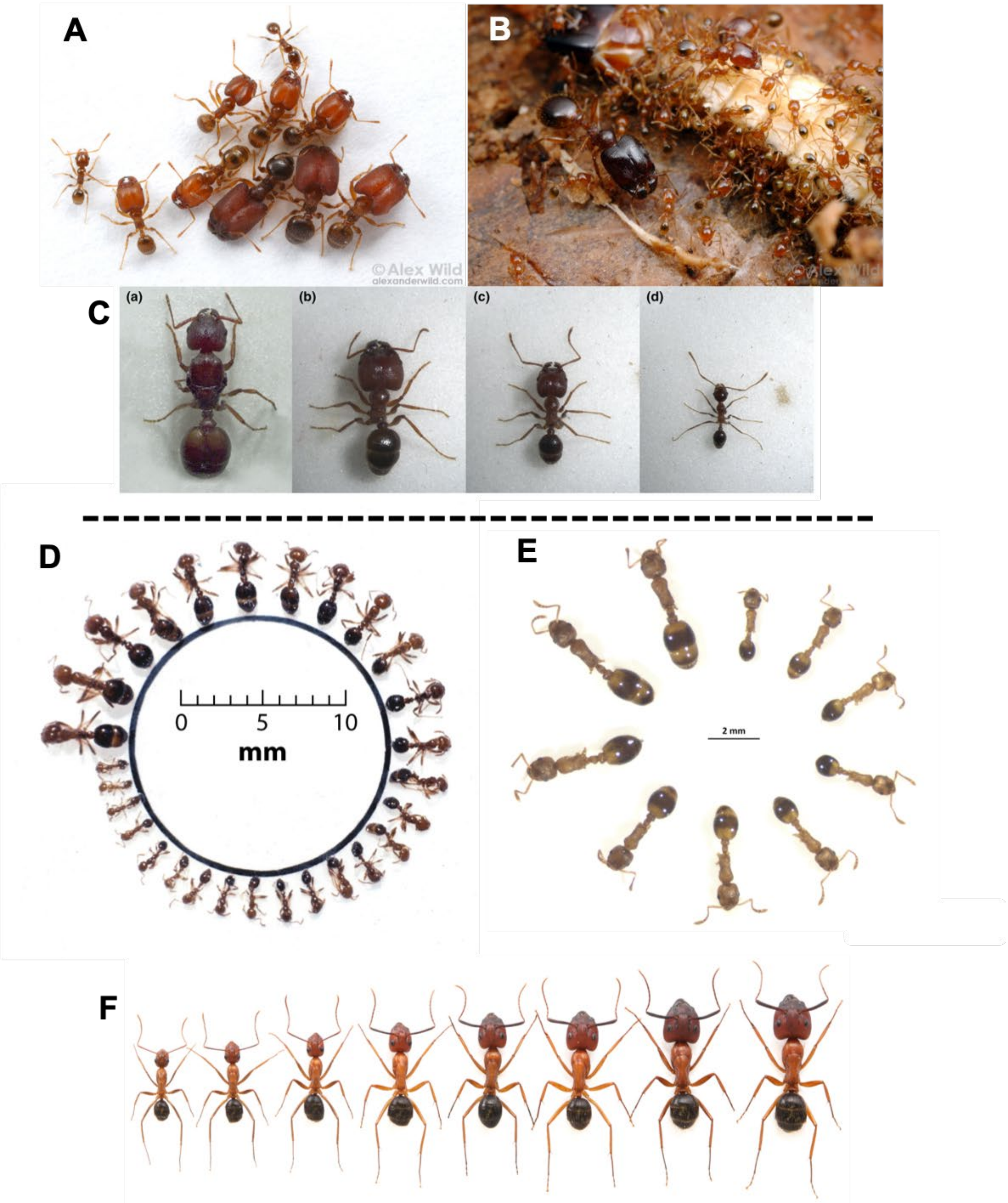

Figure 4 : Variation de la taille au sein des colonies de fourmis, soit discrète (A, B et C) ou continue (D, E et F). A : *Pheidole tepicana* (©Alex Wild), B : *Carebara affinis* (©Alex Wild), C : *Pheidole obtusospinosa* (issue de Lillico-Ouachour & Abouheif, 2017) : (a) queen (b) supersoldier (c) soldier and (d) minor worker), D : *Solenopsis invicta* (issue de Wills et al., 2018), E : *Temnothorax nylanderi* (issue de Honorio, Doums, & Molet, 2020), F : *Camponotus floridanus* (issue de Alvarado et al., 2015).

Mesure du caractère adaptatif de la taille

Wills et al. (2018) ont résumé les trois approches possibles permettant de déterminer le caractère adaptatif et donc bénéfique de la taille et de sa diversité intra-coloniale. L'approche la plus courante est d'évaluer l'efficacité d'une tâche réalisée par un individu focal par rapport aux autres membres de la colonie et de déterminer comment les différences de tailles peuvent influencer l'efficacité globale de la société (Dornhaus & Powell, 2010; Hölldobler & Wilson, 1990). Cette approche a été établie par la théorie ergonomique proposée par Oster & Wilson (1978), cherchant à expliquer l'évolution adaptive de la taille du corps dans l'optimisation de la réalisation des tâches et de son « rendement » au sein de la colonie. La prédiction phare est que les avantages adaptatifs de la diversité de taille devraient être détectables en tant que gains d'efficacité chez les individus focaux par rapport aux autres membres de la colonie.

La seconde approche, moins courante, consiste à quantifier la performance fonctionnelle de la gamme de taille des individus dans des contextes écologiques contrastés (voir par exemple (Powell, 2009). Cette approche repose sur les nouvelles capacités fonctionnelles dans des contextes écologiques particuliers (Irschick, 2003). La prédiction générale ici est que les membres focaux apportent un avantage adaptatif s'ils remplissent une fonction maximale dans les conditions écologiques rencontrées.

Enfin, la dernière méthode possible vise à mesurer directement l'impact de la variation de taille sur la *fitness* de la colonie. Cette méthode prédit que si la diversité est adaptative, alors tout changement dans la distribution de taille devrait entrainer un changement détectable dans la *fitness* des colonies. Cependant, la mesure de la *fitness* reste un défi majeur chez les insectes sociaux et particulièrement chez les fourmis. Une mesure complète de la *fitness* est difficilement quantifiable. Des variables indicatrices de la *fitness*, appelées *proxy*, sont souvent utilisées pour avoir une idée du succès d'une colonie, telles que le nombre et la qualité des futurs reproducteurs produits (gynes et males).

Bien qu'elles abordent différentes approches pour mesurer l'adaptativité de la taille et de la variation de taille, ces trois méthodes nécessitent une profonde connaissance des taxons ciblés (Wills et al., 2018). Pour la première approche, il est nécessaire de connaitre le rôle supposé de l'individu focal en lien avec sa morphologie dans son contexte écologique naturel, afin de tester le bon comportement ou la bonne fonction. Pour la seconde approche, il faut avoir identifié la gamme d'interactions écologiques possible de l'espèce et l'implication de la taille de l'individu dans ces interactions. Enfin, la dernière méthode implique également une connaissance approfondie de l'espèce pour déterminer quelles variables traduisent le succès d'une colonie et notamment quand les mesurer, par exemple l'âge de maturité d'une colonie où elles commencent à investir davantage dans les sexuées. Ainsi, il n'est jamais aisé de déterminer de manière fiable le caractère adaptatif de la taille et de sa diversité chez les insectes sociaux, et ces trois estimations peuvent être complémentaires afin d'avoir une mesure optimale.

Relation fort polymorphisme – division du travail

Si l'on se focalise chez les espèces présentant un fort polymorphisme, à savoir la présence de sous-caste (soldats/*major* et ouvrières) ou bien ayant une large gamme de variation de taille, les travaux étudiant le lien division du travail en relation avec la taille et efficacité font relativement consensus. A titre d'exemples, une large diversité de taille améliore les capacités de fourragement (Arnan, Ferrandiz-Rovira, Pladevall, & Rodrigo, 2011; Bernadou, Felden, Moreau, Moretto, & Fourcassie, 2016; Davidson, 1978 – mais voir tout de même (Rissing & Polloek, 1984) ainsi que la résistance à la dessiccation chez les fourmis granivores (Kaspari, 1993) ; la communication chez *Atta* (Evison, Hart, & Jackson, 2008) ; la défense du nid chez *Cephalotes* et *Pheidole* (Powell, 2009; Wilson, 1984) ; la tolérance thermique chez *Eciton* et *Cataglyphis* (Baudier & O'Donnell, 2018; Cerdá, Retana, & Cerda, 1997) ; et la production de couvain chez *Solenopsis* (Porter & Tschinkel, 1985a, 1986).

Contraste chez les espèces modérément / faiblement polymorphe

Chez les espèces présentant un plus faible polymorphisme, ayant une variation de taille dite modérée ou faible, les résultats sont plus contrastés, parfois même pour un même paramètre étudié. Par exemple, Billick & Carter (2007) ont trouvé une relation positive entre la diversité de taille et les capacités de fourragement chez *Formica obscuripes*, alors que Westling, Harrington, Bengston, & Dornhaus (2014) n'ont pas trouvé d'effet de la diversité chez *Temnothorax rugulatus*. Ce contraste sur le rôle de la diversité de taille est retrouvé pour la tolérance thermique, avec un effet positif chez *Leptothorax acervorum* (Heinze, Foitzik, Fischer, Wanke, & Kipyatkov, 2003) et aucun effet chez *T. curvispinosus* (Yilmaz et al., 2019). Billick (2002) avec *F. neorufibarbis* et Modlmeier & Foitzik (2011) avec *T. longispinosus* n'ont détecté aucun effet de la diversité de taille sur le nombre de cocons produits par ouvrières et sur le poids de la colonie. Modlmeier, Foitzik, & Scharf (2013) ont trouvé un effet positif sur la résistance à la famine chez *T. nylanderi*, tandis que chez la même espèce Colin et al. (2017) ont réduit expérimentalement la diversité de taille et n'ont détecté aucune différence de performances entre les colonies à diversité réduite et les non-manipulées dans les différents challenges proposés en laboratoire (déménagement de nid, fourragement, comportement hygiénique, résistance à un choc thermique …). Ainsi, chez les espèces avec un polymorphisme plus restreint, le caractère adaptatif de la taille et de sa variation n'est pas toujours évident, même au sein d'une même espèce. De plus, jusqu'à présent, peu d'études ont pu quantifier de manière fiable et exhaustive le rôle de la taille dans la *fitness* coloniale, c'est-à-dire mesurer les conséquences de la diversité morphologique sur le terrain face aux fortes pressions naturelles.

4. Objectifs de la thèse

Cette thèse s'articule autour de l'évolution de la taille et de sa diversité au sein des colonies de fourmis considérées modérément polymorphe. Un des objectifs principaux est de déterminer le rôle de cette diversité de taille intra-coloniale, certes réduite par rapport à d'autres espèces, mais toutefois bien présente. A cela nous avons ajouté la composante sociale, afin d'évaluer l'influence de l'environnement social dans cette diversité et face aux contraintes environnementales. Cette thèse se décompose en trois chapitres majeurs.

Le premier chapitre quantifiera le rôle de la diversité de taille chez les espèces sociales avec un polymorphisme limité. Chez ces espèces, le caractère adaptatif de la taille et de la variation de taille intra-coloniale ne fait pas consensus à travers les différentes études sur le sujet. De plus, ces mêmes études ont souvent été réalisées en laboratoire, ne permettant pas d'avoir une réelle estimation de la valeur sélective de la colonie. Afin d'avoir une mesure exhaustive de l'impact de la diversité de taille sur la *fitness* des colonies, nous avons réalisé des expériences en milieu semi-naturel sur une longue période. Nous avons réduit expérimentalement la diversité de taille et manipulé la taille moyenne au sein des colonies de la fourmi *Temnothorax nylanderi*. Cette expérience a été répétée lors de la saison de croissance et lors de la phase d'hibernation afin de couvrir le cycle complet de vie du modèle biologique. Grâce à notre dispositif et à l'espèce étudiée, nous avons pu quantifier l'effet de notre manipulation sur la croissance, le succès reproducteur et la survie des colonies expérimentales.

Le second axe vise à déterminer le rôle de l'environnement social dans la diversité morphologique. Chez les fourmis, très peu de preuves empiriques de l'effet de l'environnement social dans la régulation de la nourriture fournie aux larves sont connues à ce jour. Cet axe vise également à montrer, à l'inverse, comment les larves peuvent se développer lorsqu'elles ne sont pas sous contraintes des ouvrières. En d'autres mots, nous cherchons à identifier les mécanismes sociaux pouvant entrainer de la diversité de taille au sein des colonies : soit provenant du contrôle des ouvrières, soit provenant des tentatives individuelles des larves. Pour combler ce manque empirique, nous avons manipulé l'environnement de développement des larves de la fourmi *Mystrium rogeri* en laboratoire. Nous les avons nourries manuellement afin de quantifier les phénotypes produits, en comparaison au groupe contrôle dépendant des ouvrières pour se nourrir. Cet axe permet de mettre en lumière d'éventuelles sources de variations de la taille, et ouvre la voie aux démonstrations empiriques de la manipulation sociale et du conflit larve – ouvrière chez les fourmis.

Le troisième et dernier chapitre abordera le rôle de l'environnement social dans la réponse à de nouvelles contraintes. L'urbanisation apporte de nouvelles perturbations aux populations et engendre la mise en place de mécanismes adaptatifs permettant aux colonies de perdurer dans ces environnements changeants. Ici, nous avons voulu démêler les rôles de l'environnement social et des facteurs intrinsèques des larves dans la résistance aux facteurs environnementaux stressants. Pour ce faire, nous avons réalisé un *cross-fostering* de larves de populations de ville et de forêt par des ouvrières issues de la population des larves ou de l'autre, et élevé en laboratoire les différents croisements sous jardin commun en exposition ou non à un polluant, le cadmium. A nouveau, nous avons utilisé la fourmi *T. nylanderi.* Nous avons mesuré l'impact de l'exposition au polluant sur la survie et la croissance des colonies en fonction des différents croisements. Nos différents croisements n'ont pas permis de retrouver une réponse différentielle au cadmium entre les colonies de ville et de forêt, exceptée (marginalement) dans la taille des mâles produits. Ces résultats inattendus pourraient résider dans la phénologie décalée des fourmis de villes et de forêts, entrainant une sensibilité différente au cadmium en fonction des saisons. Cette dernière étude met toutefois en évidence les potentielles limites du tampon social des sociétés d'insectes face aux pressions environnementales.

CHAPITRE 1

Rôle de la diversité de taille chez les espèces de fourmis modérément polymorphes.

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Manipulation of worker size diversity does not affect colony fitness under natural conditions in the ant *Temnothorax nylanderi*.

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Abstract

In social insects, within-colony worker diversity has long been thought to improve colony fitness. Recent studies start to question this assertion in species with moderate diversity. However, demonstrating an absence of effect is difficult as this absence could be due to the measurement of only few life history traits or to artificial conditions. In order to circumvent these limitations, we experimentally decreased worker size diversity within colonies of the ant *Temnothorax nylanderi*, with or without affecting mean worker size and we reintroduced them in the field for four months (spring and early summer). We then thoroughly measured their fitness based on survival, growth and reproductive success. Our results show that our manipulation did not affect colony fitness. In addition, colonies did not restore diversity to its initial level, further suggesting that worker size diversity is not a key parameter to them. We found the classically observed positive relationship between colony size, colony growth and reproductive success. Overall, our results confirm that worker size diversity within colony is not necessarily adaptive in species where it is moderate. We discuss the alternative mechanisms that could explain the evolutionary persistence of moderate worker size diversity.

Significance statement

Organisms that live in groups can greatly benefit from the emergence of novel group-level traits. For instance, social insects show significant variability in worker size within colonies. This size diversity increases the division of labour among workers and improves colony fitness. However, in species where size diversity is moderate, this relationship may not always be verified. Here, we manipulated both worker size diversity and mean worker size within colonies of the ant *Temnothorax nylanderi*, we reintroduced them in the field, and we extensively measured colony fitness after four months. We found no impact on colony survival, growth and reproduction. We discuss how social life and its associated cooperation and conflicts could cause variation in worker size, without any positive effect on colony fitness.

Key-words: colony size, social insects, mean body size, survival, growth, reproductive success

Introduction

Animal societies can exhibit genetic, behavioral, physiological and morphological diversity among individuals of the same group and this is thought to provide benefits to the group (Sendova-Franks and Franks 1999; Robinson 2009). One of the most obvious and ecologically important traits in social insects is size diversity of individuals within colonies (bees: Brand & Chapuisat 2012; Linksvayer et al. 2011 - wasps: Kovacs et al. 2010 - ants: Molet et al. 2012). Worker size diversity improves division of labour and colony efficiency in many species (e.g. bumble bee: Peat et al. 2005; stingless bee: Ramalho et al. 1998). Among social insects, ants are the most morphologically diverse group and the one where the highest level of morphological diversity within colonies can be observed (Wheeler 1991). In ant species with high worker polymorphism (including discrete worker and soldier castes), studies support a general positive relationship between worker size diversity and colony performance (Davidson 1978; Wilson 1984; Yang et al. 2004; Evison et al. 2008; Powell 2009; Arnan et al. 2011 - but see: Rissing and Polloek 1984; Calabi and Traniello 1989). However, most species have moderate worker size diversity (with an intra-colonial coefficient of variation lower than 0.2

(Fjerdingstad and Crozier (2006)), and in this case the link between worker size diversity and colony performance is less clear. (Beshers and Traniello 1994, 1996) suggest that the adaptiveness of size variation in moderately polymorphic ants is unrelated to division of labor, and size could be driven by other pressures which increase survival or colony growth. Some studies show a positive relationship (Porter and Tschinkel 1985a, 1986; Heinze et al. 2003; Billick and Carter 2007; Modlmeier et al. 2013), others do not (Billick 2002; Modlmeier and Foitzik 2011; Westling et al. 2014; Yilmaz et al. 2019). In ant species with moderate worker size diversity, within group morphological diversity could therefore be non-adaptive at the colony level.

However, it is difficult to reliably rule out any adaptive value of worker size diversity as the presence or the lack of positive effects could always result from the limited number of traits investigated or the rearing setup under artificial laboratory conditions. Indeed, some studies focus on a single trait, such as foraging (Billick and Carter 2007; Westling et al. 2014), brood production (Porter and Tschinkel 1985a, 1986; Billick 2002; Modlmeier and Foitzik 2011), thermal tolerance (Heinze et al. 2003; Yilmaz et al. 2019) or resistance to starvation (Modlmeier et al. 2013). Accordingly, trade-offs with other traits that could be negatively affected by size diversity may have been overlooked, resulting in a biased assessment of fitness. Moreover, some of these studies are correlative, so they do not prove a causal link between size diversity and colony efficiency (e.g. Modlmeier and Foitzik 2011; Westling et al. 2014). Finally, all the experimental studies were performed in the laboratory where realistic environmental pressures are lacking (e.g. Porter and Tschinkel 1985a; Colin et al. 2017). Indeed the distinct environmental conditions (photoperiod, temperature, food availability, social environment) of laboratory and field experiments cause variation in the behavior of both vertebrates and invertebrates (Campbell et al. 2009).

Size diversity and mean worker size increase with colony size (respectively: Ferguson-Gow et al. 2014; Porter and Tschinkel 1985b). Only colonies with sufficient resources can produce large workers and enhance worker size diversity. It is important to disentangle these two traits in order to properly conclude about the adaptive value of size diversity and mean worker size within colonies of social insects. Indeed, a recent study on bumblebee colonies highlighted a positive correlation between colony performance and mean worker size, but no link with worker size diversity (Herrmann et al. 2018).

In this study, we aimed at quantifying the impact of within-colony worker size diversity and worker mean size on colony fitness using a long-lasting field-based experiment with extensive measures of fitness (survival, growth and reproductive success). We focused on an ant species lacking discrete worker subcastes and exhibiting moderate worker size diversity, *Temnothorax nylanderi* (see Appendix A1). In *Temnothorax* species, worker size does not determine worker specialization (Pinter-Wollman et al. 2012), task-allocation is highly flexible (Robinson, Feinerman, & Franks 2009) and it relies on physiological parameters (Robinson et al. 2012). Colin et al. (2017) manipulated worker size diversity within *T. nylanderi* colonies and challenged colonies in the laboratory with various tasks (nest emigration, foraging, hygienic behavior, cold shock, etc.). They did not find any differences in performance between colonies with reduced size diversity and control colonies with unmanipulated diversity. Moreover, neither mean size nor size diversity correlate with per-capita productivity (Modlmeier and Foitzik 2011). This suggests that worker size diversity is not adaptive in this species, although larger individuals survive longer to cold temperature (Heinze et al. 2003) and starvation (Modlmeier et al. 2013). We tested whether this holds true in the field by manipulating the size of workers in colonies in the laboratory. Importantly, we manipulated both within colony worker size diversity (decrease) and mean worker size (increase, decrease or no change) in order to disentangle their effects. We subsequently re-implanted colonies in the field for four months to expose them to natural biotic and abiotic selective pressures, and we subsequently measured colony fitness extensively. In *Temnothorax* species, brood production is highly synchronized (Headley 1943; Kipyatkov 1993) so, in addition to colony survival, we were able to quantify two additional fitness parameters, namely colony growth (based on the production of new workers) and reproductive success (based on the production of sexuals). In addition, because workers control larval development (Linksvayer et al. 2011), we expected colonies to compensate for the size of the removed individuals and restore diversity by producing workers of the very same size if size diversity is somewhat important for colony fitness.

Material and methods

Ants

Colonies of *Temnothorax nylanderi* nest in twigs or acorns and are thus easy to pick up from the forest leaf litter. One hundred and forty-six colonies of *T. nylanderi* were collected in February 2019 in the ‘Bois de Vincennes’ forest (Paris, France, 48° 50′ 22.14″ N, 2°26′ 51.96″

E). In the laboratory, each colony was installed in an artificial nest consisting of two microscope slides separated by a 1-mm auto-adhesive plastic foam with three chambers. The nest was placed in a plastic box (11.5 × 11.5 × 5.5 cm) providing a foraging area (see Appendix A2). During their two-week stay in the laboratory, colonies were kept in a climatic chamber at 10-12°C with a natural photoperiod mimicking the natural conditions at this period. Water was provided *ad libitum* in a tube plugged with cotton. Colonies were fed once with a freshly killed mealworm (*Tenebrio molitor*). Workers and brood were counted.

Manipulation of worker size

Out of the 146 colonies, 80 colonies were selected for the experiment based on the presence of one queen and at least 70 workers. Four treatments were performed and, in all treatments, 50% of workers were removed from the 20 colonies. In treatment 25S25L, we decreased worker diversity without changing mean worker size by removing the 25% smallest workers and the 25% largest workers. In the two other treatments, we decreased worker diversity but also either increased mean worker size by removing the 50% smallest workers (treatment 50S) or decreased mean worker size by removing the 50% largest workers (treatment 50L). The last treatment was the control (treatment 50R) as we removed 50% of workers randomly. The worker removal protocols were similar to Colin et al. (2017) and based on the apparent global body size: the sorting of large and small workers was done by eye under a stereoscopic microscope (Zeiss®, x50 magnification) whereas the removal of random workers was done without a microscope to make sure that worker size could not be evaluated. This method was proven to be reliable (Colin et al. 2017). Colonies were assigned to the four treatments based on their number of workers in order to keep a similar distribution of colony sizes among the four treatments Workers remaining in the colonies after manipulation constituted the initial workforce of the colonies (67.8 workers ± 22.5). Colonies initially contained 91.6 larvae ± 27.7.

Colony rearing in the field

We manufactured artificial nests to make them match natural nests as closely as possible, so that colonies could easily live in them after reintroduction in nature. For that purpose, we used 2.5x 2cm truncated cone corks. We drilled a 4, 5 or 6mm-wide chamber from the larger side of the cone cork, and plugged this side with a glue gun to seal the chamber. Then, a 1mm-wide entrance gallery was pierced from the smaller side of the cone cork using a pointed plier in order to connect the chamber to the outside. In the laboratory, six corks (two of each size) were placed inside each plastic box, and we induced the emigration of colonies into the corks of their choice by removing the cover glass of the original nest. Then, the six corks from each plastic box (one containing the colony) were reintroduced in the 'réserve ornithologique du Bois de Vincennes' in a semi-buried (10cm deep) 40 x 35cm bucket with a pierced bottom (for water draining) and containing local soil (see Appendix A3). The bucket lid was cut off into a ring shape and the bottom side was covered with fluon®, a slippery coating, to prevent ant escape while retaining a wide entrance. The six corks were place randomly. This allowed colonies to relocate to the nest of their choice (size and location) inside the bucket whenever they wanted to. Indeed, *T. nylanderi* colonies readily switch nest depending on environmental conditions (Foitzik et al. 2003). Because the 80 buckets had been in place for a year, the soil and litter in the buckets were very similar to those of the surrounding forest; many arthropods and soil organisms could come in and out of the buckets. A preliminary food-supplementation experiment showed that adding food into the buckets did not increase colony survival and growth, so the 40cm-wide foraging area provided enough food for the colonies. Colonies were left in the buckets from March 26th to July 16th, and subsequently collected and brought back to the laboratory. Corks were collected in the early morning, when workers were inactive because of cool temperature, in order to make sure that complete colonies were collected. Cork nests were destroyed and colonies were forced to move to artificial microscope-slide nests. Workers, sexual and worker pupae and larvae were counted. There were no sexual adults yet. Brood production is highly synchronized during Summer (July-August) in *Temnothorax* (Headley 1943; Kipyatkov 1993; Modlmeier and Foitzik 2011), therefore the individuals that we recovered as pupae most likely reflected the total production of the colonies. We allowed pupae to molt into adults in order to assess the sex of the brood. To do so, colonies were kept in the laboratory at room temperature (21°C ± 2°C) with a natural photoperiod. Water was provided *ad libitum* and colonies were fed once a week with half a freshly killed mealworm. Emerging adults were killed by freezing and then stored in 96° ethanol. The workers, males and gynes counted as 'newly produced' by colonies were individuals that emerged in the laboratory after developing into pupae in the field. All adults had emerged by August 16th and all remaining individuals were killed.

We considered that colonies had survived if they were recovered in July and if their queen was still alive. This loose definition of survival is an over-estimate relative to the proper life-history trait 'colony survival', as some colonies may actually have escaped from the bucket (entirely or only a fragment containing the queen, although this is highly unlikely because the bottom side of the lid was covered with a slippery coating – see Methods), and some colonies could have recovered from orphanage by later adopting a new fertilized queen (Foitzik and Heinze 2000). Queenless colonies were excluded from the analyses. Mortality rate of adult workers was computed as 1 - (number of workers retrieved on July 16th / initial workforce). Indeed, it is very unlikely that any workers were produced during this period (Headley 1943; Kipyatkov 1993; Modlmeier and Foitzik 2011). We also counted and measured the workers and sexuals (gynes and males) produced out of the pupae brought back to the laboratory. Traits related to colony growth were the mortality rate of adult workers, the number of workers produced, the mean size of the workers produced and its coefficient of variation (CV). Traits related to reproductive success were the number of produced sexuals (gynes and males), the mean size of the sexuals, and the sexual investment (number of sexuals / number of workers produced). All three main fitness components of colonies were thus measured.

Morphological measurements

The workers and sexuals that emerged in the laboratory were measured. Heads were separated from bodies. Heads were stuck on double-sided tape and photographed under a Discovery.V12 Zeiss stereomicroscope connected to a computer via a Zeiss AxioCam ICc 5 video camera. Head width, a standard measurement of body size in ants (Tschinkel et al. 2003), was measured with ImageJ 1.8 (Abràmoff et al. 2004) and measurements were blind to treatment.

Statistical analysis

We investigated the effect of treatments (predictor variable, four levels) on colony survival, traits related to colony growth (mortality rate of adult workers, number of new workers produced, size of the produced workers and CV of the size of produced workers) and reproductive success (number of produced sexuals, size of the sexuals and its CV, sexual investment). The mortality rate of adult workers and the number of produced individuals were also used as predictor variables in some models (Tables 1 and 2). So, in addition to treatments, we considered four other predictor variables: the initial workforce (number of workers in a colony after the experimental removal of 50% of workers), the initial number of larvae in March, the mortality rate of adult workers and the number of produced workers and sexuals. All first-order interactions between treatment and these variables were also included.

All statistical analyses were carried out with R v3.6.1 (www.r-project.org). All plots were generated using ggplot2 (Wickham 2009). First, we tested the effect of predictor variables on colony-level dependent variables (mortality rate of adult workers, number of produced workers and males, sexual investment, and size and CV of the produced individuals) using generalized linear models (GLMs). Counting data (number of produced individuals and sexual investment) were analyzed using quasipoisson distribution. Mortality rate of adult workers and CV were analyzed using Gaussian distribution. Finally, we tested the effect of predictor variables on the head size of emerged individuals, an individual-level dependent variable, using linear mixed models (LMMs, package *nlme*; Pinheiro et al 2016), with colony as random factor. All models are summarized in Table 1 and 2.

For each analysis, the minimum adequate model was selected using a backward stepwise approach where predictor variables were removed one by one from a full model based on a log likelihood ratio test. We used log likelihood ratio tests, i.e. F-tests for Gaussian distribution or Chi^2 tests for quasi-poisson distribution, to obtain the *P*-values for each predictor variable by comparing the minimum adequate model with a model excluding or including the variable of interest (according to whether the variable was present in or absent from the minimal adequate model respectively). Normality of the residuals and homogeneity of variances were checked visually following Pinheiro and Bates (2000); no transformation of the data was necessary.

Results

Effect of treatments on colony survival

Only five of the 80 manipulated colonies could not be recovered during colony collection in July (three colonies 50S, one colony 50L and one colony 25L25S). Six of the 75 recovered colonies had lost their queen (three colonies 50R and three colonies 50L). These queenless colonies were excluded from the statistical analysis. Our final dataset consisted of 69 queenright colonies.

Effect of treatments on growth

The mortality rate of adult workers depended on the initial number of larvae in interaction with the treatment (treatment x initial larvae interaction, $F_{57:60} = 2.95$, $P = 0.04$). The mortality rate of adult workers decreased with the initial number of larvae with a steeper slope in treatment 50S (figure 2a). The mortality rate of adult workers did not differ among the treatments ($F_{57:66} = 1.94$, $P = 0.063$; figure 1a). Predictors that had a marginal effect (0.05 < P-value < 0.07) and could therefore slightly affect the dependent variable were retained in the minimum adequate model.

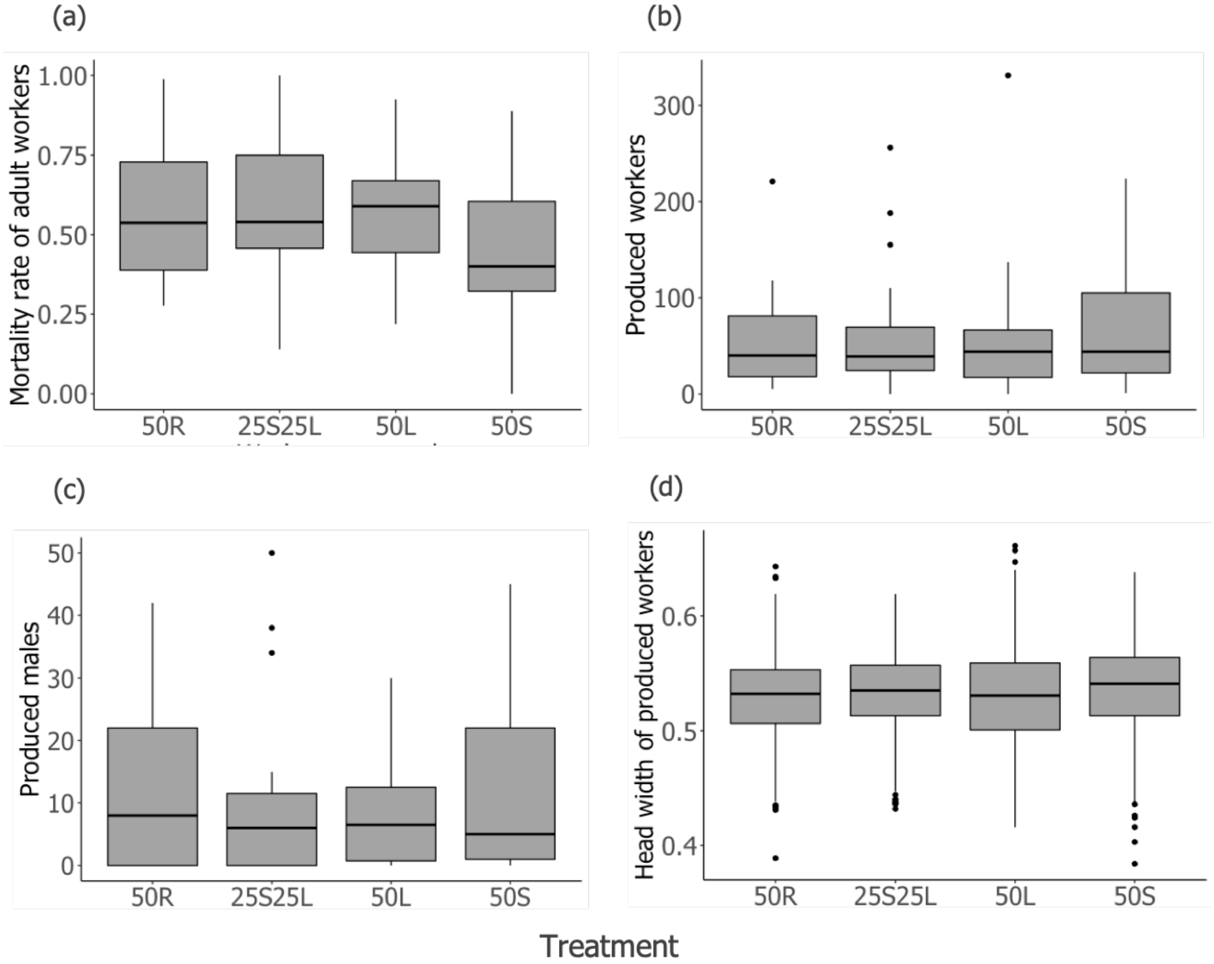

Figure 1: Boxplots comparing the effects of the four treatments (50R: 50% of randomly removed workers; 25S25L: the 25% smallest and the 25% largest removed workers; 50L: the 50% largest removed; 50S: the 50% smallest removed) on four life history traits (a) mortality rate of adult workers, (b) number of produced workers, (c) number of produced males and (d) head size of produced workers (mm). Boxes show median, quartiles and extremes. Statistics are presented in Table 1 for workers and Table 2 for males.

A total of 4 341 workers emerged in the laboratory from 66 queenright colonies (3 colonies did not produce any worker). The number of produced workers was positively correlated with the initial workforce ($F_{66:67}$ = 51.81, P < 0.001; figure 2b) and negatively correlated with the mortality rate of adult workers ($F_{66:67}$ = 4.44, P = 0.04), without significant interaction with the treatments (see Table 1). The number of produced workers did not differ among the treatments ($F_{66:63}$ = 0.38, P = 0.8; figure 1b). The coefficient of variation of the size of produced workers did not differ among the treatments ($F_{63:60}$ = 0.39, P = 0.8), and it was not explained by any other predictor (see Table 1). The size of produced workers increased with the number of produced workers (mixed effect model: χ^2_1 = 38.51, P < 0.001; figure 3a) and with the initial number of larvae (χ^2_1 = 3.79, P = 0.05). The treatments had no effect on the size of produced workers (χ^2_3 = 3.25, P = 0.3; figure 1d). Detailed statistics are presented in Table 1.

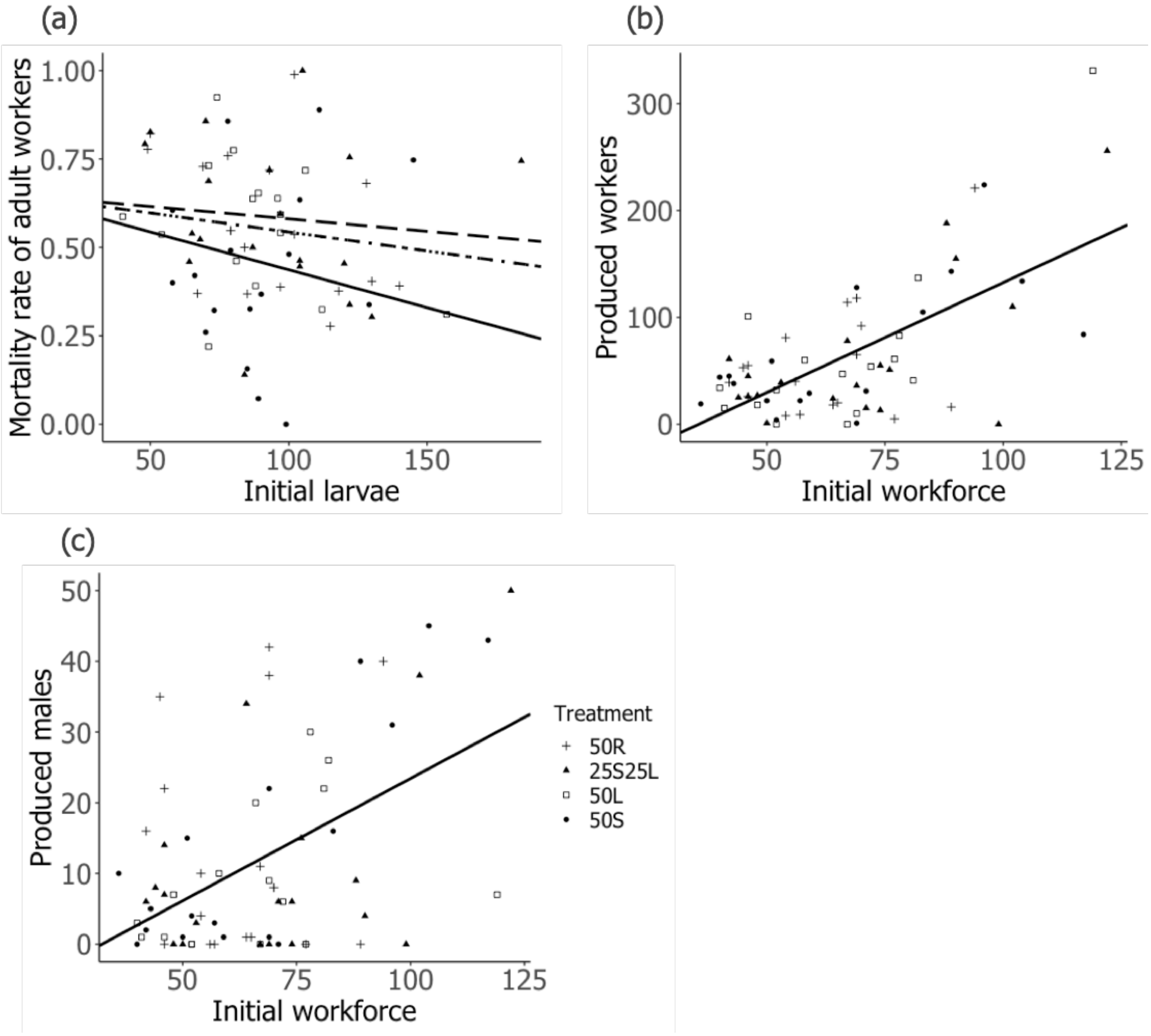

Figure 2: Correlation between the initial larvae and workforce with three different traits. When the initial larvae increased, (a) the mortality rate of adult workers (50S: solid line type, estimate= $-2.141e^{-3} \pm 1.14e^{-3}$; 50R: dotdash, estimate = $-1.072e^{-3} \pm 1.08e^{-3}$; 50L: dotted, estimate = $-1.074e^{-3} \pm 1.16e^{-3}$; 25L25S: longdash, estimate = $-0.701e^{-3} \pm 1.03e^{-3}$). When the initial workforce increased, (b) the number of produced workers increased similarly for all treatments, and (c) the number of produced males increased similarly for all treatments. The regression lines are drawn from the coefficients of the different models. Statistics are presented in Table 1 for workers and Table 2 for males.

Effect of treatments on reproductive success

No gynes were produced except for three in one colony of treatment 50S. However, 57 queenright colonies produced 809 males. The number of males was positively correlated with the initial workforce ($F_{67:68} = 20.11$, $P < 0.001$; figure 2c) but was not affected by the treatment ($F_{67:64} = 1.22$, $P = 0.3$). The size of produced males was positively correlated with the number of males ($\chi^2_1 = 4.03$, $P = 0.04$; figure 3b). The sexual investment (number of sexuals / number of produced workers) and the CV of the size of produced males were not explained by any predictor. Detailed statistics are presented in Table 2.

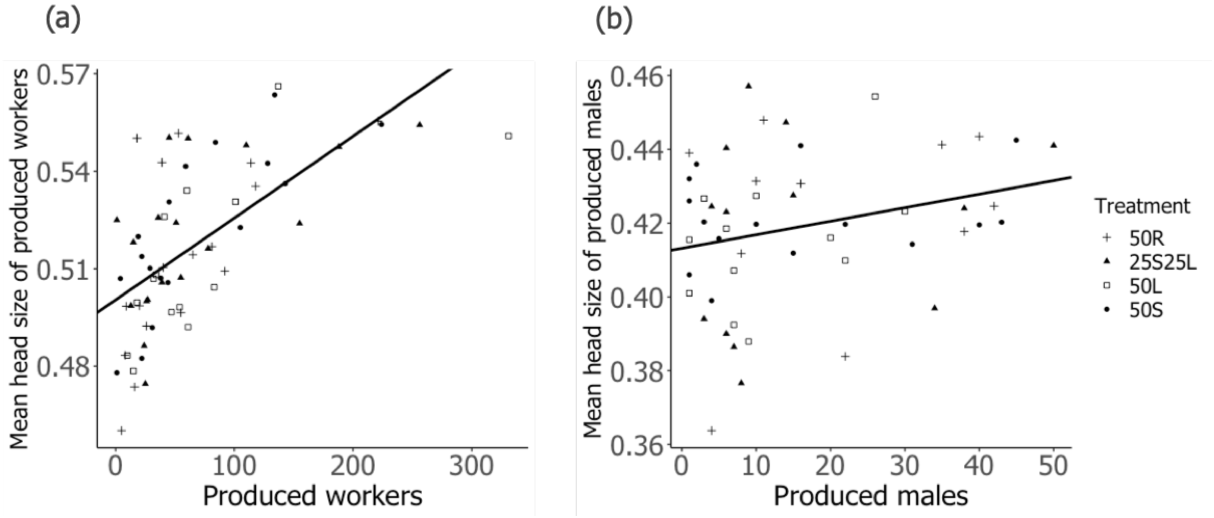

Figure 3: Correlation between the mean head size (mm) and the number of (a) produced workers and (b) produced males. The regression lines are drawn from the coefficients of the models and did not differ among treatments. Statistics are presented in Table 1 and Table 2.

Table 1: Models and statistics related to colony growth. 'Minimum model' means that the predictor was retained in the minimum model selected. A mixed effect model with colony as random factor was used for the size of produced workers in order to control for the colony of origin of workers.

Predictors	**Response variables**			
	Mortality rate of adult workers	Produced workers	CV of produced workers	Size of produced workers
Treatment	Minimum model $F_{57:66} = 1.94$, $P = 0.063$	$F_{66:63} = 0.38$, $P = 0.8$	$F_{63:60} = 0.39$, $P = 0.8$	$\chi^2_3 = 3.25$, $P = 0.3$
Initial workforce	Minimum model $F_{57:61} = 2.31$, $P = 0.069$	**Minimum model** **$F_{66:67} = 51.81$,** **$P < 0.001$**	$F_{63:62} = 0.02$, $P = 0.9$	$\chi^2_1 = 0.002$, $P = 0.9$
Treatment - initial workforce interaction	Minimum model $F_{57:60} = 2.52$, $P = 0.067$	$F_{66:63} = 0.50$, $P = 0.7$	$F_{63:59} = 0.43$, $P = 0.8$	$\chi^2_4 = 4.24$, $P = 0.4$
Initial larvae	Minimum model $F_{57:61} = 2.28$, $P = 0.07$	$F_{66:65} = 1.94$, $P = 0.2$	$F_{63:62} = 0.22$, $P = 0.6$	**Minimum model** **$\chi^2_1 = 3.79$, $P = 0.05$**
Treatment - initial larvae interaction	**Minimum model** **$F_{57:60} = 2.95$,** **$P = 0.04$**	$F_{66:62} = 0.55$, $P = 0.7$	$F_{63:59} = 0.54$, $P = 0.7$	$\chi^2_3 = 4.42$, $P = 0.22$
Mortality rate of adult workers		**Minimum model** **$F_{66:67} = 4.44$,** **$P = 0.04$**	$F_{63:62} = 0.05$, $P = 0.8$	$\chi^2_1 = 0.49$, $P = 0.5$
Treatment - mortality rate of adult workers interaction		$F_{66:63} = 0.40$, $P = 0.7$	$F_{63:59} = 0.33$, $P = 0.8$	$\chi^2_4 = 4.95$, $P = 0.3$
Produced workers			$F_{63:62} = 0.26$, $P = 0.6$	**Minimum model** **$\chi^2_1 = 38.51$, $P < 0.001$**
Treatment - produced workers interaction			$F_{63:59} = 1.01$, $P = 0.4$	$\chi^2_3 = 3.30$, $P = 0.3$

Table 2: Models and statistics related to reproductive success. 'Minimum model' means that the predictor was retained in the minimum model selected. A mixed effect model with colony as random factor was used for the size of produced males in order to control for the colony of origin of males.

Predictors	**Response variables**			
	Males produced	Sexual investment	CV of produced males	Size of produced males
Treatment	$F_{67:64} = 1.22$, $P = 0.3$	$F_{65:62} = 0.23$, $P = 0.9$	$F_{45:42} = 0.32$, $P = 0.8$	$\chi^2_3 = 0.22$, $P = 0.9$
Initial workforce	**Minimum model** $\boldsymbol{F_{67:68} = 20.11}$, $\boldsymbol{P < 0.001}$	$F_{65:64} < 0.001$, $P = 0.3$	$F_{45:44} = 0.20$, $P = 0.6$	$\chi^2_1 = 0.38$, $P = 0.5$
Treatment - initial workforce interaction	$F_{67:64} = 1.11$, $P = 0.3$	$F_{65:61} = 0.39$, $P = 0.9$	$F_{45:41} = 0.37$, $P = 0.8$	$\chi^2_4 = 1.15$, $P = 0$ .9
Initial larvae	$F_{67:66} = 0.19$, $P = 0.7$	$F_{65:64} < 0.001$, $P = 0.9$	$F_{45:44} = 2.54$, $P = 0.1$	$\chi^2_1 = 0.02$, $P = 0.9$
Treatment - initial larvae interaction	$F_{67:63} = 1.28$, $P = 0.3$	$F_{65:61} = 0.24$, $P = 0.9$	$F_{45:41} = 1.08$, $P = 0.4$	$\chi^2_4 = 0.46$, $P = 0.9$
Adult worker mortality rate	$F_{67:66} = 0.07$, $P = 0.8$	$F_{65:64} = 0.36$, $P = 0.3$	$F_{45:44} = 0.08$, $P = 0.8$	$\chi^2_1 = 0.72$, $P = 0.4$
Treatment - adult worker mortality rate interaction	$F_{67:63} = 0.51$, $P = 0.7$	$F_{65:61} = 0.73$, $P = 0.7$	$F_{45:41} = 0.08$, $P = 0.9$	$\chi^2_4 = 1.78$, $P = 0.8$
Males produced			$F_{45:44} = 1.32$, $P = 0.3$	**Minimum model** $\boldsymbol{\chi^2_1 = 4.03}$, $\boldsymbol{P = 0.04}$
Treatment - males produced interaction			$F_{45:41} = 0.43$, $P = 0.8$	$\chi^2_3 = 0.12$, $P = 0.9$

Discussion

In this study, we aimed at quantifying the impact of worker size and size diversity within colony on colony fitness using a laboratory manipulation followed by a reintroduction in the field. Because brood production is synchronized in *Temnothorax nylanderi* (Headley 1943; Kipyatkov 1993), we were able to measure both colony growth and sexual investment in addition to survival, and thus to extensively quantify fitness. In agreement with a previous laboratory study (Colin et al. 2017), reducing worker size diversity did not affect colony fitness. Colony survival, reproductive success (the number of males and the investment in sexuals) and colony growth (the number of produced workers) did not differ among the four treatments (removal of large, small, large and small, or random workers). Surprisingly, treatments that reduced or increased mean worker size did not affect fitness either. We also found that colonies did not compensate for the size of the removed workers by producing workers of the same size, which further suggests that worker size diversity has no strong effect on colony fitness.

The initial workforce of colonies had a major impact on colony fitness in our experiment, which is not the case in other species of the *Temnothorax* genus (Dornhaus et al. 2008; Bengston and Dornhaus 2013). Indeed, we found a positive relationship between the initial workforce and the number of produced workers and males. This confirms that in *T. nylanderi*, large groups grow and reproduce better (Foitzik and Heinze 2000; Foitzik et al. 2003). Only one colony produced gynes. This might be a consequence of the experimental removal of half of the workers from each colony, which resulted in a loss of workforce and a redirection of resources towards workers and males, as already known in *T. nylanderi* (Foitzik and Heinze 2000; Foitzik et al. 2003).

Our results are in line with Colin et al. (2017), who found no impact of reducing worker size diversity on colony performance in the laboratory in the same species, and Modlmeier & Foitzik (2011) who found no correlation between worker size diversity and per capita productivity in the field in *T. longispinosus*. Modlmeier & Foitzik (2011) also found no effect of the mean worker size on colony productivity. Our experimental manipulation of mean worker size confirms that it does not affect colony fitness. Moreover, colonies do not compensate for the size of the removed workers, reinforcing the idea that worker size diversity is not of primary value to them. Billick (2002) found a similar pattern in *Formica neorufibarbis*. He collected 45 workers from field colonies, and removed the 15 largest workers (7% of colony size). He followed colony development for 35 days and found no consequence on the number of pupae produced per worker. In addition, although a positive relationship between initial workforce and both mean worker size and worker size diversity is usually found in ants (Porter and Tschinkel 1985a; Ferguson-Gow et al. 2014), we did not find such a correlation. Our results hence confirm that this trait is not of primary importance for colonies. For the first time, our study proves the absence of effect of both mean worker size and worker size diversity in the field, over a long period of time, and with an extensive measure of colony fitness (three main life-history traits), making it a highly reliable result. We discuss three non-mutually exclusive hypotheses that could explain why within-colony worker size diversity still occurs.

First, worker size diversity could only bring advantages in the context of harsher environmental conditions. In *T. nylanderi*, large workers resist better to starvation (Modlmeier et al. 2013). In the closely related *Leptothorax acervorum*, larger individuals survive longer in cold temperature (Heinze et al. 2003). Based on our results, such individual-level response (individual survival) does not seem to translate to the group level (colony survival). Further experiments are needed to fully explore the role of size diversity in the context of harsher environmental conditions (e.g. during overwintering) or over a full life cycle.

Second, size diversity could result from relaxed selection. Group living can buffer selective pressures on individual development because (1) phenotypes can diverge and still be viable under the protection of the social environment (Molet et al. 2012) and (2) workers themselves can control the environment of developing larvae and adjust their development if needed (Smith et al. 2008; Segers et al. 2015). This could thus decrease the level of canalization (the ability of development to remain unchanged despite environmental or genetic variation; Debat and David 2001), and thereby increase worker size diversity. The cost of maintaining developmental canalization (Van Buskirk and Steiner 2009) could be outbalanced by the lack of benefits that it brings in a social context. Accordingly, size diversity, in species where it is moderate, could be not adaptive *per se* but just economical to avoid the costs of canalization (Hunt et al. 2011; Colin et al. 2017).

Third, larvae could exhibit selfish strategies (Amor et al. 2011, 2016). Larvae may seek to receive more resources in order to become larger individuals, and increase their direct fitness by laying male eggs (Stroeymeyt et al. 2007). Heinze & Oberstadt (1999) showed that large workers of the ant *Leptothorax gredleri* (closely related to *T. nylanderi*) have higher social ranks than small ones, so they are more likely to be the ones that lay male eggs. It would be interesting to compare larval development in the presence or absence of workers to shed light on this potential conflict. In *Ectatomma tuberculatum*, larval begging does not fully reflect the nutritional status, suggesting that larvae do cheat to receive more food (Sauvaget 2017).

The ecological success of *T. nylanderi* (up to 10 colonies per m^2: Heinze et al. 1996 - with a wide distribution throughout Western European forests, woods and parks: Csosz et al. 2015; Khimoun et al. 2020), probably originates from unassessed traits other than mean worker size or worker size diversity, such as behavioral diversity. Indeed, *Temnothorax* ants demonstrate highly flexible and context-dependent task specialization, (Pinter-Wollman et al. 2012) and they can quickly respond to changes in the required tasks (Robinson et al. 2009). Colonies with high within-colony behavioral diversity are more productive than colonies with less variation (Modlmeier et al. 2012). This may apply more generally to social insects. Indeed, in a ponerine ant lacking size diversity, age-associated division of labour is highly flexible. Foragers can revert to nursing and egg laying (Bernadou et al. 2015). Moreover, the functioning of honeybee societies is based on the coexistence of physiologically specialized individuals that perform fixed functions such as nectar processing, and physiologically plastic individuals that are also more flexible behaviorally (Johnson 2003). To summarize, the ecological success of social insects with high size-diversity could come from their sharp division of labour in relation with workers morphology (with the exception of *Veromessor pergandei* and *Pheidole dentata*, respectively Rissing and Polloek 1984; Calabi and Traniello 1989), whereas the success of species with moderate size diversity could result from their behavioral ability to quickly adapt to specific contexts.

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Author’s contributions

RH designed the study, collected and reared ants, performed the experiment and statistical analyses, and wrote the manuscript. CD designed the study, contributed to statistical analyses and wrote the manuscript. MM designed the study, wrote the manuscript and supervised the project. All authors read and approved the final manuscript.

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Data availability

The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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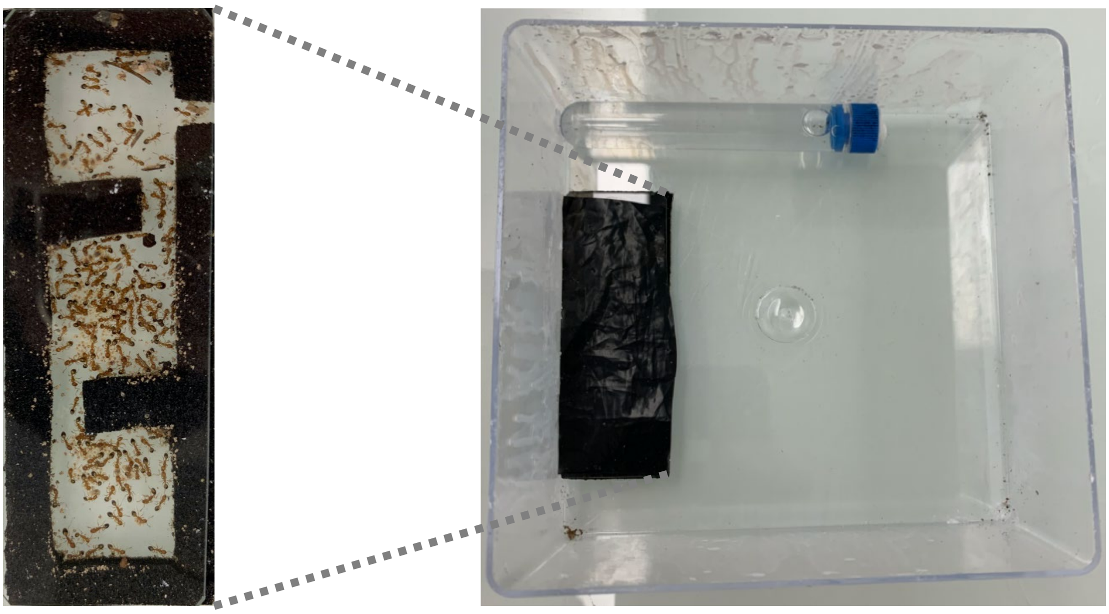
Appendix

Appendix A1: Worker size diversity within one colony of *Temnothorax nylanderi*. In our experiment, the head width of the smallest worker produced was 0.384mm, that of the largest was 0.661mm.



Appendix A2: Laboratory rearing setup. The nest consisted of two microscope slides separated by a 1-mm auto-adhesive plastic foam with three chambers, covered with black paper for darkness. It was placed in a plastic box (foraging area) with water (tube plugged with cotton) and food (mealworm).

Appendix A3: Field rearing setup. Eighty semi-buried buckets were installed in the 'réserve ornithologique du Bois de Vincennes'. Each bucket contained local soil and was pierced at the bottom for water draining. Six nests (drilled corks) were available for the colony to choose. The foraging area was restricted to the bucket. Cut-off lids are not present on the photos.

Article 2 : en cours de révision après une première soumission.

Worker size has no effect on overwintering success under natural conditions in the ant *Temnothorax nylanderi*.

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Abstract

Winter is a difficult period for animals that live in temperate zones. It can inflict high mortality and it has consequences on performance during the growing season. Social groups include individuals of various ages and sizes. This diversity may improve the ability of groups to buffer winter disturbances such as starvation or cold temperature. Studies focusing on the buffering role of worker size under winter conditions are mainly performed in the laboratory and investigate the effect of starvation or cold separately. Here, we experimentally decreased worker size diversity and manipulated worker mean size in order to study the effect on overwintering survival in the ant *Temnothorax nylanderi*. Colonies were placed under natural conditions during winter. We found a relatively high colony survival after winter. We found no effect of worker size diversity and mean worker size. However, the number of workers in colonies had a positive effect on brood survival. Our results show that the higher resistance of larger individuals against cold or starvation stresses applied separately in the laboratory does not transpose to the colony-level in the field. We discuss the mechanisms that could maintain non-adaptive size diversity and the factors that enhance winter survival in this social species.

Key-words: social insects, mean body size, size diversity, survival, winter, colony size

Introduction

Winter is a season of resource scarcity, desiccation and cold temperatures that strongly affects the physiology of individuals. It causes drastic decreases in metabolic reserves (Hahn & Denlinger, 2007; Sinclair, 2015), water content (Danks, 2000) or immune defenses (Vesterlund *et al.*, 2014; Ferguson & Sinclair, 2017). To cope with winter, poikilotherm organisms such as insects have developed different strategies. They can either migrate to milder habitats, or produce specific winter-adapted individuals that differ from the summer individuals when the life cycle includes several generations, or finally overwinter (Leather *et al.*, 1995). Overwintering involves physiological modifications that lead to growth interruption or slowdown and to resistance to low temperatures, freezing, and other winter-associated constrains (Tauber *et al.*, 1986; Leather *et al.*, 1995). For example, some insects synthesize cryoprotectants that restrict freezing to the extracellular matrix (Bale & Hayward, 2010; Toxopeus & Sinclair, 2018), while others produce antifreeze proteins (Graether & Sykes, 2004; Bale & Hayward, 2010). Overwintering also has indirect consequences on the future survival and reproductive success of individuals as it strongly affects the amount of energy available at the end of winter for the growing and reproductive seasons (Sinclair, 2015; Williams, Henry, & Sinclair, 2015).

Social life could play a buffering role against harsh winter conditions. First, in social groups, the exchange of food (trophallaxis; Heinze, Stahl, & Hölldobler, 1996; Crailsheim, 1998; Machida *et al.*, 2001; Rueppell & Kirkman, 2005) or the role of brood as food reserve (Schmickl & Crailsheim, 2001; Rueppell & Kirkman, 2005; Chouvenc, 2020) can reduce the impact of starvation during hibernation. Several studies showed that individuals survive starvation better in group than alone (Heinze *et al.*, 1996; Boulay *et al.*, 1999; Modlmeier, Foitzik & Scharf, 2013). For instance, workers in colonies with a mix of brood and adults survive starvation for a longer period (Modlmeier *et al.*, 2013). Larger individuals or workers with specific morphology can store food (Hasegawa, 1993; Børgesen, 2000; Yang, 2006) and are known to survive longer to starvation in ants (Heinze *et al.*, 2003; Modlmeier *et al.*, 2013). In addition to starvation, social life could also improve resistance to cold temperatures. Some social species build elaborate nests (review in: Theraulaz, Bonabeau, & Deneubourg, 1998) that can buffer external disturbances. Moreover nest temperature can be regulated with specific individual behaviors (review in: Jones & Oldroyd, 2006 - Tan *et al.*, 2012; Kadochová & Frouz, 2013; Fuller & Postava-Davignon, 2014; Jandt & Dornhaus, 2014). For instance, individuals in colonies are able to form clusters to protect adults or offspring from cold temperature (Heinze *et al.*, 1996; Stabentheiner *et al.*, 2003). Finally, the size of workers could also affect their resistance to temperature. In *Solenopsis invicta*, smaller individuals freeze at lower temperature (Hahn, Martin, & Porter, 2008) while in *Leptothorax acervorum*, larger workers survive longer to cold temperature (Heinze *et al.*, 2003).

Although previous studies show that worker size diversity can improve resistance to starvation and cold temperature as isolated stresses in laboratory experiments (e.g. Francke, Cokendolpher & Potts, 1986; Heinze *et al.*, 2003; Quarles, Kostecke & Phillips, 2005; Hahn *et al.*, 2008; Couvillon & Dornhaus, 2010; Modlmeier *et al.*, 2012, 2013; Oyen, Giri & Dillon, 2016; Baudier & O'Donnell, 2018; Oyen & Dillon, 2018; Sánchez-Echeverría *et al.*, 2019; Yilmaz *et al.*, 2019) it is still unclear whether it could provide colony-level benefits during realistic multifactorial overwintering conditions. Hibernation can induce both worker mortality (Herbers & Johnson, 2007; Sorvari, Haatanen & Vesterlund, 2011; Mitrus, 2013) and even high colony mortality in ants and honey bees (Morrill, Martin & Sheppard, 1978; Herbers & Johnson, 2007; Currie, Pernal & Guzmán-Novoa, 2010; Vanengelsdorp *et al.*, 2011). To our knowledge, few studies have focused on the role of individual size in the overwintering performance of social insects. They are all based on founding queens of annual wasp and bee species, and they found that larger queens survive winter better, presumably because they have more metabolic reserves (wasp: Cervo *et al.*, 2008, Kovacs & Goodisman, 2012 - bee: Owen, 1988; Beekman, Stratum, & Lingeman, 1998; Bosch & Kemp, 2004; Inoue, 2011). However, in non-annual social insects such as ants, the whole colony hibernates together. This can increase survival by up to 190% compared to an isolated queen (in the laboratory: Shiroto, Satoh, & Hirota, 2011). Therefore, as mentioned above, workers and their diversity probably play an important role against harsh conditions. It is important to assess the role of worker size diversity in a natural environment because different stresses applied simultaneously can trigger trade-off among life-history traits or have opposite effects (e.g. starvation has a positive effect on cold tolerance in silkworm larvae: Mir & Qamar, 2018).

The ant *Temnothorax nylanderi* is an appropriate model to explore this topic because colonies nest above ground in hollow acorns and twigs and are thus exposed to the external weather conditions (Foitzik *et al.*, 2004). Colonies have moderate worker size diversity (Honorio, Doums, & Molet, 2020), lacking discrete worker subcastes. In *T. nylanderi* and in a closely-related species (*Leptothorax acervorum*), larger workers survive longer to starvation and cold (Heinze *et al.*, 2003; Modlmeier *et al.*, 2013). In *Temnothorax* ants, overwintering in the field can lead to 50% queen mortality, and up to 70% worker mortality (Herbers & Johnson, 2007; Mitrus, 2013 - but see Mitrus (2015) where almost no mortality occured). We manipulated both mean worker size and worker size diversity within colony to disentangle the two effects. The study took place in a natural environment for several months to quantify the impact of worker size on colony survival, and to test whether individual-level size-related resistances to cold and starvation observed in the laboratory transpose to colonies as a whole in the field. Recently, Honorio *et al*. (2020) manipulated worker size within colonies of this species in early spring. They found no effect on survival, growth and reproductive success in the field during the growing season. In contrast, during the harsh winter season, we hypothesized that decreasing worker mean size and diversity would decrease worker and colony survival. We also predicted that colonies with more workers or more brood would survive better because colony size buffers disturbances and larvae could be used as food resources.

Material and methods

Study model

One hundred and fifty colonies of the tiny acorn ant *Temnothorax nylanderi* were collected in October 2019 in the "Bois de Vincennes" forest (Paris, France, 48° 50′ 22.14″ N, 2°26′ 51.96″ E). In the laboratory, each colony was transferred to an artificial nest consisting of two microscope slides separated by a 1-mm auto-adhesive plastic foam with three chambers. The nest was placed in a plastic box (11.5 × 11.5 × 5.5 cm) providing a foraging area. Colonies were kept for two weeks in a climatic chamber at 10-12°C with a natural photoperiod mimicking the natural conditions at this time of the year. Water was provided *ad libitum* in a tube plugged with cotton. Workers and brood were counted.

Manipulation of worker size

Out of our 150 colonies, we excluded 31 queenless colonies, four polygynous colonies and seven colonies parasitized by a cestode (Scharf *et al.*, 2012). We selected 80 colonies containing one queen and at least 70 workers. Workers were counted under a binocular microscope. Our manipulation included four treatments that consisted in the removal of 50% of workers from colonies. Each treatment involved 20 colonies. In treatment 25S25L, we decreased worker diversity without changing mean worker size by removing the 25% smallest workers and the 25% largest workers. In treatments 50S and 50L, we decreased worker diversity but also respectively increased mean worker size by removing the 50% smallest workers or decreased mean worker size by removing the 50% largest workers. The last treatment was the control (treatment 50R) where we removed 50% of workers randomly. The worker removal protocols were similar to Colin *et al.* (2017) and Honorio *et al.* (2020) and based on the apparent global body size: the sorting of large and small workers was done by eye under a stereoscopic microscope (Zeiss®, x50 magnification) whereas the removal of random workers was done without a microscope to make sure that worker size could not be evaluated. This method was proven to be reliable (Colin et al. 2017). Colonies were assigned to the four treatments based on their number of workers in order to keep a similar distribution of colony sizes among the four treatments Workers remaining in the colonies after manipulation constituted the initial workforce of the colonies (69.1 workers ± 25.3). Colonies initially contained 79.7 larvae ± 28.3. By removing half of the colony workers to manipulate worker diversity without removing larvae, we initially decreased the worker to brood ratio (initial workforce / initial larvae) within colonies. The worker to brood ratio after manipulation was between 0.47 and 2.21 (mean = 0.90, median = 0.87). Colonies were fed once with half a freshly killed mealworm (*Tenebrio molitor*) before reintroduction into the field.

Colony rearing in the field

We manufactured artificial nests to make them match natural nests as closely as possible, so that colonies could easily live in them after reintroduction in nature (Honorio *et al.*, 2020). For that purpose, we used 2.5x 2cm truncated cone corks. We drilled a 4, 5 or 6mm-wide chamber from the larger side of the cone cork, and plugged this side with a glue gun to seal the gallery. Then, a 1mm-wide entrance tunnel was pierced from the smaller side of the cone cork using a pointed plier in order to connect the chamber to the outside. In the laboratory, six corks (two of each size) were placed inside each plastic box, and we induced the emigration of colonies into the corks of their choice by removing the cover glass of the original nest. Then, the six corks from each plastic box (one containing the colony) were reintroduced in the "réserve ornithologique du Bois de Vincennes" in a semi-buried (10cm deep) 40 x 35cm bucket with a pierced bottom (for water draining) and containing local soil. The bucket lid was cut off into a ring shape and the bottom side was covered with fluon®, a slippery coating, to prevent ant escape while retaining a wide entrance. The six corks were place randomly. This allowed colonies to relocate to the nest of their choice (size and location) inside the bucket whenever they wanted to. Indeed, *T. nylanderi* colonies readily switch nest depending on environmental conditions (Foitzik, Strätz & Heinze, 2003). Because the 80 buckets had been in place for one and a half years, the soil and litter in the buckets was very similar to that of the surrounding forest; many arthropods and soil organisms could come in and out of the buckets. Colonies were left in the buckets from October 30th to March 16th, and subsequently collected and brought back to the laboratory. Corks were collected in the early morning, when workers were inactive because of cool temperature, in order to make sure that complete colonies were collected. Cork nests were destroyed and colonies were forced to move to artificial microscope-slide nests. Workers and larvae were counted.

We considered that colonies had survived if they were recovered in March and if their queen was still alive. This loose definition of survival is an over-estimate relative to the proper life-history trait 'colony survival', as some colonies may actually have escaped from the bucket (entirely or only a fragment containing the queen, although this is highly unlikely because the bottom side of the lid was covered with a slippery coating – see Methods), and some colonies could have recovered from orphanage by later adopting a new fertilized queen (Foitzik and Heinze 2000). Seven colonies lost their queen during overwintering (four 50S colonies, one 25L25S colony, one 50L colony and one 50R colony) and were excluded from the analyses of worker and brood survival. These colonies were mainly the ones that lost the most workers and larvae, suggesting imminent colony death. The variation rate of the number workers was computed as: ((number of workers retrieved on March 16th – initial workforce) / initial workforce) x 100. The variation rate of number of larvae was computed as: ((number of larvae retrieved on March 16th – initial larvae) / initial larvae) x 100.

Statistical analysis

We investigated the effect of our manipulation (predictor variable, four levels: 25S25L, 50S, 50L, 50R) on two dependent variables: the variation rate of worker number and the variation rate of larva number. In addition to treatment, we considered two others predictor variables: the initial workforce and the worker to brood ratio. Because of strong collinearity (Variance inflation factor > 5; Fox & Weisberg, 2019), we did not add the predictor variable 'initial larvae' which was highly correlated with 'initial workforce' (Spearman correlation, $r_s = 0.76$, $P < 0.001$). The interaction between treatment and initial workforce was also included. Regarding the variation rate of larva number, we included the variation rate of worker number as a predictor variable because it reflects the loss of workforce available for larval rearing at the end of the experiment. The two models are summarized in table 1.

All statistical analyses were carried out with R v3.6.1 (www.r-project.org). We performed a Fisher's exact test (for small sample size) to compare colony survival among treatments. For other traits (variation rates of worker number and larva number), we used generalized linear models (GLMs), with Gaussian distribution (by visually checking data distribution). Normality of the residuals and homogeneity of variances were checked visually following Pinheiro & Bates (2000); no transformation of the data was necessary. For each analysis, the minimum adequate model was selected using a backward stepwise approach where predictor variables were removed one by one from a full model based on a log likelihood ratio test (Fisher test). We used log likelihood ratio tests to obtain the *P*-values for each predictor variable by comparing the minimum adequate model with a model excluding or including the variable of interest (depending on whether the variable was present in or absent from the minimal adequate model respectively). All plots were generated using ggplot2 (Wickham, 2009).

Results

We recovered 78 colonies out of 80 at the end of the experiment; the two missing colonies (50L) underwent external disturbances (corks pulled out of the bucket by an animal). Seven colonies lost their queen during overwintering (see Material and Methods for details). Colony survival was therefore very high and did not differ among treatments (Fisher's exact test, $P = 0.45$). The seven queenless colonies were excluded from the statistical analyses. Our dataset for subsequent analyses thus consisted of 71 queenright colonies. Colonies lost on average 4.4% (± 20.2% sd) of workers. Quite surprisingly, 39% of queenright colonies gained workers (with five colonies gaining more than 20% workers). The variation rate of worker number did not differ among treatments ($F_{70;67} = 0.87$, $P = 0.5$; figure 1a) and was not explained by any predictor variable (table 1). Colonies gained on average 0.53% (± 32.0%) of larvae. The variation rate of larva number increased with the worker to brood ratio, meaning that colonies with a higher number of workers relative to larvae managed to increase larva survival ($F_{68;69} = 9.64$, $P = 0.002$; figure 2a). It also increased with the variation rate of worker number, meaning that colonies with higher worker survival also had higher larva survival ($F_{68;69} = 70.9$, $P < 0.001$;

figure 2b). However, the variation rate of larva number did not differ among treatments ($F_{68;65}$ = 0.48, P = 0.7; figure 1b). Statistics are detailed in table 1.

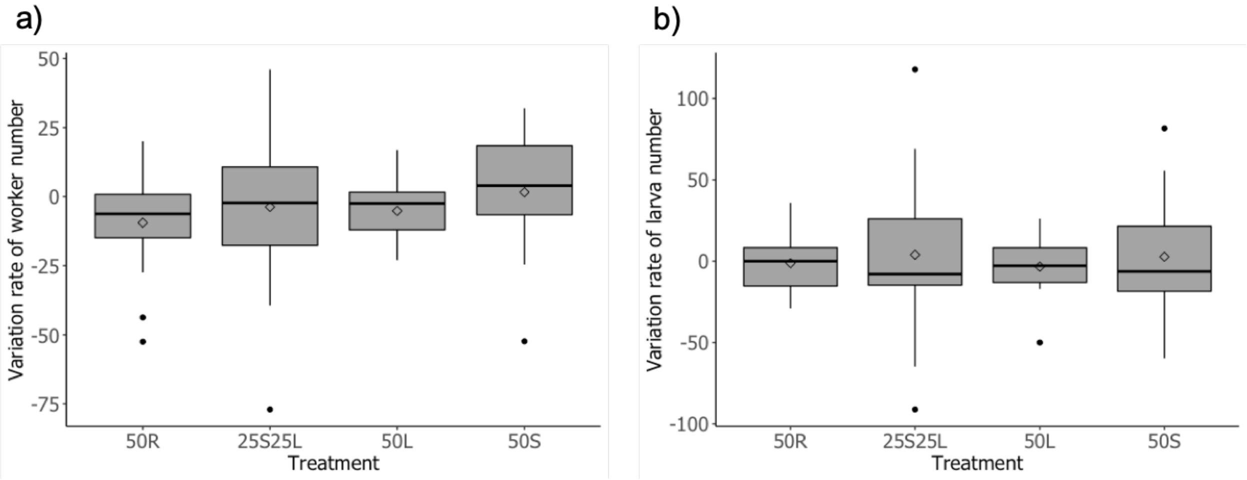

Figure 1: Boxplots comparing the effects of manipulation (four levels) on (a) the variation rate of worker number and (b) the variation rate of larva number. 50R: random removal of 50% of workers; 25S25L: removal of the 25% smallest and the 25% largest workers; 50L: removal of the 50% largest workers; 50S: removal of the 50% smallest workers. Boxes show median, quartiles and extremes. Mean is represented by empty diamond. Treatment had no effect. Statistics are presented in table 1.

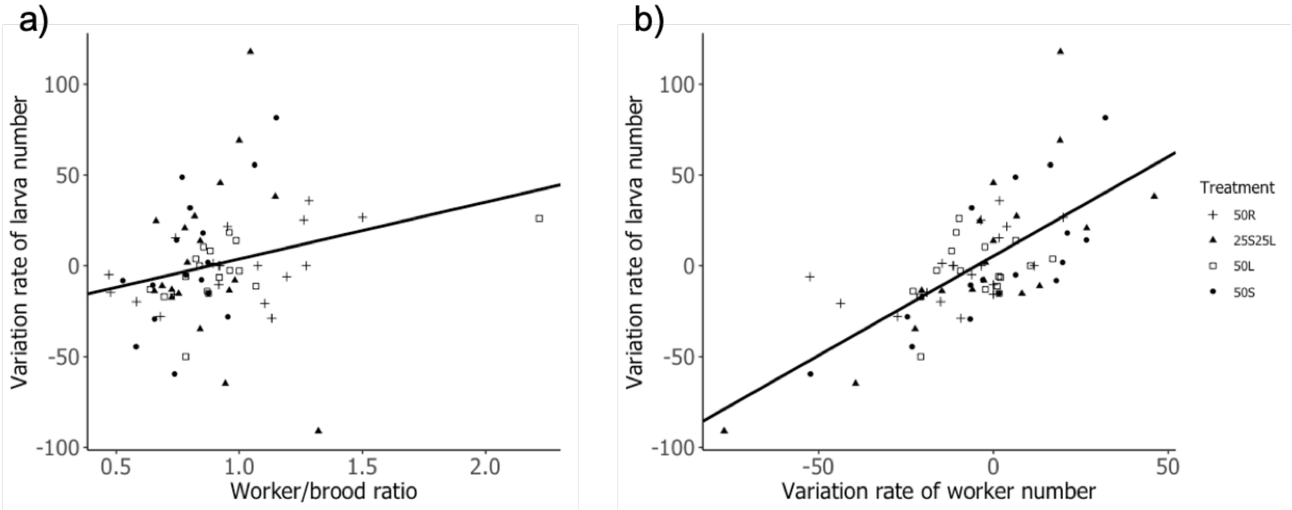

Figure 2: Correlation between the variation rate of larva number and two social traits. The variation rate of larva number increased with (a) the worker to brood ratio (intercept = -27.59, slope = 31.35) as well as (b) the variation rate of worker number (intercept = 5.35, slope = 1.09). The regression lines are drawn from the coefficients of the model. Statistics are presented in table 1.

Table 1: Models and statistics related to the variation rates of worker and larva numbers. 'Minimum model' means that the predictor was retained in the selected minimum model.

Predictor variables	**Dependent variables**	
	Variation rate of worker number	Variation rate of larva number
Treatment	$F_{70;67} = 0.87$, $P = 0.5$	$F_{68;65} = 0.48$, $P = 0.7$
Initial workforce	$F_{70;69} = 0.10$, $P = 0.7$	$F_{68;67} = 0.06$, $P = 0.8$
Treatment - initial workforce interaction	$F_{70;66} = 0.92$, $P = 0.4$	$F_{68;64} = 0.35$, $P = 0.8$
Worker/brood ratio	$F_{70;69} = 0.002$, $P = 0.9$	**Minimum model** $\mathbf{F_{68;69} = 9.64}$, $\mathbf{P = 0.003}$
Variation rate of worker number		**Minimum model** $\mathbf{F_{68;69} = 70.9}$, $\mathbf{P < 0.001}$

Discussion

In this study, we experimentally manipulated mean worker size and worker size diversity within colonies of *Temnothorax nylanderi* to quantify the consequences on colony and worker survival during winter in the field. After overwintering, we found an unexpectedly high colony survival rate (91% were recovered and still queenright after overwintering) and an unexpectedly high worker survival (96% on average) in all treatments. Subsequently, we found no effect of the manipulation on colony survival and on the variation rate of worker and larva numbers, highlighting that worker size (mean and diversity) is not a key component to get through winter. However, we found a positive relationship between the variation rate of larva number and (1) the worker to brood ratio, meaning that larvae survive better with more workers and (2) the variation rate of worker number, highlighting that worker survival is positively linked with larva survival. This suggests that brood survival is mostly determined by the workforce available rather than worker size and diversity.

The lack of effect of worker size (mean size and diversity) on overwintering performance is consistent with two other studies performed in the same species: i) a field-based experiment showing no impact on colony survival, growth and reproductive success during the growing season (Honorio *et al.*, 2020) ii) a laboratory study that found no difference in colony performance between colonies with unmanipulated or reduced worker size diversity (Colin *et al.*, 2017). Honorio *et al.* (2020) found that initial workforce was the most impactful social parameter as it was positively linked with colony growth and reproductive success. In contrast with our predictions, initial workforce had no impact on colony mortality during winter. The lack of effect of the number of workers on colony survival is also found in other studies on *Temnothorax* (Rueppell & Kirkman, 2005; Herbers & Johnson, 2007; Mitrus, 2016). The nesting microhabitat seems to be a better predictor for survival in the genus *Temnothorax*; nesting 5cm underground can reduce mortality by half compared to nesting above ground (Mitrus, 2013). Moreover*,* *Temnothorax* colonies have a great ability to scout and assess the quality of potential nests, and they readily relocate to the best one (Pratt & Pierce, 2001; Dornhaus *et al.*, 2004). These relocations can even take place in winter: in *T. curvispinosus*, 20 to 35% of the colonies change nest during cold months (Herbers & Johnson, 2007). Together, our findings and previous studies suggest that neither worker size (diversity and mean), nor colony size had an effect on colony survival during overwintering. However, we must remain cautious and not overlook the fact that the low mortality in our study may have somewhat diluted the potential effects of worker size or colony size.

The high survival of both queens and workers during winter is unexpected. Two previous studies on *Temnothorax* species reported relatively high queen and worker mortality (Herbers & Johnson, 2007; Mitrus, 2013). However, Mitrus (2015) found a very low variation rate of workers during winter with almost no queen mortality. Overwintering mortality could vary greatly over the years depending on how harsh winter is, because *T. nylanderi* colonies nest above ground and directly face external temperatures (Herbers & Johnson, 2007). During our experiment, winter was particularly mild (3°C above average, although temperatures still dropped lower than 0°C on eight nights; source: Météo France). This could explain the low mortality. However, high winter temperatures are not necessarily beneficial for overwintering colonies. Indeed, they could cause an increase in the consumption of metabolic reserves, leading to their early depletion and subsequent mortality (in ants: Sorvari *et al.*, 2011; Haatanen, Ooik & Sorvari, 2015) - more generally in insects: Bale and Hayward, 2010 - and terrestrial organisms: Williams *et al.*, 2015). Nevertheless, overwintering above ground enables *Temnothorax* colonies to become active earlier and to be better prepared for spring activity (MacLean *et al.*, 2017).

Even more surprisingly, we found that some colonies grew over winter. Our field devices prevent the escape of ants from the experimental colonies within the buckets but they remain accessible to ants coming from the outside. A few workers were observed on the bucket lids during the experiment. Ants from foreign colonies could thus enter our experimental colonies. Worker drifting and colony fusion are very common processes in *T. nylanderi*, especially in winter, because of low nest availability (Foitzik & Heinze, 1998, 2000; Foitzik *et al.*, 2007); also observed in various social insects (wasp: Sumner *et al.*, 2007 – bumble bee: O'Connor, Park, & Goulson, 2013 – honey bee: Pfeiffer & Crailsheim, 1999). Although increases in colony size after overwintering could result from the production of new workers in *T. Crassispinus* as Mitrus (2015) suggested, we discard this hypothesis because (1) brood production is a highly synchronized summer process in *T. nylanderi* (Headley, 1943; Kipyatkov, 1993) (2) our colonies contained no nymphs neither before nor after overwintering; and (3) Penick *et al.* (2017) showed that higher temperatures are required for brood development in *Temnothorax*.

The brood played no role in the survival of adults in our study. Rueppell & Kirkman (2005) highlighted that brood in *Temnothorax* can be used as food storage and be cannibalized to increase colony survival during starvation periods. The very high survival rate of workers suggests that they did not need to eat larvae as extra food. Conversely, workers in our study played a role on larval survival, as the positive variation of larva number increased with the worker to brood ratio within colonies (it peaked for a ratio of 1.04) and with the variation of worker number through the winter. Similarly, having a worker to brood ratio of 1 led to better larval survival than a ratio of 0.5 or 5 in *Formica selysi* (Purcell, Brütsch, & Chapuisat, 2012). Twenty eight of our 71 queenright colonies produced new larvae (+1% to +117%) during the winter. This suggests that colonies could use internal resources to produce additional brood without access to food, as observed in the pharaoh's ant *Monomorium pharaonis* (Børgesen, 2000).

Overall, the higher resistance of larger individuals against starvation or cold temperature observed in the laboratory (respectively in *T. nylanderi* and *L. acervorum*: Heinze *et al.*, 2003; Modlmeier *et al.*, 2013) did not transpose to the colony level in the field, highlighting the need to directly evaluate the adaptive value of colony-level traits. Our winter study, coupled with Honorio *et al.*'s growing season study (2020), further questions the role of worker size (mean and diversity) in *T. nylanderi*, and more generally in ant species with moderate worker size diversity. Worker size apparently does not enable better division of labor during the growing season or higher resistance to harsher conditions in winter. In the absence of clear adaptive value, size diversity could result either from selfish larval attempts at developing into larger individuals with developed ovaries (Heinze & Oberstadt, 1999) to get higher inclusive fitness, or from low developmental canalization in a social context (Molet, Wheeler, & Peeters, 2012; Londe *et al.*, 2015; Honorio *et al.*, 2020). Unlike solitary life, social life could buffer external disturbances (e.g. Straub *et al.*, 2015; Molet *et al.*, 2017; Crall *et al.*, 2019) and thus reduce the selective pressures on individuals within the society.

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CHAPITRE 2

Rôle de l'environnement social dans la diversité morphologique intra-coloniale.

Article en préparation pour soumission en *short note* à *Insectes Sociaux*

Development without social coercion: what happens to ant larvae fate?

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Abstract

In social insects, conflicts occur between adults and the young for larval fate. Social environment is known to highly affect produced phenotypes, both in terms of caste and size. However, empirical evidence of this social influence is relatively scarce and indirect, especially in ants where larvae are extremely dependent on workers during development. In this study, we followed the larval development without worker coercion to investigate the larval fate. We used the ant *Mystrium rogeri* where larvae can feed on their own. We performed two treatments: "alone" larvae where manually fed without worker interference, while "workers" larvae where dependent on workers for food intake. We measured weight gain weekly over the larval development. In parallel, natural larval development was followed in an unmanipulated nest. Although our experiment caused high larval mortality, with no pupation, "alone" larvae gained significantly more weight than "workers" larvae from the first week and until the end of experiment. Our data suggest that "alone" larvae can also become fatter than larvae within unmanipulated nests, highlighting possible larger phenotypes than those produced in natural colonies. To our knowledge, this is the first empirical study that removes the social environment during larval development in ants. It shows how the social control of food intake can constrain the produced phenotypes. We discuss the possible consequences of the worker – larva conflict on morphological diversity within colonies.

Key-words: larval development, food intake, plasticity, conflict, produced phenotypes, social environment

Introduction

Animal societies are often likened to models of perfect harmony, where all individuals cooperate for the good of the group. However, although individuals are often strongly related within societies, there are many sources of conflict (Ratnieks et al. 2006). Even high relatedness is not sufficient to avoid individual selfishness. In insect societies, the conflict often lies in the fact that one or few individuals (generally the queen(s)) monopolize reproductive function while the others (workers) are restricted to ergonomic tasks therefore forgoing direct reproduction. Theory predicts that female larvae have greater interest in developing into a queen to obtain higher fitness (Bourke and Ratnieks 1999; Reuter and Keller 2001; Wenseleers et al. 2003). This constitutes a strong conflict regarding female caste fate: larvae may attempt to develop into reproductive individuals in spite of colony interests (Bourke and Ratnieks 1999; Wenseleers et al. 2003, 2005; Wenseleers and Ratnieks 2004; Ratnieks et al. 2006). This attempt to cheat in fate is called selfish larval strategy. For example, this can lead to queen overproduction: up to 21% in *Melipona* bees although only few queens are needed for swarming (Moo-Valle et al. 2001; Wenseleers and Ratnieks 2004), and up to 69% of potential queens are killed immediately after emergence in some *Cataglyphis* ants (Amor et al. 2011). Nevertheless, in the majority of cases, this self-determination is prevented by constraint or coercion that force individuals to develop into workers (Ratnieks et al. 2006).

Constraint and coercion exerted by other colony members (queen or workers) on larvae are mostly genetic and nutritional (food given during larval development (Ratnieks et al. 2006) respectively. These two majors mechanisms govern caste determination, and the importance of each one varies greatly among species (reviewed in Schwander et al. 2010). For instance, caste determination in *Pogonomyrmex* ants has a strong genetic basis. Workers result from a hybridization of cryptic species while queens are from a pure lineage (Helms Cahan et al. 2002; Julian et al. 2002; Volny and Gordon 2002). Conversely in honey bee (*Apis mellifera*), caste determination is mainly influenced by the quality of food provided in the development cell (Haydak 1943; Allsopp et al. 2003; Kamakura 2011). By manually feeding honey bee larvae, (Linksvayer et al. 2011) revealed the existence of intermediate phenotypes between queen and worker (see also Haydak 1943; Weaver 1955; Slater et al. 2020), and highlighted the social control of workers to prevent the production of theses intercastes. Workers may also show aggressive behaviors towards selfish larvae (Wenseleers et al. 2004; Penick and Liebig 2012), possibly even killing them (Villalta et al. 2016).

Although the control expressed by workers over the development of individuals is strong, larvae can sometimes escape this coercion. Such selfish larval strategy is known in several taxa. In stingless bees, dwarf queens are recorded in nine species (Ribeiro et al. 2006). Dwarf queens are smaller than "normal-sized" queens and they develop in worker-intended cells (Ribeiro et al. 2006). For instance, in *Frieseomelitta varia*, worker-intended larvae can bite the neighboring cell and consume the enclosed reserves to develop into queen (Faustino et al. 2002). In some *Cataglyphis* ants, there are two types of queens: short-winged and wingless (Amor et al. 2011). Amor et al. (2016) showed that larvae that developed into short-winged queens received more protein food than those that developed into wingless queens and workers. They suggest that wingless queens, which received the same food quality as workers, are selfish individuals that escape nutritional determination (Amor et al. 2016). Another way to cheat for ant larvae is to beg for extra food from workers. Larval begging signals appear to be an honest communication of larval hunger to nursing workers (Creemers et al. 2003; Kaptein et al. 2005). However, in the context of reproductive conflicts, larval begging is supposed to be a dishonest signal used to manipulate workers into giving more food, to improve their later reproductive opportunities (Creemers et al. 2003; Kaptein et al. 2005).

In case of failure to become a queen, larvae may also have an interest in being large. Indeed, if the colony becomes orphaned, some species set up a hierarchy among workers to determine which one will lay male eggs (Heinze 2008; Blacher et al. 2010; Yagound et al. 2014; Honorio et al. 2019). In *Leptothorax gredleri*, large workers have higher social ranks than small ones, so they are more likely to be the ones that produce males (Heinze and Oberstadt 1999). Moreover, worker size and fertility are positively correlated in several ant species (e.g. Clémencet, Rome, Fédérici, & Doums, 2008; Gobin & Ito, 2003; Modlmeier, Pamminger, Foitzik, & Scharf, 2012; Smith, Schoenick, Anderson, Gadau, & Suarez, 2007). Giehr et al. (2020) highlighted that 25 to 30% of *Temnothorax crassispinus* males are produced by workers in natural populations, suggesting that even in queenright conditions, workers can increase their direct fitness. Thus, this positive relationship between size and fertility could explain worker size diversity within colony in some species. For instance, in *Temnothorax nylanderi*, this size diversity does not improve colonial fitness during the growing season and overwintering (Honorio et al. 2020; Honorio et al. submitted), and remains still unexplained. Therefore, this non-adaptive size could result from a selfish larval strategy to achieve higher direct fitness. Overall, the fate conflict between workers and larvae during larval development could be a driver of morphological diversity within colony. However, empirical evidence quantifying the potential diversity produced by the fate conflict is scarce, due to the technical difficulty to remove the social environment during larval development in highly social species where larvae strongly depend on worker care and feeding.

Here, we aimed at quantifying how larval weight gain throughout development would change in the absence of worker coercion for the access to food in an ant species. This development without worker coercion is relatively unknown in ants. Until now, that type of quantification has only been carried in eusocial (or facultatively) species where larvae develop in cells and feed on the amount of food deposited in those cells, and where it is easier to remove social control. Several studies already manipulated food intake in honey bee *Apis mellifera* (Allsopp et al. 2003; Linksvayer et al. 2011; Slater et al. 2020), sweat bee *Lasioglossum zephyrum* (Roulston and Cane 2002) or paper wasp *Polistes metricus* (Karsai and Hunt 2002; Judd et al. 2015). However, in these species, size diversity is limited compared to ants, where development without closed brood cells allows for a wider range of possible phenotypes (Molet et al. 2012; Londe et al. 2015). We used the ant *Mystrium rogeri* because colonies erratically produce intercastes (Molet et al. 2009; Yoshimura and Fisher 2014; Londe et al. 2015), highlighting possible intermediate phenotypes between queen and worker. Theses intercastes are described as mosaic phenotypes (Molet et al. 2012; Londe et al. 2015 - see also : Heinze 1998), and could be the result of selfish larvae trying to develop into reproductives. Larvae of this species have the advantage of feeding directly on freshly killed prey (Molet, pers com), making manual feeding feasible. We performed two treatments: one where larvae can feed without worker control, and another where larvae are dependent of workers for food intake. We measured larval weight gain weekly. We expected a larger weight gain for larvae that fed in the absence of social control. In parallel, we followed the development of larvae inside unmanipulated colonies. In this case, we also predicted a higher mean weight for larvae fed alone, whereas larvae fed by workers and in the complete colony would have the same mean larval weight across weeks of experiment.

Material et methods

Ants

We used the ant *Mystrium rogeri*. In this species, workers hunt centipedes (Molet et al. 2007) and then place larvae directly on the freshly killed prey. Workers do not perform trophallaxis, so we can strictly control the feeding received by larvae. Workers cannot provide food if there is no prey available. Also, this species shows a clear dimorphism between queen and workers, leading to a wide range of possible intermediate phenotypes (Londe et al. 2015). Seven colonies were collected in a rainforest (21°30’37.3”S, 47°24’29.7”E ± 500m, 989m) in Andrambovato, Madagascar, in November 2017. Four colonies were queenright (BLF40600, BLF40608, BLF40612, BLF40621) and three colonies were queenless (BLF40610, BLF40611, BLF40930). BLF40930 was the unique colony having one intercaste. Colonies contained 57.1 ± 32.7 workers, brood was also counted in the laboratory. Colonies were installed in the laboratory in a 9cm diameter petri dish, made of red plastic to keep ants in the dark. This artificial nest was then placed in a 23 x 17cm plastic box representing the foraging area. The box and the nest were covered with a plaster floor, to maintain high humidity (from 70 to 90% relative humidity). Ants were placed in a climatic chamber heated at 25°C with a natural day-night cycle. Colonies were fed daily with fresh mealworms (*Tenebio molitor*), and had water *ad libitum*.

Experimental protocol

Larvae from original nests were individually isolated in 3.5cm diameter petri dishes, with plaster floor. They were divided in two treatments. Either each larva ate alone (called treatment “alone”, 15 larvae), having the opportunity to store as many resources as she wished; or each larva ate in worker presence (called treatment “workers”, 14 larvae), therefore dependent on workers for feeding. The feeding protocol was as follows: at 7:30am, the larvae “alone” were placed on a half mealworm (muscular part) until 7:30pm. Then, food was removed and three workers from the original nest were introduced in the petri dish until 7:30am the next morning. In parallel, at the first 7:30am, the larvae “workers” were also placed with a half mealworm (also muscular part) and three workers. At 7:30pm, food and workers were removed and the larvae were left alone in their individual petri dish until 7:30am the next morning. This protocol was repeated over two consecutive days. On the third day, larvae from both treatments were placed on a half mealworm with three workers in the petri dish, considered as a day off to reduce the stress of manipulation and isolation. Workers placed in petri dishes came each time from the original nest, and were returned to the nest at the end of each phase. This cycle of two days of treatment and one day off (figure 1) was repeated until the larvae pupated. Larvae were weighed weekly, using an Ohaus Pioneer PA64C (0.1mg) scale. Larval weight gain was computed each week as the current larval weight minus the weight of the previous week. Simultaneously, weight of larvae of the same developmental stage in a complete colony (independent of the experiment) was followed weekly to study development without disturbance, representing a third treatment ("nest"). However, in this case we were not able to follow individually the larvae, no marking was possible.

7 :30am 7 :30pm 7 :30am 7 :30pm 7 :30am 7 :30am

Larvae « Workers »	Food + 3 workers	Nothing	Food + 3 workers	Nothing	Food + 3 workers
Larvae « Alone »	Food	3 workers	Food	3 workers	Food + 3 workers

Figure 1: Timeline of larval feeding protocol. This cycle was repeated until the larvae pupated.

Statistical analyses

Statistical analyses were carried out with R v3.6.1 (www.r-project.org) and plots were generated using ggplot2 (Wickham 2009). Due to small sample size, we conducted Wilcoxon tests to compare larval weight gain between treatments "alone" and "workers" each week. We also conducted Wilcoxon tests to compare mean larval weight (due to the impossibility of individual following) between the two treatments "alone" and "workers" with the unmanipulated larvae "nest". A test was performed for each week comparing two by two the different modalities (nest, alone or workers).

Results

Our experiment led to a high mortality of larvae. One larva of each treatment died the first week. Two "alone" and three "workers" larvae died the second week. Two "alone" and one "workers' larvae dead the third week. Four "alone" and three "workers' larvae dead the fourth week. At the fifth week, we only had one "alone" and three "workers" larvae. No larvae developed into pupae.

From the first week, the "alone" larvae, which fed without worker control, gained significantly more weight than the "workers" larvae, which depended on workers for food (Wilcoxon test, W = 24.5, P = 0.002). We found the same result for the second week (W = 17.5, P = 0.009), the third week (W = 14, P = 0.018) and the fourth week (W = 28, P = 0.017; figure 2). After the fourth week, we could not perform tests because of larval mortality.

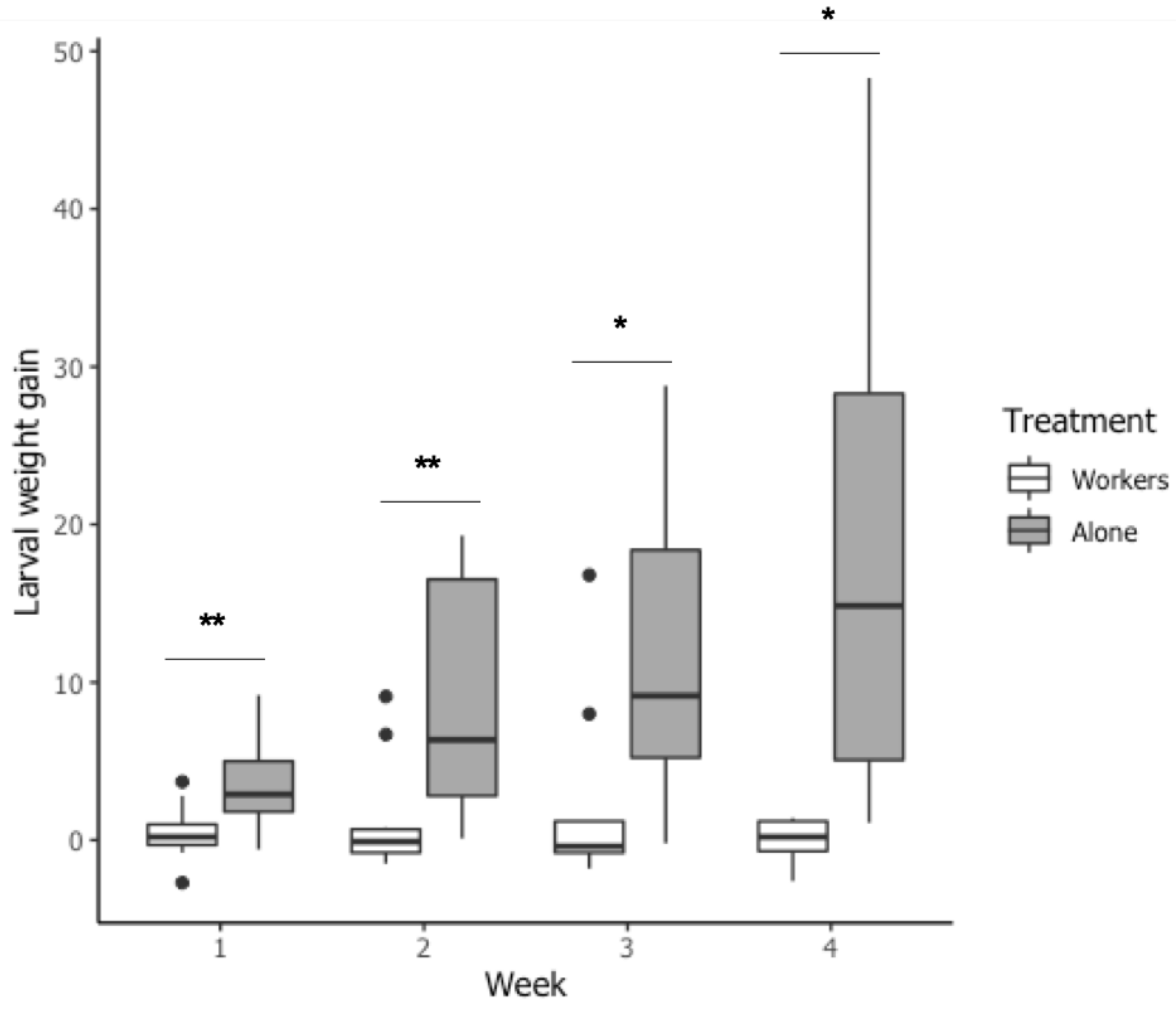

Figure 2: Boxplot showing the larval weight gain effect through the weeks of experiment, depending on the treatment. Boxes show median, quartiles and extremes. Significance of the Wilcoxon tests is indicated by $**p < 0.01$ and $*p < 0.05$.

The mean larval weight between larvae in experiment (“alone” and “workers”) and larvae in unmanipulated colony (“nest”) did not differ through weeks of experiment, excepted for the comparison between “nest” and “workers” at the week one (W = 229, P = 0.003) and four (W = 41, P = 0.01; figure 3). The absence of significant difference could also result from the low sample size due to larval mortality over the experiment. Comparing graphically the larval weight as a function of weeks (figure 3), “alone” larvae can gain more weight and faster than “nest” larvae. An “alone” larva grew up to 67mg while “nest” larvae weighed no more than 48mg in complete colonies at the final stage. The larval mortality remains high in unmanipulated colony, but four cocoons were produced at the fifth week.

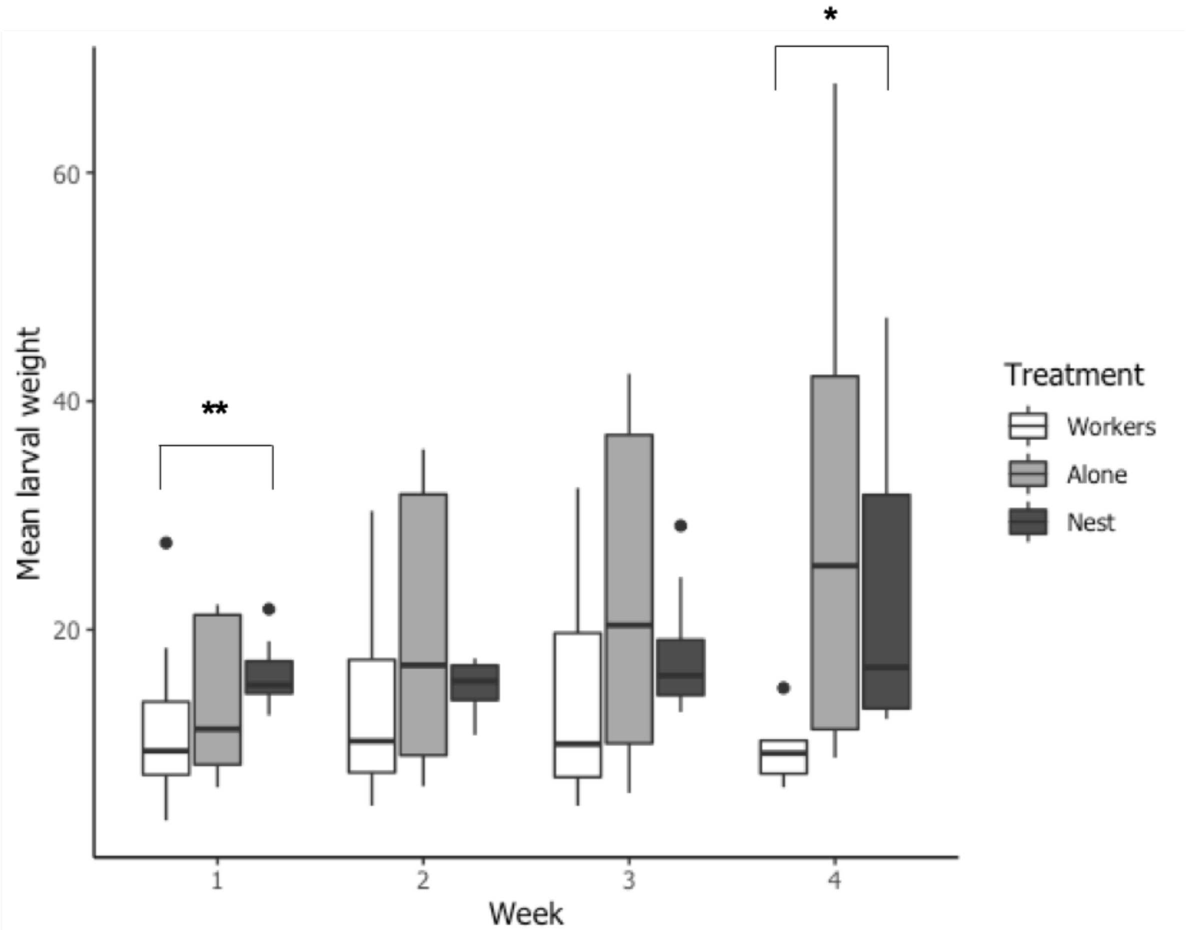

Figure 3: Weight (mg) of larvae in a complete colony (“nest”) compared to the larvae “alone” and “workers”. Boxes show median, quartiles and extremes. Significance of the Wilcoxon tests is indicated by **p < 0.01 and *p < 0.05.

Discussion

In this study, we investigated larval development without social coercion. We let larvae grow without food restriction ("alone") or under worker control for food ("workers", mimicking conditions inside nest). We also followed the larval development within unmanipulated colonies ("nest"). At the final stage (after pupation), we expected manually reared larvae to have larger bodies, especially thorax (mesonotum) and gaster, and more developed ovaries, i.e. traits associated with the queen phenotype (Londe et al. 2015, 2016). Our results showed significantly higher weight gain for larvae developing alone, and this from the first week. In addition, even if the mean larval weight did not differ between alone larvae and the two others treatments, larvae that developed alone can grow much more than larvae that were left inside the nest with the whole colony. These data indicate that ant larvae can grow larger when they have the possibility to escape from worker coercion than in natural conditions in the presence of workers. In *M. rogeri*, the major coercion for larval development would be the limitation of food access by workers.

In *M. rogeri*, the mechanisms behind intercaste production have been discussed based on morphometric and behavioral studies that reveal their mosaic nature (Molet et al. 2009, 2012; Londe et al. 2015). However, the determinism itself remains poorly known. We based our work on studies in ants showing a role of diet on caste determination (e.g. Amor et al., 2016; Smith et al., 2008; Wheeler, 1986) and on worker size (Smith et al., 2008; Wheeler, 1991). Nevertheless, this evidence is indirect (using isotopic measurements for instance; Amor et al., 2016; Smith et al., 2008; Smith & Suarez, 2010). The high larval mortality in our experiment shows the difficulty of isolating the brood from workers during development, and could explain the lack of empirical evidence in ants. It also highlights the role of workers in brood care and in the regulation of food intake, and how deeply rooted it could be in the developmental process of larvae. The largest larvae may have died of morbid obesity because they were too fat. The food intake manipulation is more easily achievable in social insects where larvae develop in cells, and are more autonomous during the development. For instance in honey bees *Apis mellifera*, Linksvayer et al. (2011) used *in vitro* rearing to study larval development without social regulation. By giving a queen diet to worker-destined larvae (and conversely), they obtained a continuum of intermediate phenotypes between queen and worker. Slater et al. (2020) manipulated more finely the quantity and the quality of diet given to honey bee larvae and also obtained a panel of intermediate phenotypes. They especially found that diet quantity influenced queen traits while diet quality did not.

These two studies in honey bees coupled with our results in one ant hint at the fact that environmental phenotype determination in eusocial hymenoptera does not result from a polyphenism as classically described (e.g. Nijhout 2003; Simpson et al. 2011; Libbrecht et al. 2013), that is the ability of a single genotype to generate multiple discrete phenotypes when the environment varies discontinuously. Polyphenism may arise from two different ways: (1) from switches in developmental pathways that produce a discontinuous reaction norm, or (2) from a reaction norm when the environment is either discontinuous or only sampled at discrete times or places (Nijhout 2003). Evidence from Londe et al. (2015) and these studies on honey bees support that a reaction norm links the worker phenotype to the queen phenotype in a continuous fashion. The production of spontaneous intermediate phenotypes in natural colonies could be prevented by workers during larval development (Linksvayer et al. 2011). Workers would discretize the environment in order to produce only two extremes phenotypes.

Our study also shows the potential for larval cheating, i.e. their development into adults that have an enhanced fertility. Indeed, in *M. rogeri*, intercastes could result from an attempt of selfish larvae at becoming reproductives, thus maximizing their direct fitness (Bourke and Ratnieks 1999; Wenseleers et al. 2003; Ratnieks et al. 2006). The mode of larval feeding (i.e. directly on dead prey) makes them less dependent on workers (compared to trophallaxis), and could increase potential cheating attempts. Cheating is further strengthened by the fact that intercastes can possibly mate and lay diploid eggs (Londe et al. 2016). Therefore, selfish larvae can reasonably expect to get higher fitness by developing into queen or intercaste. More broadly, selfish strategy can lead to intermediate phenotypes between queen and worker, and in an evolutionary approach this can lead to new castes or sub-castes (Molet et al. 2012). This has been suggested in *Cataglyphis* ants, where selfish larvae could develop into a new morphology of worker-like queens: brachypters (Amor et al. 2011, 2016). In the same vein, we can also mention the dwarf queen in stingless bees (Wenseleers et al. 2005). In termites, the hemimetabolous development allows all individuals to become potential reproductives, and paves the way for selfish strategies. Juveniles gradually develop into adults without metamorphosis. In *Reticulermes*, juveniles have the potential to become neotenic reproductives (Matsuura et al. 2018). Although intercastes may originate from selective pressures on selfish individual reproductive potential, they may also provide group-level benefits. Molet et al. (2012) suggest that intercastes can improve colony defense, food storage or reproduction. For instance, they can accompany the evolution of independent colony foundation to dependent foundation (review in Cronin et al. 2013), which does not require a mating flight nor winged queens. Colony-level selection could further modify intercastes and lead to the evolution of new castes such as ergatoid queens or soldiers (Molet et al. 2012).

To a lesser degree, selfish strategy could increase the variability of worker size within colonies. In some species where size diversity is moderate but does not increase colony fitness, this non-adaptive size could also result from cheating attempts by larvae. For instance, in *Temnothorax nylanderi*, worker size diversity and mean worker size do not improve colony performance in the laboratory (Colin et al. 2017) or colony fitness in the field during the growing season (Honorio et al. 2020) and overwintering (Honorio et al. submitted). Furthermore, in this species, intercastes are also found (Plateaux 1970; Okada et al. 2013), suggesting a possible selfish strategy of larvae as we mentioned above. There could be a balance between costs and benefits to keep the phenotypes produced in a certain range (called canalization; Debat and David 2001). Thus, in *T. nylanderi*, the cost of maintaining the produced phenotypes in a very strict range would outweigh the benefits associated with reduced diversity and optimal phenotype only. Moreover, the cost of producing divergent phenotypes is supported by the whole colony (Molet et al. 2012). Thus, the production of divergent phenotypes does not inflict significant costs on colony fitness (Colin et al. 2017). Since these costs are diluted, this reduces the selective pressures acting on individuals to produce new phenotypes within a very strict range. This feedback-loop can reinforce a lower canalization of the phenotypes produced (Colin et al. 2017) and increase the frequency of cheating larvae. As a result, size diversity could vary without any direct fitness benefit to the colony.

In conclusion, in this study we highlighted the role of worker control on the phenotypes of individuals produced. The difficulty in manipulating the social environment to rear ant larvae may explain the lack of direct empirical evidence for this taxon. However, our data suggest that larvae have the ability to grow larger than what workers usually allow in natural colonies, which could lead to a much wider range of phenotypes than those normally produced. Selfish larvae associated with the relaxed selective pressures in a social environment could be responsible for both the production of intermediate phenotypes and the existence of non-adaptive size diversity in some species.

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CHAPITRE 3

Rôle de l’environnement social dans la réponse aux contraintes environnementales.

Article en préparation. Une première version a été écrite pour cette thèse, la version finale sera co-écrite conjointement par les deux premiers auteurs.

Disentangling the role of social environment and intrinsic factors in cadmium resistance in the ant *Temnothorax nylanderi*.

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Abstract

Urbanization brings new pressures for individuals to deal with. Among them, trace elements such as cadmium are important stressors. Highly toxic, they tend to bioaccumulate in the food chain. A recent study highlights a weaker effect of cadmium for city ant colonies relative to forest counterparts. This better resistance could result from social life which can buffer external disturbances, and/or from adaptive changes that allow a better development under stressful conditions. In this study, we seek to quantify the contribution of both social environment and individuals to the improved resistance to cadmium in an urban ant. We performed a cross-fostering experiment of larvae from city and forest populations resulting in four crossings, we reared them under a common garden setup. Colonies were fed by food containing cadmium or not (control) during two months. The consequences of cadmium exposition were quantified on four response variables: adult worker mortality and larval development (duration of development, emergence rate, and size after emergence). Cadmium had a very negative effect on all variables as already observed. Surprisingly, we did not confirm the previous results showing a better resistance to cadmium for city ants. As we did not find a differential response between the two populations, we were not able to disentangle the social effect from the intrinsic factors in cadmium resistance as initially expected. Independently of feeding treatment, forest colonies produced better offspring, both in quality of worker larvae (through possible maternal or epigenetic effects) and male production (through social environment). Finally, the colony size did not improve survival against cadmium, suggesting a limit of social buffering against external disturbances. Sensitivity to cadmium could differ depending on seasons, due to shifts in phenology between urban and rural ants.

Key-words: trace element, size, colony size, common garden, cross-fostering, urbanization.

Introduction

Cities are ‘natural laboratories for understanding eco-evolutionary dynamics in an urbanizing world’ (Alberti, Marzluff, & Hunt, 2017). Urbanization and human-induced environmental changes are forcing organisms to face many new pressures (Grimm et al., 2008), including changes in impervious cover land and increases in temperature (including the ‘urban heat island’ - Levermore et al. 2018; Chick et al. 2019). Pollution is also a major component of urbanization (be it sound, light, chemical…), especially trace elements which are persistent pollutants that bioaccumulate in the food chain. For example, cadmium is especially toxic (Järup & Åkesson, 2009) and can be found in concentrations ten times higher in cities than in rural soils (Foti et al., 2017). These emerging or growing pressures are leading to changes in life history traits of individuals, both plastically and evolutionarily (Johnson & Munshi-South, 2017). For instance, urban organisms exhibit earlier timing of reproduction (plants: Jochner and Menzel 2015; ants: Chick et al. 2019), lower corticosterone stress response (birds: Partecke et al. 2006), higher thermal tolerance (ants: Diamond et al. 2017), decreased migratory behavior and aggressiveness (birds, respectively: Evans et al. 2012; Hasegawa et al. 2014), and smaller size (Daphnia: Brans et al. 2017; Spider: Dahirel et al. 2019). Thus, urbanization can modify all types of life history traits: physiology, morphology, phenology, behavior, etc. (review in Alberti et al. 2017).

Although many organisms face these pressures alone, individuals that live in groups benefit from a filter provided by the society against external disturbances. In particular, eusocial insects have better resilience to stressors than solitary individual (Straub, Williams, Pettis, Fries, & Neumann, 2015). Colony size plays a role in buffering the effects of temperature (cold: Heinze et al. 1996; warm: Molet et al. 2017), infection of parasites (Scharf, Modlmeier, Beros, & Foitzik, 2012), seasonality (Kaspari & Vargo, 1995), starvation (Heinze et al., 1996; Modlmeier, Foitzik, & Scharf, 2013), and exposure to pesticides (Crall, de Bivort, Dey, & Versypt, 2019). In addition to workers, larvae could also improve resistance in harsh periods, such as in starvations (Modlmeier et al., 2013; Rueppell & Kirkman, 2005). It is therefore important to consider the whole social environment within colonies to study social insects. The social environment is defined by the colony composition, i.e. the queen(s), the colony size (number of workers), the brood, the phenotype and proportion of each one (Wills, Powell, Rivera, & Suarez, 2018). Social life could thus confer a better tolerance to the urbanization pressures. Furthermore, the social environment highly modulates brood development in insect societies such as ants (Linksvayer, 2006; Wills et al., 2018) or bees (Linksvayer et al., 2011), and therefore it can influence the produced phenotypes within colonies. The social dimension is a key component to be considered when studying the response to external pressures.

Recently, a study showed a better tolerance of city colonies to cadmium in the laboratory: the size of produced workers and the survival of larvae are less negatively affected by cadmium in city colonies than in forest colonies (Jacquier et al., 2020), although underlying mechanisms are unknown. Indeed, cadmium tolerance could result from social environment that buffers external disturbance or from larval intrinsic factors that allow a better development under stressful conditions. A cross-fostering experiment would be useful to disentangle the effects of larval intrinsic factors from those of the social environment factor. This experimental approach would provide a causal complement to the correlative work from Jacquier et al. (2020) who found no link between resistance to cadmium and social environment effect (colony size and worker size). However, Khimoun et al. (2020) found that city and forest populations are not genetically differentiated, suggesting a lack of adaptive differentiation between the two populations (even though some loci differ between the two populations). Thus, a cross-fostering experiment mixing larva and worker origins is necessary to clarify the mechanisms that improve resistance to trace metal.

In this study, we aimed to investigate the role of social environment and larval intrinsic factors in the resistance to a trace metal, the cadmium. We used the tiny acorn ant, *Temnothorax nylanderi*, largely widespread in city and forest locations (Khimoun et al., 2020). We performed a cross-fostering experiment under common garden which has already been used in ants to disentangle the contribution of social parameters and individual factors in the response to urbanization (Chick et al., 2019; Chick, Waters, & Diamond, 2020; Diamond, Chick, Perez, Strickler, & Zhao, 2018; Jacquier et al., 2020). We crossed larvae from city colonies with workers from forest colonies, and vice versa. We also performed two control crossings where city larvae were reared by city workers from foreign colonies, and the same for forest larvae and workers. These four crossings were subjected to a cadmium-enriched or cadmium-free (control) food. The consequences of this exposure were measured on adult worker mortality and larval development (emergence rate, duration and size after emergence). Following the better resistance to cadmium for city colonies (Jacquier et al., 2020), we predict that exposure to cadmium would have a lower negative impact on adult mortality, larval emergence rate, development time and size of produced individuals in the presence of city workers (social effect). In the same way, we expected that city larvae would be less affected by cadmium compared to forest larvae (intrinsic factor effect). We also expect a positive relationship between colony size and larval development (emergence rate, development time and size after emergence).

Material and methods

Ants rearing

Temnothorax nylanderi is a tiny ant, nesting in acorns or twigs, widespread in western and central Europe and living in small colonies (few dozen of workers; Foitzik, Strätz, & Heinze, 2003). We harvested 72 queenright colonies in 'Parc des Buttes-Chaumont' (48°52'47.1"N; 2°23'45.6"E) in Paris for city population. Seventy colonies were collected in Chantilly forest (49°10'59.8"N; 2°28'43.6"E) for forest population. Colonies were harvested in April 2019 and then installed in the laboratory. The artificial nest consisted of two microscope slides separated by a 1-mm auto-adhesive plastic foam with 3 chambers. The top slide was covered with dark paper to protect the colony from the light. The nest was placed in a plastic box (11.5 x 11.5 x 5.5cm) for foraging. Workers and larvae in each colony were counted. Colonies were acclimated for seven days in the laboratory before the experiment began. We only kept the stage 2 larvae (Plateaux, 1970) in the colonies for our study.

Experimental design

The experiment was carried out in order to disentangle the effect of social environment (worker origin) from the intrinsic effect of larvae (larva origin) in resistance to cadmium, a trace metal.

To do this, we constituted a treatment based on worker origin, with two levels: city or forest. A second treatment based on larva origin was done, with the same levels: city or forest. These two treatments led in a four-crossing experiment. Two crossing exchanged brood between colonies belonging to distinct populations: crossing "CF" (the first letter corresponding to worker origin, the second one to larva origin) with each city colony receiving brood from one forest colony, and conversely crossing "FC" with each forest colony receiving brood from one city colony. The two others crossings exchanged brood between colonies belonging to the same original population: crossing "CC" with two city colonies exchanging their brood and crossing "FF" with two forest colonies exchanging their brood. "CC" and "FF" were performed to verify that our results are not simply due to the exchange of foreign brood. Colonies were matched in pairs for brood exchange, depending on the number of workers and larvae. Larvae of the two paired colonies were exchanged simultaneously, larvae remained isolated for a maximum of 15 minutes without workers.

Each colony was then exposed to a feeding treatment with two conditions: control or cadmium. Colonies were dispatched between feeding treatment based on their colony size, in order to obtain a similar distribution of colony sizes in the two-feeding treatment. Control colonies were fed three times a week with a mixture of diluted honey, soya yogurt and dried crickets. Cadmium colonies were fed with the same mixture containing 100µg/g cadmium (Jacquier et al., 2020). We had 15 "CC", "FC" and "FF" colonies, and 16 colonies "CF" with control food. We assigned more cadmium colonies to compensate for the potential risk of mortality, so we had 21 "CC" and "FF" colonies, 19 "CF" colonies and 20 "FC" colonies with cadmium food.

Colonies were reared in a climatic chamber (reference CTS TP10/600) at a temperature cycle of 22-27°C – 12:12h with natural photoperiod for 64 days, so that larvae could develop into pupae and then emerge as adults (Molet et al., 2017). Workers that emerged in the laboratory (called "produced workers") were identified based on their pale color and collected once they started to move. They were anesthetized by cooling down and stored in 96° ethanol for morphological measurements (see below). Newly laid eggs were removed to prevent the hatching of foreign larvae at the crossing.

Adult workers born in the field were removed from the colonies when they died, and counted to compute the adult mortality rate. The larva emergence rate was computed as the ratio between the number of larvae that developed to adulthood and the number of larvae initially given to the colony. For each produced worker, gyne and male, we also measured the development time (from the beginning of the experiment to emergence), the head size and the coefficient of variation (CV) of the size of produced individuals. Finally, the sexual investment ratio was computed as the ratio between the number of produced sexuals and the total number of produced individuals (sexuals and workers).

Size measurement

For morphological measures, heads were separated from bodies. Heads were stuck on double-sided tape and photographed under a Discovery.V12 Zeiss stereomicroscope connected to a computer via a Zeiss AxioCam ICc 5 video camera. Head width, a standard measurement of body size in ants (Tschinkel, Mikheyev, & Storz, 2003), was measured with ImageJ 1.8 (Abràmoff, Magalhães, & Ram, 2004).

Statistical analyses

We investigated the effects of worker origin (forest/city), larva origin (forest/city), feeding treatment (cadmium/control) and the initial colony size on the adult mortality rate, the larva emergence rate, the development time of produced individuals (sexuals and workers), the sexual investment ratio, the size and the CV of produced individuals. We included a triple interaction between feeding treatment, larva origin and worker origin. If the triple interaction was not significant, we decomposed into three-first order interactions. We also included the interaction between the feeding and the initial colony size in models to investigate whether larger colonies were less affected by cadmium than smaller ones.

All statistical analyses were carried out with R v3.6.1 (www.r-project.org). All plots were generated using ggplot2 (Wickham, 2009). First, we tested the effect of predictor variables on colony-level dependent variables (adult mortality rate, larva emergence rate, sexual investment ratio and size CV of the produced individuals) using generalized linear models (GLMs). Rate data (adult mortality and larva emergence) were analyzed using quasibinomial distribution. Sexual investment ratio and CV were analyzed using Gaussian distribution. Finally, we tested the effect of predictor variables on the development time and the head size of emerged individuals, two individual-level dependent variables, using linear mixed models (LMMs, package *nlme*; Pinheiro, Bates, DebRoy, & Sarkar, 2016), with colony as random factor. All models are summarized in Table 1.

For each analysis, the minimum adequate model was selected using a backward stepwise approach where explicative variables were removed one by one from a full model based on a log likelihood ratio test. Fisher or Chi^2 tests (according to the type of model used) and associated *P*-values for each predictive variable were obtained to obtain the *P*-values for each predictor variable by comparing the minimum adequate model with a model excluding or including the variable of interest (according to whether the variable was present in or absent from the minimal adequate model respectively). When necessary, we used Wilcoxon test to compare two by two means among the different crossing of the response variable. Normality of the residuals and homogeneity of variances were checked visually following Pinheiro & Bates (2000); no transformation of the data was necessary.

Results

Six colonies lost their queen during the experiment (one 'FF', 'FC' and 'CC' colony; three 'CC' colonies) and were excluded from data analyses. Our final dataset consisted of 136 queenright colonies. A total of 1 688 workers and 1 092 males emerged and were measured. We also obtained one intercaste and 134 gynes from only 15 colonies (13 control colonies: four 'FF' colonies, three 'CC', 'CF' and 'FC' colonies; two cadmium colonies: one 'CF' and one 'FF' colony), so we did not include gyne production in our dataset. Detailed statistics are presented in Table 1.

Adult mortality rate

The worker origin influenced the adult worker mortality, city workers ('CC' and 'CF') had a higher mortality ($F_{131:132} = 7.7$, $P = 0.006$; figure 1a). The mortality rate marginally increased with the initial colony size in cadmium feeding, while it decreased with the initial colony size in control feeding (feeding x initial colony size interaction, $F_{131:132} = 3.7$, $P = 0.057$; figure 2a).

Larva emergence rate and development time

The worker origin had no effect on larva emergence rate, but forest larvae ('CF' and 'FF') had a higher emergence rate ($F_{131:132} = 7.5$, $P = 0.006$; figure 1b). Under cadmium, the larva emergence rate was lower ($F_{131:132} = 107.9$, $P < 0.001$; figure 1b).

The worker origin had no effect on development time. Nevertheless, workers from forest larvae developed faster into adults ($\chi^2_1 = 4.2$, $P = 0.04$; figure 1c), while the development time of males did not differ according to the larva origin. Under cadmium, the development time of produced individuals was longer (workers: $\chi^2_1 = 7.1$, $P = 0.007$; figure 1c - males: $\chi^2_1 = 64.7$, *P*

< 0.001; figure 1d). The development time of produced individuals increased with the initial colony size (workers: χ^2_1 =6.2, P = 0.01; males: χ^2_1 = 5.1, P = 0.02).

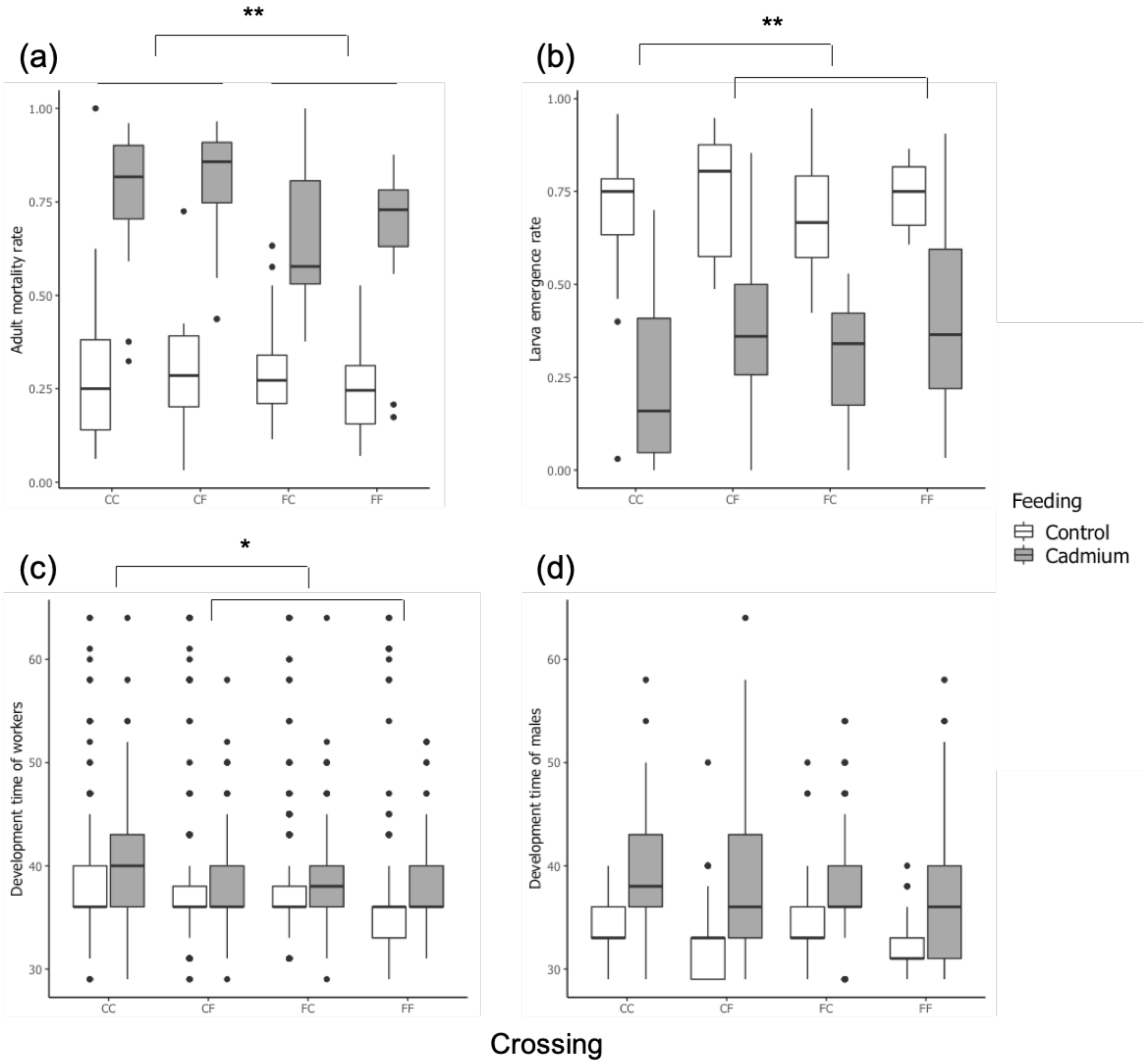

Figure 1: Boxplots comparing the effects of the worker and larva origins (crossing) and feeding on (a) the adult worker mortality rate, (b) the larva emergence rate, the development time of (c) workers and (d) males. Boxes show median, quartiles and extremes. Significance is indicated by $^{**}p < 0.01$ and $^{*}p < 0.05$. Feeding had a significant effect on all variables (not shown in the figure for clarity). Statistics are presented in Table 1.

Sexual investment ratio

Neither the worker origin nor the larva origin had an effect on the sexual investment ratio. The sexual investment ratio (number of produced sexuals / number total of produced individuals) depended on the initial colony size in interaction with feeding (feeding x initial colony size interaction, $F_{127:128} = 4.3$, $P = 0.04$). The sexual investment ratio increased with the initial colony size with a steeper slope in control feeding (figure 2b).

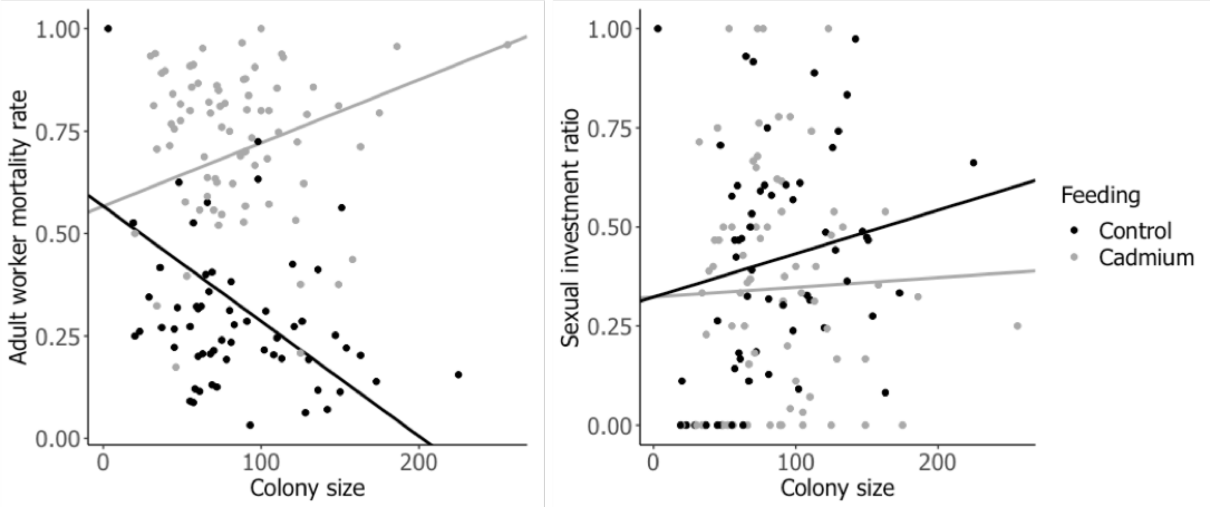

Figure 2: Correlation between the initial colony size with the adult worker mortality rate and the sexual investment ratio. When the initial colony size increased, (a) the adult mortality increased for cadmium feeding and decreased for control feeding, (b) the sexual investment ratio increased (black line: control, estimate = $1.096e^{-3} \pm 6.47e^{-4}$; grey line: cadmium, estimate = $0.246e^{-3} \pm 6.30e^{-4}$).

Size of produced individuals

The CV of produced males resulted from a triple interaction among feeding, worker and larva origins (feeding x worker origin x larva origin interaction, $F_{87:91} = 2.4$, $P = 0.050$; figure 3b). The negative impact of cadmium was less strong when city workers (‘CC’) reared males from city larvae resulting in a lower CV than those reared by forest workers (‘FC’) (W = 23, $P = 0.046$). The worker origin influenced the size of produced males, city workers (‘CC’ and ‘CF’) produced marginally smaller males relative to forest workers (‘FC’ and ‘FF’; $\chi^2_1 = 3.6$, $P = 0.059$; figure 3d).

Under cadmium, the CV of produced individuals was larger (workers: $F_{117:118} = 8.8$, $P = 0.004$; figure 3a – males: $F_{87:92} = 8.8$, $P < 0.001$; figure 3b), and the produced individuals were smaller (workers: $\chi^2_1 = 157.9$, $P < 0.001$; figure 3c - males: $\chi^2_1 = 156.2$, $P < 0.001$; figure 3d). The CV of produced workers increased with the initial colony size ($F_{117:118} = 4.8$, $P = 0.03$).

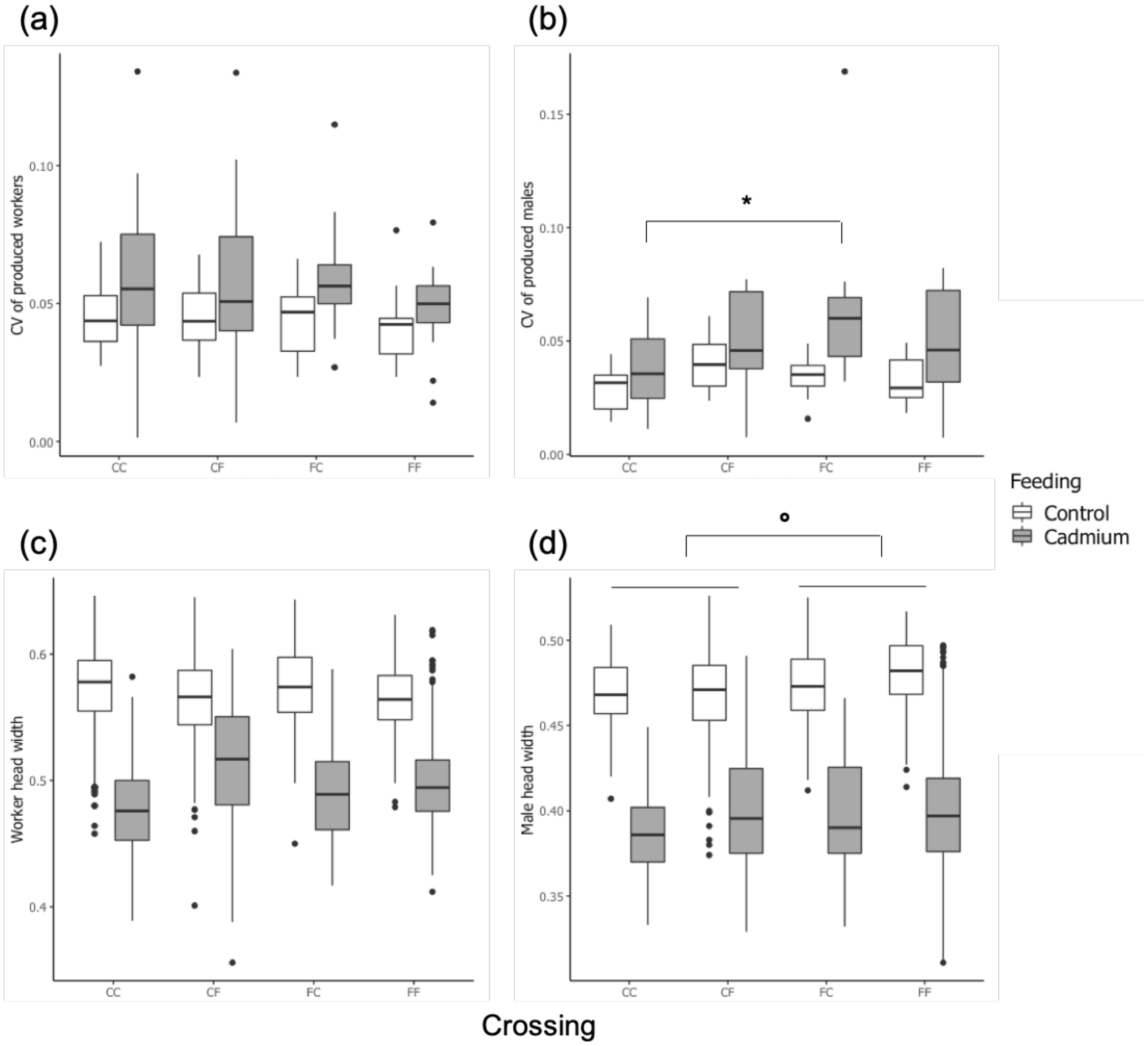

Figure 3: Boxplots comparing the effects of the worker and larva origins (crossing) and feeding on the CV of produced (a) workers and (b) males, and on the head width of produced (c) workers and (d) males. Boxes show median, quartiles and extremes. Significance is indicated by $*p < 0.05$ and $0.05 < °p < 0.06$. Feeding had a significant effect on all variables (not shown in the figure for clarity). Statistics are presented in Table 1.

Table 1: Models and statistics for the different response variables. "Minimum model" means the predictor was retained in the minimum model selected. A mixed effect model with colony as random factor was used for the development time and head size of produced individuals, in order to control the origin of workers.

Predictors	**Response variables**								
	Adult mortality rate	Larva emergence rate	Worker development time	Male development time	Sexual investment ratio	CV of worker size	CV of male size	Worker head size	Male head size
Feeding	**Minimum model $F_{131:133} = 92.4$, $P < 0.001$**	**Minimum model $F_{131:132} = 107.9$, $P < 0.001$**	**Minimum model $\chi^2_1 = 7.1$, $P = 0.007$**	**Minimum model $\chi^2_1 = 64.7$, $P < 0.001$**	**Minimum model $F_{127:129} = 2.5$, $P = 0.09$**	**Minimum model $F_{117:118} = 8.8$, $P = 0.004$**	**Minimum model $F_{87:92} = 4.9$, $P < 0.001$**	**Minimum model $\chi^2_1 = 157.9$, $P < 0.001$**	**Minimum model $\chi^2_1 = 156.2$, $P < 0.001$**
Worker origin	**Minimum model $F_{131:132} = 7.7$, $P = 0.006$**	$F_{130:131} = 0.8$, $P = 0.4$	$\chi^2_1 = 0.5$, $P = 0.5$	$\chi^2_1 = 0.5$, $P = 0.4$	$F_{126:127} = 0.5$, $P = 0.4$	$F_{117:118} = 0.6$, $P = 0.4$	**Minimum model $F_{87:92} = 2.1$, $P = 0.06$**	$\chi^2_1 = 0.6$, $P = 0.4$	**Minimum model $\chi^2_1 = 3.6$, $P = 0.059$**
Worker origin - feeding Interaction	$F_{130:131} = 2.9$, $p = 0.087$	$F_{129:131} = 0.6$, $P = 0.5$	$\chi^2_2 = 2.0$, $P = 0.4$	$\chi^2_2 = 4.4$, $P = 0.1$	$F_{125:127} = 0.5$, $P = 0.6$	$F_{115:117} = 0.3$, $P = 0.7$		$\chi^2_2 = 0.6$, $P = 0.7$	$\chi^2_1 = 0.03$, $P = 0.9$
Initial colony size	**Minimum model $F_{131:133} = 2.8$, $P = 0.066$**	$F_{130:131} = 1.6$, $P = 0.2$	**Minimum model $\chi^2_1 = 6.2$, $P = 0.01$**	**Minimum model $\chi^2_1 = 5.1$, $P = 0.02$**	**Minimum model $F_{127:129} = 2.7$, $P = 0.07$**	**Minimum model $F_{117:118} = 4.8$, $P = 0.03$**	$F_{66:87} = 1.1$, $P = 0.3$	$\chi^2_1 = 1.0$, $P = 0.3$	$\chi^2_1 = 0.2$, $P = 0.6$
Initial colony size - feeding interaction	**Minimum model, $F_{131:132} = 3.7$, $P = 0.057$**	$F_{129:131} = 2.3$, $P = 0.1$	$\chi^2_1 = 0.01$, $P = 0.9$	$\chi^2_1 = 0.8$, $P = 0.4$	**Minimum model $F_{127:128} = 4.3$, $P = 0.04$**	$F_{116:117} = 1.1$, $P = 0.3$	$F_{85:87} = 2.1$, $P = 0.1$	$\chi^2_2 = 3.6$, $P = 0.2$	$\chi^2_2 = 0.2$, $P = 0.9$
Larva origin		**Minimum model $F_{131:132} = 7.5$, $P = 0.006$**	**Minimum model $\chi^2_1 = 4.2$, $P = 0.04$**	$\chi^2_1 = 2.8$, $P = 0.1$	$F_{126:127} = 1.7$, $P = 0.2$	$F_{116:117} = 0.6$, $P = 0.4$	**Minimum model $F_{87:92} = 1.9$, $P = 0.1$**	$\chi^2_1 = 0.4$, $P = 0.5$	$\chi^2_1 = 0.07$, $P = 0.8$
Larva origin - feeding interaction		$F_{130:131} = 0.9$, $P = 0.3$	$\chi^2_1 = 1.2$, $P = 0.3$	$\chi^2_2 = 3.2$, $P = 0.2$	$F_{125:127} = 1.2$, $P = 0.3$	$F_{115:117} = 0.3$, $P = 0.7$		$\chi^2_2 = 2.6$, $P = 0.3$	$\chi^2_2 = 0.3$, $P = 0.8$
Triple interaction worker origin - larva origin - feeding		$F_{126:131} = 0.4$, $P = 0.8$	$\chi^2_5 = 3.7$, $P = 0.6$	$\chi^2_6 = 8.7$, $P = 0.2$	$F_{121:127} = 0.5$, $P = 0.8$	$F_{111:117} = 0.5$, $P = 0.8$	**Minimum model $F_{87:91} = 2.4$, $P = 0.050$**	$\chi^2_6 = 6.0$, $P = 0.4$	$\chi^2_5 = 2.8$, $P = 0.7$

Discussion

In this study, we wanted to identity the mechanisms responsible for the better resistance to trace metal in urban ant populations. We aimed to disentangle the effect of social environment from the effect of larval intrinsic factors. Our results confirm the very negative impact of cadmium exposure on colony development in ants (Jacquier et al., 2020) and more generally in various taxa (Cervera, Maymó, Sendra, Martínez-Pardo, & Garcerá, 2004; Demenesku et al., 2014; den Besten, Herwig, Zandee, & Voogt, 1989; Gomot, 1998). We found a negative effect of cadmium on adult worker survival, larva emergence rate, sexual investment ratio, development time and size of produced individuals. Cadmium also increased the coefficient of variation (CV) of produced individuals, as already observed in this species for another stressful environment (warm temperature : Molet et al., 2017). Surprisingly, we did not confirm the results of Jacquier et al. (2020) showing a weaker effect of cadmium in city colonies. They found that under cadmium, emergence rate of larvae and head width of produced workers were less affected for city ants, in four different locations (four city/forest pairs). As we did not find a difference in resistance to cadmium between forest and city colonies, we could not test our hypothesis about the contribution of social environment and larval intrinsic factors.

We predicted a social effect in cadmium tolerance, meaning a lower negative impact on our response variable in the presence of city workers. We detected only one triple interaction, between the feeding treatment and the origin of workers and larvae, for the CV of produced males. In cadmium feeding, the CV of city larvae is higher when they were reared by forest workers, highlighting a less negative impact of cadmium for city larvae when they were reared by city workers. Thus, it seems that in stressful conditions forest workers produced males with a more variable size and would be less able to maintain male phenotypes in a certain range when external environment is more stressful. In contrast with Jacquier et al. (2020), our colonies produced a non-negligible quantity of males, possibly due to difference in timing of experiment between the two studies. In *T. nylanderi*, brood production is highly synchronized (Headley, 1943; Kipyatkov, 1993), but males are produced more quickly than workers (our study and Jacquier et al., 2020). As we started our study later (in April, compared to February – March for Jacquier et al.) and winter was milder, colonies were probably more advanced in the process of male production. The lack of gyne production is not surprising, given that resource allocation can be redirected towards workers and males after a large worker loss in our species (Foitzik & Heinze, 2000; Foitzik et al., 2003; Honorio, Doums, & Molet, 2020).

Although males emerged from city larvae reared by forest workers had a higher CV under cadmium feeding, males reared by forest workers were marginally larger (1,7% larger than those produced by city workers). These larger males are thus supposed to have greater mating success and sperm production in ants (Brown & Keller, 2006; Wiernasz, Sater, Abell, & Cole, 2001). Thus, when forest workers reared city larvae under stressful conditions, they produced more variable males; but overall, they produced larger males whatever the origin of larvae and feeding treatment. As it is a weak effect, it may also be due to higher adult worker mortality for city workers, so fewer workers were available during the experiment to care and feed larvae. Indeed, adult city workers unexpectedly had a higher mortality rate. Jacquier et al. (2020) found no difference in adult mortality between city and forest colonies. This suggests that cadmium resistance is not necessarily quantifiable based on adults, but only on the individual in development (the larvae).

We expected that city larvae would be less affected by cadmium and thus a role of larval intrinsic factors in cadmium resistance. However, we only found a limited effect of the origin of larvae, independently of feeding treatment and contrary to our predictions. Forest larvae had a higher emergence rate and workers produced from forest larvae had a shorter development time. Forest larvae appeared to be of better quality, although this is not reflected with the final size of emerged workers. Our study does not allow to further substantiate the process(es) involved, but two potentials mechanisms can be proposed. Difference in egg provisioning though maternal or epigenetic effects can generate difference in development and emergence rate between larvae. For instance, juvenile hormone and ecdysteroid transmitted to brood influence development and emergence rate in ants (Cahan, Graves, & Brent, 2011; Libbrecht et al., 2013; Schwander et al., 2008 - see also : LeBoeuf et al., 2018, 2016). Forest queen can therefore lay better quality eggs, which develop faster and with a higher emergence rate. Genetic does not seem to be involved given that the city and forest populations are not differentiated in this species, but this hypothesis cannot be totally discard considering the few discriminated loci between the two populations (Khimoun et al., 2020).

Another more general social component is the colony size, regardless of worker origin. Depending on the received food (with or without cadmium), colony size was correlated with the adult worker mortality rate and the reproductive ratio. Under control feeding, the adult mortality decreased when colony size increased. Conversely, under cadmium, the adult mortality increased when colony size increased. Insect societies can buffer external disturbances (Crall et al., 2019; Kaspari & Vargo, 1995; Modlmeier et al., 2013; Molet et al., 2017; Scharf et al., 2012), but our study highlights a potential limit to this social buffering. When the disturbance is too high, larger colonies do not survive disturbances better, but instead be more destabilized and collapse. Due to their large numbers, each colony member (especially foragers) can be affected by different stressors (pathogens, parasites, pollutions, etc) and can transmit them to other individuals within society. Thus, compared to solitary organisms, insect colonies could be exposed to more environmental stressors, which can interact and make colonies more vulnerable. In honey bees, colonies could be more sensitive to sublethal effects of pesticides and pathogens and cause colony collapse disorder (Barron, 2015). Regarding sexual investment, colony size was more strongly correlated with sexual investment ratio under control feeding than under cadmium feeding. Larger colonies invest more in sexual production, as classically found in this species (Foitzik & Heinze, 2000; Foitzik et al., 2003; Honorio et al., 2020). The development time of produced individuals (both workers and males) also increased with colony size within colonies, consistent with the loss of per-capita productivity as a function of colony size, found in many ants species (review in Kramer, Scharf, & Foitzik, 2014).

Lastly, colony size was positively correlated with the CV of produced workers. In this species, colony size within colonies could increase worker size diversity, as found in some ant species (Ferguson-Gow, Sumner, Bourke, & Jones, 2014; Tschinkel, 1988, 1993). With the exception of cadmium which affects worker size and its CV, no other predictor variable influences the size of produced workers. This could be explained by the possible non-adaptivity of worker size in this species (Colin, Doums, Péronnet, & Molet, 2017; Honorio et al., 2020; Honorio et al., submitted). In *T. nylanderi* and more widely in social species showing moderated or limited size diversity, worker size could be independent of colony fitness. Other individual variables that are more associated with colony fitness should be considered, such as physiological (metabolic rate : Chick et al., 2020) or immune traits (Demenesku et al., 2014).

In this study, we did not confirm the previous discovery highlighting a better resistance for cadmium in city colonies (Jacquier et al., 2020). Conversely, forest worker larvae would have better resources to develop faster and with a higher emergence rate. Forest adult workers would rear larger males, suggesting a higher reproductive success. These results are not in line with the less negative effect of cadmium for urban ants (Jacquier et al., 2020) or more broadly to urbanization (Diamond, Chick, Perez, Strickler, & Martin, 2018; Diamond, Chick, Perez, Strickler, & Zhao, 2018; Diamond et al., 2017). In particular, the differential responses observed between our study and Jacquier et al. (2020) suggest a variation in sensitivity to cadmium depending on the season. At the end of overwintering, city colonies could be stronger in cadmium resistance compared to forest colonies; while during spring, city colonies would no longer be able to better resist cadmium. Further studies are needed to investigate the possible seasonal effect on cadmium tolerance between the two habitats, since urban and forest ants have a slightly shifted phenology (Chick et al., 2019), and overall to disentangle the social effect from the intrinsic factors in cadmium resistance.

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DISCUSSION GENERALE ET PERSPECTIVES

Une question centrale en écologie évolutive est de comprendre le rôle et la détermination de la taille des organismes. L'objectif majeur de cette thèse était de déterminer le rôle de la diversité de taille des ouvrières au sein des colonies de fourmis, en se focalisant sur les espèces à diversité modérée. Le second objectif était de quantifier l'influence de l'environnement social sur cette diversité de taille intra-coloniale et face aux perturbations externes. Plus précisément, le premier chapitre avait pour but de quantifier le caractère adaptatif de la diversité de taille des ouvrières et donc du potentiel intérêt pour le groupe de cette diversité. A l'inverse, le second chapitre visait à explorer l'intérêt individuel, et notamment l'impact du conflit larves – ouvrières dans la production des phénotypes et de la diversité morphologique que cela peut engendrer au sein de la colonie. Enfin, notre troisième chapitre évaluait la part de l'environnement social dans la résistance à de nouvelles contraintes environnementales représentées par le cadmium.

Ces trois chapitres apportent de nombreuses informations améliorant notre compréhension sur le rôle de la taille chez les insectes sociaux, sur les mécanismes pouvant générer de la diversité de taille mais également sur le tampon social résultant de la vie en colonie. Cette dernière partie vise à synthétiser et mettre en lien les résultats obtenus, ainsi qu'à discuter de diverses perspectives ouvertes grâce à cette thèse.

La diversité de taille modérée : absence de bénéfices pour le groupe ?

Avec le chapitre 1, nous avons voulu éclaircir le rôle de la diversité de taille chez les espèces ayant une diversité modérée. Il est toujours difficile d'affirmer une absence d'effet, quel que soit le paramètre étudié, qui pourrait être issue d'un nombre limité de traits testés ou des conditions expérimentales ne reflétant pas les pressions écologiques. Pour pallier à cela et répondre au mieux à notre question, nous avons réduit expérimentalement la diversité au sein de colonies de fourmis et réalisé une étude sur le long terme (plusieurs mois) en milieu semi-naturel, afin d'avoir une mesure exhaustive et fiable des conséquences de cette réduction de diversité sur la *fitness* des colonies expérimentales. Notre manipulation a clairement mis en évidence l'absence d'effet de la diversité de taille sur la survie, la croissance et le succès reproducteur des colonies de la fourmi *Temnothorax nylanderi*. Les colonies dont la diversité avait été réduite n'ont pas restauré la taille des individus prélevés, soulignant que cette diversité n'est pas forcément primordiale pour le fonctionnement de ces colonies. De plus, dans le but d'étayer ce résultat, nous avons répété cette même expérience durant les conditions de l'hiver.

A nouveau, la diversité de taille n'influe pas sur la survie coloniale durant l'hivernage. Nous avons dans le même temps manipulé la taille moyenne des ouvrières (augmentation ou diminution) au sein des colonies, et de manière surprenante nous ne trouvons aucun effet sur toutes nos variables mesurées lors des deux études. Cumulé aux travaux en laboratoire se focalisant également sur l'intérêt de cette diversité (Colin, Doums, Péronnet, & Molet, 2017), nous pouvons affirmer avec une forte probabilité que la taille et sa diversité ne sont pas adaptatives chez notre modèle d'étude. Plus largement, la question de l'intérêt de la diversité de taille se pose lorsque celle-ci est modérée, où les résultats sont contrastés. Nous discuterons plus en détails dans la section suivante des mécanismes qui pourraient maintenir cette diversité.

Un paramètre important à prendre en compte dans l'évaluation du rôle de la taille est l'impact de la société dans son ensemble. Des différences inter-individuelles basées sur la taille sont observées en laboratoire, par exemple pour la résistance à la famine (Modlmeier, Foitzik, & Scharf, 2013) ou au froid (Heinze, Foitzik, Fischer, Wanke, & Kipyatkov, 2003), mais ces différences ne sont pas transposées à l'échelle du groupe. Ainsi, il est fondamental d'évaluer l'importance d'un trait sur la *fitness* en considérant la réponse de la colonie dans son ensemble et en intégrant toutes les composantes sociales. Les larves, notamment, peuvent améliorer la résistance à la famine chez *Temnothorax* (Modlmeier et al., 2013; Rueppell & Kirkman, 2005) lorsqu'elles sont présentes dans un groupe. De plus, le ratio ouvrières – larves peut également influencer la taille des individus produits (Purcell, Brütsch, & Chapuisat, 2012). Plus largement le couvain a un rôle important dans le fonctionnement et la dynamique des sociétés d'insectes (revue dans Schultner, Oettler, & Helanterä, 2017). Afin d'avoir une estimation optimale des conséquences d'un trait sur les performances, l'étude doit concerner l'ensemble de la colonie, autrement dit tout l'environnement social qui peut interagir dans cette société.

Si la taille n'améliore pas la *fitness* des colonies, leur succès écologique pourrait résider dans leur flexibilité comportementale. Les fourmis modérément polymorphe présentent généralement une grande flexibilité au niveau du comportement (Hölldobler & Wilson, 1990), et notamment les fourmis du genre *Temnothorax* qui démontrent une spécialisation des tâches en fonction du contexte (Pinter-Wollman, Hubler, Holley, Franks, & Dornhaus, 2012) et réagissent rapidement aux changements dans les tâches requises (Robinson, Feinerman, & Franks, 2009). Le succès écologique des insectes sociaux à fort polymorphisme pourrait ainsi résider dans l'étroite relation liant la division du travail avec la morphologie des ouvrières, tandis que le succès des espèces à diversité de taille moins prononcée pourrait provenir de leur capacité comportementale à s'adapter rapidement à des contextes spécifiques.

Des intérêts individuels pour expliquer le maintien de cette diversité ?

La diversité de taille au sein des colonies peu polymorphes n'améliore ainsi ni la division du travail, ni la survie ou la croissance. Toutefois, si elle est bien présente, son intérêt réside potentiellement dans d'autres sources. Avec le chapitre 2, nous avons abordé le conflit ouvrières – larves, qui pourrait constituer une source de variation de la taille des individus produits. En effet, les larves en développement ont davantage intérêt à se développer en individu reproducteur afin d'obtenir une *fitness* inclusive plus importante (Bourke & Ratnieks, 1999; Ratnieks, Foster, & Wenseleers, 2006; Wenseleers & Ratnieks, 2004; Wenseleers, Ratnieks, & Billen, 2003). Dans le cas de la détermination environnementale des castes, les larves pourraient ainsi tenter de devenir une reine plutôt qu'une ouvrière en obtenant davantage de ressources pour se développer. A défaut de devenir des reproducteurs, les larves peuvent aspirer à devenir de grandes ouvrières, qui sont très souvent corrélées à une plus grande fertilité chez de nombreuses espèces de fourmis (Clémencet, Rome, Fédérici, & Doums, 2008; Dietemann, Hölldobler, & Peeters, 2002; Frumhoff & Ward, 1992; Gobin & Ito, 2003; Modlmeier, Pamminger, Foitzik, & Scharf, 2012; Smith, Schoenick, Anderson, Gadau, & Suarez, 2007). En utilisant la fourmi *Mystrium rogeri* dont les larves peuvent se nourrir en autonomie directement sur des proies capturées, nous avons manuellement nourri ces larves, les laissant se développer sans contrainte sociale. Nous avons en parallèle élevé des larves dépendantes des ouvrières pour la prise alimentaire, également en isolement. Bien que préliminaire, notre étude suggère que lorsque le contrôle social est absent lors de la prise alimentaire, les larves peuvent potentiellement se développer en individus plus grands que ce que les ouvrières autorisent dans les colonies non manipulées. Ainsi, des variations dans le contrôle social pourraient être une source de diversité de taille sans que celle-ci soit forcément adaptative. Les stress externes sont connus pour influencer les phénotypes produits, par exemple une forte température (Molet, Péronnet, Couette, Canovas, & Doums, 2017) ou une exposition à un élément trace (Jacquier et al., 2020). Dans la même veine, une perturbation sociale pourrait laisser l'opportunité à des larves de se développer de manière égoïste en individus plus grands en dépit de la régulation sociale habituelle, via la nourriture par exemple, ce qui pourrait expliquer la production rare d'individus atypiques tels que des intercastes (Londe et al., 2015; Molet, Wheeler, & Peeters, 2012)

La vie sociale peut en quelque sorte autoriser une variation de taille sans que celle-ci soit bénéfique à la société. Outre les capacités de tampon des perturbations externes citées plus haut, les sociétés d'insectes peuvent supporter les coûts liés à la production des phénotypes divergents (Molet et al., 2012). Le coût lié à la production d'individus atypiques est dilué sur l'ensemble des membres et représente *in fine* un coût très faible à l'échelle coloniale. Ainsi, la vie sociale, de par sa capacité à tamponner les perturbations externes et les phénotypes divergents, pourrait réduire les pressions de sélection agissant sur les individus au sein des colonies d'insectes sociaux. Ce tampon social pourrait également réduire la canalisation du développement des individus, c'est-à-dire la capacité à maintenir la production de phénotypes constant en dépit de variations environnementales (Debat & David, 2001). Le coût à maintenir une forte canalisation serait trop important au regard des bénéfices potentiels à produire des phénotypes dans une gamme restreinte. Ce processus pourrait amplifier ou du moins autoriser une certaine diversité de taille (Hunt et al., 2011), sans que celle-ci ne soit liée à un quelconque avantage en terme de fitness pour la société.

Enfin, les progrès en génétique et génomique nous permettront sans doute de mettre en lumière de manière plus fine les bases de la variation de taille, comme suggéré par Wills et al. (2018). De même, les connaissances sur l'écologie des espèces doivent être améliorées et davantage prises en compte afin d'intégrer les données écologiques dans la diversité et ainsi déterminer l'imbrication possible de ces facteurs. Par exemple, certaines contraintes écologiques pourraient forger la diversité de taille (Powell, 2009; Powell & Franks, 2006) à l'instar de l'adaptation extrême que représente la phragmose (Hasegawa, 1993; Powell, Price, & Kronauer, 2020).

La taille, une bonne mesure de réponses chez les organismes sociaux ?

Si la taille des individus n'est pas adaptative pour la colonie et résulte plutôt dans des intérêts individuels, se pose alors la question de son utilisation en tant que variable réponse. En effet, la taille est communément utilisée en tant que telle chez *Temnothorax nylanderi* (Jacquier et al., 2020; Molet et al., 2017) ou plus largement chez les fourmis et autres insectes sociaux (par exemple : Bernadou, Römermann, Gratiashvili, & Heinze, 2016; Kelemen & Dornhaus, 2018; Purcell, Pirogan, Avril, Bouyarden, & Chapuisat, 2016). La pertinence de cette mesure de réponses peut être remise en cause par nos travaux (chapitre 1). Puisque que cette taille n'améliore pas nécessairement la *fitness* des colonies, la production d'individus de tailles différentes en fonction d'un paramètre étudié ne refléterait par forcément une conséquence significative sur la *fitness* coloniale. Ainsi, en fonction du caractère adaptatif ou non d'un trait, ici la taille, il faudrait adapter les variables mesurées afin d'évaluer fiablement la réponse des sociétés d'insectes à un paramètre défini.

Cette remise en question devrait s'appliquer toutefois qu'aux ouvrières. Bien que nous n'ayons pas testé cette hypothèse, la phase solitaire (dans le cas de fondation indépendante) des individus reproducteurs durant l'accouplement et la fondation nécessite d'importantes ressources internes, spécifiquement pour les gynes, les mâles mourant rapidement après l'accouplement (Keller & Passera, 1989; Sundström, 1995; Wagner & Gordon, 1999). En effet, la taille des reproducteurs est généralement corrélée au succès d'accouplement et de fondation de nouvelle colonie (Abell, Cole, Reyes, & Wiernasz, 1999; Fjerdingstad & Keller, 2004; Wagner & Gordon, 1999; Wiernasz & Cole, 2003; Wiernasz, Sater, Abell, & Cole, 2001).

Les potentielles limites des sociétés animales

Les sociétés animales et particulièrement les colonies d'insectes peuvent faire face à de fortes contraintes et de nombreuses perturbations, notamment via leur mode de vie en groupe et leur nid élaboré (Straub, Williams, Pettis, Fries, & Neumann, 2015; Theraulaz, Bonabeau, & Deneubourg, 1998). Généralement, les colonies les plus populeuses sont moins affectées par les stress environnementaux (Crall, de Bivort, Dey, & Versypt, 2019; Kaspari & Vargo, 1995; Molet et al., 2017; Straub et al., 2015). Cependant, notre chapitre 3 démontre que ce tampon social a ses limites. Nous avons étudié les conséquences d'une exposition à un élément trace, le cadmium. Des études ont déjà montré que les sociétés de fourmis pouvaient atténuer les effets de la contamination à des éléments traces (cadmium, zinc, plomb, cuivre …). Les concentrations mesurées diffèrent entre les castes (mâles, reines et ouvrières) et les stades de développement (larves, nymphes ou adultes), mais également entre les différents groupes d'ouvrières (revue dans Grześ, 2010). Les fourrageuses présentent notamment des concentrations en éléments traces plus importantes, à l'inverse les nymphes et les reines ont des concentrations plus faibles (Maavara, Martin, Oja, & Nuorteva, 1994; Migula & Głowacka, 1996). Toutefois, les mécanismes permettant de limiter la contamination des membres restant au sein de la colonie ne sont pas encore connus (Grześ, 2010). Ici, notre étude montre que le cadmium induit une plus forte mortalité des adultes chez les colonies les plus populeuses. Nous proposons deux explications possibles à cette perte de tampon social face au cadmium. D'une part, la concentration utilisée dans la nourriture pourrait être trop importante pour que les colonies tamponnent cette exposition. En effet, la concentration utilisée correspond à une teneur physiologique entrainant 50% de mortalité (CL_{50}) sur une période donnée, mais cette concentration n'est pas forcément pertinente sur le plan écologique (Jacquier et al., 2020). Cette concentration est environ 40 fois plus élevée que la concentration en cadmium retrouvée dans les sols urbains (Foti et al., 2017). Toutefois, une exposition récurrente aux polluants peut entrainer une bioaccumulation, et mener à de fortes concentrations en cadmium chez les fourmis *Formica polyctena* et *F. pratensis* équivalente à notre concentration CL_{50} utilisée (Starý & Kubizňáková, 1987). Ainsi, la concentration utilisée, bien que forte, pourrait se trouver dans la fourchette haute des valeurs quantifiées sur le terrain (Jacquier et al., 2020).

Une autre explication possible est l'effondrement des sociétés (*societal collapse*). Les propriétés émergentes des systèmes sociaux complexes peuvent apporter une meilleure résilience mais dans le même temps conduire à une sensibilité accrue aux perturbations (Middleton & Latty, 2016). Du fait de leur grand effectif, les colonies d'insectes pourraient être plus sujettes aux différentes perturbations de l'environnement, telles que des pathogènes, parasites, polluants, pesticides etc. Ces perturbations peuvent entrainer la mort des individus ou l'incapacité à fourrager correctement, conduisant par exemple à un recrutement de plus jeunes individus novices et moins efficaces en la matière chez les abeilles (Perry, Søvik, Myerscough, & Barron, 2015). Cela peut être une stratégie efficace face à certains stress aigus, mais en revanche peut accélérer l'effondrement de la société face à des perturbations chroniques (Barron, 2015; Perry et al., 2015). A titre d'exemple, les colonies d'abeilles sont particulièrement vulnérables aux effets subléthaux des agents pathogènes et pesticides qui conduisent à un effondrement des colonies (*colony collapse disorder*). En effet, une moyenne de 30% de perte a été répertoriée depuis 2007 en Amérique du nord (Barron, 2015). Ainsi, en étant soumises à différentes pressions et de manières durables, les colonies d'insectes pourraient être plus susceptibles de péricliter. Le large nombre d'individus constituant ces sociétés sont autant de sujets possibles pouvant être impactés par ces stress et les transmettre par la suite aux autres membres.

Perspectives

Nous l'avons déjà partiellement évoqué, l'absence d'effet est toujours difficile à affirmer. Ici, nos différentes études sur le terrain (chapitre 1) ajoutées à celle de Colin et al. (2017) en laboratoire démontrent une absence claire d'effet de la taille et de sa diversité sur la *fitness* et la performance des colonies de la fourmi *Temnothorax nylanderi*. Ces résultats pourraient être appuyés de manière encore plus soutenue en étudiant les conséquences de la manipulation de la taille sur un cycle complet continu ou bien sur plusieurs années afin d'éviter les potentiels biais saisonniers (un hiver moins rude par exemple). Nos résultats devront être également comparés avec une mesure exhaustive de la *fitness* chez les espèces sociales à diversité modérée. Par exemple, des études pourraient se focaliser sur d'autres espèces de fourmis où la diversité de taille ne semble pas apporter d'avantages sur les paramètres testés, telles que d'autres fourmis du genre *Temnothorax* (*longispinosus* (Modlmeier & Foitzik, 2011; Modlmeier, Liebmann, & Foitzik, 2012), *rugulatus* (Westling, Harrington, Bengston, & Dornhaus, 2014), *curvispinosus* (Yilmaz et al., 2019)), ou chez d'autres genres tels que *Formica* (Billick, 2002; Tawdros, West, & Purcell, 2020). Nos questionnements mériteraient également d'être étendus plus largement aux insectes sociaux, une étude sur le bourdon *Bombus impatiens* a déjà montré une absence d'effet de la taille sur les performances coloniales (thermorégulation et comportement hygiénique (Jandt & Dornhaus, 2014) ou dans la résistance au froid (Oyen & Dillon, 2018)). Ces études comparatives à travers différents taxons permettraient d'accroitre les connaissances sur l'évolution de la taille et de ses potentiels bénéfices liés. Toutefois, obtenir une mesure des trois composantes de la *fitness* (survie, succès reproducteur, croissance) est rarement aisé, notamment en fonction des traits d'histoire de vie des espèces et du dispositif et protocole à élaborer.

Il serait possible d'aller plus loin dans la distinction entre intérêts de la taille pour le groupe ou pour les individus. En reprenant notre dispositif expérimental du chapitre 1, une expérience basée sur un apport supplémentaire en nourriture permettrait d'investiguer dans quelles composantes les colonies investissent davantage. Nous avions tenté de réaliser cette expérience sur le terrain, en utilisant à nouveau la fourmi *Temnothorax nylanderi*, avec un enrichissement alimentaire d'une à quatre fois par mois (20 colonies par réplicats). Toutefois, notre dispositif n'était pas encore au point et ne nous a permis de récolter tous les individus produits à l'issue de cette expérience, et ainsi d'avoir des résultats robustes. Si la taille ou la diversité de taille des ouvrières est importante, les colonies pourraient investir dans les ouvrières plus grandes ou plus diverses ; à défaut elles pourraient produire un plus grand nombre d'individus. Sinon, elles pourraient également investir davantage dans la production d'individus reproducteurs, gynes ou males, avec potentiellement des tailles plus importantes et ayant ainsi un meilleur succès reproducteur (voir exemples cités dans la section « La taille, une bonne mesure … »). Au sein de notre espèce modèle, l'allocation des ressources a déjà été étudiée en fonction de la qualité et de la densité des nids, de l'apparentement et de l'effectif de la colonie (Foitzik & Heinze, 2000; Foitzik, Strätz, & Heinze, 2003) mais la taille des ouvrières en fonction des ressources disponibles n'a pas encore été quantifiée. Chez la fourmi polymorphe *Pheidole flavens*, une supplémentation en nourriture accroit la production de soldats par rapport aux ouvrières *minor* plus petites (McGlynn & Owen, 2002). Il serait donc intéressant de manipuler la disponibilité des ressources, afin de déterminer à la fois plus finement l'intérêt de la taille chez les espèces à diversité modérée (savoir si cette diversité de taille est constitutif ou non), mais aussi d'identifier de possibles sources de variations de tailles des ouvrières.

L'identification des sources de variation de taille dans le contingent des ouvrières est également une manière de découpler la part de l'intérêt colonial de l'intérêt individuel. Chez les insectes sociaux, le nombre de larves est très souvent corrélé au nombre d'ouvrières. Le ratio ouvrières – larves pourrait être une source de variation de taille, sans que celle-ci soit forcément adaptative. La disponibilité en ouvrières pour élever un certain nombre de larves pourrait moduler la possibilité de triche et d'expression de comportement égoïste des larves. Chez *F. selysi* où les larves sont nourries par trophallaxie de la part des ouvrières, un fort ratio ouvrières/larves conduit à une production d'ouvrières plus grandes (Purcell et al., 2012). Toutefois, ce résultat pourrait être inversé chez des espèces où des larves sont plus autonomes et se nourrissent directement sur des proies. C'est le cas chez la fourmi *Cataglyphis cursor* qui mêle trophallaxie et nourriture sur proies fraiches. La manipulation de l'environnement social, tel que le ratio ouvrières – larves, serait ainsi une intéressante étude complémentaire. D'une manière générale, l'avancée des recherches en génomiques nous servira à comprendre les bases génétiques, moléculaires et développementales des variations de taille inter- et intra-spécifiques, et ainsi appréhender au mieux le rôle de la taille chez les fourmis (Wills et al., 2018).

Une autre étape importante serait de clarifier la diversité de taille chez les fourmis. Ici, nous avons utilisé les travaux de (Fjerdingstad & Crozier, 2006) comme indicateur de variation intra-spécifique. Il serait judicieux d'effectuer la comparaison de la diversité de taille à plus large échelle étant donné que le nombre d'espèces de fourmis ne cesse de croitre (plus de 16 000 espèces à ce jour ; antweb.org). Autant la distinction entre les espèces à variation de taille discrète (présence de castes soldat et ouvrière) est claire, une clarification devrait être apportée pour les espèces à diversité continue. L'utilisation d'un indicateur commun, tel que le coefficient de variation (mesure de la variance indépendamment de la taille, calculée comme le rapport entre l'écart type et la mesure moyenne) ou le facteur de variation (rapport entre la mesure la plus grande et la mesure la plus petite), faciliterait la catégorisation entre espèces fortement, modérément ou faiblement polymorphes.

Une fois la diversité de taille plus clairement définie, une autre distinction plus fine serait nécessaire : celle du rôle de la taille moyenne d'un côté, et de la diversité de l'autre. Souvent, les deux effets sont confondus. Il serait donc pertinent de découpler à plus large échelle si l'intérêt réside dans le fait d'avoir en moyenne de grandes ouvrières, ou bien d'avoir une répartition équitable de grandes et petites ouvrières pour bénéficier des subtilités de chaque morphologie. Dans le cadre du chapitre 1, nous avons manipulé à la fois la taille moyenne et la diversité de taille des ouvrières, permettant ainsi de découpler l'effet de chacun. Nos résultats indiquent une absence d'effet de ces deux paramètres. Toutefois, d'autres études sur d'autres espèces devraient être effectuées afin de comparer et découpler finement le rôle de la taille moyenne et de la diversité. Par exemple, chez le bourdon *B. impatiens*, la taille moyenne des ouvrières améliore les performances coloniales, tandis qu'il n'y a pas d'effet de la diversité de taille (Herrmann, Haddad, & Levey, 2018 - voir aussi Jandt & Dornhaus, 2014).

Enfin, cette thèse ouvre la voie à de nouvelles tentatives de démonstration empirique du rôle de l'environnement social dans le contrôle des phénotypes produits chez les fourmis. Nos données préliminaires sur *M. rogeri* (chapitre 2) ne permettent pas d'affirmer fiablement le développement en phénotypes plus grands que ceux retrouvés en colonies naturelles, mais suggèrent qu'une manipulation artificielle est possible chez ce groupe hautement social et dont les larves dépendent fortement des ouvrières durant le développement. En raison du nombre limité de colonies à notre disposition et de la difficulté à les récolter, nous n'avons pas pu affiner notre protocole afin d'avoir des résultats plus robustes. La forte mortalité larvaire suggère qu'un équilibre doit être trouvé entre nourrissage manuel et soins aux larves délivrés par les ouvrières. Un compromis serait d'utiliser une espèce qui mêle trophallaxie et nourrissage sur des proies, mais cela pourrait biaiser la prise alimentaire des larves (les larves pourraient être sous-nourries par les ouvrières pour compenser les apports du nourrissage manuel). Quoi qu'il en soit, ces manipulations de l'environnement social méritent d'être poursuivies pour tenter de démontrer les théories communément admises mais seulement partiellement démontrées empiriquement chez les fourmis.

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ANNEXE

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Pre-existing differences in putative fertility signals give workers the upper hand in ant reproductive hierarchies

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In social groups, competition often gives rise to conflicts, which are regulated through a variety of mechanisms. In several social insect species, the conflict for male production that takes place between workers after queen loss, is regulated through the establishment of a reproductive hierarchy. A recent study of *Neoponera apicalis* showed that workers differ in their fertility levels in the presence of the queen and proposed that such idiosyncratic differences might influence access to the top of the hierarchy after queen loss. In this study, we therefore sought to characterize the influence of the initial heterogeneity in ovarian development and its chemical and behavioural correlates on the establishment of reproductive hierarchies among orphaned workers, which can only produce males. We monitored the chemical profile before and after hierarchy establishment in four groups of orphaned workers of *N. apicalis* morph 6. The analysis of the cuticular profiles showed that tricosane (n-C_{23}) was highly correlated with ovarian development and could consequently act as a fertility signal in this ant. The relative amount of tricosane on the cuticle, both before and after the establishment of the hierarchy, was also correlated with the rank achieved within the hierarchy and with the expression of agonistic behaviours. Thus, our study experimentally shows that idiosyncratic differences in a putative fertility signal (and therefore presumably in ovarian activity) between workers in the queen's presence reliably predict the outcome of reproductive conflict after queen loss. We propose that this signal (together with an increased agonistic motivation of the more fertile workers) could play a major role in the regulation of dominance/submission behaviours, enabling the most fertile individuals to rapidly access top ranks and monopolize reproduction, thereby maximizing the global reproductive success of all colony workers while minimizing the costs associated with the expression of agonistic behaviour.

Reproductive hierarchies often appear in social hymenopteran species when the queen of the colony disappears or her reproductive potential decreases. In most species, workers, although they cannot mate, maintain an ability to develop their ovaries and lay unfertilized male-destined eggs (Yagound, 2014). In these species, the establishment of reproductive hierarchies through ritualized agonistic interactions regulates the overt conflict for male production (Heinze, Holldobler, & Peeters, 1994; Oliveira & Hölldobler, 1990). An individual's rank stems from several factors that are classically described as ‘intrinsic’ and ‘extrinsic’ and which are intertwined in a network of feedback loops. Intrinsic traits refer to the state of each individual (e.g. neuroendocrine titres, reproductive status and motivation to fight, as well as potential chemical cuticular correlates) that determine its absolute fighting ability (so-called resource-holding power, or RHP, after Parker, 1974). Extrinsic factors that play a role in establishing hierarchies include the effects of past experiences (Rutte, Taborsky, & Brinkhof, 2006) and social environment whose causal role is exerted through the modification of intrinsic factors, which in turn modify future experiences. These influences are notably reflected in winner–loser effects where the outcome of an encounter (victory or defeat) induces changes in the neuroendocrine titres (Hsu, Earley, & Wolf, 2006), thus influencing individual behaviour and the outcome of future encounters (Dugatkin & Earley, 2004; Sasaki et al., 2016).

In ants, cuticular hydrocarbons (CHCs) are well known for indicating colonial affiliation, but they also convey more subtle social

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information about species, sex, caste, hierarchical status and reproductive status (Greene & Gordon, 2003; Liebig, 2010), thus constituting unique individual chemical profiles. This chemical signal results from quantitative or qualitative differences (or both) of endogenous and exogenous origins between one or more compounds across individuals, castes and colonies (d'Ettorre & Lenoir, 2010). The signal can therefore allow the recognition of a congener's idiosyncratic characteristics, and many studies have demonstrated the involvement of CHCs in fertility and/or dominance signalling (Abril et al., 2018; Holman et al., 2013, 2016; Smith et al., 2009, 2015). The perception of the signal modifies the behaviour of potential partners (reviewed in Leonhardt, Menzel, Nehring, & Schmitt, 2016). When it reflects RHP and/or fertility, the signal is thought to be honest, and workers are accordingly expected to follow their own interests in response to the signal and promote their inclusive fitness (Keller & Nonacs, 1993; Heinze & D'Ettorre, 2009). Thus, in the case of reproductive hierarchies in a queenless colony, the most fertile worker should be selected to access the top of the hierarchy and produce males. Selection of the reproductive individuals stems from a fine balance between direct fitness costs, indirect fitness benefits and relatedness (Keller & Nonacs, 1993). Yagound, Blacher, Fresneau, Poteaux, and Châline (2014) have shown that workers of a Neotropical ant species, *Neoponera apicalis*, can use CHCs as an index of rank in workers' established reproductive hierarchies, the quantity of certain compounds functioning as a reliable signal of both individual ovarian development and social status.

Here, we studied the establishment of reproductive hierarchies after queen loss in workers of *N. apicalis*. In this species, workers cannot mate and therefore are unable to produce female progeny (Fresneau, 1994). While queenright workers do not usually lay eggs, they start producing males soon after being orphaned (Dietemann & Peeters, 2000). Because there is no production of new workers, which would care for the brood or adopt a new queen, males must be produced rapidly after the queen's death or no nurses will be available. Behavioural mechanisms exploiting interindividual differences in queen presence for both reproductive physiology and chemical signalling (Yagound et al., 2015) would allow the rapid establishment of a reproductive hierarchy after queen loss and hence meet the evolutionary pressure to rapidly solve the conflict between workers over male production (Dietemann & Peeters, 2000). Namely, we propose that the most fertile workers could be more motivated to enter the reproductive race, and that fertility signalling could help resolve the ritualized agonistic encounters. Such behavioural processes would ensure the most fertile workers lead the hierarchy, thereby maximizing the inclusive fitness of the whole worker collective (Hamilton, 1964; Keller & Nonacs, 1993). To test our hypothesis, we first correlated variation in cuticular profiles and ovarian development among workers to determine which compound might be the putative fertility signal in *N. apicalis* (Liebig, Peeters, Oldham, Markstadter, & Holldobler, 2000; Monnin, Malusse, & Peeters, 1998; Yagound et al., 2015). We then jointly monitored the development of this compound (as a noninvasive proxy for ovarian development) and of ritualized agonistic behaviours by workers, from queen loss to the stabilization of the reproductive hierarchy. We predicted that the workers most fertile in the presence of the queen would be more active during the establishment of the hierarchy and therefore would access the top ranks and monopolize reproduction.

METHODS

Ethical Note

Neoponera apicalis is a common ant species in central-south American tropical forests. We obtained collection permits (No 47615) from the Chico Mendes Institute for Conservation and Biodiversity (ICMBio/SISBIO) from the Brazilian Ministry of the Environment (MMA). Our experimental design in the laboratory included the orphaning of four experimental groups of workers, the labelling and behavioural observation of individual ant workers, the monitoring of the cuticular compounds and the dissection of workers to record ovary development. Ants were kept in artificial nests which are commonly used in ant research and in which ants do not show abnormal or stereotypical behaviour. The whole range of expected behaviour was observed. Ants were manipulated with soft forceps, which prevent any damage, and marked with paint, which does not alter their behaviour in the long term. Ants were killed by freezing before dissection. All these procedures were conducted following the institutional guidelines of animal welfare of both Brazil and France.

Ants

We collected 18 colonies of *N. apicalis* in Brazil in November 2016: eight queenless and six with fewer than 20 workers (1–56 workers, mean 22.1 workers per queenright colony, SD 17.5). The fact that eight colonies were queenless thus suggests that queenlessness is common in this species. Comparison of hierarchy establishment in the *N. apicalis* species complex showed that it occurs earlier, and agonistic behaviour is more pronounced, in monogynous species (Yagound, 2014). This suggests that an increased chance of queenlessness selects for hierarchy establishment mechanisms allowing quick conflict resolution. In this study, we used four colonies: colony 1 was collected in Marituba, state of Para (1°21′18″S, 48°20′21″W), and colonies 8, 18 and 20 in Santa Barbara do Para (1°13′36″S, 48°‘17′43″W). Cytochrome C oxidase I sequence analysis revealed that our colonies belong to morph 6 of *N. apicalis* (Yagound, Savarit, Fichaux, Poteaux, & Châline, n. d.). *Neoponera apicalis* was divided into three morphospecies by Delabie, Mariano, Mendes, Pompolo, and Fresneau (2008) based on fine morphological differences in this complex of cryptic species. Ferreira, Poteaux, Delabie, Fresneau, and Rybak (2010) defined three additional morphs based on a set of morphological, acoustic, chemical and genetic data. Yagound (2014) added a seventh morph. Mackay and Mackay (2010) described morph 5 as *Neoponera cooki*, but the original numeration is kept in order to be consistent. Colonies were harvested in mid-October 2016 and installed in the laboratory a week later. The experiment started 2 months after their installation. During this acclimation period, workers remained with the queen. The ants were housed in plaster nests (18 x 14 cm) connected to an external environment of the same size. They were maintained at a temperature of 25 ± 2 °C, a relative humidity of 50 ± 10% and a day:night cycle of 12:12 h. Each colony was fed three times a week with an apple–honey mixture and thawed crickets (*Acheta domestica*), as well as water ad libitum.

Based on the study by Yagound, Blacher, Chameron, and Châline (2012), which showed that workers close to the queen were the first to reproduce at the onset of hierarchical competition, we assumed that the most fertile workers would stay next to the queen within the nest. We selected and individually marked 20 workers in the vicinity of the queen in each experimental colony. Each of these workers received a number label glued on the thorax and two coloured dots (Uni-ball marker). The chemical profile of ants before orphaning was extracted using SPME (see below). The individuals were then released into the original nest. The following week, 15 of these 20 selected workers were isolated and placed in another artificial nest of the same type, to mimic an orphaning process. We recorded agonistic interactions, that is, antennal boxing and bites, in the nest (see Dominance Hierarchy below). Antennal boxing consists of repeated and rapid strokes of one ant by another with the antennae. This behaviour is typical of many ant species and is often observed during the establishment of hierarchies. In established hierarchies, dominant individuals perform antennal boxing against subordinate individuals (Blacher, Lecoutey, Fresneau, & Nowbahari, 2010; Monnin & Peeters, 1999; Yagound et al., 2014). Biting occurs when the individual uses its mandibles to grip a part of another individual's body. In most instances, biting was prolonged, thereby immobilizing the other individual with no apparent damage or cuts, and we consider this behaviour ritualized biting. On the 10th day of the experiment, workers were frozen for later extraction of their chemical profile after orphaning and measurements of their ovarian development (Fig. 1). Of the initial 60 ants, 59 survived to this stage.

Extraction and Analysis of Chemical Profiles

The individuals' chemical profiles were analysed before they were orphaned and after the establishment of the reproductive hierarchy. The initial chemical profile (before orphaning) was obtained by solid-phase microextraction (Monnin et al., 1998). This involved rubbing an SPME fibre (polydimethylsiloxane 100 μm) on the first segment of the abdomen for 2 min. The fibre was then desorbed in a Varian 3900 gas chromatograph with flame ionization detection (GC-FID). The carrier gas used was helium at 1 ml/min, with hydrogen streams at 30 ml/min and air at 300 ml/min. The programme was as follows: the initial temperature was 70 °C for 1 min, then it rose from 40 °C/min for 4 min to 250 °C, then increased by 1 °C/min for 8 min to 258 °C and finally increased from 40 °C/min to 320 °C and stabilized at 320 °C for 3 min. The temperature of the injector was maintained at 280 °C and that of the FID at 340 °C. Profiles were extracted with the Varian system control software Star Chromatography workstation version 6.2 (Varian, Palo Alto, CA, U.S.A.). The compounds were identified based on their retention time (Appendix Table A1), comparing them to standard hydrocarbons already identified in a gas chromatograph coupled to a mass spectrometer (GC-MS) as well as chromatograms of Yagound (2014) for *N. apicalis* morph 6. The advantage of this method was that it was not invasive; however, it was time consuming and did not allow quantification of compounds.

Owing to time constraints, the chemical profile after orphaning was obtained by a liquid phase extraction. The head and thorax of each dissected ant were soaked in 200 μl of pentane containing 4 ng/μl of compound n-C_{17} (representing our internal standard) in a vial tube for 5 min. The tube was then left to evaporate. After the solution was completely evaporated, the sample was analysed by GC–MS (Agilent A7890), by injecting 2 μl of the extract resuspended in 80 μl of solvent (pentane), with electron impact ionization at 70 eV. The carrier gas was helium at 1 ml/min. The same analysis programme as above was applied. The chemical profiles were integrated using the MSD ChemStation software version E.02.01.1177 (Agilent Technologies Inc., Santa Clara, CA, U.S.A.). The compounds were identified by comparing their retention time and spectra with already known compounds. The internal standard allowed us to translate peak areas to absolute quantities for the related compounds.

Dominance Hierarchy

Antennal boxing and bites were recorded, together with the identities of the interacting ants. The loser was the ant showing submissive behaviour, that is, hunching or dodging. Twelve observation sessions were carried out (15 h total) per colony: two of 1.5 h on the first, second and third days after being orphaned, then two of 1 h on the fourth and fifth days and finally one of 1 h on the eighth and 10th days. During these sessions, all boxing and bites were recorded. Observations were made under red light to avoid biasing the ants' behaviour in the interior of the nest (Depickère, Fresneau, & Deneubourg, 2004).

The hierarchical rank of each worker in the orphaned colonies was obtained using the 'Glicko-rating' method, which is a dynamic matched comparison model that calculates a score for each individual, based on the outcome of each individual's interactions (victory or defeat; Glickman, 1999). From this score, a ranking can be determined to deduce the hierarchy. The Glicko-rating algorithm includes a positive constant 'c', which governs the size of the standard deviation over time. This constant is defined by the user, an increased value of 'c' leading to a greater average deviation per individual over time. In our study, following the guidelines of Glickman (1999; and see So, Franks, Lim, & Curley, 2015), we used a value of 1 for 'c'. We checked the impact of the 'c' value on our results by replicating the calculation over a range of 1–10. We obtained similar results for the hierarchical rankings over the whole range. Glicko-rating calculations were performed with the PlayerRatings package v1.0 (Stephenson & Sonas, 2014) in R 3.4.1 (R Development Core Team, 2017). Data were compiled in chronological order of dyadic interactions. The same coefficient was attributed to antennal boxes and bites, so that in the calculation of the hierarchy the two types of agonistic behaviours had the same power.

Fertility Measurement

With a graduated 10× binocular microscope, we measured the ovarian development of the ants. The length of the three basal

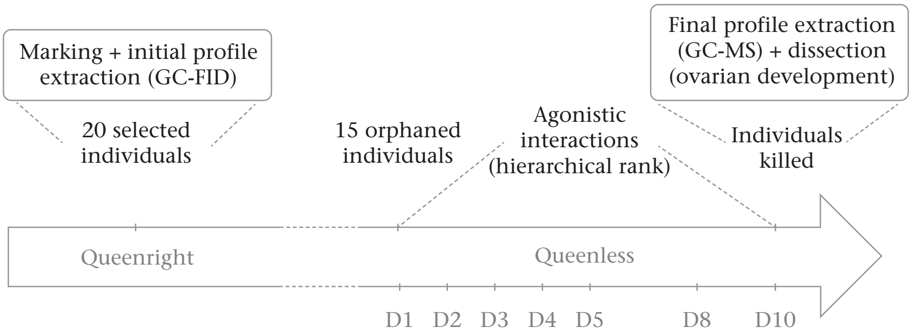

Figure 1. Timeline of the experiment. The queen was removed from each colony 7 days after individuals were marked and the experiment ran for 10 days (D1 - D10). 'Initial profile' corresponds to the workers' chemical profile in the presence of the queen (determined by flame ionization detection, GC–FID); 'final profile' corresponds to the chemical profile at the end of the experiment (determined by gas chromatography–mass spectrometry, GC–MS). Agonistic interactions (antennal boxing and bite) were used to calculate the hierarchical rank of the 15 orphaned individuals per colony.

oocytes of the ovarioles of each ovary was measured. A fertility index was calculated by summing the lengths of the six basal oocytes (Yagound et al., 2014). We present this below as mean ± SD.

Statistical Analysis

Establishment of the hierarchy

The distribution of the average number of agonistic interactions per hour of observation was compared between colonies to compare the dynamics of hierarchy establishment. For this, a two-sample Kolmogorov–Smirnov test was performed between each pair of the four colonies. To compensate for multiple comparisons, P values were then adjusted to P' values following Holm (1979).

The linearity 'h' within our four colonies was calculated between the 15 orphaned workers by the de Vries (1995) method using software R (package compete, Curley, 2016).

To verify whether worker isolation led to the establishment of a reproductive hierarchy, we investigated the link between the hierarchical rank and fertility of individuals using Spearman correlations for the 59 orphaned ants dissected at the end of the experiment.

Chemical data analysis

Although some intercolonial heterogeneity is expected in the proportion of each compound in the cuticular profiles, a principal coordinate analysis (PCo) and an analysis of similarity (ANOSIM) were performed to verify whether our experimental colonies (59 workers) shared a similar chemotype, due to the potential presence of cryptic morphs, differing in chemical profiles. For this, we used the PERMANOVA+ for PRIMER software (Anderson, Gorley, & Clarke, 2008) using a Euclidean distance matrix calculated on square-root-transformed percentages.

Using two different methods to extract the chemical profiles was a potential source of methodological variability. To verify whether our methods were reliable, we used Spearman correlations to compare the profiles before and after orphaning using the proportions of the major compounds, namely n-C_{21}, $C_{23:1}$ and n-C_{23}, with a Bonferroni–Holm adjustment for multiple tests on the same data set (Holm, 1979). Significant correlations would indicate reliability of the two methods (even if distinct methods can generate a slight chemical distance between the profiles before and after orphaning). This calculation could be done only for three colonies (see Results for details). In addition, two individuals from colony 20 could not be included in these chemical analyses because of a technical problem when acquiring the profile before orphaning (missing data). In colony 20 there were only 14 individuals because a worker died during the experiment. We thus analysed a total of 42 workers. For these three colonies we also performed a Mantel test (package ecodist in R) between the Euclidean distance matrix of the square-root-transformed percentages of the chemical profiles before and after orphaning to evaluate global concordance between the two methods.

To identify the cuticular compound(s) potentially acting as a fertility signal in our study, we used Spearman rank correlation (on 44 workers) to assess the relationship between the fertility index measured at the end of the experiment and the final quantities of cuticular compounds. Once the putative fertility signal was identified, we also checked for correlations with the proportions before orphaning to verify pre-existing heterogeneity between the workers in the presence of the queen (42 workers). P values were adjusted to account for multiple testing of the same data (Holm, 1979).

Last, we investigated the presence of a correlation between the putative fertility signal and the observed behaviour using Spearman correlation. The deviation from the mean quantity of fertility-related compound(s) (within each colony) was correlated with the different behaviours expressed by each individual (42 workers). Using a mean deviation index allowed us to buffer the effect of intercolonial variation in the quantity of compound.

RESULTS

Setting up Reproductive Hierarchies

Dynamics of agonistic behaviours

Despite some variability among colonies in the intensity of agonistic displays (Fig. 2), the dynamics of agonistic behaviour did not differ significantly between them. No two by two comparison between colonies was significant (Kolmogorov–Smirnov test with Bonferroni–Holm correction: colony 1–8: $D = 0.571$, $P' = 0.203$; colony 1–18: $D = 0.571$, $P' = 0.203$; colony 1–20: $D = 0.571$, $P' = 0.212$; colony 8–18: $D = 0.571$, $P' = 0.203$; colony 8–20: $D = 0.571$, $P' = 0.203$; colony 18–20: $D = 0.429$, $P' = 0.575$). Agonistic interactions rose rapidly within the first 2 days of being orphaned and then returned to basal level.

Establishment of hierarchies

Hierarchies established in colonies 8, 18 and 20 had linearities of $h' = 0.52$ ($P = 0.001$), $h' = 0.66$ ($P < 0.001$) and $h' = 0.65$ ($P < 0.001$),

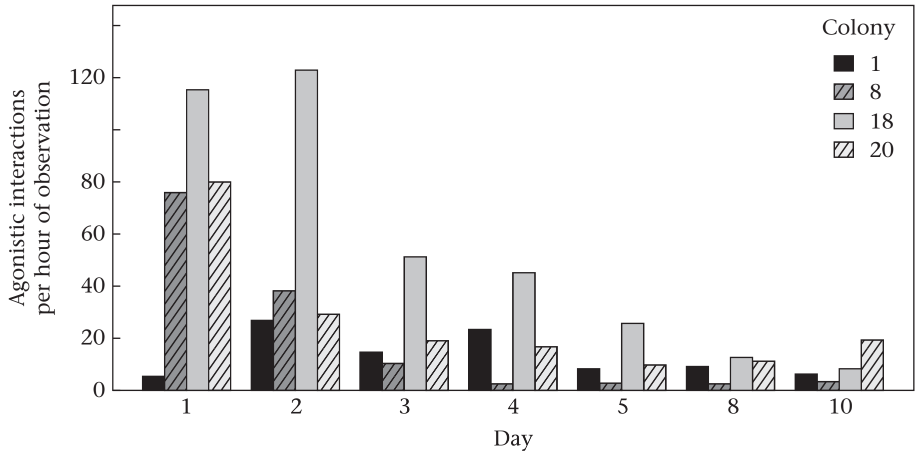

Figure 2. Number of agonistic interactions per hour of observation as a function of observation day during the 10 days after the queen was removed.

respectively. The hierarchy in colony 1 did not show a significant linearity ($h' = 0.19$, $P = 0.48$).

Rank and ovarian development

All but 10 of our 59 workers had activated ovaries. The average ovarian development, measured at the end of the experiment, was highest for colony 18 (4.16 ± 2.59 mm), followed by colony 8 (3.72 ± 3.10 mm), colony 1 (3.42 ± 2.25 mm) and finally colony 20 (2.97 ± 2.06 mm). Within each colony the fertility index of workers was significantly correlated with their hierarchical rank from the first day of being orphaned (Table 1).

Chemical Profiles, Fertility and Behaviours

Chemical analyses of profiles after orphaning

The chemical profiles were grouped by colony in the PCo and with the ANOSIM, suggesting the existence of a characteristic colonial signature (Appendix Fig. A1). Considering the average chemical distance calculated between colonies, colony 18 was very different from the others (0.75 on average with colony 18, against 0.20 between the other three colonies; Appendix Table A2).

The cuticular profile of each ant was composed of 28–30 peaks and included several series of n-alkanes, branched mono and dimethyl-alkanes and alkenes, with carbon atom numbers ranging from 19 to 33. The majority of compounds were linear alkanes and alkenes. Consistent with the chemical distance results, colonies 1, 8 and 20 displayed a qualitatively distinct chemotype from colony 18 (Appendix Fig. A2). Colony 18 was thus excluded from correlation analysis with the chemical profiles. The chemical profiles of the workers from colony 18 were heterogeneous, some appearing separated and others represented among the other colonies' profiles (Appendix Fig. A1). As several morphs of *N. apicalis* occur in the collection area, this unusual result could thus be a consequence of a chance hybridization between two morphs (i.e. a male from another morph), which cannot be detected using nuclear DNA. Although interesting, we have no additional means to explain this discrepant chemotype.

Table 1
Spearman correlations between an individual ant's fertility index and hierarchical rank on the first, second and 10th (final) day of the experiment

	r_S	P'	N
Day 1 rank	−0.3	0.03	59
Day 2 rank	−0.39	0.006	59
Day 10 rank	−0.44	0.002	59

To compensate for multiple comparisons, P values were adjusted to P' values following Holm (1979).

Table 2
Spearman correlations between the proportions of the three main compounds within individual ants' chemical profiles before and after queen removal

Cuticular hydrocarbons	r_S	P'	N
n-C_{21}	0.48	0.001	42
$C_{23:1}$	0.34	0.03	42
n-C_{23}	0.74	2.1e-8	42

To compensate for multiple comparisons, P values were adjusted to P' values following Holm (1979).

Reliability between the two chemical extraction methods

The proportions of the three major compounds (n-C_{21}, $C_{23:1}$ and n-C_{23}) in the SPME samples analysed by GC–FID before orphaning were significantly correlated with those analysed by GC–MS after orphaning (Table 2). The Mantel test between the two chemical profile matrices before and after orphaning were significantly correlated ($P < 0.001$) with a Mantel value of 0.70, which, considering the potential variation due to fertility and environmental changes between the two analyses, is sufficient to validate the use of the two methods. Initial and final n-C_{23} proportions were also correlated (Spearman correlation: $r_s = 0.75$, $P < 0.001$).

Correlation between chemical profiles and fertility

The amount of the alkane tricosane (n-C_{23}) after orphaning was significantly correlated with the fertility index for each individual (Spearman correlation: $r_s = 0.63$, $N = 44$, $P < 0.001$; Fig. 3a), as was the proportion of n-C_{23} in the chemical profile before orphaning (Spearman correlation: $r_s = 0.69$, $N = 42$, $P < 0.001$; Fig. 3b). This compound was the component of the chemical profile that best correlated with ovarian development. Correlation values of the other compounds are presented in Appendix Table A3.

Fertility and behaviour

We focused on n-C_{23} which was the best correlated compound with fertility. Both the number of fights won by an ant and the number of interactions it was involved in were significantly correlated with the quantity of n-C_{23} after orphaning (Table 3). The same results were obtained when considering only the first 2 days of interaction. Last, behaviours at the beginning of the experiment (first 2 days) were highly correlated with all behaviours observed during the whole 10 days of the experiment (Table 4), showing that the hierarchy was established during the first 48 h after queen loss.

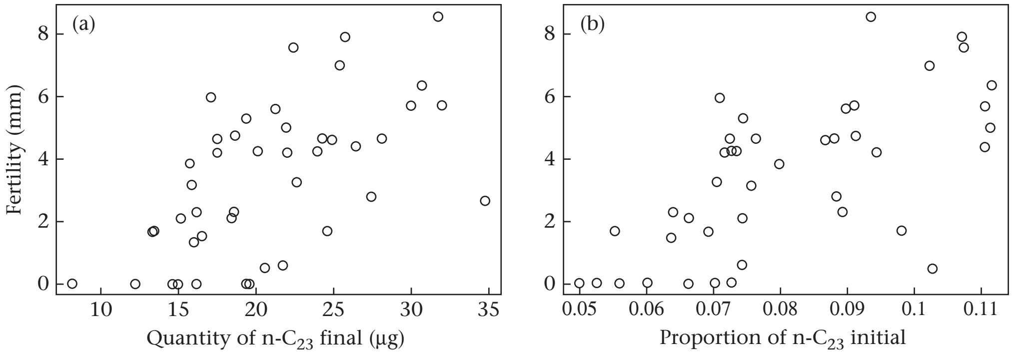

Figure 3. Variation in the fertility index (summed lengths of the six basal oocytes in the ovary, mm) as a function of (a) the amount of tricosane in the ants' chemical profile after queen removal and (b) the proportion of tricosane in the profile before queen removal.

Table 3
Spearman correlations between the behaviours expressed after (final n-C_{23}) and before (initial n-C_{23}) queen removal and the mean deviation in amount of n-C_{23} between individuals of the same colony

	r_S (final n-C_{23})	P'	r_S (initial n-C_{23})	P'	N
Final hierarchical rank	−0.52	0.002	−0.48	0.003	42
No. of fights won during days 1 and 2	0.46	0.008	0.53	0.002	42
Total no. of fights won	0.53	0.001	0.59	2.5e-4	42
Percentage of fights won during days 1 and 2	0.37	0.01	0.43	0.008	42
Total percentage of fights won	0.44	0.008	0.44	0.008	42
Fight number during days 1 and 2	0.45	0.008	0.51	0.002	42
Fight number total	0.5	0.002	0.59	2.2e-4	42

To compensate for multiple comparisons, P values were then adjusted to P' values following Holm (1979). ‘Final’ and ‘total’ correspond to the behaviours expressed from day 1 to day 10 of being orphaned. ‘Fight number’ corresponds to the number of fights an individual was involved in. Data dispersions are presented in Fig. A3.

DISCUSSION

Our results confirmed the rapidity of hierarchy establishment over a period of 48 h after queen loss. The number of agonistic behaviours decreased drastically after this period, which is typical of a stabilized hierarchy. The cuticular profiles analysis showed tricosane to be highly correlated with ovarian development, therefore putatively acting as a fertility signal. Interestingly, the relative amounts of tricosane on the cuticle both before and after the establishment of the hierarchy were also correlated with (1) the rank achieved within the hierarchy and (2) the frequency of the agonistic behaviours displayed.

These results constitute the first experimental evidence that differences in ovarian activity (estimated by an indirect method) between workers in the presence of the queen accurately predict the outcome of the reproductive hierarchy, which is a consequence of a tournament between workers. They also fully support our hypothesis that physiological differences between workers are mirrored in modulated motivations to fight (Stevenson, Hofmann, Schoch, & Schildberger, 2000). The outcome of the dominance/submission could then be facilitated with chemical signalling (Yagound et al., 2015).

Fertility signals have been identified in other morphs of *N. apicalis* (Yagound, 2014), but not in morph 6. In three colonies (the fourth having a different chemotype) tricosane was the cuticular compound best correlated with fertility. Thus, tricosane is the most probable fertility signal in these colonies. We used tricosane as a proxy for the fertility signal, but we are aware that it may also be part of a mixture of compounds used for fertility recognition. This does not, however, change the conclusions of our study. In his comparative study of fertility signalling in the *N. apicalis* complex, Yagound (2014) found that an alkene was best correlated with fertility in morph 6 ($r_s = 0.75$, $P < 10^{-4}$). However, Yagound could analyse only one colony and he also found that tricosane was correlated with ovarian development ($r_s = 0.51$, $P < 0.01$). This finding and the larger sample that we analysed legitimize the interpretation of tricosane as a putative signal of fertility for *N. apicalis* morph 6. Other compounds were also correlated with fertility in our sample but tricosane appeared to be the best correlated compound with both fertility and behavioural parameters (the expression of agonistic behaviours and the social ranks achieved) during the establishment of the hierarchy. This consistency between physiological and behavioural data fulfils the condition for tricosane to be an index of fertility. The correlation between the putative fertility signal and the hierarchical ranks also strengthens the idea of it being an honest signal (Heinze & D’Ettorre, 2009). A reliable index of fertility allows appropriate reproductive decision making, depending on individual interests in terms of inclusive fitness (Yagound, 2014). This signal would allow workers to identify the best potential reproducer within the colony.

Table 4
Spearman correlations between behaviours during days 1 and 2 combined and from day 1 to day 10 of the experiment

	r_S	P	N
Hierarchical rank	0.86	<2.2e-16	59
No. of fights won	0.96	<2.2e-16	59
Percentage of fights won	0.90	<2.2e-16	59
No. of fights	0.95	<2.2e-16	59

The initial heterogeneity between ants in queenright colonies could be amplified during subsequent agonistic interactions. Idiosyncratic variations could initially reflect the differences in workers' ages affecting their physiological and hormonal states, and hence their ovarian activation (Yagound et al., 2015). Workers with an already partially active ovarian system would have a clear advantage during the establishment of hierarchies. Lamba et al. (2007) hypothesized that in other eusocial insects (wasps) fighting could be used not to exclude the other females from dominance, but rather to speed up the development of the ovarian system of the future reproductive (via an action on biogenic amines) and so facilitate the monopolization of colony reproduction. Aggressive behaviours also lead to a decrease in juvenile hormone titre (usually positively correlated with fertility) in subordinate individuals (Tibbetts, Fearon, Wong, Huang, & Tinghitella, 2018). Physical contact between workers in the ant *Diacamma* has also been shown to affect dopamine secretion in the worker's brain and to regulate reproduction inside the nest (Shimoji et al., 2017). Agonistic interactions in *Neoponera* may thus impact ovarian development through similar neuroendocrine changes.

Hierarchical status discrimination based on the putative fertility signal can generate a linear hierarchy. Fertility signalling would be involved in both the establishment (Yagound et al., 2015) and the maintenance (Heinze, Stengl, & Sledge, 2002) of the reproductive hierarchy. Agonistic interactions acting on the physiological and hormonal secretions would reinforce the pre-existing differences in fertility between individuals, and this would accelerate cooperation within the nest. Subordinates would maximize their fitness by quickly resolving conflicts by reducing their ability to reproduce (Tibbetts et al., 2018).

Interestingly, our results showed a strong correlation between fertility and the number of fights an individual is involved in (whatever the outcome). This result suggests two mutually nonexclusive hypotheses. First, tricosane could be correlated with both fertility and motivation to fight and/or involvement in the colony's hierarchy. Biogenic amines such as octopamine or dopamine could possibly be involved in this process. Indeed, biogenic amines mediate changes in dominance behaviour linked with fertility in the ant *Harpegnathos saltator* (Penick, Brent, Dolezal, & Liebig, 2014). Moreover, it has been demonstrated in the cricket *Gryllus bimaculatus* that these bioamines are necessary to trigger aggressive behaviour (Stevenson et al., 2000). Second, tricosane could act as a fertility signal and thus attract aggression from competitors attempting to gain dominance. Such behaviour where workers attack congeners that display fertility signals has been shown, for example, in the context of worker policing in social insects (ants: Hartmann, D'Ettorre, Jones, & Heinze, 2005; Monnin & Peeters, 1999; Smith et al., 2009; bees: Visscher & Dukas, 1995; wasps: Wenseleers, Tofilski, & Ratnieks, 2005). This mechanism could thus ensure the fertility signal has similar functions in the contexts of worker policing and establishment of the reproductive hierarchy, namely regulating reproduction at the level of the worker collective.

The fact that tricosane was also correlated with the percentage of fights won favours the first explanation. Attacked individuals in the case of worker policing are indeed more likely to be defeated (and their reproductive activity suppressed; Monnin & Peeters, 1999), while highly motivated animals could have an advantage in a tournament system. Tricosane could thus both reflect ovarian development and be correlated with a network of neuroendocrine activity that ensures fighting motivation and, maybe more generally, the ability to mobilize resources (RHP; Parker, 1974). One mechanism ensuring the honesty of the fertility signal (and its role in the reproductive hierarchy) could be the strong links between the neuroendocrine networks involved in the regulation of reproduction, agonistic behaviour and fighting abilities. A second mechanism could be that individuals 'motivated' to fight, but lacking the skills required to occupy the top of the hierarchy, would be defeated by other workers. This mechanism would be in line with theories proposing that the costs (both physiological and social) of maintaining a signal ensure its honesty (Zahavi, 1975; Heinze & D'Ettorre, 2009).

The loss of the queen probably lifts an inhibition for already fertile individuals which very quickly start competing to reproduce. The highest motivation for fighting of these individuals probably drives the expression of ritualized agonistic encounters within the colony. The impact of social experience and especially winner–loser effects would then help amplify the pre-existing differences at the physiological (Oliveira, Silva, & Canário, 2009), cognitive and behavioural (Hsu & Wolf, 2001; Rutte et al., 2006) levels. The social system would then develop from the queenright state, where all workers refrain from reproducing, to the establishment of the reproductive hierarchy based on self-organized processes. After a short period of social perturbation with intense fighting behaviour, the social system stabilizes again with the selection of a new reproductive individual and the disappearance of agonistic interactions.

To our knowledge, this is the first study to monitor the development of the chemical profile from the queenright state to the stabilization of a reproductive hierarchy by orphaned ant workers. Our study supports the hypothesis that the pre-existing fertility differences between individuals in the queenright condition predict the destiny of workers in the reproductive hierarchy. The most fertile workers reach the high ranks and produce males. The selective pressures are strong after queen loss, with a short time window for producing the last batch of reproductive ants (Dietemann & Peeters, 2000). In response to these strong ecological constraints, ants have developed a recognition system based on cuticular hydrocarbons related to ovarian development and acting as a fertility signal (Yagound et al., 2015). This fertility signal, already perceptible in the presence of the queen, makes it possible for workers to evaluate the interindividual differences and, subsequently, agonistic interactions help to establish and stabilize the reproductive hierarchy (especially with winner–loser effects; Chase, Tovey, Spangler-Martin, & Manfredonia, 2002). All these mechanisms allow a quick resolution of the overt conflict and ensure division of reproductive and ergonomic tasks inside orphaned colonies to allow the production of males.

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Appendix

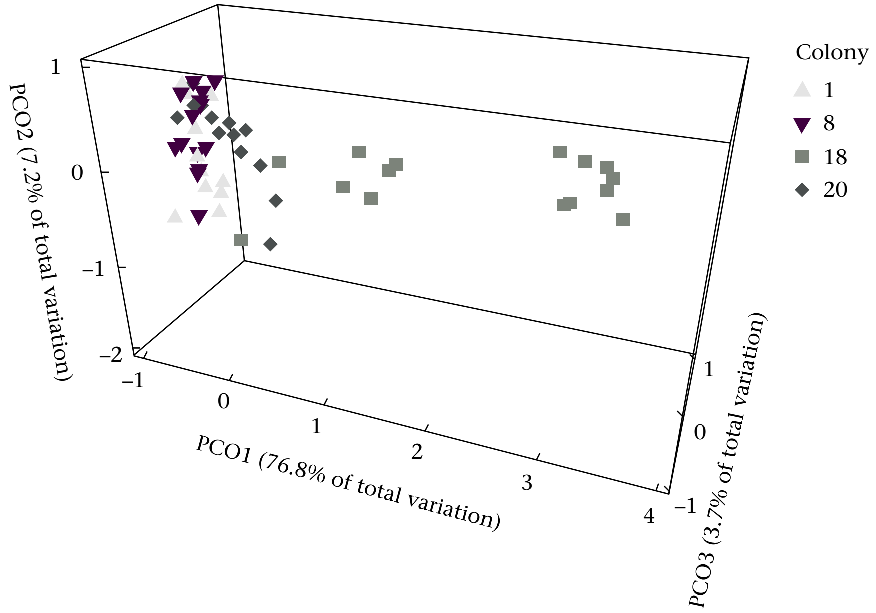

Figure A1. Principal coordinate analysis (PCO) of the chemical profiles of the four colonies (based on the Bray–Curtis similarity matrix calculated with the square-root-transformed proportions). $N = 15$ individuals in each colony.

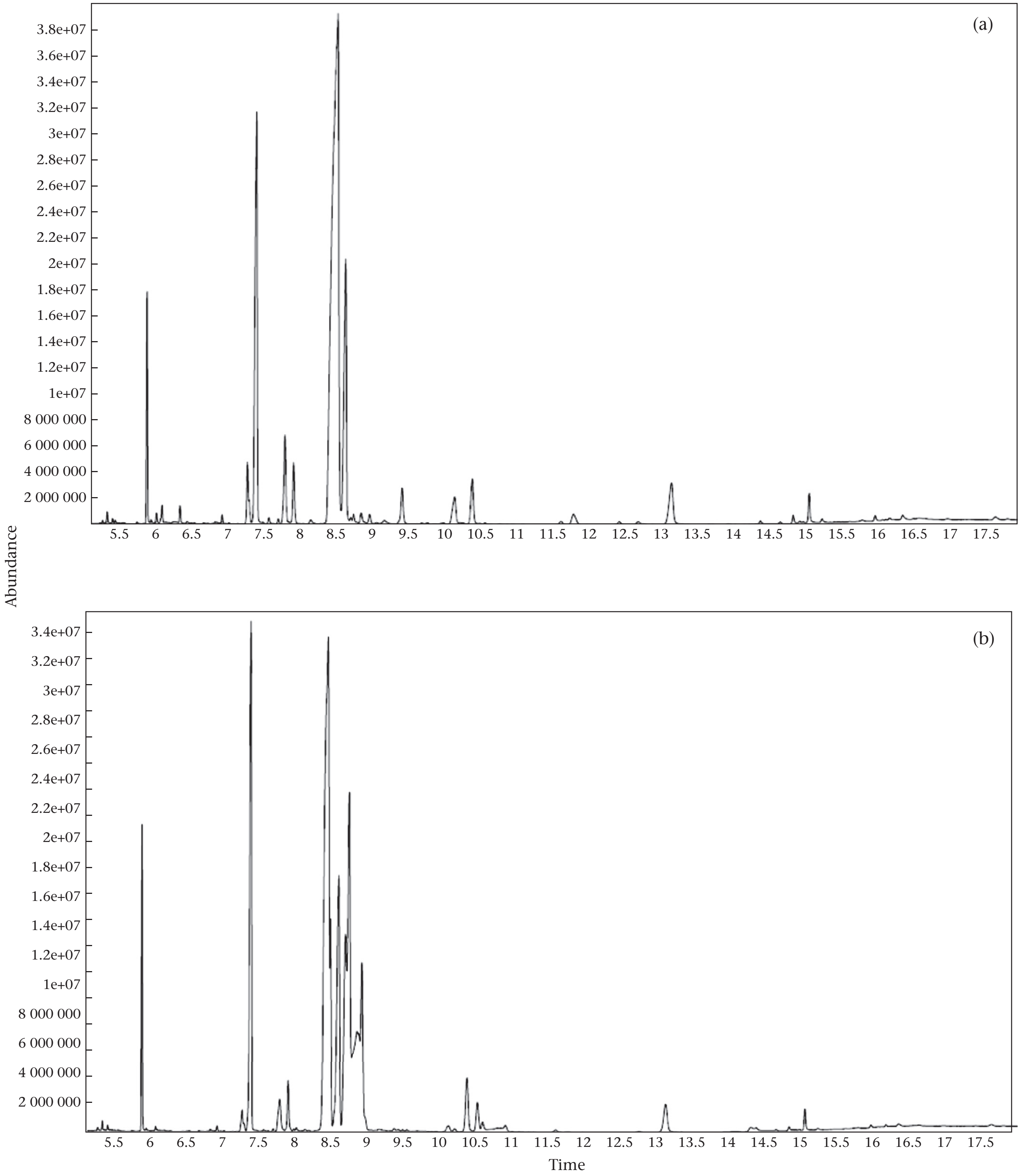

Figure A2. Representative examples of the chemical profiles from (a) colony 1 (chemotype A) and (b) colony 18 (chemotype B).

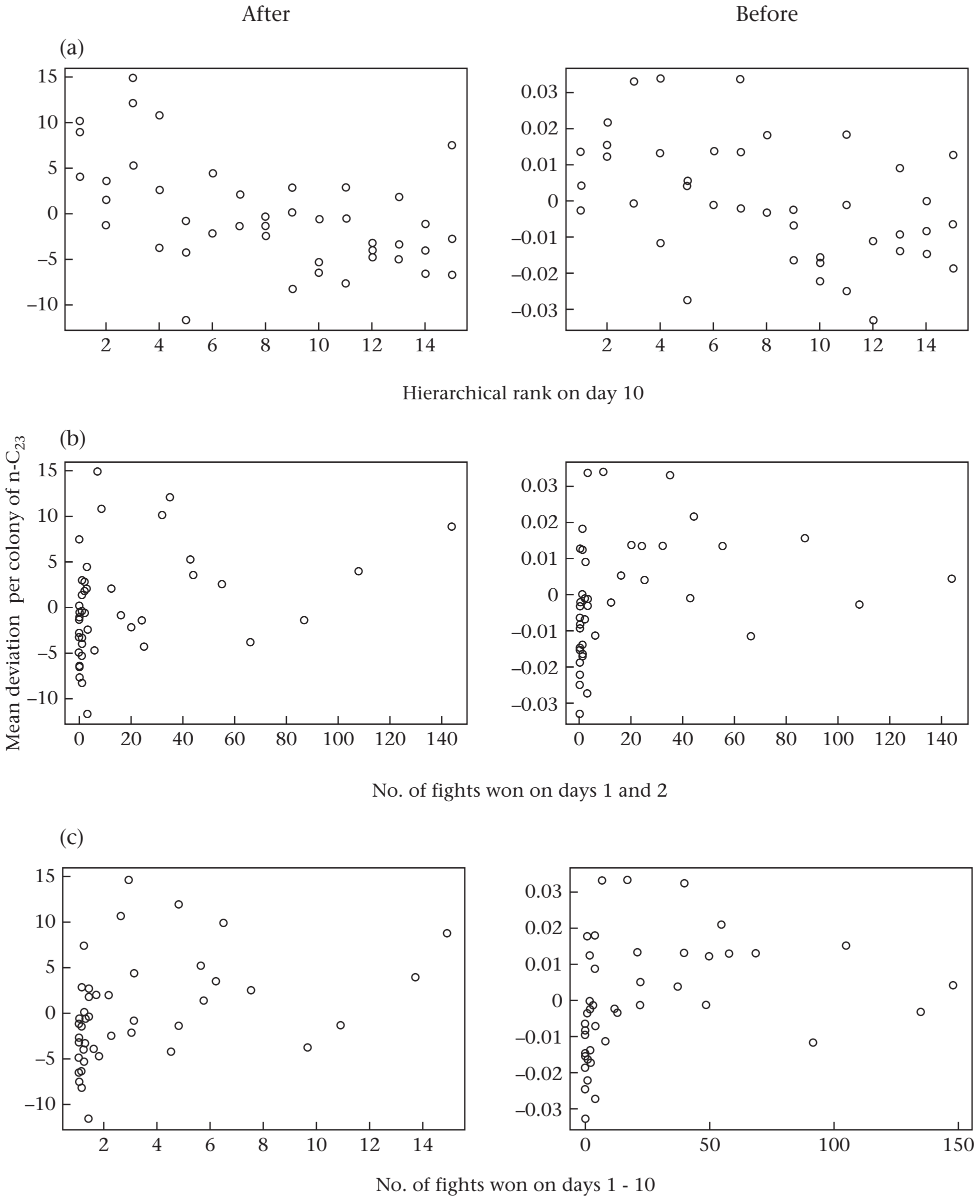

Figure A3. Plot of the data used in the Spearman correlations presented in Table 3. Correlations are shown between the recorded behaviours and the mean deviation of tricosane ($n\text{-}C_{23}$) per colony after (left) and before (right) queen removal. (a) Hierarchical rank on the 10th day (the end of the experiment), (b) the number of fights won on days 1 and 2, (c) the total number of fights won from day 1 to day 10, (d) the percentage of fights won on days 1 and 2, (e) the total percentage of fights won from day 1 to day 10, (f) the number of fights individuals were involved in on days 1 and 2 and (g) the total number of fights individuals were involved in from day 1 to day 10.

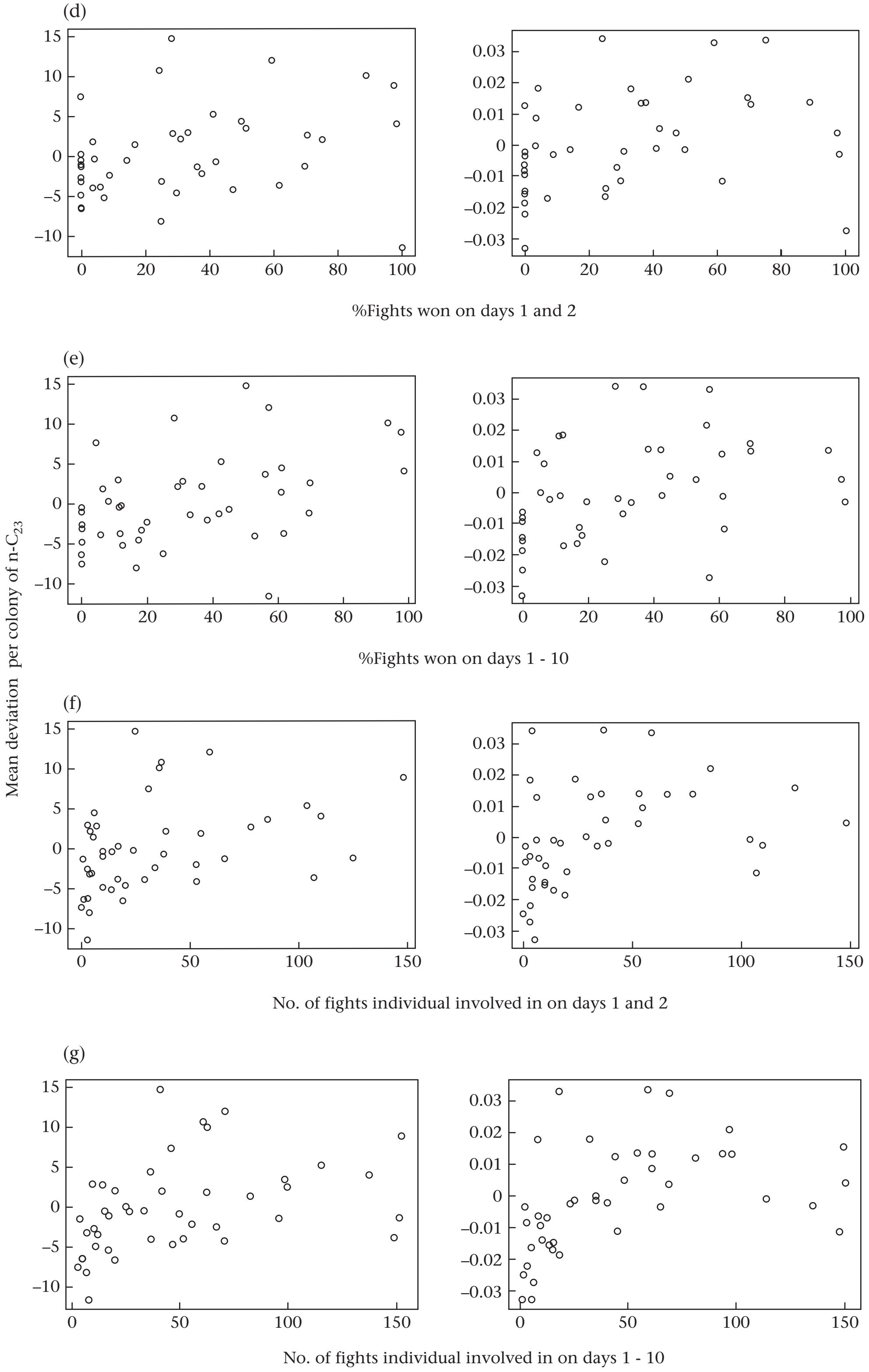

Figure A3. (*continued*).

Table A1

Identification of cuticular hydrocarbons (CHCs) on *N. apicalis* morph 6 for a moderately fertile individual (corresponding to chemotype A in Fig. A2a)

Peak	Retention time	Relative abundance	Characteristic fragments	CHC ID
1	5.876	–	–	n-C_{17} (internal standard)
2	6.532	0.05	268	n-C_{19}
3	6.915	0.08	282	n-C_{20}
4	7.265	0.7	294	$C_{21:1}$
5	7.399	14.85	296	n-C_{21}
6	7.563	0.06	140 196 295	9-MeC_{21}
7	7.694	0.07	70 267 295	4-MeC_{21}
8	7.785	1.41	308	$C_{22:1}$
9	7.906	0.97	310	n-C_{22}
10	8.139	0.07	169 182 309	11-MeC_{22}
11	8.523	62.42	322	$C_{23:2}$ + $C_{23:1}$
12	8.633	11.13	324	n-C_{23}
13	8.847	0.19	168 196 323	11-MeC_{23}
14	8.962	0.16	85 252 281 323	5-MeC_{23}
15	9.167	0.09	336	$C_{24:1}$
16	9.371	0.07	338	n-C_{24}
17	10.135	0.99	350	$C_{25:1}$
18	10.392	1.4	352	n-C_{25}
19	11.606	0.14	366	n-C_{26}
20	12.756	0.04	378	$C_{27:1}$
21	13.168	2.45	380	n-C_{27}
22	14.374	0.12	394	n-C_{28}
23	14.824	0.33	365 393	2-MeC_{28}
24	14.918	0.06	406	$C_{29:1}$
25	15.046	0.83	408	n-C_{29}
26	15.225	0.24	168 196 252 281 407	11-16-diMeC_{28}
27	15.961	0.2	393 421	2-MeC_{30}
28	16.065	0.04	434	$C_{31:1}$
29	16.174	0.09	436	n-C_{31}
30	16.352	0.32	168 196 224 252 281 309 435	11-13-15-MeC_{31}
31	17.634	0.44	168 308 337 463	11-MeC_{33}

Table A2

Analysis of similarity between the chemical profiles of the four colonies

Groups	*R*	*P*	Permutations
1, 8	0.148	0.01	9999
1, 18	0.758	0.0001	9999
1, 20	0.208	0.0002	9999
8, 18	0.771	0.0001	9999
8, 20	0.281	0.0001	9999
18, 20	0.747	0.0001	9999

The global test of the analysis of similarity gives a global *R* of 0.549 ($P = 0.0001$, number of permutations = 9999). Pairwise test results are given in the table.

Table A3

Spearman correlations (with Bonferroni–Holm adjustment for multiple comparisons) between the fertility index and the compounds present in the chemical profile after orphaning of colonies 1, 8 and 20 ($N = 44$)

Cuticular hydrocarbons	r_S	P'
n-C_{19}	**−0.51**	**0.009**
n-C_{20}	−0.15	1
$C_{21:1}$	−0.23	1
n-C_{21}	0.18	1
9-MeC_{21}	−0.28	1
4-MeC_{21}	**0.44**	**0.05**
$C_{22:1}$	0.07	1
n-C_{22}	0.48	0.023
11-MeC_{22}	0.41	0.14
$C_{23:2}$ + $C_{23:1}$	0.27	1
n-C_{23}	**0.63**	**1.5e-4**
11-MeC_{23}	0.07	1
5-MeC_{23}	**0.61**	**2.9e-4**
$C_{24:1}$	0.34	0.42
n-C_{24}	0.3	1
$C_{25:1}$	**0.45**	**0.05**
n-C_{25}	**0.48**	**0.03**
n-C_{26}	0.27	1
$C_{27:1}$	−0.01	1
n-C_{27}	0.41	0.13
n-C_{28}	0.16	1
2-MeC_{28}	0.08	1
$C_{29:1}$	−0.22	1
n-C_{29}	0.24	1
11-16-diMeC_{28}	0.12	1
2-MeC_{30}	0.05	1
$C_{31:1}$	−0.11	1
n-C_{31}	−0.22	1
11-13-15-MeC_{31}	0.05	1
11-MeC_{33}	−0.06	1

For colony 18, only the compound n-C_{27} was correlated with fertility ($r_S = -0.5$, $N = 15$, $P' = 0.057$). To compensate for multiple comparisons, *P* values were adjusted to P' values following Holm (1979). Significant values are highlighted in bold.

La diversité de taille des ouvrières au sein des colonies de fourmis : intérêts du groupe ou des individus ?

Chez les insectes sociaux, la diversité de taille des individus dans les colonies est supposée améliorer la division du travail et ainsi augmenter la *fitness* des colonies. Cela fait relativement consensus chez les espèces à forte diversité de taille continue ou bien avec la présence de plusieurs castes non reproductrices. En revanche, chez les espèces à diversité plus limitée, représentant la grande majorité des insectes sociaux, les résultats sont plus contrastés. Cette thèse s'est donc focalisée sur l'intérêt de la taille des ouvrières au sein des colonies de fourmis à diversité modérée. Nous avons dans un premier temps démontré que la taille moyenne et la diversité de taille des ouvrières ne sont pas forcément adaptatives chez notre espèce d'étude, *Temnothorax nylanderi*, à travers des manipulations expérimentales en milieu semi-naturel durant la période de croissance et durant l'hibernation. Face à ce manque d'intérêts de la taille des individus pour la colonie, nous avons investigué les potentiels intérêts pour les individus. En utilisant la fourmi *Mystrium rogeri*, nous avons manipulé la prise alimentaire des larves et ainsi étudié le développement des larves sans contraintes de la part des ouvrières. Ces données sur le développement larvaire suggèrent le développement de phénotypes plus grands en l'absence de coercion des larves par les ouvrières. Cela sous-entend à la fois que l'environnement social contrôle fortement la taille des individus produits, mais également qu'une perturbation de cet environnement social et/ou des comportements égoïstes des larves peuvent générer de la diversité de taille dans les colonies de fourmis. Dans un dernier chapitre, nous avons quantifié la contribution de cet environnement social dans la résistance à un perturbateur externe, en utilisant un élément trace. L'idée était de découpler la part sociale représentée par les ouvrières de la part intrinsèque des larves dans la résistance au cadmium en utilisant des colonies de la fourmi *Temnothorax nylanderi* provenant de villes et de forêts. De manière surprenante, notre étude n'a pas montré de réponses différentielles au cadmium entre ces deux populations concernant les ouvrières et nous n'avons pu tester notre hypothèse initiale que sur les mâles. En revanche, cette dernière étude met en lumière les limites à la résilience des sociétés d'insectes, qui pourraient être sujettes à davantage de stress et de manière plus chronique par rapport aux individus solitaires. Plus globalement, cette thèse ouvre la voie à reconsidérer le rôle de la taille chez les insectes sociaux et la place que prennent les intérêts individuels dans sa détermination. L'amélioration en profondeur des connaissances sur les déterminismes générant la diversité de taille, notamment via la génétique et génomique, aidera à la distinction entre intérêts du groupe et/ou des individus et ainsi à déterminer plus finement le rôle de la taille chez les insectes sociaux.

Mots clés : polymorphisme, adaptation, plasticité phénotypique, insectes sociaux, environnement social, tampon social, conflits sociaux, *Temnothorax*, *Mystrium*.