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Lauren Jacquier

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# Sorbonne Université

Ecole doctorale 227

*Sciences de la Nature et de l'Homme : écologie et évolution*

## **Réponse à l'urbanisation d'un insecte eusocial : interconnexion des réponses génétiques, plastiques, et rôle de l'environnement social**

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Thèse de doctorat d'Ecologie

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# Sommaire

Remerciements .....	3
Sommaire .....	7
Introduction .....	9
1- Les écosystèmes urbains, des milieux anthropisés en pleine expansion appliquant d'importantes pressions de sélection sur leurs populations .....	9
a- Croissance, caractéristiques et intérêts scientifiques des milieux urbains .....	9
b- Les réponses des espèces à l'urbanisation .....	11
c- Réponse à l'urbanisation : génétique ou plasticité ? .....	19
2 - La socialité : un avantage face aux changements environnementaux ? .....	23
a- L'environnement social impacte le phénotype des individus produits au sein d'une société .....	23
b- Les bénéfices de la vie en société pour faire face aux changements environnementaux .....	24
c- Les coûts à la vie en société face aux changements environnementaux .....	25
3 - Importance de la variabilité temporelle dans la réponse aux changements environnementaux chez les espèces eusociales .....	27
a- Cycle de vie des insectes eusociaux en milieu tempéré .....	27
b- Variations temporelles des stressseurs environnementaux .....	30
c- Variations temporelles dans la susceptibilité aux stressseurs chez les espèces sociales .....	31
4- Objectifs de la thèse et modèle d'étude .....	33
Chapitre un : Divergence de traits d'histoire de vie entre populations urbaines et forestières chez la fourmi <i>T. nylanderii</i> .....	34
Chapitre deux : Déterminisme de la réponse à l'urbanisation, rôles des facteurs génétiques et de l'environnement social .....	35
Chapitre trois : Variation intra-annuelle des traits d'histoire de vie entre populations urbaines et forestières .....	36
Chapitre 1 : Divergence de traits d'histoire de vie entre populations urbaines et forestières chez la fourmi <i>Temnothorax nylanderii</i> .....	37
Article 1 : Urban colonies are more resistant to a trace metal than their forest counterparts in the ant <i>Temnothorax nylanderii</i> .....	37
Article 2 : Urban colonies are less aggressive but forage more but than their forest counterparts in the ant <i>Temnothorax nylanderii</i> .....	68
Chapitre deux : Déterminisme de la réponse à l'urbanisation, rôles des facteurs génétiques, plastiques et de l'environnement social .....	101
Article 3 : Hibernation Conditions Contribute to the Differential Resistance to Cadmium between Urban and Forest Ant Colonies .....	101
Article 4 : Disentangling the roles of social and individuals effects on cadmium tolerance in the ant <i>Temnothorax nylanderii</i> .....	125
Chapitre trois : Variation intra-annuelle des traits d'histoire de vie entre populations urbaines et forestières .....	149



Article 5 : Cadmium tolerance in city and forest colonies is higher in spring than at the end of hibernation in the ant <i>Temnothorax nylanderi</i> .....	149
Conclusion.....	172
1- La divergence des traits d’histoire de vie entre populations urbaines et rurales met en évidence un filtre urbain s’exerçant à l’échelle des populations.....	172
a- Les populations urbaines tolèrent mieux les métaux traces.....	172
b- Les colonies urbaines fourragent plus, mais sont moins agressives que les colonies forestières .....	174
2- L’origine de ces divergences de traits : rôle limité de l’environnement social ? ....	176
a- Le rôle de la génétique et de l’environnement abiotique dans la divergence urbain-forestier de la réponse au cadmium.....	176
b- L’environnement social semble jouer un rôle limité dans la tolérance aux stressseurs ..	177
3- Des divergences de traits stables dans le temps ? L’impact potentiel de la variation intra-annuelle des dynamiques de colonie sur la divergence urbain-rural.....	179
4- Perspectives .....	181
Bibliographie.....	185

# Introduction

## **1- Les écosystèmes urbains, des milieux anthropisés en pleine expansion appliquant d'importantes pressions de sélection sur leurs populations**

### **a- Croissance, caractéristiques et intérêts scientifiques des milieux urbains**

Dans ce manuscrit, nous définirons les environnements urbains comme des milieux construits et habités par l'Homme, à la surface majoritairement couverte par des constructions et à forte densité de présence et/ou d'activité humaine (Foti et al., 2017; Lambert et al., 2021). Cette définition inclut donc un certain nombre d'environnements relativement variés, tels que des zones résidentielles, des centres urbains, des zones d'activité commerciale ou industrielle. Les milieux urbains représentent actuellement environ 3% de la surface terrestre, mais sont également ceux connaissant le taux de croissance actuel le plus rapide (Lamb and DiLorenzo, 2014) du fait de l'augmentation de la population globale et de la migration des populations des zones rurales vers urbaines. Différentes projections estiment que d'ici 2050, environ 75% de la population mondiale vivra en ville (Zlotnik, 2017).

L'urbanisation se fait à des rythmes différents selon les pays et les régions du globe (Seto et al., 2012). L'organisation globale des villes et leur écologie peut également varier d'un pays et même d'une ville à l'autre, en fonction de l'âge de cette ville, sa taille, la nature de ses activités économiques, son plan d'urbanisme et autre. Cependant, les milieux urbanisés présentent tous un certain nombre de caractéristiques partagées, menant à des phénomènes d'homogénéisation biotique. Les modifications environnementales causées par l'urbanisation peuvent être divisées en quatre grandes catégories : la modification ou la création de nouvelles surfaces, des modifications de l'environnement physico-chimique, la modification des assemblages biotiques et des interactions entre ces assemblages, et une modification des régimes de perturbation (McDonnell et al., 2008). La modification et la création de surfaces bétonnées ou goudronnées augmente fortement la fragmentation de l'habitat. Les modifications dans les interactions biotiques peuvent être causées par l'extinction de populations locales ne pouvant survivre à l'urbanisation, par l'introduction massive d'espèces exotiques (surtout végétales) ou invasives dans les milieux urbains (Blair, 1996; Riley et al., 2005) ou par des changements de traits dans les populations ou les espèces en interaction. Par exemple, en milieu urbain les plantes ont

tendance à fleurir plus précocement dans l'année comparé à leurs homologues ruraux, ce qui peut en conséquence impacter les populations de pollinisateurs se nourrissant sur ces fleurs (Neil and Wu, 2006). La modification importante des sols cause des changements dans les cycles biogéochimiques locaux, menant par exemple à des inondations beaucoup plus fréquentes (Zhou et al., 2017). Parmi les modifications de l'environnement physico-chimique, les milieux urbanisés présentent en général des températures plus élevées (+2°C en moyenne) que les environnements ruraux adjacents, à cause des effets d'Ilots de Chaleur Urbaine, (Bornstein, 1968), une forte pollution acoustique du fait des activités humaines (trafic routier, ferroviaire ou aérien, chantiers de construction, activités industrielles), une forte pollution lumineuse avec les éclairages de nuit (Falchi et al., 2016) ou encore une pollution chimique (perturbateurs endocriniens hormonaux, plastiques, métaux traces) importante (Dris et al., 2015; Foti et al., 2017; Salapasidou et al., 2011). Notamment, du fait des activités industrielles souvent proches, de l'utilisation massive de matériaux de construction et du trafic routier important, de nombreuses villes présentent des taux de pollution importants en métaux traces tels que le plomb, le zinc, le cuivre et le cadmium (Foti et al., 2017; Moreno et al., 2011).

Les métaux traces (anciennement appelés « métaux lourds ») sont définis comme des métaux trouvés en faible concentration, de l'ordre du ppm ou moins, dans un substrat spécifié (Duffus, 2002). Ces métaux traces peuvent être nécessaires pour l'organisme mais toxiques à haute dose (exemple : zinc, fer), ou n'avoir aucune utilité biologique et être un polluant quelle que soit sa concentration (exemple : le plomb, le cadmium). Parmi les métaux traces présents en quantité importante en milieu urbain par rapport à des environnements non-pollués, on trouve notamment le cadmium : si l'on prend l'exemple de la ville de Paris, les concentrations en cadmium sont environ 10 fois plus importantes dans les sols des urbains (2.45mg/kg) par rapport à ceux des environnements ruraux, situés en moyenne à une cinquantaine de kilomètre du centre de Paris (0.30 mg/kg, Foti et al., 2017). Le cadmium est un métal trace n'ayant aucune fonction biologique mais ayant de nombreux effets toxiques sur les organismes. En effet celui-ci interagit avec les mitochondries, dérégule les processus de prolifération et de mort cellulaire, interfère avec les mécanismes de réparation de l'ADN ou avec d'autres ions essentiels au fonctionnement biologique de l'organisme tels que le calcium, le zinc ou le fer (Martelli et al., 2006; Rani et al., 2014). Ces interférences mènent par exemple à la formation de protéines mal repliées ou inactives qui s'accumulent, augmentant ainsi le stress oxydatif dans les cellules. Il peut également interférer avec la régulation calcique, un point central de transduction du signal dans les cellules (Thévenod, 2009). L'exposition au cadmium a donc des effets négatifs tant à l'échelle cellulaire (apoptose, stress oxydatif, dommages à l'ADN) qu'à l'échelle des tissus et

de l'organisme (dommages neuronaux, malformations ou morts des embryons, non éclosion des œufs, problèmes immunitaires, den Besten et al., 1989; Demenesku et al., 2014; Jin et al., 2015). Il s'agit donc d'un bon candidat potentiel pour l'étude d'une éventuelle adaptation des populations urbaines à la pollution. De plus, il présente également l'intérêt de présenter une toxicité croissante en fonction de la dose, sans effet de seuil on/off ou de courbes en U de toxicité (c'est-à-dire, des polluants pour lesquels des valeurs extrêmes hautes et basses de concentration sont toxiques, mais pour lequel des valeurs intermédiaires de concentration sont sans conséquence, Attar and Maly, 1982; Belliardo, 2018; Karntanut and Pascoe, 2002).

Les milieux urbains, de par leur présence mondiale, permettent également de réaliser des études avec de très nombreux réplicats, qui s'avèrent nécessaire pour mettre au jour des mécanismes généraux de réponse à l'urbanisation. En effet, bien que les villes partagent des caractéristiques communes et tendent à causer une homogénéisation biotique (Knop, 2016; McKinney, 2006), celles-ci sont également très variables en terme non seulement de paramètres purement dépendants de leur localisation spatiale (climat, saisonnalité...) mais également en terme de paramètres anthropiques tels que les densité de population, l'âge de la ville, l'importance des activités socio-économiques, culturelles ou industrielles, la nature des échanges économiques qui peuvent à leur tour impacter d'autres paramètres de l'environnement, biotique ou abiotique. Par exemple, deux études faites à une année d'intervalle sur la même cohorte d'espèces mais dans des pays différents (Suisse et Allemagne) ont donnée des résultats opposés, avec des bourdons plus grands en ville dans une étude (Allemagne, Theodorou et al., 2021) et plus petits dans l'autre (Suisse, Eggenberger et al., 2019). Une autre étude menée sur les communautés d'oiseaux et de papillons a montré que le degré d'homogénéisation biotique causée par les villes variait selon leur latitude (Blair, 2001). La possibilité de mener des études sur plusieurs réplicats est donc d'une importance cruciale avant de tirer des conclusions générales sur l'effet de l'urbanisation.

### **b- Les réponses des espèces à l'urbanisation**

Face aux nombreuses contraintes imposées par le milieu urbain, les communautés biotiques sont modifiées, avec des variations de la richesse spécifique (par exemple, déclin de la richesse spécifique dans le groupe des tardigrades (delBarco-Trillo, 2019), des oiseaux (McKinney, 2008), des abeilles (Matteson et al., 2008), mais augmentation de la richesse spécifique pour les plantes dans la majorité des cas, (McKinney, 2008), mais voir Jim and Chen, 2009). Il est également observé des modifications de la composition (arthropodes : Piano et al., 2017, Gibb

and Hochuli, 2002) et de l'abondance des espèces (qui peut augmenter, Clergeau et al., 1998; McIntyre, 2000; Steinberg et al., 1997, ou diminuer, Fenoglio et al., 2020, 2021; Kurta and Teramino, 1992; McIntyre, 2000) dans les communautés. L'urbanisation impacte également les traits des organismes appartenant à certaines communautés. Par exemple, quelques études ont montré une modification de la taille des espèces d'arthropodes, qui étaient en général plus petites en milieu urbain probablement du fait de la moindre grande disponibilité en ressources ou de la chaleur plus importante qui accélère le développement et augmente le taux métabolique de manière plus coûteuse pour les gros organismes (Merckx et al., 2018). Cependant le pattern opposé peut aussi être observé pour les espèces où la taille joue un rôle clé dans la dispersion ou pour qui les aires de fourragement sont très étendues, c'est à dire où les grands organismes sont plus avantagés (Merckx et al., 2018; Theodorou et al., 2021). D'autres études semblent indiquer que les organismes généralistes survivent également mieux en milieu urbain, possiblement à cause de l'homogénéisation des conditions biotiques et abiotiques causée par l'urbanisation (McKinney, 2006; Sorace and Gustin, 2009). Ces études suggèrent donc la présence de filtres opérant en milieu urbain, sélectionnant les espèces les mieux adaptées aux conditions de vie en milieu urbanisé. Une telle sélection peut également s'appliquer sur les individus à l'échelle des populations, sur laquelle nous allons nous attarder un peu plus et qui fera l'objet de cette thèse.

De nombreuses espèces ont des populations réparties à la fois en milieu urbain et non urbain, permettant l'étude des modifications des traits d'histoire de vie en réponse à certains stressors urbains (dans cette thèse, nous définirons comme « stressor » tout facteur entraînant, pour l'organisme y étant exposé, une diminution de sa probabilité de survie et de ses capacités de reproduction, Bijlsma and Loeschke, 2005). Ces études sont très inégalement réparties selon les différents groupes taxonomiques. Ainsi, les vertébrés représentent près de la moitié des études en écologie urbaine, et les oiseaux à eux-seuls représentent environ 1/5<sup>ème</sup> ces études (McDonnell and Hahs, 2015; Rivkin et al., 2019). Au sein d'un groupe taxonomique, certains ordres sont également beaucoup plus étudiés que d'autres, par exemple parmi les arthropodes, les lépidoptères et les coléoptères représentent presque 50% des études tandis que les hyménoptères en représentent à peine 10% (McIntyre, 2000). La table 1 donne une liste des groupes auxquels appartiennent des espèces pour lesquelles une divergence de traits d'histoire de vie entre populations urbaines et non urbaines a été démontrée. Ces études ont souvent été menées sur plusieurs couples de populations urbain-rural, mettant ainsi en évidence un syndrome urbain (c'est-à-dire, un ensemble de caractéristiques communes qui tendent à être favorisée en milieu urbain) et une réponse globale à l'urbanisation. **Malgré leur importance**

**écologique (biomasse, régulation des populations, ingénieurs écologiques, pollinisateurs) et économiques (agriculture, espèces invasives) (Angulo et al., 2021; Farji-Brener et al., 2010; Ghaly and Edwards, 2011; Klatt Björn K. et al., 2014; Lach et al., 2010; Milligan et al., 2016; Philpott and Armbrrecht, 2006), il est intéressant de noter que les espèces eusociales sont relativement peu étudiées.**

**Tableau 1.** Résumé des différentes espèces pour lesquelles une divergence entre populations urbaines et forestières a été observé. Pour chaque espèce, le trait impacté est indiqué, ainsi que le mode de détermination de cette divergence (génétique, plastique ou inconnue), et son effet supposé ou avéré sur la valeur sélective.

Groupe	Trait	Détermination	Effet sur la valeur sélective	Référence
Arthropodes	Taille : plus gros (bourdon, araignées) ou plus petits en ville selon les espèces (bourdons)	Génétique (scan génomique) et plastique	Non-testé	Eggenberger et al., 2019; Theodorou et al., 2021
	Changement de phénologie (production plus tôt de sexuées, <i>Temnothorax curvispinosus</i> )	Génétique (jardin commun), plasticité	Non-testé	Chick et al., 2019
	Tolérance thermique (fourmis : <i>T. curvispinosus</i> , <i>Atta sexdens</i> , demoiselles : <i>Coenagrion puella</i> )	Génétique (jardin commun, <i>T. curvispinosus</i> , demoiselle), inconnue ( <i>Atta sexdens</i> )	Oui pour <i>T. curvispinosus</i> Non testé pour les autres	Angilletta et al., 2007; Diamond et al., 2018; Tüzün et al., 2017
	Changement de couleur (phalène du bouleau <i>Biston betularia</i> )	Génétique (éléments transposables)	Oui	Hof et al., 2016; Kettlewell, 1955

	Comportement (forme des toiles, <i>Araneus diadematus</i> ), fourragement plus actif chez l'abeille <i>Tetragonula carbonaria</i> , période de fourragement étendue et exploitation de nouvelles ressources chez le bourdon <i>Bombus terrestris</i> .	Génétique (hypothétique, non testé mais suggéré par la réponse cohérente à grande échelle) et plasticité phénotypique (variation à petite échelle géographique)	Probablement (contrebalance des effets négatifs locaux de l'urbanisation sur la fécondité, meilleure productivité pour les autres)	Dahirel et al., 2019; Kaluza et al., 2016; Stelzer et al., 2010
	Métabolisme : taux métabolique plus haut ( <i>T. curvispinosus</i> )	Génétique (jardin commun)	Probablement mal-adaptatif	Chick et al., 2021
	Communication : augmentation de la fréquence des chants (cigale <i>Cryptotympana takasagona</i> )	Plasticité phénotypique	Non-testé	Shieh et al., 2012
Oiseaux	Maturité sexuelle précoce (Merle noir <i>Turdus merula</i> ) , ponte d'œuf plus tôt dans la saison (passereaux)	Génétique (jardin commun) et plasticité phénotypique	Non testé	Chamberlain et al., 2009; Partecke Jesko et al., 2004
	Changement dans les niveau de glucocorticoïdes (Merle noir <i>T. merula</i> , Junco ardoisé <i>Junco hyemalis</i> )	Génétique ou effet maternel (jardin commun)	Non testé	Atwell et al., 2012; Partecke et al., 2006



			Non testé (Junco, bruant)	
Comportement : agressifs et audacieux (Junco Ardoisé <i>J. hyemalis</i> , Bruant chanteur <i>Melospiza melodia</i> ), anxiété (Merle noir <i>T. merula</i> )	Génétique ou effet maternel (jardin commun), génétique avec polymorphisme d'un gène SERT (anxiété)	Probablement adaptatif pour le merle (sélection convergente sur 10 des 12 populations testées)		Atwell et al., 2012; Mueller et al., 2013; Scales et al., 2011
Migration (merle noir <i>T. merula</i> )	Génétique (jardin commun)	Hypothétique (saison de reproduction plus longue)		Evans et al., 2012; Partecke and Gwinner, 2007
Chant plus court ou plus haut (mésange charbonnière <i>Parus major</i> , merle noir <i>T. merula</i> )	Plasticité phénotypique	Non testé		Mendes et al., 2011; Slabbekoorn and den Boer-Visser, 2006
Morphologie : corps et ailes plus petits (moineau domestique <i>Passer domesticus</i> ), queue plus courte (mésange charbonnière <i>P. major</i> )	Génétique (jardin commun)	Non testé		Liker et al., 2008; Senar et al., 2014

	Modifications génétiques associées aux concentrations en plomb ( <i>P. domesticus</i> )	Génétique (scan de génome)	Non testé	Andrew et al., 2019
Plantes	Phénologie (floraison précoce en ville, <i>Prunus</i> sp, <i>Robinia pseudoacacia</i> , <i>Lepidium virginicum</i> )	Inconnu ( <i>Prunus</i> sp et <i>R. pseudoacacia</i> )	Non testé	Lu et al., 2006; Yakub and Tiffin, 2017
		Génétique pour <i>L. virginicum</i>	Adaptatif ( <i>L. virginicum</i> )	
	Morphologie (des graines, <i>Crepis sancta</i> , ou des feuilles, <i>Lepidium virginicum</i> )	Génétique (modèles de génétique quantitative)	Oui pour les deux	Cheptou et al., 2008; Yakub and Tiffin, 2017
	Croissance ( <i>Digitaria ciliaris</i> )	Génétique (jardins communs)	Oui	Fukano et al., 2020
Champignon	Tolérance thermique (croissance améliorée à haute température, ralentie aux basses, <i>Chrysosporium pannorum</i> )	Génétique (jardin commun)	Oui	McLean et al., 2005
Poissons	Résistance à la pollution ( <i>Fundulus heteroclitus</i> )	Génétique (scan génomique)	Oui	Reid et al., 2016
	Changement morphologique ( <i>Gasterosteus aculeatus</i> , <i>Semotilus atromaculatus</i> )	Génétique (scan génomique)	Oui (baisse des taux de prédation)	Kern and Langerhans, 2018; Kitano et al., 2008

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Reptiles	Comportement : évitement des prédateurs, locomotion (lézard <i>Lampropholis guichenoti</i> )	Inconnu	Non testé	Prosser et al., 2006
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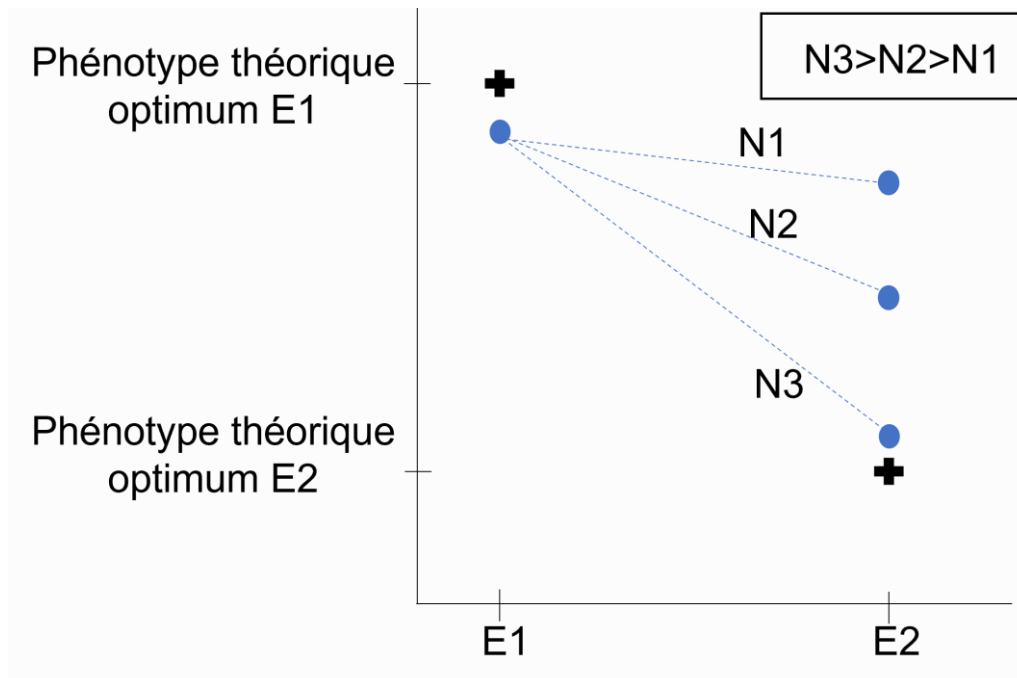
### **c- Réponse à l'urbanisation : génétique ou plasticité ?**

Les réponses à l'urbanisation peuvent être génétiquement déterminées ou bien plastiques. Le type de stress auquel l'organisme répond impacte également le type de réponse à ce stress. L'adaptation au sens strict peut être définie comme un changement dans la séquence génétique conférant un avantage de fitness à l'individu la portant comparé aux autres individus en étant dépourvus, et augmentant en fréquence au cours des générations. Cependant, certaines études élargissent ce terme en y incluant la plasticité phénotypique, c'est-à-dire la capacité à exprimer différents phénotypes à partir d'un même génotype en fonction de l'environnement (Snell-Rood et al., 2018), du moment que celle-ci confère une meilleure fitness aux individus concernés et augmente en fréquence dans la population concernée (on parle alors de plasticité adaptative, Ghalambor et al., 2007). Le type de stress urbain considéré va influencer la probabilité de réponse plastique ou génétique. En effet, les modifications de l'environnement causées par l'urbanisation peuvent être divisées en deux grandes catégories, celles ayant un impact aigu (c'est à dire limité dans l'espace et dans le temps, et potentiellement non prédictibles, telles que la pollution lumineuse ou sonore) et celles à impact chronique (très diffusées spatialement, temporellement stables, tels que la pollution chimique ou les effets d'îlot de chaleur urbaine, McDonnell and Hahs, 2015). Il a été proposé que les stress chroniques, parce qu'ils sont stables et présents sur de longues périodes de temps, étaient plus susceptibles de mener à une adaptation génétique des populations comparés aux stress aigus (McDonnell and Hahs, 2015). Les stress aigus sont, eux, plus susceptibles de mener à des réponses plastiques, qui pourront ensuite éventuellement être elles aussi soumises à sélection et évolution (McDonnell and Hahs, 2015). Des études faites en jardin commun (c'est-à-dire, en élevant des individus issus de différentes populations dans le même environnement naturel, semi-naturel ou en laboratoire) permettent de mettre en évidence des réponses à base génétique ou plastique. Si la divergence de traits continue de s'exprimer entre les différentes populations, cela suggère une origine génétique dans cette divergence de traits ; si elle disparaît, alors cette divergence est d'origine plastique. Cependant, les effets maternels, c'est-à-dire le fait que le phénotype de la mère impacte le phénotype de ses descendants (par exemple, via des effets épigénétiques), ne peuvent la plupart du temps pas être totalement exclus de la réponse, ceux-ci pouvant avoir des effets sur plusieurs générations.

Les réponses génétiquement déterminées mènent à une modification des fréquences alléliques des populations urbaines par rapport aux non-urbaines. L'adaptation des populations à un environnement donné a été vue classiquement comme un processus lent de modification des fréquences alléliques, agissant sur de longues périodes. Cependant, l'étude de l'adaptation en milieu urbain a permis de mettre en évidence des phénomènes d'évolution rapide sur des populations données, aussi appelé microévolution (McDonnell and Hahs, 2015; Miranda et al., 2013). Un exemple classique d'adaptation en milieu urbain est celui de la phalène du bouleau. Cet insecte vit principalement dans les forêts de bouleau et possède deux morphes, l'un blanc et l'autre noir. En Angleterre, lors de la révolution industrielle, Kettlewell avait remarqué une modification dans la fréquence de ces deux morphes dans les forêts proches des villes. Le morphe blanc, majoritaire avant l'industrialisation massive, avait tendance à disparaître au profit du morphe noir au fil des années. (Kettlewell, 1955) avait émis l'hypothèse d'une sélection différentielle des deux morphes en fonction de leur couleur : en effet, le morphe blanc, bien camouflé sur les troncs blancs de bouleau avant la révolution, était désormais visible sur les troncs noircis par les fumées des usines. Les oiseaux les repérant très facilement, ce morphe était bien plus sujet à prédation que le morphe noir. Au contraire, le morphe auparavant contre-sélectionné était désormais avantageux. Avec les années, la pollution de l'air dégagée par les usines s'est grandement atténuée, et les troncs de bouleau sont redevenus peu à peu blancs, avantageant alors à nouveau les morphes blancs (Cook and Saccheri, 2013). Cet exemple d'évolution en milieu urbain est le plus ancien connu, mais d'autres études récentes mettent en évidence des processus d'évolution sur d'autres organismes tels que des poissons, des fourmis ou des champignons (Table 1).

Les organismes peuvent également répondre de manière plastique. Cette plasticité est particulièrement importante en milieu urbain, car elle peut permettre à des populations de se maintenir dans un environnement stressant suffisamment longtemps pour que des processus de sélection génétiques agissent (Ghalambor et al., 2007; Lambert et al., 2021). Par exemple, la plasticité comportementale est vue comme particulièrement importante dans la réponse à l'urbanisation, et les espèces parvenant à survivre et/ou proliférer en milieu urbain sont généralement celles montrant de hauts niveaux de plasticité comportementale, leur permettant d'exploiter les nouvelles ressources propres au milieu urbain (Miranda, 2017). Selon la balance (ou trade-off) entre le coût de cette plasticité pour l'organisme et le degré auquel cette plasticité permet son maintien dans l'environnement, deux grandes tendances peuvent être dégagées : une sélection vers une plus grande plasticité phénotypique, ou au contraire une sélection vers une réduction de cette plasticité, si la réponse plastique est mal-adaptative. Ces réponses

plastiques peuvent impacter plusieurs générations successives, par exemple via des effets maternels (on parle alors de plasticité transgénérationnelle). Ainsi, la plasticité peut jouer un double rôle dans l'adaptation à l'environnement urbain. D'un côté, elle peut permettre à des populations de se maintenir dans cet environnement, laissant éventuellement du temps pour une adaptation génétique (Ghalambor et al., 2007). D'un autre côté, la réponse plastique peut au contraire freiner la sélection génétique si elle maintient des individus proche d'un optimum phénotypique sans coût important et ainsi empêche une sélection efficace d'individus porteurs d'allèles plus avantageux (Buskirk; Thibert-Plante and Hendry, 2011). Il est également important de noter que la plasticité est elle-même soumise à des processus de sélection : la plasticité peut évoluer en réponse aux changement d'environnement, par exemple si la sélection agit sur les normes de réactions, c'est-à-dire le degré de plasticité (Figure 1; Diamond et al., 2018; Miranda, 2017). Les espèces eusociales ont la particularité de montrer des niveaux importants de plasticité phénotypique. Par exemple, à partir d'un même génotype, une larve pourra donner une ouvrière, une reine ou encore une soldate (Figure 2). **Malgré cette caractéristique particulière des espèces eusociales, leur mode de réponse (génétique ou plastique) et leur degré de réponse à l'urbanisation n'a encore été que relativement peu étudié.**



**Figure 1.** Les normes de réaction exprimées dans le cadre de la plasticité phénotypique peuvent être sous sélection. E1, E2 : environnement théorique 1, environnement théorique 2. Les croix noires symbolisent les optimums phénotypiques théoriques dans chaque environnement. Le point bleu présente un phénotype théorique présent dans ces

environnements. Si on suppose que plusieurs normes de réaction sont possibles en réponse à un changement d'environnement 1 vers 2 (N1, N2, N3 : normes de réaction 1, 2, 3), alors la norme de réaction N3, qui est celle qui rapproche le plus le phénotype du nouvel optimum, sera plus avantageuse que la N2, elle-même plus avantageuse que la N1.



**Figure 2.** Plasticité phénotypique chez les espèces eusociales. Au-dessus : photo de l'intérieur d'une ruche d'*Apis mellifera*, où la reine est distincte morphologiquement des ouvrières. Les ouvrières sont semblables entre elles. En bas : différentes castes de femelles de la fourmi *Messor barbarus* (©Claude Lebas).

## 2 - La socialité : un avantage face aux changements environnementaux ?

### a- L'environnement social impacte le phénotype des individus produits au sein d'une société

De très nombreuses espèces vivent en groupe, mais le degré de socialité de ces structures varie, ainsi que la stabilité temporelle de ces groupes. L'eusocialité correspond au niveau le plus développé de structure sociale, avec un système de division du travail aussi connu sous le nom de castes (par exemple division du travail reproductif avec reine vs ouvrières). Plusieurs générations coexistent au sein d'un même nid, et les générations plus anciennes coopèrent pour élever la nouvelle. Dans certaines espèces, la morphologie des individus varie selon leur rôle dans la colonie (caste soldate des termites, ouvrières major ou minor des fourmis du genre *Messor* par exemple). Dans d'autres, la morphologie ne varie pas beaucoup d'un individu à l'autre si ce n'est la taille, et un individu donné pourra exercer différents rôles dans la colonie au cours de sa vie. Par exemple, chez les abeilles, les jeunes adultes sont responsables du soin au couvain puis basculent vers un rôle de fourrageuse une fois un âge limite atteint (polyéthisme d'âge, Winston, 1991).

Outre les facteurs génétiques et environnementaux classiques, la détermination du phénotype chez les espèces eusociales implique aussi un rôle important de l'environnement social, c'est-à-dire l'ensemble des facteurs liés à la composition de la colonie (par exemple, le nombre total d'individus dans cette colonie, leur degré d'apparentement, la diversité de taille des individus ou encore les proportions relatives entre les différentes castes). Par exemple, il a été montré que chez la fourmi *Pheidole obtuspinosa*, qui possède deux castes distinctes morphologiquement (ouvrières à petite tête et soldates à grosse tête), les ouvrières pouvaient contrôler le phénotype des futures émergentes de manière à maintenir une proportion d'environ 90-95% d'ouvrières pour 5 à 10% de soldates (Rajakumar et al., 2018). Chez les abeilles, le développement d'une larve en adulte de phénotype reine ou en adulte de phénotype ouvrière dépend du type de nourriture avec laquelle la larve a été nourrie, les larves étant nourries à la gelée royale se développant en reine (Wheeler, 1986). Chez d'autres espèces, la taille de la colonie influence la taille des ouvrières, avec les grosses colonies produisant de plus gros individus (par exemple chez les fourmis, *T. nylanderii*, Molet et al., 2017, ou *Cataglyphis cursor*, Clémencet and Doums, 2007) ou au contraire avec de grosses colonies produisant de plus petits individus chez plusieurs espèces de bourdons (Castillo et al., 2015). Enfin, la nature même des ouvrières ou des larves peut impacter le phénotype des autres membres de la colonie. Par exemple,



(Linksvayer, 2007) a montré que la taille des individus produits chez certaines espèces de *Temnothorax* était contrôlée par les ouvrières, avec des tailles à l'émergence variable en fonction de l'espèce de *Temnothorax* élevant des larves issues d'une même colonie. D'autres études ont montré que les larves régulaient la diapause de la reine chez la fourmi *Leptothorax acervorum* (Kipyatkov et al., 1997), ou encore que les ouvrières de *Myrmica rubra* étaient, en fonction de leur état physiologique, capables ou incapables de maintenir des larves en état de diapause (Brian, 1955). Ainsi, chez les espèces sociales, l'environnement social joue un rôle clé dans la détermination du phénotype des individus.

### **b- Les bénéfices de la vie en société pour faire face aux changements environnementaux**

La vie en groupe permet de tamponner certaines variations environnementales, comme celles liées à la température. Par exemple, chez le manchot empereur, la formation de groupes compacts permet de réduire la dépense énergétique de quasiment 40% face au froid arctique (Gilbert et al., 2008). Ce rôle de la vie en groupe dans la thermorégulation a été montré chez de nombreuses autres espèces (marmotte alpine, chauve-souris, lémuriniens, macaques, abeilles, Arnold, 1988; Campbell et al., 2018; Ostner, 2002; Willis and Brigham, 2007). La formation de groupes compacts permet aussi de limiter les pertes hydriques liées à un assèchement du milieu, comme montré chez l'isopode *Porcellio scaber* (Broly et al., 2014). Enfin, la coopération entre individus permet la construction de nids complexes, qui permettent une régulation des conditions climatiques à l'intérieur de ceux-ci indépendamment des variations externes (Singh et al., 2019).

Chez les organismes eusociaux les colonies les plus grandes sont celles qui résistent le mieux à certains stressseurs. Par exemple, chez les fourmis, les plus grandes colonies sont aussi celles qui résistent le mieux à une famine prolongée (Modlmeier et al., 2013), aux fortes températures (Molet et al., 2017), aux parasites (Scharf et al., 2012) ou aux variations saisonnières dans les quantités de nourriture disponible (Kaspari and Vargo, 1995). Chez les abeilles, plusieurs études montrent un lien entre la taille de colonie et la résistance aux pesticides, avec les plus grandes colonies résistant mieux (Crall et al., 2019; Rundlöf et al., 2015), à l'échelle de la communauté comme à l'échelle de l'espèce. Un autre exemple connu de l'avantage de l'eusocialité face à un changement d'environnement est celui de l'espèce de fourmi invasive, *Linepithema humile*. Introduite par accident dans de nombreuses aires géographiques telles que l'Amérique du Nord, l'Europe ou encore l'Australie et la Nouvelle Zélande, cette espèce fait

des dommages considérables et parvient à s'implanter dans des environnements totalement nouveaux, entrant en forte compétition voir éradiquant les populations en place (Ward, 2009). Cette forte capacité d'adaptation serait due à la formation de gigantesques colonies composées de plusieurs millions d'individus coopérant les uns avec les autres, surclassant alors les espèces locales en termes compétitifs (Liang and Silverman, 2000; Suhr et al., 2009; Van Wilgenburg et al., 2010). Il a d'ailleurs été montré chez cette espèce que la compétitivité augmentait avec la taille de la colonie (Holway and Case, 2001), et que des colonies différentes pouvaient de surcroît coopérer par manque de marqueurs odorants leur permettant de discriminer les individus issus du même nid ou d'un nid différent (Björkman-Chiswell et al., 2008). L'eusocialité pourrait être un facteur favorisant le caractère « invasif » de certaines espèces, et donc améliorer leur capacité à s'implanter dans un environnement nouveau grâce à leur forte compétitivité (Chapman and Bourke, 2001).

Ces études soutiennent la théorie de résilience des superorganismes, selon laquelle les organismes eusociaux avec les plus grandes colonies (et éventuellement des colonies polygynes) sont également ceux qui sont les plus susceptibles de résister aux perturbations environnementales, grâce à un effet tampon de la perte d'ouvrière et une moins grande sensibilité à la stochasticité (Straub et al., 2015). Il est intéressant de noter qu'une autre étude a montré que les pressions de sélection négatives avaient tendance à être moins importantes chez les espèces à haut niveau de complexité sociale et formant de grandes colonies (Imrit et al., 2020), ce qui pourrait suggérer également un effet tampon de la socialité.

### **c- Les coûts à la vie en société face aux changements environnementaux**

Les espèces eusociales pourraient être particulièrement sensibles aux changements environnementaux. La vie en société nécessite un certain nombre de caractéristiques telles qu'une bonne communication entre les membres d'une même colonie, la reconnaissance des congénères, un système de castes ou une immunité sociale (Fisher et al., 2021, 2019). Ces traits peuvent être impactés très fortement par les changements environnementaux (Fisher et al., 2021, 2019). Par exemple, la communication et la reconnaissance se fait principalement via les hydrocarbures cuticulaires (CHCs) chez les fourmis. La signature en CHCs de chaque individu varie selon la génétique mais aussi selon les conditions environnementales rencontrées. La tendance à l'homogénéisation biotique de certains territoires (notamment urbains, McKinney, 2006) pourrait augmenter le risque de cleptoparasitisme (le fait qu'un parasite se nourrisse sur

les réserves accumulées par d'autres) chez ces espèces à cause des problèmes de reconnaissance des individus induit par l'homogénéisation des CHCs, laissant la voie libre à l'entrée dans le nid pour certains cleptoparasites (Fisher et al., 2021, 2019).

La relaxation de la pression de sélection négative observée par (Imrit et al., 2020) peut aussi s'expliquer par une taille efficace de population plus restreinte chez les organismes sociaux. En effet, le fonctionnement d'une colonie parfois composée de plusieurs milliers d'individus peut demander des ressources et des territoires plus importants que le fonctionnement d'un individu solitaire, alors que le nombre d'individus reproducteurs au sein de cette même colonie reste faible. Cette taille effective réduite pourrait alors mener à une dérive génétique plus forte et à une sélection moins efficace, ralentissant les processus d'adaptations à des perturbateurs environnementaux.

La concentration de nombreux individus au même endroit peut également mener à des problèmes de compétition, notamment pour l'accès à la nourriture. Cette forte densité de population, parfois composée d'individus proches génétiquement les uns des autres, peut aussi favoriser la propagation de virus ou de parasites (Fisher et al., 2019), bien que des mécanismes d'immunité collective existent (Cremer et al., 2007; Ugelvig and Cremer, 2007). Chez les espèces eusociales, les grandes colonies composées de nombreux individus pourraient aussi être plus exposées aux maladies ou aux polluants (pesticides...) à cause de la forte probabilité de rencontre entre un individu de la colonie et un de ces stressseurs (Barron, 2015). La perte d'efficacité chez les individus touchés par ces stressseurs peut mener à un effondrement de la colonie, sans même que les individus la composant ne décèdent, par simple désorganisation. (Naug, 2009) a aussi mis en évidence que l'hétérogénéité des réseaux d'interaction entre individus augmente aussi avec la taille de colonie, chez une espèce de guêpe eusociale (*Ropalidia marginata*). Ainsi, les « nœuds » de communication sont concentrés sur quelques individus, et la perte stochastique de ces quelques individus peut mener à une désorganisation importante de la colonie, dont ne souffrent pas les colonies plus petites possédant des réseaux d'interactions plastiques et homogènes.

Enfin, les espèces sociales pourraient être particulièrement vulnérables à un phénomène connu sous le nom d'effet Allee (Angulo et al., 2018; Courchamp et al., 1999, 2008). L'effet Allee correspond à un lien entre la densité d'une population et la fitness individuelle de chaque individu composant cette population, grâce à des propriétés liées à la vie en groupe, telles que la plus grande probabilité de rencontre d'un partenaire ou la modification de l'environnement par les individus eux même. Par exemple, Allee et ses collaborateurs ont montré que chez une espèce de poisson, une densité importante de population améliorerait la survie grâce à une

modification de l'environnement par les sécrétions de ces poissons (jusqu'à un point de bascule où la compétition devient trop forte, Allee, 1938; Allee and Bowen, 1932). Chez les espèces sociales, l'effet Allee peut être envisagé à l'échelle de la population, mais également à l'échelle du groupe, c'est-à-dire de la colonie chez les insectes eusociaux (Angulo et al., 2018). La relation densité de population – probabilité d'extinction pourrait être particulièrement important chez les espèces sociales, où un nombre minimum d'individus est nécessaire au fonctionnement de la colonie. Par exemple, une modélisation a montré que chez l'abeille domestique, les colonies étaient capables de tamponner la perte d'un petits nombre de fourrageuses, mais passé un certain seuil, la perte de fourrageuse ne peut plus être compensée et mène à un colony collapse disorder, c'est-à-dire un effondrement brut et soudain de l'effectif de la colonie (Perry et al., 2015).

**Les espèces eusociales sont donc potentiellement plus résistantes aux changements environnementaux, du fait de leur vie en société. Mais cette même socialité pourrait s'avérer être un fardeau pour faire face à ces changements, à cause des traits propres aux espèces eusociales. Il est donc crucial d'étudier la façon dont les sociétés répondent aux perturbations, ainsi que d'évaluer les niveaux de stress auxquels les sociétés peuvent se montrer résistantes, afin de pouvoir prédire la réponse de ces espèces aux perturbations environnementales en cours et futures.**

### **3 - Importance de la variabilité temporelle dans la réponse aux changements environnementaux chez les espèces eusociales**

#### **a- Cycle de vie des insectes eusociaux en milieu tempéré**

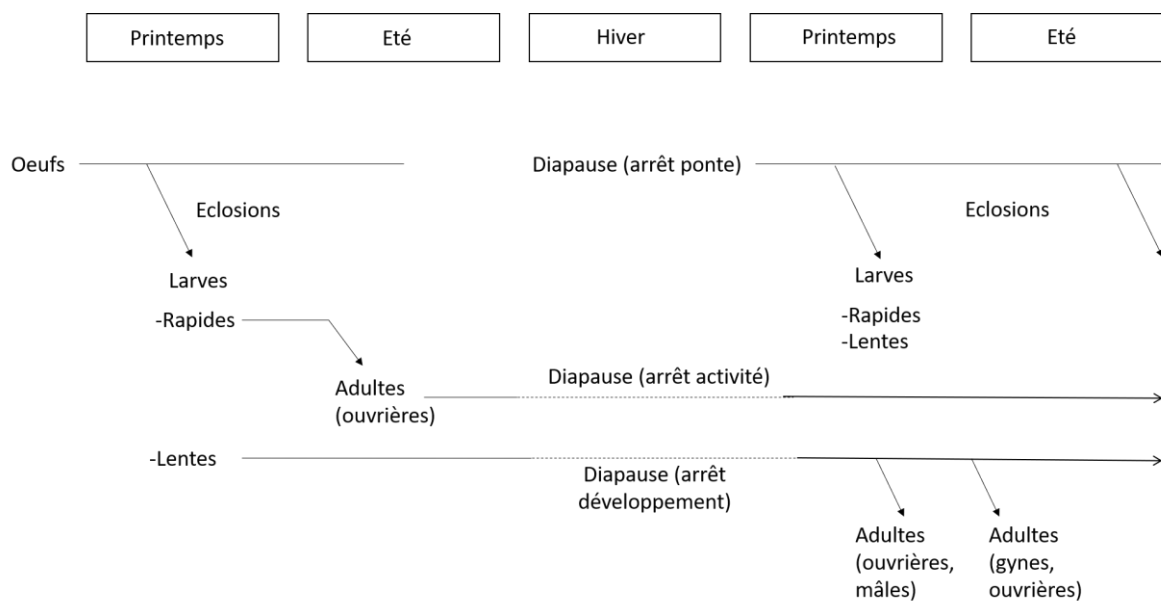
Les insectes sociaux, en tant que « superorganismes » ont, en plus du cycle de vie individuel (œufs, larve, pupa, adulte), un cycle de vie dit colonial. Ce cycle consiste aussi en un développement des différents individus composant la colonie, mais ne peut pas être considéré comme une simple somme de ces développements. Des mécanismes de régulation sociale ont lieu entre les différents membres de la colonie, contrôlant le développement et l'état physiologique des individus en fonction des besoins de la colonie. Cette régulation collective du développement est absente chez les organismes solitaires et constitue donc un trait propre aux espèces sociales, qui détermine les spécificités du développement saisonnier chez ces espèces.

Il existe chez les insectes eusociaux des cycles de vie dit annuels (par exemple, chez les bourdons) et des cycles de vie dit pluriannuels (plus répandus, par exemple chez l'abeille domestique ou les fourmis) (Kipyatkov, 1993). Dans le cas d'un cycle de vie annuel, la reine qui a passé l'hiver en hibernation commence à pondre et à élever ses œufs au début du printemps. Ces œufs éclosent en larves qui deviennent alors des adultes, qui assurent ensuite les tâches de maintenance de la colonie telles que la construction du nid, le fourragement ou le soin au couvain, tandis que la reine arrête de s'occuper des larves pour pondre uniquement. Les premières ouvrières émergentes sont souvent de taille très réduite par rapport à celles qui émergeront ensuite et qui seront élevées par leur sœur et non par leur mère. A l'été, la nouvelle génération de sexués émerge et s'accouple au cours du vol nuptial, et la future reine nouvellement fécondée construira un nid sommaire pour passer l'hiver en hibernation. La colonie dont elle est issue mourra pendant l'hiver.

Les espèces pluriannuelles partagent le même pattern général pour les belles saisons (printemps, été), mais divergent en hiver. Contrairement aux espèces annuelles, les espèces pluriannuelles ont des colonies qui survivent à l'hiver, avec la reine mais aussi une partie des ouvrières et parfois du couvain. Ce cycle de vie est dit hétérodynamique (à variation saisonnière), par opposition à homodynamique dont on qualifie généralement les espèces tropicales (Kipyatkov, 2001), ou de quasi-hétérodynamique (espèces tropicales étant plus ou moins adaptées à des régions tempérées, Kipyatkov, 2001) et dont nous ne parlerons pas dans le cadre de cette thèse. Chez les espèces hétérodynamiques, la colonie alterne des phases de croissance au printemps et en été avec des phases d'arrêt du développement en hiver appelées phases de diapause (Kipyatkov, 2001; Lopatina, 2018). Il s'agit d'un état contrôlé d'activité métabolique faible, associée à une activité, une croissance et un développement à l'arrêt ou très faible, qui augmente la résistance à des conditions environnementales non favorables, ici l'hiver et ses faibles températures pour les espèces tempérées (Popović et al., 2015). Cet état nécessite des étapes physiologiques pour sa mise en place et n'est pas juste induit par le froid : par exemple, Plateaux a mis en évidence chez la fourmi *Temnothorax nylanderi* qu'une colonie collectée en été et placée à 4°C mourrait (ouvrières, reine et couvain), contrairement à ce qui est observé en hiver sur le terrain où les colonies survivent mieux (Plateaux, 1970). La diapause est associée à la sécrétion de nombreux facteurs anti-stress (King and MacRae, 2015; Popović et al., 2015). Chez la majorité des espèces de fourmi vivant en milieu tempéré, il existe deux types de couvains résultant de ces alternances de phases d'activité et de diapause : le couvain « lent » et le couvain « rapide ». Le couvain lent correspond aux œufs qui ont été pondus et ont éclot en larves en été, avant de passer l'hiver en diapause sous forme larvaire. Ces larves reprendront leur

développement au printemps suivant (Figure 3), avec les larves les plus grosses donnant en général les sexuées et étant les premières à reprendre leur développement. Le couvain dit « rapide » a été pondu, a éclot et se développe totalement en adulte durant la belle saison, et ne rentre jamais en diapause. Les sexués ne peuvent émerger que du couvain dit « lent » (Vargo and Passera, 1992; Wheeler, 1986). Certaines espèces ne possèdent qu'un seul de ces types de couvain (par exemple, seulement du couvain lent chez la fourmi *Leptanilla japonica*, Masuko, 1990). Les proportions de couvain rapide et lent peuvent varier au sein d'une même espèce selon les facteurs environnementaux rencontrés ou selon les années plus ou moins favorables (Lopatina, 2018; Plateaux, 1970), montrant ainsi une certaine plasticité et adaptabilité de ce système.

Enfin, chez les fourmis du genre *Formica*, aucun individu ne passe l'hiver sous forme de larve, on parle de stratégie d'élevage de couvain concentré (Kipyatkov, 1993, 2001). Nous ne détaillerons pas ce type de cycle ici, car il ne correspond pas à celui de notre modèle biologique.



**Figure 3.** Cycle de vie et de croissance des individus au sein d'une colonie en fonction des saisons. Chez les espèces hétérodynamiques, deux types de larves peuvent cohabiter au sein d'une même colonie. Les traits pleins correspondent aux périodes de présence du stade développemental au sein de la colonie. Les flèches correspondent aux changements de stade développemental.

## **b- Variations temporelles des stressseurs environnementaux**

Les conditions physico-chimiques des environnements sont soumises à des variations, à l'échelle de plusieurs années ou au sein d'une même année. L'amplitude de ces variations varie d'un écosystème à l'autre, selon les conditions physico-chimiques qui leur sont propres. Par exemple, en milieu tempéré la température varie annuellement, avec des écarts de température entre été et hiver de l'ordre d'une trentaine de degrés, mais également entre années, avec des années sujettes par exemple à des épisodes de canicule ou d'autre de froids extrêmes, et un réchauffement global du climat année après année (rapport du GIEC, V. Masson-Delmotte, P. Zhai, H. O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. et al.). En milieu tropical, les températures varient beaucoup moins annuellement mais les précipitations peuvent avoir de fortes saisonnalités, avec alternances de saison sèche et de saison humide. Ces variations temporelles ont des impacts sur les phénotypes des individus, par exemple chez le papillon *Bicyclus anynana*, deux morphes de pattern de coloration des ailes existent, différents selon que la larve ait effectué sa métamorphose durant la saison sèche ou humide. Les précipitations présentent également des variations annuelles et interannuelles, changeant la disponibilité en eau et l'hygrométrie des milieux au cours du temps (Brakefield, 1997).

Ces variations climatiques peuvent créer à leur tour d'autres types de variations, comme les ressources disponibles pour les organismes. Par exemple, la disponibilité en insectes ou en graines dans les milieux tempérés varie (insectes absents l'hiver à cause des températures froides induisant leur hibernation dans des endroits protégés des prédateurs, graines enfouies dans le sol). La qualité de la nourriture au cours de l'année varie également, en termes d'abondance ou de nature, menant par exemple chez les abeilles à des saisons plus ou moins favorables à l'élevage de larves qui pourront se développer en gynes, avec une période riche en protéine et donc favorable en été, et plutôt défavorable en hiver (Hoover et al., 2006). Pour les herbivores, la nature des végétaux présents au cours de l'année varie, ainsi que leur composition en chlorophylle ou tanins (Iason et al., 1995).

Les concentrations en métaux traces dans les sédiments, les sols ou les cours d'eau varient également de manière saisonnière, avec par exemple des concentrations en métaux traces plus fortes dans certaines rivières durant l'été (Li and Zhang, 2010; Mohiuddin et al., 2012), ou des concentrations moins fortes dans les sédiments et les rivières durant la mousson (Inde, Alagarsamy, 2006). Les sols et l'atmosphère sont également sujets à des variations saisonnières des concentrations en métaux traces, notamment dans les milieux urbains (Haritash and Kaushik, 2007; Peña-Fernández et al., 2015). Des variations interannuelles existent également,

avec par exemple de l'accumulation de métaux traces au fur et à mesure des années (Peña-Fernández et al., 2015).

Les activités humaines peuvent également être à l'origine de variations saisonnières ou pluriannuelles des stressseurs. Par exemple, des déversements légaux ou illégaux d'eaux usées issus de l'agriculture, des égouts ou d'activités industrielles sont régulièrement effectués, menant à des épisodes de pics de pollution chimique dans l'eau, l'air ou les sols (pesticides, métaux lourds, plastique, hydrocarbures..., Laubier, 2004; McCahon and Pascoe, 1990; Peña-Fernández et al., 2015; Zhang et al., 2011) ou de pollution physique (modifications des températures de l'eau dans les rivières par exemple, Sinokrot and Gulliver, 2000).

**Tous ces stressseurs connaissent donc des fluctuations à longue échelle, à la fois saisonnières ou sur plusieurs années. Ces variations ne sont pas forcément synchronisées, ce qui peut donc amener de la superposition de plusieurs hauts niveaux de stressseurs à certains moments de l'année et à leur absence à d'autre. Ceci peut rendre parfois difficile les prédictions de la capacité des populations à faire face à ces stressseurs multiples (Henry et al., 2014, 2017).**

### **c- Variations temporelles dans la susceptibilité aux stressseurs chez les espèces sociales**

Les espèces sociales présentent des réponses aux stressseurs qui peuvent être variable selon le cycle de vie de l'individu (larve, adulte) ou selon le cycle saisonnier et donc le statut physiologique de l'individu ou de la colonie. Par exemple, chez l'abeille domestique *Apis mellifera*, les ouvrières adultes sont relativement moins sensibles à l'exposition à un pathogène (BQCV, Black Queen Cell Virus) que les larves chez qui ce virus induit des taux de mortalité jusqu'à 90% (Doublet et al., 2015). Cet effet est également observé pour une exposition à ce pathogène en combinaison avec un pesticide (le thiaclopride). Ceci pourrait être dû aux variations physiologiques dans la sensibilité aux infections au cours du cycle de vie chez les abeilles. Chez la fourmi *Myrmica rubra*, il a été montré que la sensibilité à un métal trace, le zinc, dépendait à la fois de la population dont était issue la colonie mais aussi du stade de vie de l'individu considéré, avec des adultes qui résistent mieux à l'exposition au zinc que les larves (Grześ, 2010a). De plus, les adultes montrent une augmentation de la résistance au zinc quand les populations viennent de milieux de plus en plus pollués initialement, alors que cette



résistance croissante ne se retrouve pas chez les larves, montrant l'importance de considérer les différents stades de vie pour l'étude de l'adaptation à un stress.

Les espèces sociales montrent aussi des variations saisonnières dans la réponse aux stressseurs de type polluants. Chez les fourmis, l'accumulation de métaux traces montre des variations saisonnières, avec des concentrations plus faibles au printemps et en automne possiblement en lien avec le cycle de vie colonial et l'activité plus forte des colonies en été, pour la production des sexuées (Rabitsch, 1997). Chez les abeilles, une étude a montré que la sensibilité à une catégorie de pesticides, les néonicotinoïdes, varie selon la température (Henry et al., 2014), soulevant le problème de l'étude de la résistance à un stressseur sans prendre en compte les interactions éventuelles avec des variations environnementales naturelles comme les variations de température.

Toujours chez les abeilles, la prévalence de l'infection à un parasite, *Nosema ceranae*, varie en fonction des saisons, avec une plus forte mortalité induite par ce parasite au printemps, et ce bien que le pathogène en question soit présent en quantités relativement constante tout au long de l'année (Traver et al., 2012). Ce pattern de variation saisonnière des infections par des parasites a aussi été documenté chez des espèces de fourmi, en lien avec des variations dans les taux d'agressivités des ouvrières (D'Etorre et al., 2004; Valles et al., 2010). Une étude a montré que selon le cycle de vie de la colonie (en phase de production de sexuées ou d'ouvrières), l'apparition saisonnière de phénomènes de floraison massive (en lien avec les activités agricoles) n'avait pas le même impact, avec une production d'ouvrières accrue mais plus rarement une production de sexués qui varie selon la période à laquelle ces floraisons massives ont lieu (Hovestadt et al., 2019).

Des variations dans la résistance aux températures ont aussi été montrées, avec des limites thermiques maximum et minimum variables selon les saisons ou le type de nourriture disponible. Les saisons les plus chaudes correspondent aux limites thermiques maximum les plus importantes chez les individus testés (Bujan and Kaspari, 2017; Bujan et al., 2020).

**L'amplitude et la nature des stressseurs varie également en milieu urbain, en fonction non seulement des variations naturelles (climat, saisons) mais aussi anthropiques (variation dans les niveaux d'activité humaines, par exemple chauffage plus important en hiver, trafic routier variable sur la journée et l'année...). Pourtant, à notre connaissance, aucune étude ne s'est encore intéressée à l'interconnexion entre ces variations des stressseurs et les variations des cycles de vie des espèces eusociales dans la réponse de ces espèces à l'urbanisation.**

#### 4- Objectifs de la thèse et modèle d'étude

Cette thèse a pour objectif de déterminer comment une espèce eusociale peut répondre à des changements environnementaux rapides et importants, à travers l'exemple de la réponse à l'urbanisation, et comment les caractères liés à cette socialité peuvent impacter cette réponse, en termes d'environnement social mais également en termes de variations dans le cycle de vie de la colonie. Le modèle d'étude choisi est la fourmi *Temnothorax nylanderi*. Cette espèce de fourmi eusociale possède des populations présentes à l'échelle de tout le territoire, en ville comme en forêt (Figure 4). Sa grande densité de population permet d'effectuer des études au pouvoir statistique fort. C'est une espèce formant de petites colonies qui vont d'une dizaine à une centaine d'ouvrières avec le plus souvent une reine unique, qui niche dans les bouts de bois morts, les châtaignes, les glands ect (Figure 4). Ce mode de vie en fait une espèce facilement récoltable et élevable au laboratoire, contrairement à de nombreuses autres espèces de fourmis.



**Figure 4.** Exemple de milieux dans lesquelles les colonies de *Temnothorax nylanderi* ont été récolté. En haut à gauche : forêt de Chantilly, en bas à gauche : Parc des Buttes-Chaumont, Paris. Droite : exemple d'un nid de *T. nylanderi*, ici au creux d'une branche morte.

## **Chapitre un : Divergence de traits d'histoire de vie entre populations urbaines et forestières chez la fourmi *T. nylanderi***

Le premier chapitre s'attache à mettre en évidence des traits d'histoire de vie différenciés entre populations urbaines et forestières de fourmi. La plupart des études s'intéressant à des différences entre populations urbaines et forestières d'organismes eusociaux tels que les fourmis s'intéressent surtout à l'évolution des traits liés à réponse aux températures en moyenne plus élevées en ville (Chick et al., 2019, 2021; Diamond et al., 2017, 2018). Le but de ce premier chapitre est donc de combler partiellement le manque d'études portant sur d'autres traits. De nombreuses études conduites sur des populations d'arthropodes (collembole, moustiques, fourmis) ont montré que les populations vivant dans des milieux très contaminés tels que d'anciens sites miniers ou d'activités industrielles montraient des signes d'adaptation à la pollution aux métaux traces (Grzes, 2010b; Mireji et al., 2008; Roelofs et al., 2009). Cependant, peu d'études ont été menées sur des populations urbaines, pourtant très exposées à cette pollution (quelques études existent cependant, en particulier sur des organismes aquatiques ou sur des oiseaux, Table 1). Nous avons donc prélevé des colonies en milieu urbain et en milieu forestier. Nous avons ensuite, en jardin commun, exposé ces colonies à du cadmium présent dans la nourriture pendant deux mois, le temps que les larves nourries avec le cadmium se développent et émergent en adulte. Après ces deux mois, différents traits ont été mesurés : taux de mortalité des ouvrières, des larves, et taille des émergentes. Certains phénomènes inattendus (arrêt de développement des larves) ont également été observés. Nous nous intéresserons également dans ce premier chapitre à une éventuelle différence de comportement entre colonies urbaines et forestières. En effet les études portant sur l'influence de l'urbanisation sur le comportement ont été dans leur très grande majorité menées sur des vertébrés, notamment les oiseaux. Ces études montrent des divergences de comportement entre populations urbaines et forestières, notamment en termes de comportements de fourragement ou d'agressivité et d'audace des individus, le milieu urbain ayant tendance à sélectionner les individus les plus agressifs et audacieux (Miranda, 2017; Miranda et al., 2013). Cependant, les conclusions sur l'effet de l'urbanisation sur le comportement de ces vertébrés ne peuvent être transposées à l'échelle des espèces sociales, et le nombre d'études portant sur la caractérisation de l'influence du milieu urbain sur ces derniers restent rares (mais voir Kaluza et al., 2016; Stelzer et al., 2010).

## **Chapitre deux : Déterminisme de la réponse à l'urbanisation, rôles des facteurs génétiques et de l'environnement social**

Le deuxième chapitre cherche à déterminer les rôles respectifs des facteurs sociaux, génétiques et plastiques dans la détermination de ces traits d'histoire de vie différenciés. En effet chez les organismes sociaux, en plus des traditionnels facteurs génétiques, l'environnement social (par exemple, la taille du groupe, le soin au couvain de la part des ouvrières, la diversité de taille des individus de la colonie...) impacte le phénotype des individus vivant dans la colonie. Le choix du trait d'histoire de vie différencié s'est ici porté sur la meilleure tolérance au cadmium des populations urbaines, de par sa robustesse apparente (effet trouvé sur plusieurs sites, plusieurs années différentes). Un premier article s'attache à mettre en le rôle de facteurs génétiques ou plastiques dans la meilleure tolérance au cadmium des populations urbaines. En effet, l'expérience du premier chapitre a été menée sur des colonies sortant juste d'hibernation. Or, les conditions d'hibernation entre milieux urbain et forestiers divergent, avec des hivers plus chauds en ville, entre autres possibles paramètres (il existe également des variations dans les degré de pluviométrie par exemple, Li et al., 2020; Rosenfeld, 2000). Ces différentes conditions auraient pu mener à différents états des colonies au sortir de l'hibernation. Par exemple chez certaines espèces les hivers plus chauds permettent une meilleure survie des larves ou des ouvrières, tandis qu'au contraire chez d'autres des hivers chauds mènent à une consommation énergétique plus importante et donc à des probabilité de survie moins grande après l'hiver (Haatanen et al., 2015; Sorvari et al., 2011; Williams et al., 2003). Nous avons prélevé des colonies en milieu urbain et forestier en automne, avant que les colonies n'entrent en hibernation, puis nous les avons fait hiberner en jardin commun à 4°C. A la fin de l'hiver, nous avons exposé ces colonies de jardin commun au cadmium et mesuré leur réponse. En parallèle, la même expérience avec des colonies ayant hiberné sur le terrain a également été faite. La réponse différentielle au cadmium entre ville et forêt a bien été retrouvée lorsque les colonies sont élevées en jardin commun, soulignant donc un rôle génétique. Cependant, étrangement, les colonies ayant hiberné sur le terrain ne montraient plus cette réponse différentielle. Une explication de ce phénomène peut tenir en un hiver exceptionnellement doux cette année-là. Cette expérience permet de mettre en évidence l'interconnexion entre facteurs génétiques et environnementaux.

La deuxième expérience portait sur le rôle de l'environnement social. La meilleure tolérance au cadmium des colonies urbaines peut s'expliquer par plusieurs facteurs : une meilleure faculté des ouvrières urbaines à s'occuper des larves en conditions stressantes, une meilleure tolérance

des larves urbaines à une exposition au cadmium, ou bien les deux. Pour démêler les rôles respectifs des larves et des ouvrières, nous avons effectué une expérience de cross fostering. Nous avons fait élever des larves de forêt par des ouvrières de ville, et vice-versa. Des contrôles ont tout de même été effectués (avec des ouvrières de ville élevant des larves de ville issues d'une autre colonie) afin de s'assurer que la réponse différentielle ville/forêt au cadmium était toujours présente. Un autre facteur social (la taille de colonie) a également été pris en compte. Malgré l'apparente robustesse de la meilleure réponse au cadmium chez les colonies urbaines, nous n'avons pas été capables de reproduire ce résultat lors du cross-fostering. Cela peut être dû au cross fostering lui-même, l'échange de larves pouvant être stressant pour la colonie. Cependant, une autre explication possible tient peut-être à la phénologie décalée de cette expérience, entraînant une sensibilité différente au cadmium en fonction des saisons. Cette expérience a tout de même permis de mettre en évidence, à travers l'étude du rôle de la taille de la colonie, les limites de l'effet protecteur du groupe face à des perturbations importantes.

### **Chapitre trois : Variation intra-annuelle des traits d'histoire de vie entre populations urbaines et forestières**

Enfin, le dernier chapitre s'est intéressé à l'effet des différentes dynamiques de colonie au cours de l'année dans l'existence de traits différenciés entre ville et forêt. En effet, l'investissement de la colonie dans les différents membres la constituant (ouvrières, mâles, gynes, reine, œufs, couvain) varie en fonction des saisons, de même que la demande en ressources (besoin protéiques important durant le printemps pour élever les sexués, besoin en sucre pour le maintien des ouvrières adultes...) ou la force et la qualité des stressors influençant la colonie. Nous avons donc comparé la réponse au cadmium chez des colonies sortant d'hibernation, peu actives, appauvries en réserves à la suite de la période d'hibernation et qui investissent surtout dans la production de nouvelles ouvrières, et celle de colonies en milieu de printemps qui sont alors en train de produire des sexués et qui possèdent également des ouvrières mieux nourries et très actives dans le fourragement. Dans ce chapitre, nous avons mis en évidence une variation saisonnière dans la susceptibilité au cadmium des colonies, avec des colonies de printemps résistant mieux. La réponse différentielle ville/forêt était toujours observée au printemps, mais ne portait pas sur les mêmes traits d'histoire de vie que ceux observés sur les colonies en sortie d'hibernation. Ceci souligne l'importance de prendre en compte le cycle de vie dans l'étude de l'adaptation d'un organisme à un changement environnemental, celui-ci pouvant avoir des effets différents au cours des saisons.

# **Chapitre 1 : Divergence de traits d’histoire de vie entre populations urbaines et forestières chez la fourmi *Temnothorax nylanderi***

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**Urban colonies are more resistant to a trace metal than their forest counterparts in the ant *Temnothorax nylanderi***

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**Keywords:** Urbanization, common garden, cadmium resistance, stress, heat, social insects

**Abstract:** Cities can be used as in situ replicates to assess the responses of organisms to environmental changes, such as increased heat or pollution. Community-level studies and more rarely population-level studies have been conducted, but they are not often based on multiple city replicates, thus preventing generalization. Our study tested whether colonies of the ant *Temnothorax nylanderi* from forest and urban populations exhibit distinct responses to pollution. Because this is a social species, we could also test whether social traits (colony size and mean size of adult workers) affected the response of colonies to urbanization. We collected

colonies from four pairs of forest and urban habitats and reared them in the laboratory under normal or cadmium-supplemented diets. We then measured the emergence rate and size of newly-produced workers as well as the mortality rate of adult workers. In all four forest/city replicates, urban colonies were less negatively affected by cadmium than forest colonies for emergence rate and size of newly-produced workers, but not for mortality rate of adult workers. We did not find any correlation between social traits and cadmium resistance, in contrast with what was found in other eusocial insects. We discuss the potential adaptive nature of this differential response.

## **Introduction**

The United Nations predict that by 2030, more than 60% of the worldwide human population will live in cities, resulting in a threefold increase of total urban areas (Seto et al., 2012). Urbanization is associated with many abiotic environmental changes, such as modified land use, habitat fragmentation, increased temperatures known as Urban Heat Island effect, noise, light, and chemical pollutions including trace metals. It is also associated with biotic environmental changes such as the introduction of exotic species (Bornstein, 1968; McDonnell et al., 1997; Pickett et al., 2001). Because these changes are quite fast and recent on the evolutionary timescale, cities can be seen as in situ laboratory replicates for understanding the responses of species that face rapid environmental changes (Buczowski and Richmond, 2012; McDonnell and Hahs, 2015). Most studies investigating the impacts of urbanization on wildlife have been conducted at the community level (Cheptou et al., 2008; McKinney, 2008). Urbanization alters the composition of communities (higher or lower species abundance), functional traits (e.g. reduced body size), and interaction networks (Alexandre et al., 2010; Blair, 2001; Clergeau et al., 1998; Germaine and Wakeling, 2001; McKinney, 2008; Merckx et

al., 2018; Pillsbury and Miller, 2008). In contrast, species-level studies are less common (McDonnell and Hahs 2015). Relative to non-urban populations, animals from urban populations express altered behavior such as songs with distinct pitches or loss of migratory behavior (Evans et al., 2012; Mendes et al., 2011), changes in physiology such as lower glucocorticoid secretion in response to stress or higher oxidative stress (Partecke et al., 2006), earlier reproduction (Chick et al., 2019; Partecke et al., 2004), faster circadian rhythm oscillations (Dominoni et al., 2013), higher parasitism rate (Bradley and Altizer, 2007) and/or differences in morphology (Liker et al., 2008; Weller and Ganzhorn, 2004). These shifts in life-history traits are often seen as a response, adaptive or not, to urban stressors. However, because cities are extremely diverse and because most studies are based on a single city and not multiple city replicates (but see Chick et al., 2019; Johnson et al., 2018), it is difficult to make general conclusions and identify a robust syndrome of urbanization for population-level traits. In addition, population studies are generally correlative (Chamberlain et al., 2009; Hof et al., 2016; Isaksson, 2010; Lazić et al., 2015; Leong et al., 2016; Liker et al., 2008; Lowe et al., 2014; Lu et al., 2006; Mendes et al., 2011; Scales et al., 2011; Slabbekoorn and den Boer-Visser, 2006), and do not quantify the respective contributions of genetics and phenotypic plasticity. So far, common garden experiments (the rearing of individuals from different origins under the same environmental condition) between urban and non-urban populations highlighted a genetic basis for the lower dispersal ability of urban plants (Cheptou et al., 2008), earlier reproduction of urban birds and ants (Chick et al., 2019; Partecke et al., 2004) and for specific behaviors of urban birds (lower migratory behavior, increased boldness and stress (Evans et al., 2012; Partecke et al., 2006)). Additional common garden experiments are thus needed to complement correlative studies and assess causality and determination of various other life-history traits associated with urban life.



Pollution is one of the main chronic urban stressors (Foti et al., 2017). For example, traffic-generated air pollution is responsible for increased oxidative stress leading to genetic damages, respiratory diseases and brain inflammation (Guxens and Sunyer, 2012; Ieradi et al., 1996; Isaksson, 2010) as well as higher susceptibility to parasites (Bradley and Altizer, 2007) in animals. Cities are also contaminated by trace metals. Cadmium concentrations can be up to ten times higher in urban soil relative to rural soil: for example, cadmium has a concentration of 0.30 mg.kg<sup>-1</sup> in soil of rural woods and 2.45 mg.kg<sup>-1</sup> in urban woods (Foti et al., 2017). Cadmium alters plant physiology by interfering with calcium uptake (Stroiński, 1999). It also impacts various life history traits, causing longer development in fishes and arthropods, decreased hatching rate in fishes, sea stars and arthropods (Den Besten et al., 1989; Cervera et al., 2004; Gomot, 1998), decreased body size in fishes (Cervera et al., 2004) and immunosuppression in rats (Demenesku et al., 2014). Ants are a good model to study the effects of trace metals because they live in long-lived colonies and they forage locally around their nest, exposing themselves to bioaccumulation (Grześ, 2010a).

Ants represent a large portion of the total earth animal biomass (on average, 15 to 20%, Schultz, 2000). They are found in a large diversity of ecosystems including urban areas and can deeply affect the functioning of ecosystems (Farji-Brener et al., 2010; Philpott and Armbrrecht, 2006; Sanders and Veen, 2011). Because of their ecological interest, many studies have investigated how urbanization can affect ants. These studies highlighted a simplification of ants communities with urbanization (lower species richness, dominance of one particular species: Buczkowski and Richmond, 2012; Lessard and Buddle, 2005; Sanford et al., 2009). At the scale of populations within one species, fewer studies have been conducted. It seems that urbanization impacts thermal tolerance, as urban workers resist high temperatures better than rural workers (Diamond et al., 2017; Angilletta et al., 2007), and also tolerate fast increase in

temperature better (Diamond et al., 2018). However, studies investigating other traits than heat response among populations of one species are, to our knowledge, lacking.

Social lifestyle could enhance robustness against environmental changes (Rubenstein and Abbot, 2017). Ants live in colonies made of few tens to millions of individuals where labor is divided among individuals (Lach et al., 2010). Under division of labor, individuals concentrate on a limited number of tasks avoiding them to suffer from the risks associated to the tasks they do not perform. Such risk dilution could allow colony to better cope with stress than solitary individuals that have to perform all tasks, therefore suffering from all associated risks. For instance, eusocial species of bees are less negatively impacted by neocotinoids than solitary species (Rundlöf et al., 2015), and models suggest that, within species, larger colonies are more resistant to pesticides (Crall et al., 2019). Similarly, the resistance to environmental changes may increase with colony size because large groups develop information networks that are redundant and interaction networks that are more resilient (Naug, 2009). In ants, larger colonies are more resistant to parasites and starvation (Modlmeier et al., 2013; Scharf et al., 2012). How could sociality enhance the resistance of ants to anthropic stressors is an open question.

The aim of this study was to test whether there is a general effect of urbanization on resistance to trace metals (cadmium) in the acorn ant *Temnothorax nylanderi*. This is a small ant species (up to six millimeters long) with small colonies from ten to few hundred workers (Foitzik and Heinze, 1998) that nests in acorns and twigs on the ground. It is broadly distributed across all western Europe. Workers forage at a short range from 20 to 100cm away from the nest (Heinze et al., 2010), so they are good models to test for the effect of local pollution on life history traits. Cadmium negatively affects life history traits in various taxa, including insects (Malakar et al., 2009; Mirčić et al., 2010). The resulting selective pressure can lead to the evolution of cadmium-tolerant populations in less than four generations ( e.g. in *Limnodrilus hoffmeisteri* , Klerks and Levinton, 1989; or in collembola, Posthuma and Van Straalen, 1993; Roelofs et al.,

2009). We tested whether colonies of *T. nylanderi* from cities are more tolerant to the negative effects of cadmium than colonies from forests. For this purpose, we fed colonies with cadmium-enriched food or normal food, in a common garden laboratory setup, and we measured several traits of the newly produced workers. We expected urban colonies to be more tolerant to cadmium because of their chronic exposition to higher cadmium concentration. Moreover, this species offers the opportunity to assess the correlation between social traits and cadmium resistance. In social species, colony size and mean worker size are known to improve colony performance (Billick and Carter, 2007; Herrmann et al., 2018; Jandt and Dornhaus, 2014; Kaspari and Vargo, 1995). This was also found in *T. nylanderi* (Molet et al., 2017). We therefore expected a weaker effect of cadmium on colonies with more workers or larger workers.

## **Material and methods**

### ***Colony collection and rearing***

We used the ant *Temnothorax nylanderi* as our biological model. Colonies are easy to collect and rear in the laboratory, and they produce new workers in two months at 22-27°C cycle (Molet et al., 2017). Two hundred and thirty-eight colonies of *T. nylanderi* were collected from eight sites at three locations in France (Paris, Bordeaux, Lyon). Each location had one pair of collection sites: a central urban park (city habitat) and a forest located between 23 and 52 km from the city (forest habitat). Two pairs were collected in Paris location from two different parks and forest sites and in two different years, 2017 and 2018 (see table 1). They are subsequently called “Paris’17” for colonies collected in March 2017 and “Paris’18” for colonies collected in March 2018. Colonies from Lyon and Bordeaux were collected in February and March 2018. After collection, colonies were brought back to the laboratory and workers were counted. We

discarded colonies with no queen or more than one queen (72 colonies) as well as colonies parasitized by cestodes (9 colonies, identified by the pale orange colour of workers). A total of 157 queenright colonies were used for the experiment and reared in foraging plastic boxes (11.5 x 11.5 x 5.5 cm) containing an artificial nest made of two microscope slides separated by a thin layer of plastic foam with hollow chambers. The top slide was covered with dark plastic to protect the colony from the light. Plastic boxes were kept in two temperature-controlled chambers (reference CTS TP10/600) at 22°C from 7pm to 7am and 27°C from 7am to 7pm. These stressful temperatures were chosen to speed up development and to amplify the effects of cadmium (Abdel-Lateif et al., 1998).

### ***Experimental design***

Colonies were experimentally exposed to a feeding treatment including two conditions: cadmium and control, applied to all locations and all pairs of habitats. We counted workers in all colonies. Colony size ranged from 5 to 260 individuals. In order to avoid any bias in colony size between control and cadmium conditions, we evenly assigned colonies to cadmium and control based on their colony size for each habitat within each location. Colonies were randomly distributed between the two temperature-controlled chambers independently of their location, habitat and treatment. All original brood were removed from the colonies except for about 30 second instar larvae. This assured us that larvae would spend most of their development under laboratory conditions (as opposed to field conditions), and they would all be the same age across colonies, locations, habitats and treatments. The 71 colonies (out of 238) that had less than 30 second instar larvae, were left with all of their second instar larvae (21 colonies had 25-30 larvae, 18 colonies had 20-25 larvae, and only four colonies had less than 10 larvae). Newly laid eggs were removed every week to avoid the hatching of extra larvae. We fed colonies for 61 days every other day with a mixture of yoghurt, diluted honey and dried crickets containing 100µg/g cadmium (cadmium condition) or no cadmium (control condition). Food was provided

in excess, whatever the size of the colony. We removed food leftovers 12h after food introduction. The cadmium concentration corresponds to the LC<sub>50</sub> (concentration at which half of the treated individuals die) of *Spodoptera littoralis* (M.A. Pottier, personal communication). The mortality rate of the adult workers present in the colony from the beginning of the experiment (i.e. workers born in the field, subsequently called field workers) was computed based on the frequent removal of corpses and defined as the total number of dead field workers at the end of the experiment divided by the total number of field workers at the start of the experiment.

The experiment was performed over two different years: 2017 for Paris'17, and 2018 for Bordeaux, Lyon and Paris'18. For both years, colonies were acclimated for 14 days in laboratory before the experiment started. The experiment lasted for 61 days, so any larva that would have escaped the egg removal protocol and hatched could not have reached adulthood before the end of the experiment. The emerged workers (subsequently called lab workers) were identified based on their pale color (*Temnothorax* larvae do not spin a cocoon) and collected once they started to move. They were anesthetized by cooling down and stored in ethanol for subsequent morphological measurements. We computed the emergence rate of larvae as the ratio between the number of emerged adults (both workers and sexuals) and the initial number of larvae. The date of emergence was recorded in order to compute development duration for each lab worker, i.e. the duration from the second instar larva to the first movements of the newly emerged lab worker. Nine queens died during the experiment (one from Bordeaux-city-cadmium, one from Lyon-city-control, three from Paris'17-city-control, one from Paris'17-city-cadmium, two from Paris'17-forest-control and one from Paris'18-forest-cadmium). We excluded these colonies from the analyses, so 148 colonies were left in.

### ***Morphological measurements***

At the end of the experiment, the heads of lab and field workers were stuck on double-sided tape and photographed at a x5 magnification under a stereomicroscope (Zeiss Discovery.V12) connected to a computer via a camera (Zeiss AxioCamICc). Head width was measured from eye to eye (just posterior to the eyes) with ImageJ (<https://imagej.nih.gov/ij/>; Abramoff et al., 2004) and used as a proxy of body size (Molet et al., 2017).

### ***Statistical analyses***

We used R v3.6.2 (R Core Team, 2015) to conduct our statistical analyses. We tested for the effect of treatment (control, i.e. fed without cadmium, and cadmium, i.e. fed with cadmium), habitat (city or forest) and location (Paris'17, Paris'18, Bordeaux, Lyon), and their two level interactions on three dependent variables (mortality of field workers; emergence rate and size of lab workers). As a recent study evidenced a lack of genetic structure at large geographic scale in *T. nylanderi*, we considered the four collections as four independent locations (Khimoun et al., 2020). We also tested for the effect of two covariates (initial colony size and mean size of field workers) on each dependent variable to investigate the effects of these social traits. To test for a buffering effect of colony size or field worker size, we included in the models these covariables as well as their interaction with treatment. For the two dependent variables measured at the colony level (mortality rate of field workers and emergence rate of lab workers), we used generalized linear models (GLMs). Because mortality and emergence rates were around 50%, using a quasibinomial model was not better than using a Gaussian model with log-transformed data, and both models provided the same qualitative results. We therefore presented data from the Gaussian model for simplicity and because this allows to get AIC information. When considering the size of lab workers as the dependent variable, we used a linear mixed-effect model with the package *lme4* (Pinheiro et al., 2016) with colony considered

as a random factor as size were measured at the individual level. This allows to control for the potential similarities among workers within the same colony and therefore avoids pseudo-replication. We visually checked the homogeneity of variance and normality of residuals. We chose the minimal adequate model based on AIC scores. The *P*-values for the explanatory variables and interactions were obtained by removing or adding the variable of interest from/to this minimal adequate model (Zuur et al., 2009) and comparing them with a log-likelihood ratio test using the function *anova*. When necessary, we used *lsmeans* to compare means among the different levels of the explanatory variables.

## **Results**

The 148 colonies produced a total of 1269 lab workers (922 for the control condition and 347 for the cadmium condition, Table 1), 215 males (176 for the control condition and 39 for the cadmium condition, Table 1), and no gynes in 61 days. Because only few males (39) emerged under cadmium condition, statistical power was lacking and we chose not to present results on male traits.

**Table 1** Number of *Temnothorax nylanderi* colonies used (in bold) and number of workers (W), males (M) and larvae that paused their development (P) collected for each location, treatment and habitat type. We also provided mean colony size (Colony size) and the mean field worker size (MFWS) for each location, treatment and habitat type.

		Treatment	NC	N	CS	MFWS	Site	GPS
Paris'17	City	Control	13	247W, 21M, 1P	76 ±38	0.57±0.01	Jardin Ecologique	48°50'59.684"N
		Cadmium	14	206W, 8M, 2P	70±41	0.58±0.02		2° 21' 40.385"E
	Forest	Control	13	213W, 6M, 10P	81±35	0.58±0.02	Fontainebleau	48° 26' 2.595"N
		Cadmium	13	87W, 1M, 0P	70±32	0.59±0.02		2°43' 52.842"E
Bordeaux	City	Control	7	39W, 12M, 46P	83±35	0.56±0.01	Jardin public	44°51'16.9"N
		Cadmium	7	12W, 1M, 111P	88±39	0.57±0.01		0°35'16.5"W
	Forest	Control	12	130W, 29M, 46P	72±49	0.54±0.02	Monfaucon	44°52'23.6"N
		Cadmium	11	11W, 11M, 193P	84±44	0.56±0.02		0°08'14.7"W
Lyon	City	Control	4	18W, 22M, 36P	49±27	0.57±0.01	Parc de la Tête d'Or	45°46'44.2"N



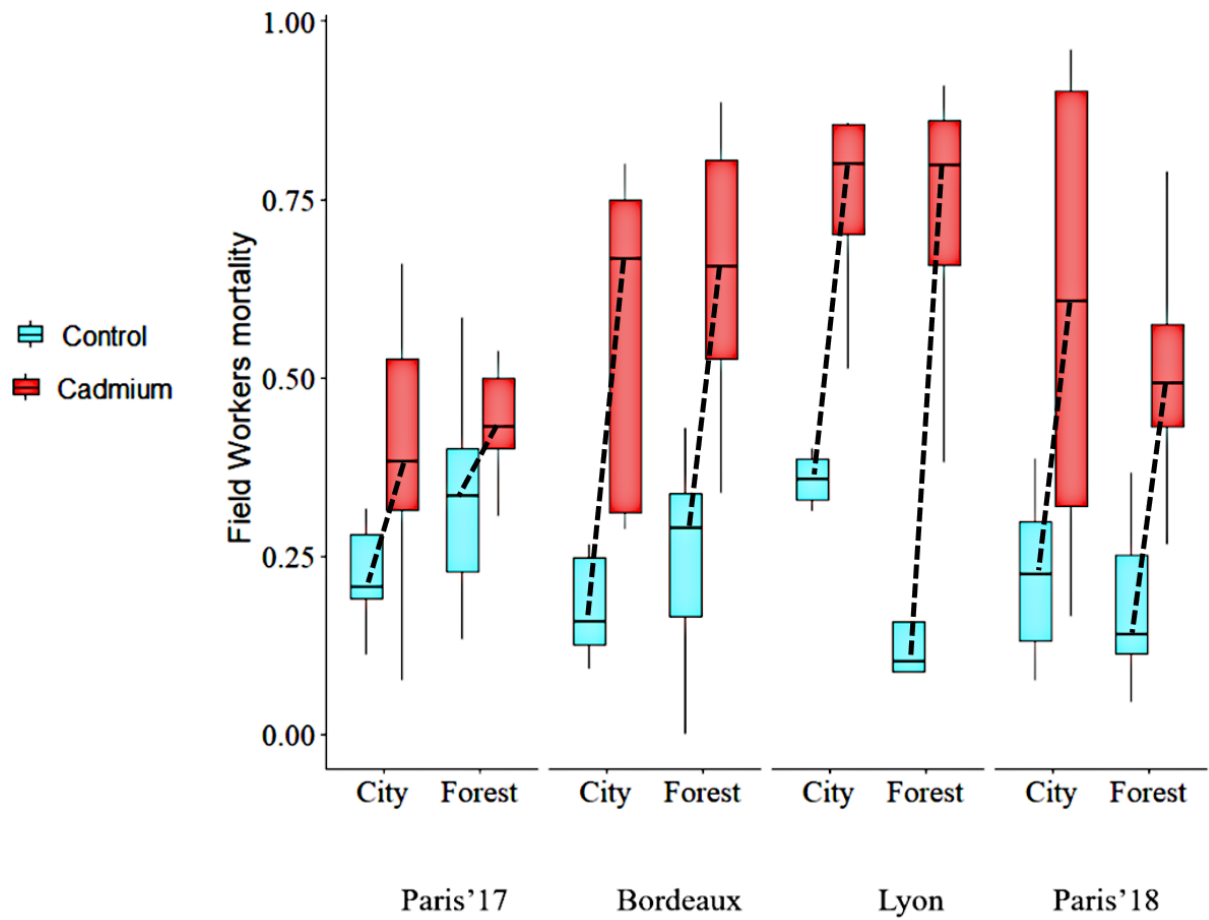
		Cadmium	5	10W, 1M, 31P	63±36	0.55±0.02		4°52'54.3"E
	Forest	Control	4	28W, 4M, 19P	82±64	0.55±0.03	Arandon	45°43'07.5"N
		Cadmium	4	7W, 3M, 26P	83±50	0.56±0.02		5°26'44.5"E
Paris'18	City	Control	10	107W, 64M, 59P	99±48	0.58±0.01	Buttes-Chaumont	48°52'47.1"N
		Cadmium	11	7W, 7M, 173P	134±71	0.59±0.01		2°23'45.6"E
	Forest	Control	10	140W, 23M, 67P	109±59	0.58±0.02	Chantilly	49°10'59.8"N
		Cadmium	10	7W, 2M, 135P	93±47	0.58±0.02		2°28'43.6"E

### ***Field workers***

Only nine queens died during the experiment (three under cadmium treatment, six under control treatment). There was no differential queen mortality between control and cadmium conditions (Fisher's exact test,  $P = 0.49$ ) nor between city and forest colonies (Fisher's exact test,  $P = 0.32$ ). The size of field workers ( $F_{142,141} = 1.76$ ,  $P = 0.187$ ) and colony size (the number of field workers by colony) were not significantly different between habitats ( $F_{143,142} = 0.024$ ,  $P = 0.87$ ). Colony size was only affected by the location ( $F_{143,146} = 4.90$ ,  $P < 0.001$ ), with larger colonies found in Paris' 18 ( $P$ -adjusted = 0.041 for Paris' 18-Bordeaux,  $P$ -adjusted = 0.014 for Paris' 18-Lyon and  $P$ -adjusted = 0.002 for Paris' 17-Paris' 18, Table 1). The size of field workers also differed among locations ( $F_{141,144} = 17.58$ ;  $P < 0.001$ ) with the bigger field workers found in Paris' 17 and Paris' 18 ( $P$ -adjusted Paris' 17-Paris' 18 = 0.95,  $P$ -adjusted Paris' 17-Bordeaux and Paris' 18-Bordeaux  $< 10^{-6}$ ,  $P$ -adjusted Paris' 17- Lyon and Paris-18-Lyon  $< 10^{-3}$ ). As expected, mean worker size was positively correlated with colony size ( $F_{131,132} = 13.10$ ,  $P < 0.001$ ; slope estimate =  $1.28 \cdot 10^{-4}$ ).

At all locations, the mortality rate of field workers was significantly higher in cadmium condition than in control condition (Treatment effect,  $F_{143,142} = 115$ , parameter estimate treatment control = -0.39,  $P < 10^{-16}$ ) but the magnitude of the effect varied among locations (Treatment-Location interaction,  $P = 0.00037$ ,  $F_{142,139} = 6.5$ , Figure 1, Table S2). Mortality rate significantly differed among locations (Location effect,  $F_{143,146} = 2.9$ ,  $P = 0.035$ ) with a higher mortality rate in Lyon ( $t = 1.824$ ,  $P = 0.07$ , figure 1). Under the hypothesis that urban colonies resist better to stressors than forest colonies, we were expecting to find a differential effect of cadmium on mortality rate depending on habitat (Treatment: Habitat interaction). However we did not find this effect (Treatment: Habitat interaction,  $F_{138,137} = 0.01$ ,  $P = 0.91$ ) and there was no

effect of habitat on mortality rate ( $F_{140,139}=0.36$ ,  $P=0.54$ ). Colony size and size of field workers as well as their interactions with treatment did not affect mortality rates (Table S2).



**Fig. 1** Boxplot of the mortality rate of field workers by habitat, location and treatment. Colonies fed with cadmium are in red, colonies fed with control are in blue. Boxes show median, quartiles and extreme values. Statistics are presented in Table S2. Dotted lines show significant treatment effects, and stars show significant treatment:habitat interactions (meaning that dotted lines are not parallel).

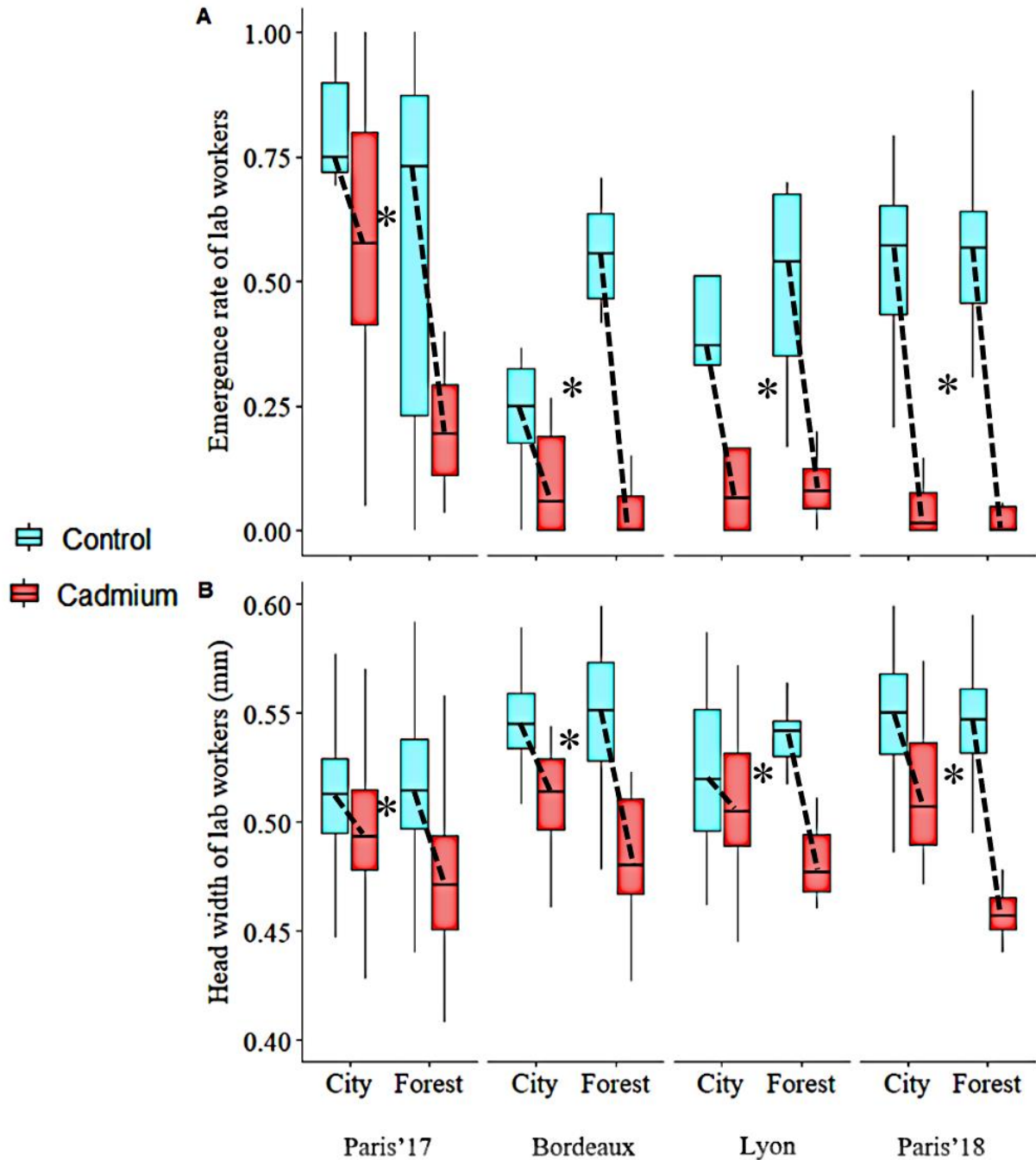
### *Laboratory workers*

In all locations, the emergence rate was significantly lower in cadmium condition than in control condition ( $F_{141,142}=104$ , parameter estimate treatment control=0.23,  $P<10^{-16}$ , figure 2A). The effect was in the same direction in all locations even though its magnitude marginally

significantly differed among locations (Treatment: Location interaction,  $F_{140,137}=2.7$ ,  $P=0.045$ , Table S2). The emergence rate significantly differed among locations ( $F_{141,144}=21$ ,  $P<10^{-10}$ ). Interestingly, when treated with cadmium, forest colonies experienced a stronger decrease in emergence rate than city colonies for all location (Treatment: Habitat interaction,  $F_{138,137}=4.22$ ,  $P=0.04$ , figure 2A, Table S2). This was associated with an increase in the number of larvae that apparently paused their development at early stages (15% in control vs 30% in cadmium condition,  $t_{134}=3.76$ ,  $P=0.00024$ ). Emergence rate in control condition was the same between city and forest habitats at each location (Paris'17:  $t_{16}=1.8$ ,  $P=0.08$ ; Lyon:  $t_6=-0.07$ ,  $P=0.9$ ; Paris'18:  $t_{19}=-0.44$ ,  $P=0.65$ ) except for Bordeaux ( $t_{10}=-2.99$ ,  $P=0.01$ ), so the observed Treatment: Habitat interaction was not caused by an initial differential emergence rate between forest and city colonies when exposed to a stressful temperature. The emergence rate was affected by the mean size of field worker (Table S2), with colonies containing larger field workers having lower emergence rate ( $F_{138,137}=8.8$ ,  $P=0.0034$ , slope estimate=-2.4) whatever the treatment (no significant interaction treatment and size of field workers ( $F_{133,134}=0.24$ ,  $P=0.62$ )).

Colonies treated with cadmium produced significantly smaller lab workers in all locations ( $X_7=48$ , parameter estimate treatment control=0.00312,  $P<10^{-11}$ ) but as previously, the effect significantly differed in magnitude among locations (Treatment: Location interaction,  $X_3=9.87$ ,  $P=0.0197$ , Figure 2B,  $P<10^{-2}$ , Table S3). Interestingly, this decrease in the size of lab workers was stronger in forest colonies than in city colonies for all location (Treatment: Habitat interaction,  $X_1=5.03$ ,  $P=0.02485$ , Table S3, Figure 2B). The size of lab workers under control condition did not differ between the two types of habitats for all location (Paris'17:  $t_{330}=-0.7$ ,  $P=0.46$ , Bordeaux:  $t_{88}=-1.5$ ,  $P=0.12$ , Paris'18:  $t_{202}=-1.4$ ,  $P=0.13$ ) except for Lyon ( $t_{28}=-2.19$ ,  $P=0.03$ ), so the observed Treatment: Habitat interaction was not caused by an initial difference in the size of lab workers between forest and city colonies when exposed to a stressful

temperature. Regarding social traits, only colony size affected significantly and positively the size of lab workers ( $X_1=12.52$ ,  $P=0.00040$ , slope estimate =  $1.7 \cdot 10^{-4}$ ) whatever the treatment (no significant interaction between treatment and colony size; Table S3 to see all statistical results).



**Fig. 2** Boxplot of worker traits by habitat, location and treatment. Colonies fed with cadmium are in red, colonies fed with control are in blue. (A) Emergence rate of lab workers. (B) Head width of lab workers (mm). Boxes show median, quartiles and extreme values. Dotted lines

show significant treatment effects, and stars show significant treatment:habitat interactions (meaning that dotted lines are not parallel).

## **Discussion**

The aim of our study was to assess whether response to cadmium differed between urban and forest colonies of the small acorn ant *Temnothorax nylanderi*. Our hypothesis was that, because urban colonies are more exposed to cadmium than forest colonies in the wild, they should be less sensitive to it. Exposure to high cadmium concentration (100mg/kg) caused an increase in adult mortality rate and a decrease in size and emergence rate of lab workers compared to the control condition, confirming that cadmium has a negative effect on life history traits in this species, as found in other insects (Malakar et al., 2009; Mirčić et al., 2010). As expected, the negative effect of cadmium was weaker on urban colonies, with a limited decrease in emergence rate and size of lab workers compared to forest colonies in all the four city/forest pairs. However, mortality rate of field workers was equally affected in urban and forest colonies. Because this differential effect of cadmium was observed in all four pairs of habitats, we can confidently conclude that urbanization leads to a better tolerance to cadmium. Whether this effect of urbanization could be considered as an adaptive response is discussed below.

Different hypotheses could explain the lower sensitivity of city colonies to cadmium. First, because urban soils often contain more cadmium than non-urban soils (on average, 2.45mg/kg for urban wood soil in Paris, and 0.3 mg/kg in rural wood soils of Ile-de-France, Foti et al., 2017), we can hypothesize that urban colonies are more used to cope with higher cadmium concentration. Although the underlying mechanism (phenotypic plasticity, maternal effects, genetic adaptation) remains unknown, such resistance has already been documented in the collembola *Orchesella cincta*, where individuals from populations of heavily cadmium-contaminated disused mining sites are more resistant to cadmium (Roelofs et al., 2009). In our

experiment, the concentration used in the food ( $100\text{mg}\cdot\text{kg}^{-1}$ ) corresponds to the  $\text{LC}_{50}$  (the concentration that triggers 50% mortality over a given period of time), a physiologically but not ecologically relevant dose. Indeed, this concentration is about fifty times higher than the typical cadmium concentration measured in urban soils. However, in the field, recurrent exposure to pollutants can lead to bioaccumulation and cadmium concentration can reach  $102\text{ mg/kg}$  in the ant *Formica polyctena*, for a soil concentration of  $2\text{ mg}\cdot\text{kg}^{-1}$  (Starý and Kubizňáková, 1987). Therefore, the concentration that we used was high but still in the upper range of real field values. Second, the resistance to cadmium found in our urban colonies could reflect a general resistance to other trace elements such as arsenic, zinc, lead or mercury that could be found at a higher concentration in urban leaf litter because of intense industrial activity and proximity with traffic. This phenomenon, called co-tolerance model, features positive correlations among resistances to multiple stressors (Lucas et al., 2013; Tlili et al., 2011). Such co-tolerance has been observed in some bacteria for trace metals and algae for trace metals and high temperature (Bozhkov et al., 2010; Díaz-Raviña et al., 1994). Accordingly, urban ants may have evolved general detoxification systems that can cope with various trace elements. Third, the co-tolerance concept could be even broader and include other type of stressors than pollutant. Our rearing temperature ( $22\text{-}27\text{ }^{\circ}\text{C}$ ) was stressful for ants as opposed to the typical  $15\text{-}20\text{ }^{\circ}\text{C}$  experienced in nature (Molet et al., 2017). Because of the Urban Heat Island effect, populations living in urban centers frequently experience higher temperatures than their non-urban counterparts. Therefore, they could be less stressed by heat (Diamond et al., 2017) and subsequently could be able to better resist to cadmium. City colonies did not perform better than forest colonies under control conditions, but this may be due to the ability of forest colonies to buffer stressors. When exposed to several stressing factors (heat + cadmium), forest colonies may however collapse whereas urban colonies would resist. Interestingly, studies performed on European black birds reared under common garden experiments showed that urban populations

have a lower stress response to human handling than their rural counterparts (Partecke et al., 2006), suggesting that urban populations can evolve a general tolerance to stressors. These conclusions should be confirmed by further experiments to assess whether the observed lower sensitivity to cadmium of city colonies in *T. nylanderi* is due to a general resistance to stress or to a more efficient detoxification system.

Despite a strong effect of cadmium on worker mortality, we did not find any effect on queen mortality in city and forest colonies, as 97% of queens survived. Some studies suggest that workers act as a filter, limiting queen exposure to toxic compounds such as cadmium (Martin, 2000). The larger body size of queens might also help them to better resist cadmium (Grześ, 2010b; Vesela and Vijverberg, 2007). An unexpected effect of cadmium was the increased frequency of larvae stopping their development. These larvae did not grow over the entire course of the experiment, but they still looked alive and were kept in the nest with the rest of the brood and cared for by workers, unlike dead larvae that were typically discarded outside. In insects, environmental factors such as cold temperature, can induce a pause in development (Andrewartha, 1952). This pause can be endogenous, or triggered by external factors such as photoperiod and temperature which also affect its occurrence and duration (Kipyatkov and Lopatina, 2009). However paused development can be induced by other non-seasonal (thus unpredictable) environmental factors, such as trace metals in adult earthworms or hypoxia in *C. elegans* embryos (Miller and Roth, 2009; Morgan and Morgan, 1993). Individuals that pause their development usually survive longer in response to a stress, for example flesh-fly larvae exposed to anoxic conditions (Kukal et al., 1991). Thus, paused development can be interpreted as a stress-avoidance mechanism (Morgan and Morgan, 1993). The higher proportion of paused *T. nylanderi* larvae under cadmium condition may be a general defense mechanism to face a wide range of stressors. It is unknown whether paused larvae can resume development or are just barely surviving and are doomed to death. This and the potential impact of paused



development on the life history traits of workers that would manage to resume development deserves further investigation.

The differential response to cadmium between urban and non-urban colonies of *T. nylanderi* could have genetic or environmental determination. In some hexapodes, populations that are tolerant to heavy metal have duplicated metallothionein genes (*Drosophila melanogaster*, Maroni et al., 1987) or an increased expression of a single metallothionein gene (*Orchesella cincta*, Roelofs et al., 2006). A recent genomic analysis (Rad-seq) based on our *T. nylanderi* colonies revealed that 19 loci are under divergent selection between urban and non-urban populations, but none of them corresponding to metallothionein genes (Janssens et al., 2009; Khimoun et al., 2020; Maroni et al., 1987). These results support the idea that, in spite of a high gene flow among populations, adaptive genetic differentiation can occur between urban and forest populations. Whether the differential response to cadmium observed in our study is genetically determined or not remain an open question.

In social groups, the phenotype of an individual is also determined by the surrounding social environment. The social environment includes colony size (number of individuals), the phenotype of other individuals (caste, size, morphology, physiology, behavior), the proportion of individual types, and complex emerging group properties including communication. In this study, we tested for a potential buffering effect of colony size and size of field workers on the response to cadmium. We found two links between social traits and the phenotype of lab workers but they were similar with and without cadmium. First, larger colonies produced larger lab workers, a typical phenomenon in ants (Molet et al., 2017; Porter and Tschinkel, 1985). Second, colonies with larger field workers had a lower emergence rate, which may be due to the longer development duration of large workers that were then exposed to cadmium for longer. In contrast, in bees, larger colonies were found to be more resistant to pollutants (Crall et al., 2019; Rundlöf et al., 2015). Social life could deeply impact the ability of ants to face

environmental changes. On one hand, it may help species to cope with environmental changes to an extent that cannot be achieved by solitary species. The social filtering of the environment can for instance protect the brood from temperature fluctuations, drought, starvation or pathogens. On the other hand, consequences of social life such as extreme task-specialization or obligatory collective behavior may make social species more vulnerable to environmental changes (Chapman and Bourke, 2001; Luque et al., 2013). Because of the ecologic and economic values of social species (Björn et al., 2014; Philpott and Armbrecht, 2006) it is crucial to understand how they react to environmental changes.

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### **Supplementary Information**

#### ***Statistical models***

For each dependent variable, we built the following complete model:

*Dependent variable* ~ *Treatment:Habitat* + *Treatment:Location* + *Treatment* + *Habitat* + *Location* + *Colony size:Treatment* + *Mean field worker size:Treatment* + *Colony size* + *Mean field worker size Treatment* (+ random Colony effect).

We compared candidate models by removing factors one by one. We tested the effect of each factor as follows: (1) Colony size:Treatment; (2) Mean field worker size:Treatment (3) Colony size; (4) Mean field worker size; (5) Treatment:Habitat; (6) Treatment:Location; (7) Treatment; (8) Habitat and (9) Location. When the Treatment:Habitat or the Treatment:Location interaction was significant, we used *lsmeans* to estimate the effect of one factor depending on the level of the other. We then chose the minimal adequate model based on AIC scores.

**Table S1. Effect of the different factors and their interactions on colony size (number of field workers) and size of field workers.** Fisher scores and associated *P*-values are from ANOVAs between generalized linear models including or excluding the factor or interaction.

	Colony size	Size of field workers
Colony size	NA	F <sub>131,132</sub> =13.10 0.00042
Treatment : Habitat	F <sub>137,138</sub> =2.13	F <sub>138,137</sub> =1.93

	<i>0.15</i>	<i>0.17</i>
Treatment : Location	$F_{137,134}=0.61$	$F_{138,141}=0.93$
	<i>0.61</i>	<i>0.43</i>
Treatment	$F_{141,142}=0$	$F_{143,142}=1.49$
	<i>0.99</i>	<i>0.22</i>
Habitat	$F_{143,142}=0.02$	$F_{142,141}=1.76$
	<i>0.8754</i>	<i>0.187</i>
Location	$F_{143,146}=4.89$	$F_{146,143}=17.6$
	$2.85 \times 10^{-3}$	$< 10^{-6}$

**Table S2. Effect of the different factors and their interactions on the mortality rate of field workers and the emergence rate of lab workers.** Fisher scores and associated *P*-values are from ANOVAs between generalized linear models including or excluding the factor or interaction.

	Mortality rate of field workers	Emergence rate of lab workers
Colony size	$F_{136,137}=1.8$ <i>0.17</i>	$F_{136,137}=0.3$ <i>0.58</i>
Size of field workers	$F_{138,137}=0.8$ <i>0.37</i>	<b><math>F_{138,137}=8.8</math></b> <b><i>0.0034</i></b>
Colony size :	$F_{136,135}=0.072$	$F_{136,135}=0.74$
Treatment	<i>0.93</i>	<i>0.38</i>
Size of field workers :Treatment	$F_{133,134}=0.023$ <i>0.87</i>	$F_{133,134}=0.24$ <i>0.62</i>

Treatment : Habitat	$F_{138,139}=0.01$	$F_{138,137}=4.22$						
	<i>0.91</i>	<i>0.041</i>						
Treatment : Location	$F_{142,139}=6.5$	$F_{140,137}=2.87$						
	$<10^{-3}$	<i>0.038</i>						
Treatment	$F_{143,144}=115$	$F_{141,142}=104$						
	$<10^{-16}$	$<10^{-16}$						
Habitat	$F_{140,139}=0.36$	$F_{138,139}=5.8$						
	<i>0.54</i>	<i>0.017</i>						
Location	$F_{143,146}=2.9$	$F_{141,144}=21.3$						
	<i>0.035</i>	$<10^{-10}$						
Lsmeans		<b>Control</b>	<b>Cadmium</b>					
Habitat   Treatment		<i>0.74</i>	<i>0.011</i>					
Lsmeans	Paris	Paris2	Lyon	Bordeaux	Paris	Paris2	Lyon	Bordeaux
Treatment   Location	$<10^{-3}$	$<10^{-3}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$

**Table S3. Effect of the different factors and their interactions on the size and the development duration of lab workers.** Chi<sup>2</sup> scores and associated *P*-values are from ANOVAs between generalized linear models including or excluding the factor or interaction.

	Size of lab workers
Colony size	$X_I=12.52$ $<10^{-3}$
Size of field workers	$X_I=1.42$ 0.23

Colony size	:	$X_1=0.08$			
Treatment		0.76			
Size of field		$X_1=0.23$			
workers :Treatment		0.62			
Treatment : Location		$X_3=9.87$			
		<b>0.019</b>			
Treatment : Habitat		$X_1=5.03$			
		<b>0.025</b>			
Treatment		$X_1=48$			
		<b><math>4.10^{-12}</math></b>			
Habitat		$X_1=4.29$			
		<b>0.038</b>			
Location		$X_3=42.13$			
		<b><math>&lt;10^{-8}</math></b>			
Treatment   Habitat	City		Forest		
		<b><math>&lt;10^{-4}</math></b>		<b><math>&lt;10^{-4}</math></b>	
Lsmeans	Paris	Paris2	Lyon	Bordeaux	
Treatment   Location	<b><math>&lt;10^{-4}</math></b>	<b><math>&lt;10^{-4}</math></b>	<b>0.0026</b>	<b><math>10^{-4}</math></b>	

## 1 **Article 2 : Manuscrit non-encore soumis**

2 Urban colonies are less aggressive but forage more than their forest counterparts in the ant

3 *Temnothorax nylanderi*

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12

### 13 **Highlights**

- 14 • City and forest colonies of *Temnothorax nylanderi* diverged in their behavior.
- 15 • City colonies performed more foraging trip than forest colonies.
- 16 • City colonies were less aggressive than forest colonies.
- 17 • Aggression level and trip number were correlated in city but not in forest colonies.

18

### 19 **Abstract**

20 Urbanization imposes new constraints for organisms living in cities, leading to changes in  
21 behavior for urban populations. Especially, foraging and aggressive behaviors are often affected  
22 by urbanization. In eusocial species, behavior can be defined at the individual level but also at  
23 the colonial level. From now on, few studies have investigated how urbanization may affect  
24 colony-level behaviors. In this study, we compared aggressive and foraging behaviors between  
25 city and forest colonies of the eusocial species *Temnothorax nylanderi*. We collected colonies

26 in three urban parks and in three forests. After two weeks of laboratory-acclimation, we  
27 investigated foraging traits (mean and maximum number of foragers feeding on the food, the  
28 food discovery time, and the number of food-to-nest trips) by introducing food in the foraging  
29 area for three hours. We also measured in another experiment the colony aggressive level by  
30 introducing the body of a frost-killed conspecific in the nest and recording the proportion of  
31 biting over five minutes of observations. We also assessed behavioral correlations between  
32 foraging and aggression. We found that city colonies performed more foraging trips than forest  
33 colonies, and that city colonies were less aggressive. Interestingly, we found a behavioral  
34 correlation between foraging and aggression level in city colonies but not in forest colonies.

35

36 **Key-words:** Behavior, behavioral syndrome, eusocial, foraging, urbanization

37

### 38 **Introduction**

39 Urbanized area spread very rapidly these last decades (Lamb and DiLorenzo, 2014; Seto et al.,  
40 2012). Urbanization is associated with a large variety of stressors for wildlife, such as increased  
41 level of chemicals pollution (Foti et al., 2017), light and noise pollution, increased habitat  
42 fragmentation or higher mean annual temperature because of the Urban Heat Island Effect  
43 (Bornstein, 1968; Grimm et al., 2008; Owens and Lewis, 2018; Pickett et al., 2001). One of the  
44 first response of wildlife to urbanization is often based on behavioral changes (Ditchkoff et al.,  
45 2006; McDonnell and Hahs, 2015; Sih et al., 2011; Wong and Candolin, 2015), probably  
46 because it is a highly plastic trait prone to rapid response to novel environmental challenges  
47 (Miranda, 2017; Miranda et al., 2013). Changes in behavior can be beneficial and allow  
48 populations to survive or even thrive under altered environmental conditions, sometimes long  
49 enough before the occurrence of genetic adaptation (Ghalambor et al., 2007; Losos et al., 2004).  
50 For example, several studies reported bolder behavior of urban individuals relative to rural

51 individuals in birds and mammals which could allow them to better exploit resources under  
52 disturbed environment such as city center (dark-eyed junco, Atwell et al., 2012; blackbirds,  
53 Partecke et al., 2006; squirrels, Uchida et al., 2020). However, behavior might also be  
54 maladaptive and lead to population decline or extinction (Ghalambor et al., 2007; Zala and  
55 Penn, 2004). For example, changes in the song frequency of birds in urban environments lead  
56 to fewer mating opportunities because of lower males attractiveness (Luther et al., 2016).

57 Some categories of behavior are often affected by environmental changes. Foraging behaviors  
58 are frequently modified in response to urbanization but not always in the same direction  
59 depending on the species or the stressor studied. For example, foraging activity increases in  
60 urban area in bats (light stressor, Jung and Kalko, 2010), damselflies (pesticides stressor, Tüzün  
61 et al., 2015), bees (in response to an increase in food availability, Kaluza et al., 2016) or birds  
62 (in response to changes in prey types, Estes and Mannan, 2003) but decreases in spiders  
63 (decrease of web surface in the web-orb spider *Araneus diadematus*, Dahirel et al., 2019).

64 Changes in aggressive behavior are also very common in response to urbanization (Miranda,  
65 2017). Birds and reptiles tend to be more aggressive in cities (Baxter-Gilbert and Whiting, 2019;  
66 Davies et al., 2018; Lacy and Martins, 2003; Phillips and Derryberry, 2018; Scales et al., 2011,  
67 but see Mitrovich et al., 2009). A study performed on a rodent species, the striped field mouse  
68 *Apodemus agrarius* reports that urban individuals are more aggressive during the night, but less  
69 aggressive during the day relative to rural individuals (Łopucki et al., 2021). Other behavioral  
70 categories such as space use (Mitrovich et al., 2009; Tigas et al., 2002), migratory behavior  
71 (Partecke and Gwinner, 2007; Tryjanowski et al., 2013) or communication (Slabbekoorn and  
72 den Boer-Visser, 2006) can also differ between urban and forest populations. Last decades,  
73 behavioral studies reported that behaviors can be correlated, a phenomenon known as  
74 behavioral syndrome (Sih et al., 2004). For example, bolder individuals are also often more  
75 aggressive (Kralj-Fišer and Schneider, 2012; Mafli et al., 2011; Wright et al., 2018).

76 Interestingly, behavioral syndromes have been shown to differ depending on the environment.  
77 For example, aggressiveness is positively associated with boldness in rural individuals, but not  
78 in urban individuals for some species (birds: Scales et al., 2011; fishes: Bell and Stamps, 2004).  
79 The vast majority of urban behavioral studies have been performed on vertebrates, especially  
80 birds (Miranda, 2017). Despite the ecological importance of eusocial insects, including in urban  
81 areas, their behavioral changes in response to urbanization have rarely been investigated.  
82 Eusocial insects such as ants or bees are commonly found in urban environment (Fenoglio et  
83 al., 2020). They live in colonies and are characterized by a division of labor among individuals.  
84 Depending on their role in the colony, individuals will display different types of behavior: for  
85 example, in *Myrmica spp*, patrollers are bold, aggressive and active whereas nursers are shy  
86 and passive (Chapman et al., 2011). Individual behavior varies depending on several factors  
87 such as age, morphological caste or social environment (Lucas and Sokolowski, 2009;  
88 Robinson, 1992; Simola et al., 2016). In eusocial species, behaviors can also be considered at  
89 the colonial level (Bengston and Dornhaus, 2014; Jandt et al., 2014) defining a colony  
90 personality. Colony personality has been shown to vary depending on environmental factors  
91 (Bengston and Dornhaus, 2015). Considering foraging activities, colonies living in  
92 environments with few nesting sites tend to forage farther and have lower foraging effort than  
93 colonies living in habitats with more nesting sites in the ant *Temnothorax rugatulus* (Bengston  
94 and Dornhaus, 2014, 2015). Considering aggressiveness, ant colonies of *T. rugatulus* are more  
95 aggressive when there are fewer nesting sites available or when parasite pressure is higher  
96 (Bengston and Dornhaus, 2015; Kleeberg et al., 2015). Behavioral correlations at the colonial  
97 scale can also vary with environmental factors. In ants, aggression level and foraging effort are  
98 negatively correlated under cold climate but positively correlated under warm climate (Segev  
99 et al., 2017). Because urbanization is associated with changes in environmental factors such as  
100 temperature, parasite pressure and nest availability, we would expect changes in colonial



101 behavior in social insects linked to urbanization. So far, only two studies have been performed  
102 at the population level, both detected a change in foraging activity. In urban bee populations,  
103 foraging activity is increased (*Tetragonula carbonaria*, Kaluza et al., 2016) and in bumblebees,  
104 the foraging-season is increased through exploitation of novel food resources (Bumblebee  
105 *Bombus terrestris*, Stelzer et al., 2010). At the species level, urbanization is associated with a  
106 “discovery-defense” foraging strategy in ants, where species with colonies that discover food  
107 more rapidly also exploit it more efficiently in a urban context (Dáttilo and MacGregor-Fors,  
108 2021). As far as we are aware of, no studies have yet been performed in ants at the population  
109 level.

110 In this study, we compared aggressiveness and foraging between urban and forest colonies of  
111 the ant *Temnothorax nylanderi* and we quantified the correlation between them. *T. nylanderi* is  
112 a small ant, nesting in acorn or small branches, commonly found in both urban and non-urban  
113 habitats. Based on previous results presented above, we expected a higher foraging effort in  
114 urban colonies. Moreover, given that urban habitats may provide fewer large, preferred nesting  
115 sites (Friedrich and Philpott, 2009), we expected a higher competition among nesting sites in  
116 urban habitats and therefore a higher level of aggressiveness (Bengston and Dornhaus, 2015).  
117 Interestingly, the correlation between aggressiveness and foraging of this species is known to  
118 vary according to temperature with a positive association in warm habitats and a negative one  
119 in cooler habitats (Segev et al., 2017). As urban environments are about 2°C warmer than the  
120 non-urban ones because of the Heat Island Effect, we therefore predict a positive correlation  
121 between foraging and aggressiveness in urban populations, but not the reverse in forest  
122 populations. To test these predictions, we collected colonies from three different urban parks  
123 and three different forests, and we measured their behavior at the colonial level: their foraging  
124 effort (discovery time, number of foraging trips, maximum and mean number of foragers on

125 the food) and their aggressive behavior in response to a non-nestmate conspecific intrusion in  
126 the nest.

127

## 128 **Material and methods**

### 129 *Colony collection and rearing*

130 Colonies nest in hollow pieces of dead twigs or acorns and usually consist of no more than 200  
131 individuals, making complete colonies (queen, workers and brood) easy to collect. We collected  
132 colonies between May 2020 and June 2020 in three urban parks and three forests (Table S1 for  
133 GPS coordinates). Urban parks have diverse deciduous tree species (hazelnut, ashes, oaks,  
134 planes, beech, birch and exotic trees such as ginkgo) and bushes. The ground is covered with  
135 ivy, leaf litter or wood chips. The areas of these parks differ greatly (Table S1). All these urban  
136 parks are surrounded by unsuitable habitats for nesting (road, sidewalk, concrete...). Forests  
137 combine oaks, pine trees and beeches, and the ground is covered with ivy or litter leaf. Summary  
138 of the number of colonies collected at each site, as well as mean and variance in number of  
139 workers is given in Table S1.

140 Colonies were brought back to the laboratory and installed in 11.5 x 11.5 x 5.5 cm plastic boxes.  
141 Nests were made of two microscopic slides (75 x 26 x 1 mm) separated by 1mm moss layer.  
142 Colony with no queens (9 urban colonies, 11 forest colonies) or more than one queen (2 urban  
143 colonies, 1 forest colonies) were discarded. Cestode-parasitized colonies (with typical yellow  
144 workers, Scharf et al., 2012) were also excluded from the experiment (0 urban colony, 2 forest  
145 colonies). We counted workers and larvae in each colony (Table S1). We chose a subset of  
146 colonies based on their number of workers and larvae in order to have similar distributions  
147 between urban and forest habitats (see statistical analysis and Table S2). We finally had a total  
148 of 36 forest and 41 city colonies to analyze. Colonies were provided ad libitum food and water  
149 for two weeks. Food was made of a mixture of yoghurt, honey and dried crickets. Colonies

150 were kept in a climatic chamber (reference CTS TP10/600) at 20-22°C – 12h : 12h cycle with  
151 natural photoperiod during those two weeks.

152

### 153 *Behavioral experiments*

154 For each colony, two experiments were performed successively. First we assessed foraging  
155 efficiency, then aggressiveness. We performed all experiments between 1:00 pm and 4:00 pm.  
156 Eight colonies were tested each day for foraging and 10 colonies for aggressiveness, so the  
157 experiments lasted 14 and 11 days respectively for the foraging and aggression experiment. We  
158 randomized the colonies used each day so that that colonies from the same site were not all  
159 tested on the same day.

160 Two weeks before the foraging experiment, we removed food from the tested colonies to  
161 stimulate their foraging activity on the day of the experiment. Water was still provided ad  
162 libitum. Two weeks of food deprivation is not too much stressful for this species, as workers  
163 can survive up to 50 days without food in their colony (Modlmeier et al., 2013). The foraging  
164 experiment started by putting a mixture of yogurt, crickets and diluted honey on a microscope  
165 glass. The microscope glass containing the food was then added into the foraging area of the  
166 colonies, ten centimeters away from the nest entrance. The foraging area was then filmed for  
167 three hours using a digital HD video camera recorder (HDR-XR500E, Sony). After three hours,  
168 the remaining food was removed from the foraging area. Four variables were measured. Based  
169 on the video, we noted the time at which the food was discovered by the first forager  
170 (“Discovery time”). We counted the number of foraging trips from the food to the nest  
171 performed during the three hours (“Trip number”) by visually tracking individuals. As  
172 individuals moved very slowly, we were able to visually track individual without the need to  
173 mark them. Finally, we recorded the number of workers feeding on the food every 10 minutes  
174 (18 values in total over three hours) and keep the value with the maximum number of foragers

175 feeding on the food at the same time (“Max foragers”). We also computed the mean number of  
176 foragers feeding on the food at the same time based on the 18 values (“Mean foragers”).  
177 At the end of the foraging experiment, we left the colonies for 15 to 18 days and we then started  
178 the aggression experiment. Colony were fed twice a week during this period. Each aggression  
179 experiment consisted in putting the body of a dead conspecific worker into the nest and video-  
180 recording the interactions of workers with the corpse during five minutes. The corpses were  
181 freshly frost-killed workers collected from a different site located in a suburban forest  
182 (Vincennes). Each experiment was videotaped with a Sony Handycam HD and the behaviors  
183 were recorded using the free software BORIS V7.4.10 (Friard and Gamba, 2016). We recorded  
184 bitings of workers towards the corpse. Bitings were recorded as durations given that workers  
185 often bit the corpse for a prolonged period of time so that the number of bitings alone could not  
186 properly reflect the aggression intensity. We computed aggression level as the ratio between  
187 biting duration and total recording duration (five minutes). We also noted carrying behaviors  
188 of the corpses in order to make sure that workers did consider the very fresh corpse as an  
189 intruder. As expected with a fresh corpse, carrying behaviors were rare ( $0.118 \pm 0.1165$ ). After  
190 the behavioral experiments, colonies were frost-killed.

191

### 192 *Morphological measurements*

193 In order to control for the effect of mean worker size and of worker size variation on the  
194 behavioral measurements, we measured worker size on a subsample of 20 workers for each  
195 colony (or as many as possible if there were less than 20 workers in a colony). We used head  
196 width, a proxy of worker size (Araujo and Tschinkel, 2010). For each worker, we separated the  
197 head from the body, we stuck the head on a double-sided tape and we photographed it under a  
198 stereomicroscope (Zeiss Discovery.V12) connected to a computer via a camera (Zeiss

199 AxioCamICc). Head width was measured as the posterior eye-to-eye distance using ImageJ  
200 (<https://imagej.nih.gov/ij/>; Abràmoff et al., 2004).

201

### 202 *Statistical analysis*

203 All analyses were performed using R v3.6.2 (Team, 2013).

204 We first controlled whether worker size, number of workers and number of larvae diverged  
205 between habitats. To do so, we used linear mixed model (lme4) with colony and site as nested  
206 random factors, and habitat as predictors for each dependent variable. We assessed the effect  
207 of habitat by removing it and comparing this reduced model with the complete model using a  
208 log-likelihood ratio test. We found no differences in worker size, number of workers and  
209 number of larvae between forest and urban colonies (Table S2).

210

211 We used generalized linear mixed models (GLMMs, lme4 R package (Bates et al., 2015)). For  
212 each model, site was used as random factor to account for potential similarities of colonies of a  
213 given site. Our dataset consisted of five response variables: four response variables related to  
214 foraging (discovery time, maximum number of foragers, mean number of foragers and trips  
215 number) and the aggressivity level. Complete models included habitat as a fixed factor and the  
216 number of workers and larvae, and mean worker size as covariables (Table S2). We tested for  
217 double way interactions between habitat effect and each of the covariables and none were  
218 significant (Table S3). We log transformed the two covariates (the number of workers and  
219 larvae) so that all predictor variables are at the same order magnitude (Table S2). According to  
220 the dependent variable, we used different residual distributions and link function. For trip  
221 number, discovery time and aggressivity level, we used a Gaussian distribution with an identity  
222 link. Regarding discovery time, we transformed it using a log transformation ( $\log(1 + \text{Discovery}$   
223  $\text{time})$ ) to achieve linearity. For the maximum and mean number of foragers, we used a quasi-

224 poisson distribution with a logit link function, as this model led to the best distribution of  
225 residuals. We visually checked the distribution of residuals using the DHARMA package  
226 (Hartig and Hartig, 2017). More details on the statistical analysis can be found in table S2. To  
227 obtain the Minimum adequate model (MAM), we used a backward stepwise procedure. We  
228 first tested colony size, then number of larvae, then mean worker size and finally habitat effect.  
229 When the P-value was higher than 0.05, the predictor variable was removed from the model,  
230 until no variable could be removed (i.e. the MAM had been obtained). Final P-values were  
231 obtained by adding or removing variable from this MAM and comparing models using a log  
232 likelihood ratio test.

233 We used spearman test to investigate correlations among variables within each habitat  
234 (aggression, food intake, maximum number of foragers, mean number of foragers and trips  
235 number), as data were not all normally distributed. All P-values for pairwise comparisons were  
236 corrected using the false discovery rate method of Q-value as described in (Chapman et al.,  
237 2011). For the significant correlations, we compared correlation coefficients between habitat  
238 using SMATR package (Warton et al., 2018). We corrected for multiple test comparison as  
239 described above.

240

## 241 **Results**

### 242 *Foraging*

243 Urban colonies performed a significantly higher number of trips (51% higher) from the nest to  
244 the food spot than forest colonies (Habitat effect,  $X^2_1=5.77$   $P=0.016$  Figure 1a). The number of  
245 trips significantly increased with the number of larvae (estimated slope=46.68  $X^2_1=14.64$   
246  $P=0.00013$ ) but not with the number of workers or with the mean worker size (Table S3).

247 The maximum number of foragers feeding on the food at the same time did not differ between  
248 forest and urban colonies (Table S3 Figure 1b). This maximum number of foragers increased

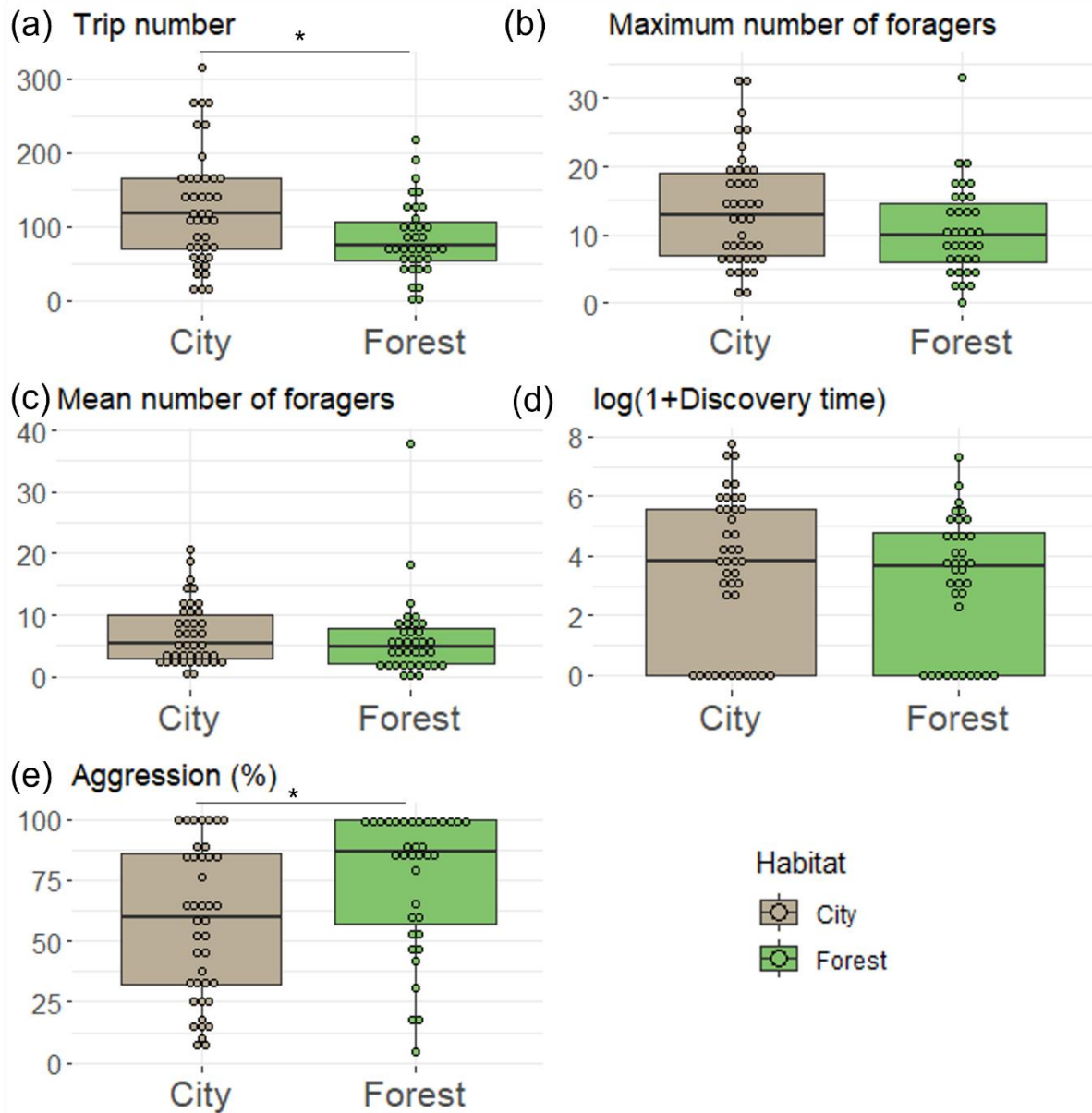
249 with the number of workers and larvae in the colony (resp. estimated slope=0.52  $X^2_1=36.70$   
250  $P<10^{-8}$ ; estimated slope=0.51  $X^2_1=32.09$   $P<10^{-7}$ ) but was not affected by the mean worker size  
251 (Table S3).

252 The mean number of foragers did not differ between urban and forest habitats (Table S3 Figure  
253 1c). It significantly increased with the number of larvae (estimated slope 0.41  $X^2_1=5.57$   
254  $P=0.018$ ) but was not affected by the number of workers or the mean worker size (Table S2).

255 The discovery time (i.e. time at which a forager touch the food for the first time) did not differ  
256 significantly between forest and urban colonies (Table S3 Figure 1d). The discovery time  
257 decreased with the number of workers (slope -1.19,  $X^2_1=4.61$   $P=0.032$ ) and with the number of  
258 larvae (slope -1.98,  $X^2_1=11.56$   $P=0.00067$ ) but was not affected by the mean worker size (Table  
259 S3).

#### 260 *Aggressive behavior*

261 City colonies were significantly less aggressive than forest colonies (28% less aggressive)  
262 (habitat effect,  $X^2_1=5.02$   $P=0.025$  Figure 1e). None of the covariate were significantly correlated  
263 with the aggressive behavior (number of workers, number of larvae or mean worker size, Table  
264 S3).



265

266 **Figure 1.** Dot-plot of the five tested traits (a: Trip number, b: maximum and c: mean number

267 of foragers, d: discovery time and e: aggression percentage), in city and forest colonies. City

268 is shown in grey, forest in green. For each box, median and quartiles are shown as well as the

269 raw data as points. (\*) shows significant differences between city and forest colonies (p<0.05).

270 Details of each site data are given in Figure S3.

271

272 *Correlation between foraging and aggression*



273 We tested whether behavioral traits were correlated within the same habitat (i.e. separately for  
 274 city and forest). For city colonies, we found seven significant positive correlations (Table 1,  
 275 Figure 2): six between pairs of foraging traits and one between one foraging trait (trip number)  
 276 and aggression level. For forest colonies, we only found three significant positive correlations  
 277 (Table 1, Figure 2) that only involved foraging traits and that were also found in city colonies.  
 278 All tested correlations are shown in figure S2.

279 For the common significant correlations between city and forest colonies (i.e. correlation  
 280 between mean and maximum number of foragers, between mean number of foragers and trip  
 281 number and between maximum number of foragers and trip number), we tested whether  
 282 correlations coefficient was the same. Correlation coefficients significantly differed between  
 283 city and forest for two correlations: mean number of foragers and trip number, and maximum  
 284 number of foragers and trip number (Table S4).

285

286 **Table 1.** p-value of correlation test (kendall) for city (upper panel) and forest (lower panel)  
 287 colonies. Significant p-values are indicated in bold. P-values indicated are the corrected p-  
 288 values for multiple testing. Correlation coefficients (R) are shown.

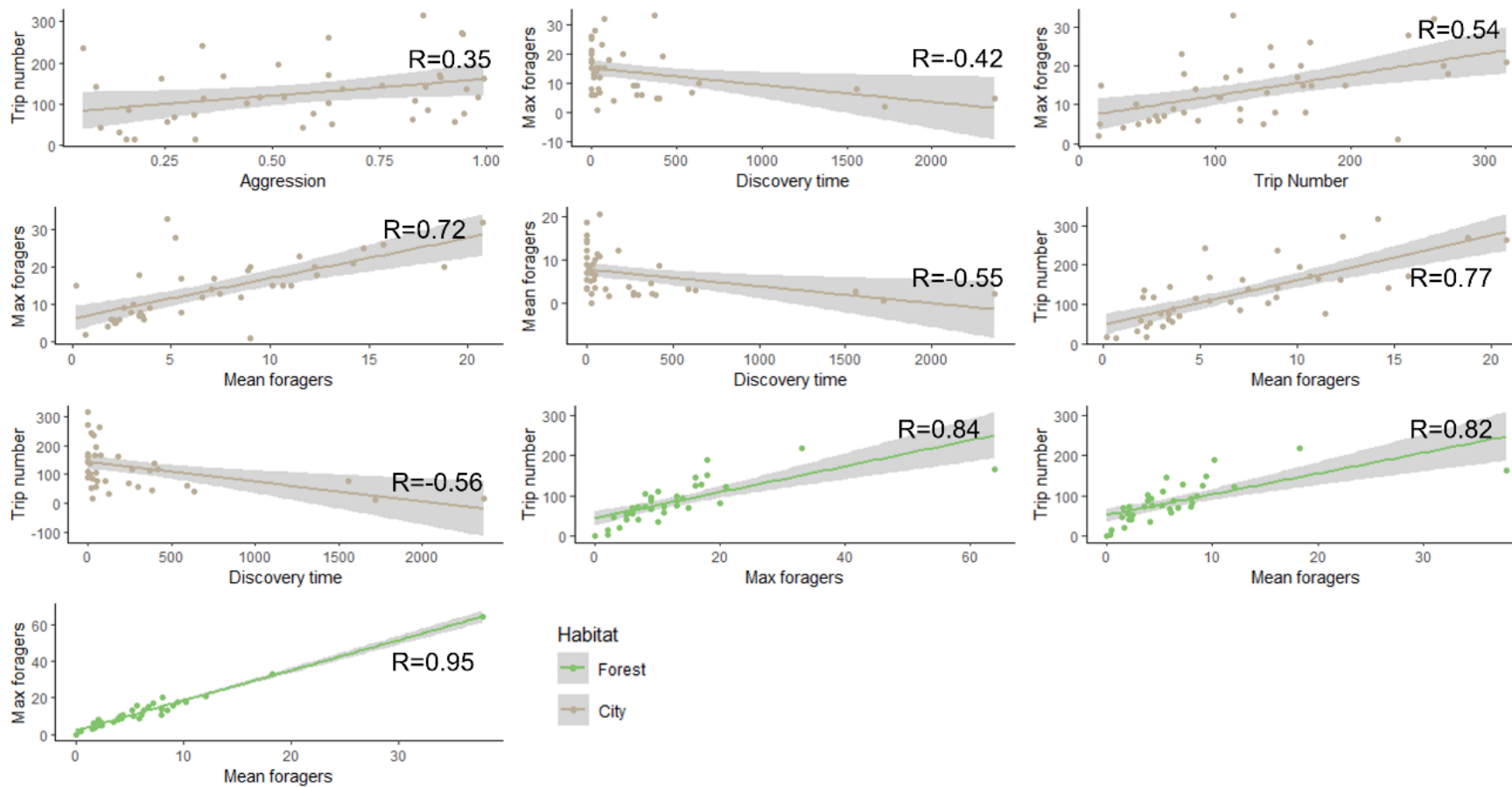
	Maximum foragers	Discovery time	Trip number	Mean foragers	Aggression
Maximum foragers	NA	<b>0.0095</b> <b>R=-0.42</b>	<b>0.00042</b> <b>R=0.54</b>	<b>&lt;10<sup>-7</sup></b> <b>R=0.72</b>	0.26
Discovery time	0.12	NA	<b>0.00030</b> <b>R=-0.56</b>	<b>0.00032</b> <b>R=-0.55</b>	0.44
Trip number	<b>&lt;10<sup>-10</sup></b> <b>R=0.84</b>	0.11	NA	<b>&lt;10<sup>-8</sup></b> <b>R=0.77</b>	<b>0.037</b> <b>R=0.35</b>
Mean foragers	<b>&lt;10<sup>-14</sup></b>	0.11	<b>&lt;10<sup>-8</sup></b>	NA	0.29

---

	<b>R=0.95</b>		<b>R=0.82</b>		
Aggression	0.40	0.61	0.40	0.50	NA

---

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291 **Figure 2.** Correlation plot of pairwise comparisons of correlations between four traits (maximum number of foragers, mean number of foragers,  
 292 discovery time, aggression and trips number), for city and forest colonies. For each plot, the R values indicates the correlation coefficient. Only  
 293 the significant correlations are shown.

## Discussion

In this study, we tested whether colonies from urban ant populations differed in foraging and aggressive behavior relative to their forest counterparts, as reported in other urban behavioral studies (Kaluza et al., 2016; Miranda, 2017; Mitrovich et al., 2009; Scales et al., 2011). We were expecting a higher foraging effort and a higher aggressiveness of urban colonies. As described in other taxa (birds: Scales et al., 2011, bees: Kaluza et al., 2016, reptiles: Mitrovich et al., 2009), we found evidence that city and forest colonies diverge in some behavioral traits related to foraging and aggressiveness. In contrast with our expectations, we found that city colonies were less aggressive than forest colonies (28% in mean). Most foraging traits did not differ between city and forest colonies, at the exception of the foraging trip number between the nest and the food which was 51% higher for city colonies. This is in line with other studies performed on other species, for which urban populations had higher foraging activity (Estes and Mannan, 2003; Kaluza et al., 2016). Unlike (Dáttilo and MacGregor-Fors, 2021), we did not find that urban colonies were able to discover food more rapidly. This may be due to our small foraging area (but *T. nylanderi* forage usually 20 cm away in nature, Heinze et al., 2010), or to the very distinct neotropical species assessed by (Dáttilo and MacGregor-Fors, 2021). This highlights the need to diversify the focus of urban studies to various biomes. We also found that some behavioral traits were correlated, but correlations differed between city and forest populations. We found positive correlations between one foraging trait (trip number) and aggression in city colonies, but no in forest colonies.

The positive correlation between foraging and aggression of city colonies differs from what was found in another *Temnothorax* species (*T. rugatulus*), where the most active foraging colonies were also the less aggressive (Bengston and Dornhaus, 2014). However, other studies

reported positive correlation between aggressiveness and boldness. Boldness can be defined as the propensity of an individual to take risks, such as reducing flight initiation distance in response to predator in birds or escaping strategies (death-fainting) in beetles. Our study corroborates that performing more foraging trips is risky and is correlated with more aggressiveness. Investigating other traits related to boldness (such as exploration behavior) would be necessary to add support to this hypothesis. Regarding foraging traits, we found the mean number of foragers, the maximum number of foragers and the trip number to be positively correlated in both city and forest colonies. However, in forest colonies, none of the tested foraging traits are related to aggression, contrary to city colonies. Why is there a behavioral correlation between aggression and foraging traits in city but not in forest colonies? Several studies reported that behavioral syndromes could differ depending on the environments (Bell and Stamps, 2004; Bengston and Dornhaus, 2015; Bengston et al., 2014; Scales et al., 2011). For example, (Bengston et al., 2014) showed that in the red-knee tarantula *Brachypelma smithi*, individuals reared in an enriched environment (with a sand-soil substrate to dig and ivy leaves to hide) showed behavioral correlations between exploratory and boldness behaviors, whereas spiders reared in a depleted environment (plastic box) did not. In birds, behavioral correlations between boldness and aggressive behaviors are found in rural but not in city populations of the song sparrow *Melospiza melodia* (Scales et al., 2011). The absence of behavioral correlations in forest populations of *T. nylanderi* could therefore result from their divergent environment. In the present study, we only tested a limited number of behaviors (foraging and aggression). It would be interesting to include additional behaviors such as boldness and exploratory behaviors, and to test whether correlations also differ between city and forest populations. Several studies reported a positive correlation between mean temperatures and aggressiveness in human (Greenberg, 1972; Kenrick and MacFarlane, 1986). The ‘Pace of Life Syndrom’ hypothesis also states that individuals living in warmer environment should have higher

metabolic rates and therefore should be more aggressive and active (Careau et al., 2008; Segev et al., 2017). A study on *T. rugatulus* reported that colonies living in environments limited in nesting sites were also more aggressive. Therefore, because cities are usually 2°C warmer than surrounding rural areas and provide less suitable nesting sites for ant species living in natural cavities (Friedrich and Philpott, 2009), we expected city colonies to be more aggressive than forest colonies. Surprisingly, we found instead that forest colonies were more aggressive toward a non-nestmate conspecific. In another ant species, *Formica acquilonia*, colonies living in sites contaminated with copper are less aggressive than colonies living in unpolluted areas (Sorvari and Eeva, 2010). As urban soils are often more contaminated in trace metal than forests soils, at least in our study area (Foti et al., 2017), it is possible that urban colonies are less aggressive because of their exposure to trace metals. Another hypothesis stands in recognition cues. In ants, recognition of nestmates and non-nestmates is based on cuticular hydrocarbons (CHCs) and aggression is higher between individuals that differ more in their CHCs (Sturgis and Gordon, 2012). The CHCs of the dead conspecifics that we used in this study could have been closest to the ones of city colonies, resulting in lower aggressive level. Indeed, the CHC profiles of ants can vary because of environmental difference (e.g. nesting material or diet, Heinze et al., 2010; Liang and Silverman, 2000). (Heinze et al., 2010) showed that in *T. nylanderi*, workers were less aggressive toward non-nestmates coming from similar or nearby environments, because their cuticular profiles were more similar to each other compared with distant colonies or colonies living in different types of wood. Dead conspecifics that we used in this study came from a suburban wood, located between 5 and 10 km away from urban parks but more than 50 km away from forests, and also heavily managed. This may have resulted in more similar environmental CHC cues between city/suburban individuals than between forest/suburban habitats. This however remains highly speculative, because we did not record in which wood type colonies from city, suburban or forest environments were nesting at the

time of their collection. It would be necessary to use corpses from a variety of habitats and compare the CHC profiles to test whether CHC differences are responsible of the different level of aggressiveness.

City colonies performed more foraging trips from nest to the food than forest colonies for a given period. This could reflect two different foraging strategies in forests and cities even though the other three measurements (i.e. mean number of foragers, maximum number of foragers and discovery time) were not different. The higher number of foraging trips could result from a higher number of foragers. However, we did not find that forest and city habitats differed in mean or maximum number of foragers; it is therefore unlikely that the higher number of foraging trip came from a higher number of foragers. Another hypothesis stands for the speed of the foragers: fastest foragers could perform more foraging trips. (Chick et al., 2021) reported that city workers run faster than rural ones because of their higher metabolic rate. The foraging strategy is linked with food availability and predictability: in a context of food abundance and high predictability, slower but more accurate individuals that can bring a large quantity of food to the nest are favored, whereas faster and less accurate foragers are favored under unpredictable and frequently changing environments (Chittka et al., 2009, 2009; Raine and Chittka, 2012). City parks can be more unpredictable because of human presence and management (littering, lawn mowing, planting of flowers, soil sweeping, litter cleaning) and this could favor faster individuals that can quickly exploit a food source.

The behavioral divergence observed between forest and city populations could be plastic or genetic. Behavior is a highly plastic traits and is often one the first trait to change in response to rapid human-induced environmental changes (Miranda, 2017; Sih et al., 2011, 2011). However, recent studies reported a genetic basis for behavioral divergence between urban and non-urban populations (Mueller et al., 2013, 2020; Salmón et al., 2021). In this study, behavioral assays were performed under a common garden laboratory setup, however colonies

did not spend much time under this condition. Performing behavioral assays on colonies reared over one year in the laboratory, and therefore consisting of laboratory-born workers under a true common garden laboratory setup, could be useful to assess whether the divergence in foraging and aggressive behaviors between city and forest colonies of *T. nylanderi* is genetically based or plastic.

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### **Author's contributions**

LJ designed the study, collected colonies, performed the experiment and the video analysis, performed statistical analyses, and wrote the manuscript. CD designed the study, contributed to statistical analyses and wrote the manuscript. MM designed the study and wrote the manuscript. All authors read and approved the final manuscript.

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### **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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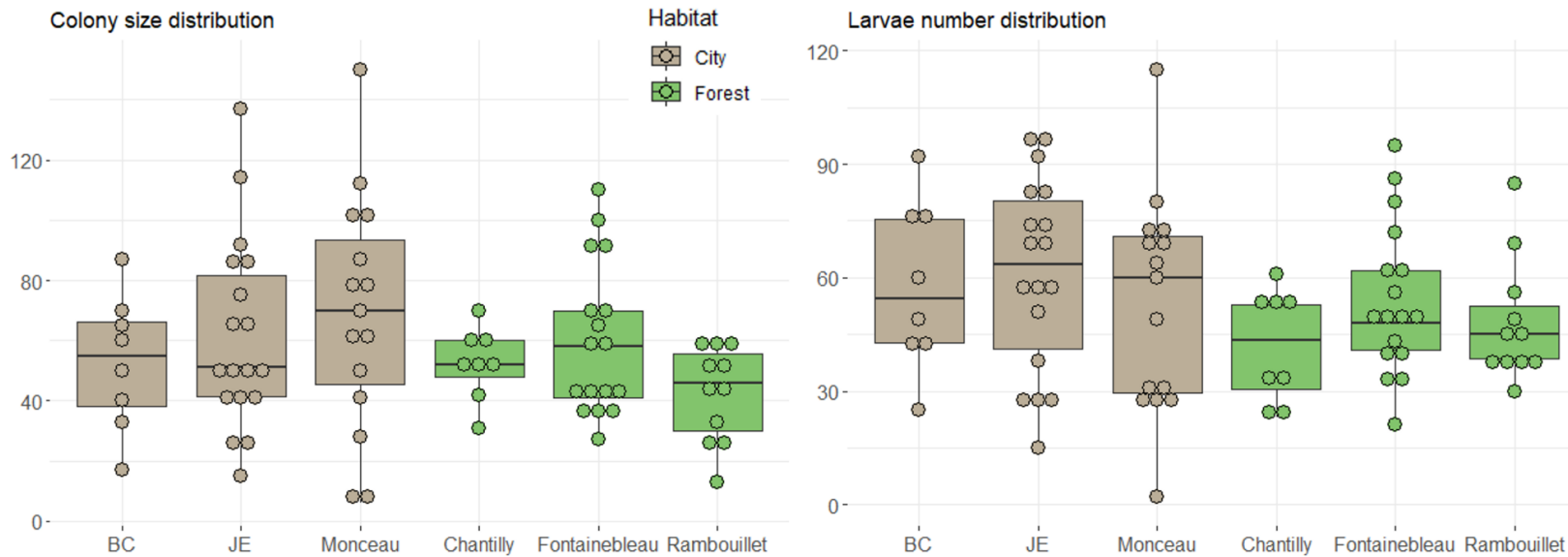
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## Supplementary Informations

**Table S1.** Area, GPS coordinate and number of colonies collected at each site (with one queen, without parasite) and number of colonies kept for statistical analysis. Mean colony size and standard deviation are shown before and after colony-subset selection. For each site, the total area is indicated.

Site	Area (hectares)	GPS	Number of colonies collected	Number of workers (mean±sd)	Number of colonies used	Number of workers (mean±sd)
Chantilly	6300	49°10'59.8"N 2°28'43.6"E	10	49.6 (±10.2)	8	52.1 (±8.9)
Fontainebleau	25 000	48° 26' 2.595"N 2°43' 52.842"E	20	56.6 (±20.6)	17	60.3 (±20.6)
Rambouillet	14 000	48° 39' 0.01"N 1° 49' 59.99"E	11	42.4 (±13.1)	11	47.7 (±9.1)
Buttes-Chaumont	25	48°52'47.1"N 2°23'45.6"E	10	59.6 (±20.6)	8	53 (±16)
Monceau	8	48° 52' 27.59" N 2° 18' 19.93" E	17	81.9 (±39.3)	15	63.3 (±26.5)
Jardins Ecologiques	1	48°50'59.684"N 2° 21' 40.385"E	22	65.6 (±20.0)	18	62.6 (±24.9)



**Figure S1.** Colony size and larvae number distribution at each site. Red: city site, blue: forest site. For each boxplot, median and quartiles are shown. BC: Buttes-Chaumont, JE: Jardins Ecologiques.

**Table S2.** Summary of statistical analysis performed on colony size, number of larvae and time before the first-contact of a forager with the food to check for equal distribution between city and forest habitats. For the first-contact time, we used a zero-inflated model as the data contained a lot of 0 (the forager was already presents if the foraging area and directly detects the food). Because of the zero-inflated model, we were not able to use the anova procedure for model comparison and just extract p-value from the model summary.

Model specification	Dependent variable	Predictor	Chisq or z-value	P-value
LMER, site as random factor	Number of workers	Habitat	$X^2_1=2.23$	P=0.13
	Number of larvae	Habitat	$X^2_1=2.33$	P=0.13

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LMER, site and colony as random factors	Mean worker size	Habitat	$X^2_1=0.093$	P=0.76
		Number of workers	$X^2_1=35.76$	P<10 <sup>-8</sup>

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**Table S3.** Statistical results for behavioral traits. Analysis of variance table for the models testing the effect of habitat, number of workers, number of larvae and worker mean size on foraging and aggression traits, as well as interactions between habitat and the other variables. Significant p-value are indicated in bold.

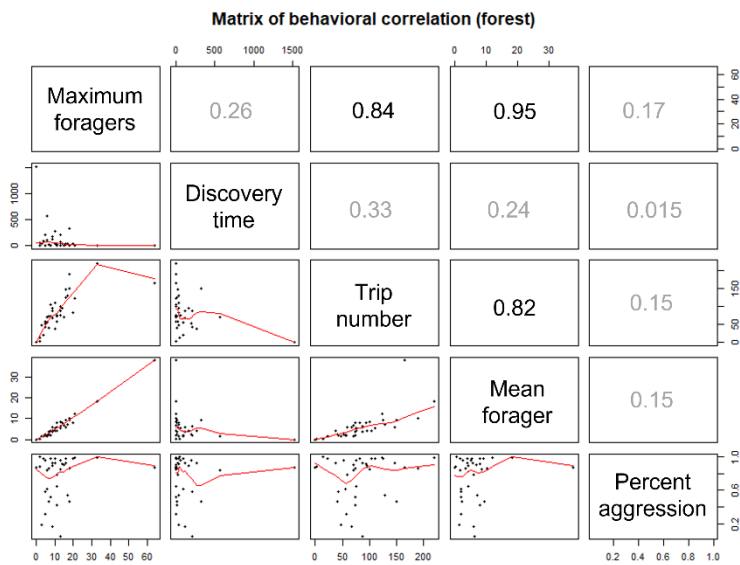
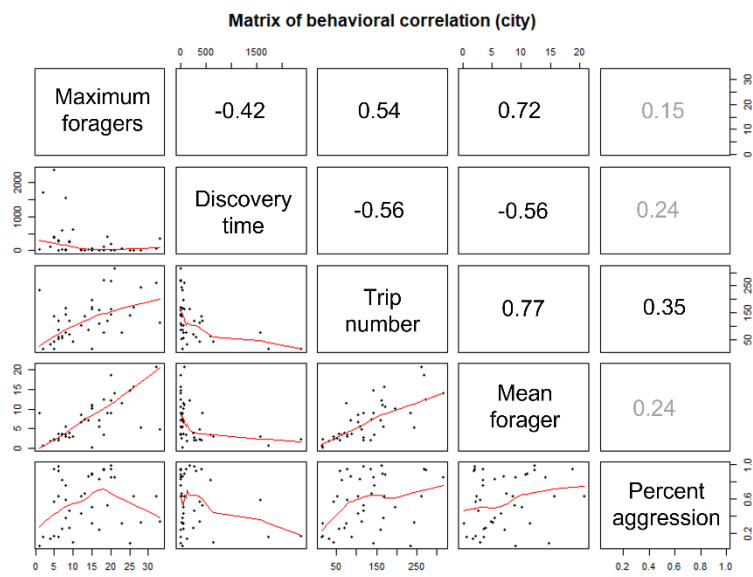
Model specification	Dependent variable	Predictor	Chisq or z-value	P-value
Linear mixed model, site as random factor	Trip number	Habitat : Number of workers	$X^2_2=5.54$	0.062
		Habitat : Number of larvae	$X^2_1=0.85$	0.36
		Habitat : Mean worker size	$X^2_2=0.17$	0.91
		Habitat	$X^2_1=5.77$	<b>P=0.016</b>
		Log(Number of workers)	$X^2_1=0.55$	P=0.45
		Log(Number of larvae)	$X^2_1=14.64$	<b>P=0.00013</b>
		Mean workers size	$X^2_1=0.084$	P=0.77
GLMs, quasipoisson, site as random factor	Mean number of foragers	Habitat : Number of workers	$X^2_1=0.032$	P=0.86
		Habitat : Number of larvae	$X^2_1=0.035$	P=0.85
		Habitat : Mean worker size	$X^2_2=0.14$	P=0.93
		Habitat	$X^2_1=0.20$	P=0.65
		Log(Number of workers)	$X^2_1=2.39$	P=0.12
		Log(Number of larvae)	$X^2_1=5.57$	<b>P=0.018</b>
		Mean workers size	$X^2_1=0.048$	P=0.82
GLMs, quasipoisson, site as random factor	Maximum number of foragers	Habitat : Number of workers	$X^2_1=0.010$	P=0.91

		Habitat : Number of larvae	$X^2_1=0.001$	P=0.99
		Habitat : Mean worker size	$X^2_2=0.86$	P=0.65
		Habitat	$X^2_1=0.21$	P=0.65
		Log(Number of workers)	$X^2_1=36.70$	<b>P&lt;10<sup>-8</sup></b>
		Log(Number of larvae)	$X^2_1=32.09$	<b>P&lt;10<sup>-7</sup></b>
		Mean workers size	$X^2_1=3.58$	P=0.058
Linear mixed model, site as random factor	Log(1+Discovery time)	Habitat : Number of workers	$X^2_2=5.59$	P=0.061
		Habitat : Number of larvae	$X^2_1=0.87$	P=0.35
		Habitat : Mean worker size	$X^2_2=1.01$	P=0.60
		Habitat	$X^2_1=0.82$	P=0.36
		Log(Number of workers)	$X^2_1=4.61$	<b>P=0.032</b>
		Log( Number of larvae)	$X^2_1=11.56$	<b>P=0.00067</b>
		Mean workers size	$X^2_1=0.029$	P=0.86
Linear mixed model, site as random factor	Aggression level	Habitat : Number of workers	$X^2_2=0.34$	P=0.84
		Habitat : Number of larvae	$X^2_2=0.83$	P=0.66
		Habitat : Mean worker size	$X^2_2=4.45$	P=0.11
		Habitat	$X^2_1=5.03$	<b>P=0.025</b>
		Log(Number of workers)	$X^2_1=0.87$	P=0.17
			$X^2_1=2.39$	P=0.12

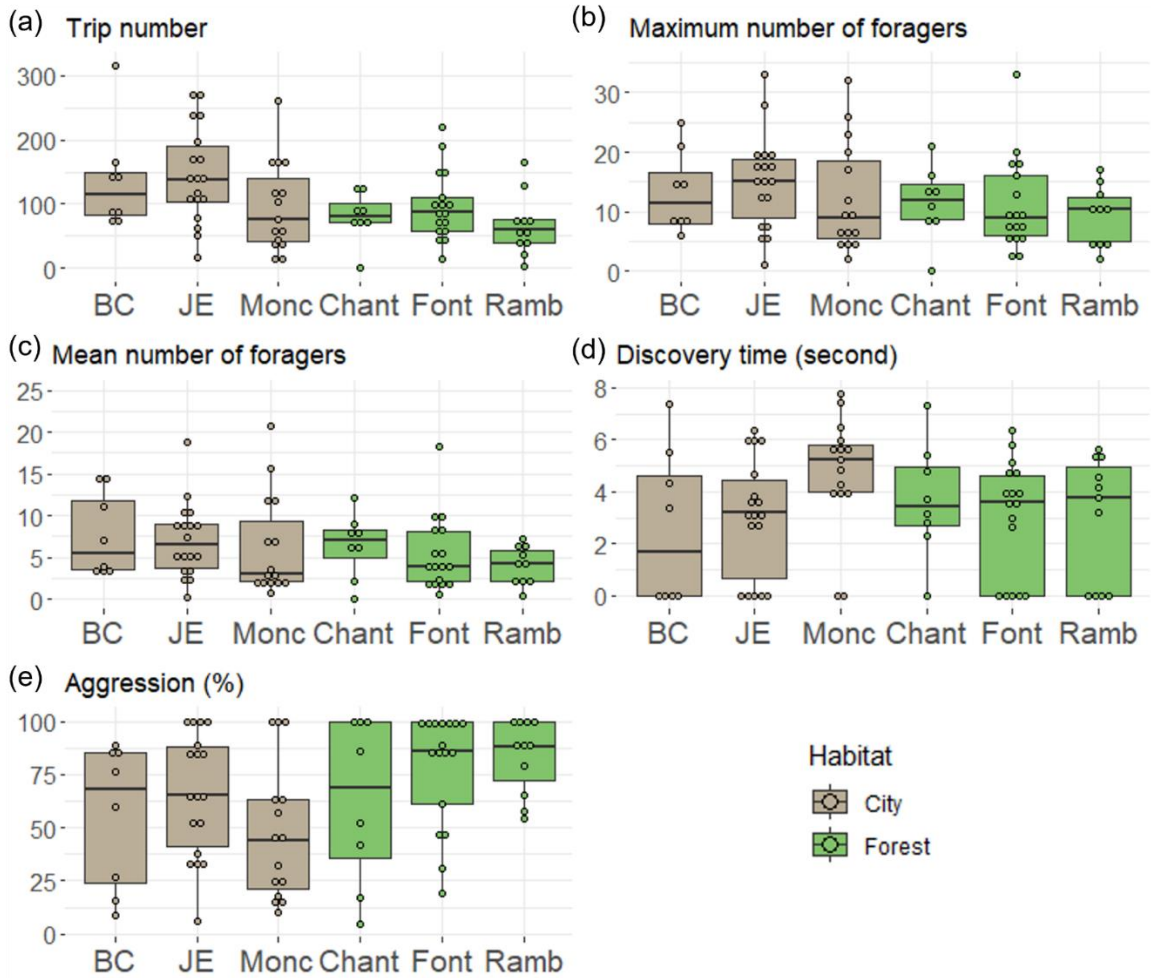
	Log(Number of larvae)	$X^2_1=0.60$	P=0.43
	Mean workers size		

**Table S4.** Corrected p-values of city-forest correlations comparison. Significant p-value ( $p < 0.05$ , in bold) indicates that the correlations coefficients are statistically different between habitats.

	Maximum foragers	Trip number	Mean foragers
Maximum foragers	NA	<b>0.0000302</b>	0.73
Trip number		NA	<b>0.00025</b>
Mean foragers			NA



**Figure S2.** Correlation plot of pairwise comparison of correlation between four traits (maximum number of foragers, mean number of foragers, trip number, discovery time and , aggression rate), for city and forest colonies. For each plot, the upper-panel values indicates the correlation coefficient R. Significant correlation are indicated in black, non-significant in grey.



**Figure S3.** Dot-plot of the five tested traits (a: Trip number, b: maximum and c: mean number of foragers, d: discovery time and e: aggression percentage), in city and forest colonies, for each site. BC= Buttes Chaumont, JE= Jardins Ecologiques, Monc = Monceau, Chant = Chantilly, Font = Fontainebleau, Ramb = Rambouillet. City is shown in grey, forest in green. For each box, median and quartiles are shown as well as the raw data as points.

# Chapitre deux : Déterminisme de la réponse à l'urbanisation, rôles des facteurs génétiques, plastiques et de l'environnement social

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**Hibernation Conditions Contribute to the Differential Resistance to Cadmium between Urban and Forest Ant Colonies**

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**Simple Summary:** The resistance of organisms to trace metals can have a genetic or a plastic origin. Indeed, differential environmental conditions experienced before the exposure to trace metals could physiologically condition organisms and plastically enhance their subsequent resistance to trace metals. In this study on the ant *Temnothorax nylanderi*, we investigated whether the better cadmium resistance of urban colonies relative to forest colonies could originate from the distinct hibernation conditions that they experienced prior to cadmium exposure. We compared the ability of urban and forest colonies to resist cadmium depending on whether they had hibernated in their respective urban or forest habitats or under a laboratory common garden setup. We found that urban colonies resisted cadmium better than forest colonies when they had hibernated under a common garden. Surprisingly, this difference was not observed between urban and forest colonies that had hibernated in the field, in contrast with

a previous study. One reason may be that winter was particularly mild on the year of our experiment. Our results therefore support the idea that urban colonies are genetically adapted to resist trace metals, but that this adaptation is only revealed under specific environmental conditions.

**Abstract:** Trace metals such as cadmium are found in high concentrations in urban environments. Animal and plant populations living in heavily contaminated environments could adapt to trace metals exposure. A recent study shows that urban populations of the acorn ant *Temnothorax nylanderi* are more resistant to cadmium than their forest counterparts. However, this study was performed using field colonies that had just come out of hibernation. Because urban and forest hibernation environments differ, the differential resistance to trace metals may originate either from differential hibernation conditions or from a different resistance baseline to cadmium. In this study, we tested these two hypotheses using laboratory common garden hibernation conditions. We let urban and forest colonies of the ant *T. nylanderi* hibernate under the same laboratory conditions for four months. After this hibernation period, we also collected field-hibernating colonies and we compared cadmium resistance between urban and forest colonies depending on the hibernation condition. We found a differential response to cadmium under common garden, with urban colonies displaying less larval mortality and lower size reduction of the produced individuals. This suggests a different resistance baseline of urban colonies to cadmium. However, unexpectedly, we did not detect the differential response between urban and forest colonies in the field, suggesting a more complex scenario involving both genetic and environmental influences.

**Keywords:** common garden; cold; trace metal; urbanization; social insects

## **Introduction**

Trace metals at high concentrations have negative impacts on wildlife as they affect cellular and physiological processes (DNA damage, higher oxidative stress, brain inflammation [1–3]) and life history traits (body size reduction, decreased birth rate and hatching rate for eggs, higher mortality rate [2–7]). High concentrations are mainly associated with human activities such as active or disused mining sites, sewage evacuations into aquatic environments, and in dense cities because of high traffic activity or home heating [8–10]. Animal populations living in such contaminated sites can be less sensitive to negative effects of trace metals because of a higher expression of stress-related genes when exposed to trace metals or because of changes in detoxification-linked turnover of proteins [11,12]. For example, in response to metal exposure, some invertebrate populations have higher expression of metallothionein (*Orchesella*

*cincta*, Collembola [12]) heat shock protein (*Chironomus tentans*, Diptera [13]) or antioxidant enzymes, more stable expression of calcium-related stress signaling (*Lumbricus rubellus*, Annelida [14]), and higher secretion or higher turnover of metallothionein protein (*Eisenia fetida*, Annelida [15], and *Hediste diversicolor*, Annelida [11]). These effects have been tested on individuals that had been reared under common garden environments in the laboratory [12,13] or on individuals collected in the field one or two weeks before the start of ecotoxicological assays [11,14,15]. Common garden (i.e., the rearing of individuals from different populations under the same environmental conditions) experiments prove that the higher resistance to trace metals in populations from heavily contaminated soils is a stable response that is not directly induced by the stressful environment and reflects a different baseline resistance that could result from genetic adaptation. However, this method does not allow for the addressing of the question of a modulation of this genetic adaptation by interactions with environmental conditions.

The above-mentioned physiological responses to trace metals are not necessarily stressor-specific and can also be induced by other stressors such as cold and freezing. For instance, a physiological mechanism allowing insects to face winter cold and freezing is “diapause”. Diapause is defined as a “hormonally mediated state of low metabolic activity associated with reduced morphogenesis, increased resistance to environmental extremes and altered or reduced behavioral activity” [16]. Interestingly, some of the anti-stress factors expressed during winter diapause are the same as those expressed in response to trace metal exposure, such as metallothionein, heat shock protein, glutathione, or antioxidant enzymes [16–19]. Temperature at which diapause occurs impacts the secretion of these anti-stress factors [16,20]. For example, in *Ostrinia nubilalis*, a lepidoptera, larvae entering diapause at cold temperature express high levels of heat shock protein and lower levels of glutathione S transferase compared with larvae that entered diapause at warm temperature, which express higher levels of glutathione S transferase, metallothionein, ferredoxin, and heat shock protein. These differential patterns of gene expression are reinforced with the time spent in the cold environment by diapausing larvae [16]. Temperature can also impact diapause stability, with higher metabolic rate when winter temperatures are warmer or more variable [21]. This negatively impacts winter survival but also long-term survival and fitness of individuals because of lower weight at the end of diapause [21,22]. Thus, winter temperature can have long-term impacts on life history traits and could ultimately affect future resistance to trace metals.

Ants are often used to monitor pollution because they are present in almost all terrestrial ecosystems and because they have long-lived colonies with local foraging, making them prone



to local bioaccumulation [23]. Ants are considered as relatively resistant to trace metals pollution, maybe because of their effective metal regulation systems [23]. Most temperate ant species enter diapause during winter, with differences regarding which life stage enters diapause depending on the species [24]. Winter temperature impacts worker survival, with higher or lower worker survival during warm winter, highlighting a sensitivity to hibernation temperature [25,26].

Cadmium is a trace metal with toxic effects on organisms because of its interference with essential metals such as calcium or zinc [27]. For example, organisms exposed to cadmium have decreased hatching rate, longer or stopped development, or reduced body size [4–6]. Cadmium is found at high concentrations in cities because of traffic and industrial activities [9,27]. For example, urban wood soils of the city of Paris are in modern times more concentrated in cadmium than adjacent rural wood soils (2.45 mg/kg vs. 0.30 mg/kg, [9]). Genetic adaptation to cadmium pollution has been documented in some arthropod species such as *O. cincta* (collembola) [12], *Drosophila melanogaster* (Diptera) [28], and *Porcellio scaber* (Isopod) [29] by comparing populations living in heavily contaminated sites (ancient mining sites, blast furnace steelworks factories [10]) and rural sites. However there is, to our knowledge, only one study assessing whether urban populations also display some adaptation to this trace metal [30]. Interestingly, a previous study showed that in the ant *Temnothorax nylanderi*, urban colonies are more resistant to cadmium than their forest counterparts, with a weaker negative impact of cadmium on larval survival and body size of emerging workers [30]. However, this study was conducted on colonies that had overwintered in the field. Therefore, the differences observed between urban and forest populations may reflect differences in hibernation environments, as this species nests aboveground and is exposed to variation in climatic environments during winter [31]. For example, in cities, winter air temperature is usually warmer because of the urban heat island effect [32], but at the same time there is less snow cover to protect soil organisms from the cold [33]. Cities also have reduced rain fall and moisture [33]. Because of these different hibernation conditions, gene expression levels of anti-stress factors may differ between city and forest populations, resulting in a differential “preparation” to post-hibernation cadmium exposure.

In this paper, we tested whether the effect of cadmium compared to a control differentially affected the colonies life history traits according to (i) the hibernation conditions (in the field or under common laboratory conditions) and (ii) the origin of the colonies (urban or forest colonies). More specifically, we tested whether the differential colony response to cadmium previously observed between urban and forest populations of the ant *T. nylanderi* [30]

is maintained under a common garden (lab) hibernation condition. This would indicate that the differential response is not caused by external environmental factors and that genetic adaptation may have occurred in city populations, even though maternal and epigenetic effects could not be ruled out. Alternatively, the absence of differential response under lab hibernation condition would suggest that the differential response is a plastic response, i.e., there is no genetic differences between urban and forest colonies, and the better cadmium resistance of urban colonies is due to different past environmental conditions (hibernation condition here) that would have enabled them to resist better to cadmium.

## **Material and methods**

The small acorn ant *T. nylanderi* is a common species in Europe, found in both urban and rural habitats. Colonies consist of a few hundred individuals who nest in pieces of dead plant material on the ground (twigs, acorns, chestnuts, etc.), making them easy to collect. Colonies were collected in a park within Paris (Buttes Chaumont, 48°50'59.684" N 2°21'40.385" E) for the urban habitat and in a wood 50 km away from Paris (Chantilly, 49°10'59.8" N 2°28'43.6" E) for the forest habitat. A previous study [9] found a mean concentration of 2.45 mg/kg of cadmium in Paris urban wood soils, and of approximately 0.30 mg/kg of cadmium in rural wood soils (a proxy of our Chantilly forest soils). We first collected colonies in October 2019 that we used for the common garden (lab) hibernation condition. Just after collection, colonies were brought back to the laboratory and we let them acclimatize for 1 week at 10–15 °C in climatic chamber (reference CTS TP10/600). After the acclimatization period, they were put under artificial overwintering at 4 °C for approximately 4 months (2 November to 9 March). For comparison, the mean temperature from November to early March was 8.1 °C for Paris and 6.9 °C for Chantilly (infoclimat data, available online: <https://www.infoclimat.fr> (accessed on 2020-12-04)). In early March, lab colonies were warmed up to 10–15 °C for 1 week. At the same time, we collected colonies in the field from the same populations, we brought them back to the laboratory, and we let them acclimatize for 1 week at 10–15 °C (“field colonies”). These colonies had therefore hibernated in the field. The experiment started at the same time for both lab colonies and field colonies after the week at 10–15 °C.

All colonies were reared in 11.5 × 11.5 × 5.5 cm plastic boxes with the lid pierced to let air circulate. Artificial nests were made of 2 microscope slides separated by a 2 mm thin moss chamber, covered by a dark plastic sheet to protect the colonies from the light. Colonies were provided with water ad libitum within a small tube plugged with cotton. We discarded colonies with no queen (41 colonies), with more than 1 queen (17 colonies), or with workers infected by

a cestode (4 colonies). Infected workers have a typical pale-yellow coloration [34]. We ultimately kept 72 lab colonies and 70 field colonies.

Two days before starting the experiments, we removed all eggs and larvae from the colonies except for the second instar (S2) larvae, which corresponds to an early developmental stage, and thus all individuals would be exposed to cadmium at the same timing of their development and for the vast majority of their development duration. We counted all workers and remaining S2 larvae. Number of larvae ranged from 2 to 51. The initial number of workers was called “colony size”. Colony size ranged from 9 to 280 workers in lab colonies, and from 11 to 225 in field colonies.

### *2.1. Experimental Design*

The experiment started when colonies were put under a 22–27 °C 12–12 h cycle and exposed to 2 different treatments (control and cadmium). Colonies were fed every other day with a mixture made of yoghurt, dried crickets, diluted honey, and vitamins, with 100 µg/g of cadmium in the food (cadmium treatment) or without cadmium (control treatment). This concentration is 50 times higher than the average cadmium concentration in urban wood soils of Paris, but corresponds to the LC<sub>50</sub> determined in a previous study [30]. Food was provided in excess (approximately 5 g of food per colony). As colonies were fed 3 times a week, this food quantity was enough to last for 2 days whatever the colony size.

We removed newly laid eggs every week. The experiment lasted for 61 days, which corresponds to the maximal duration of larvae development from second-instar to a newly emerged worker at 22–27 °C. Therefore, if some eggs went unnoticed during removal, newly hatched larvae could not have reached adulthood before the end of the experiment. We collected newly emerged workers daily (subsequently called “lab workers”) and stored them in 90° ethanol for further analysis. Those workers are recognizable by their typical pale orange color that they lose after a few days. The collection date was noted, and the total development duration was computed as the number of days between the start of the experiment and the collection date.

Colonies were evenly assigned to control or cadmium treatment so that colony size distribution was the same between the 2 treatments for both lab and field colonies.

### *2.2. Measured Variables*

Dead workers were counted every week and their corpses were removed. The worker mortality rate was estimated as the total number of dead workers divided by the number of initial workers in the colony.

As previously observed, some larvae did not grow but stayed alive during the course of the experiment, and were kept within the nest by the workers [30]. These larvae were not extra larvae hatched during the experiment as we removed all newly laid eggs from the nest on a weekly basis. We assumed that these larvae paused their development, as described in other studies, and they were therefore referred to as “paused larvae” [19]. The larval paused development rate was measured as the ratio between the number of paused larvae at the end of the experiment and the initial number of larvae. The number of emergent larvae corresponded to the number of larvae that achieved their development into adults (workers, males, and gynes) at the end of the experiment. The emergence rate was computed as the ratio between the number of emerged adults and the initial number of larvae. The number of dead larvae was obtained by subtracting the number of emerging adults and paused larvae from the initial number of larvae. The larvae mortality rate was computed as the ratio between the number of dead larvae and the initial number of larvae. Therefore, the sum of emergence rate, larval paused development rate, and larvae mortality rate was equal to 1.

We also measured the size of lab workers. We recorded the duration of their development as the number of days between the start of the experiment and their collection time. Heads of the workers were removed and adhered to a double-sided tape. Heads were photographed using a stereomicroscope (Zeiss Discovery.V12). Head width was measured as the eye-to-eye (posterior side) length, a good proxy of the worker size [35], using ImageJ (available online: <https://imagej.nih.gov/ij/>, accessed on 2018-02-03, [36]). Using a linear mixed effect model, we checked that the development duration was not different depending on hibernation conditions, treatments, or habitats so that differences in head width were not caused by different development durations.

All the above-mentioned measurements are given in table S1 (for the individual-level variables) and table S2 (for the colony level variables).

### *2.3. Statistical Analyses*

We used R v3.6.2 [37] for all subsequent statistical analysis.

We compared the response to cadmium of city and forest colonies that had overwintered in the field and colonies that had overwintered in common garden in the lab. We tested for the effect of treatment (control or cadmium), habitat (city or forest), and hibernation conditions (field or

lab) and their interactions on the 5 response variables (workers and larvae mortality rate, emergence rate, larval paused development rate, lab workers size). We also added colony size, alone or in interaction with treatment, as a covariable. If the cadmium response depends on the habitat but not on hibernation conditions, we expected to find a treatment–habitat interaction with no treatment–habitat–conditions triple interaction.

We used a generalized linear model (GLM) to analyze the effect of the factors on worker mortality rate, larvae mortality rate, larval paused development rate, and emergence rate. We used a quasibinomial logit link as a simple binomial logit link was associated with over dispersion. We built the complete model including all factors and their interactions. We used a backward stepwise procedure to choose the minimum adequate model. The complete model was compared with the same model without the variable of interest. The two models were compared with a log-likelihood test (ANOVA) using  $\chi^2$  or Fisher scores depending on the model. The minimum adequate model was obtained when no more variables could be extracted from the model without significantly changing this model. We obtained the *p*-value for each variable by adding or removing the tested variable from the minimum adequate model and comparing the 2 models using a log-likelihood test.

In contrast with the above-mentioned dependent variables, the head width of lab workers was based on several measurements within each colony. Consequently, we used a mixed model with the colony as a random factor to control for pseudoreplication, but the analyses and statistical tests were the same as above, this time using the nlme package [38].

For all linear models, we visually checked for homoscedasticity and normal distribution of the residuals using plot functions. We used ggplot2 R package [39] for data visualization.

## **Results**

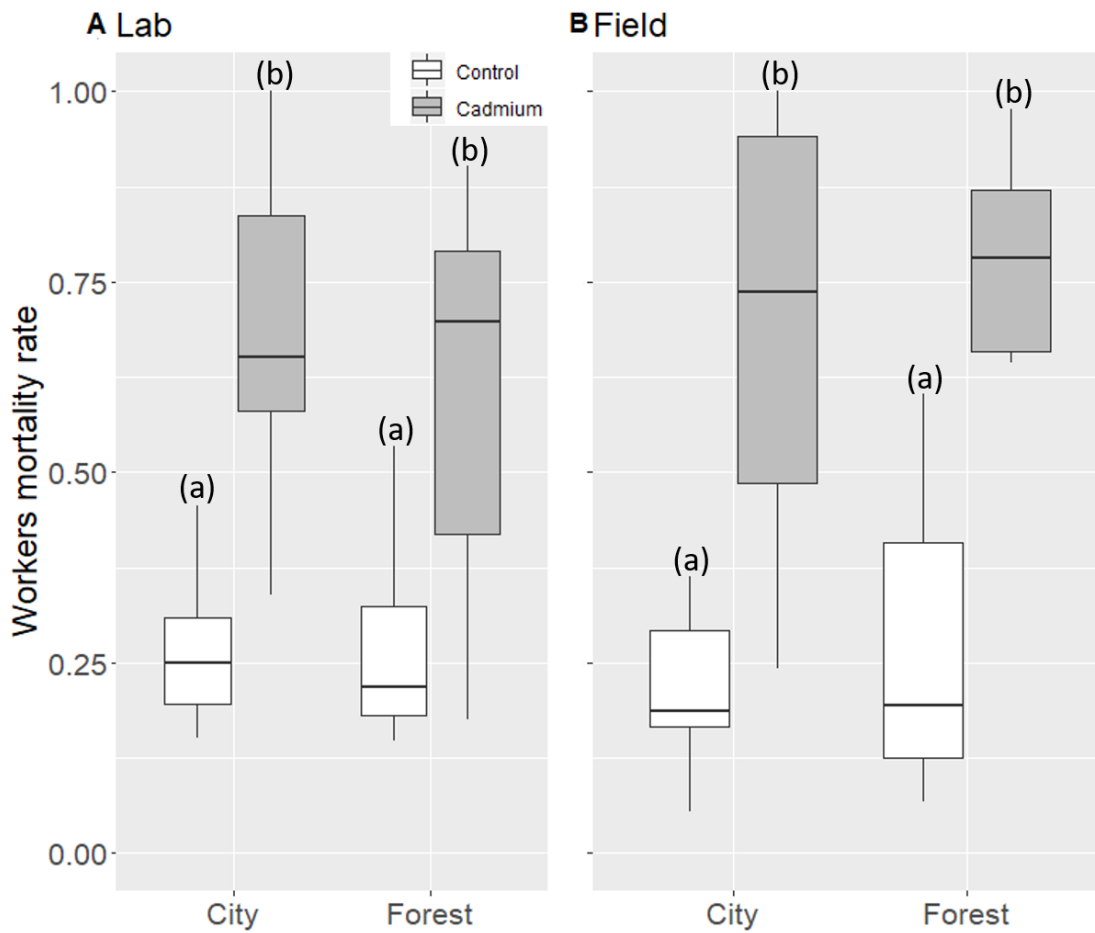
Thirteen queens died during the course of the experiment (nine lab colonies and four field colonies). We discarded these colonies at the time the queen died, and we removed them from subsequent analyses. Out of the 129 remaining colonies (63 in lab and 66 in field conditions), we collected a total of 999 workers (562 in lab and 437 in field conditions), 327 males (181 in lab and 146 in field conditions), and 87 gynes (63 in lab and 24 in field conditions). Despite this relatively high number of males and gynes, field forest colonies produced only three males and no gyne under cadmium treatment, which prevented us from analyzing data regarding sexuals.

### *3.1. Worker Mortality Rate*

Cadmium treatment significantly increased worker mortality rate (+42% in colonies treated with cadmium, Table 1 and Figure 1). This increase in worker mortality rate was similar whatever the habitat (no treatment–habitat interaction, Table 1 and Figure 1) or hibernation conditions (no significant treatment–conditions interaction, Table 1 and Figure 1). The worker mortality rate was not significantly different between the two habitats or hibernation conditions (no conditions or habitat effect, Table 1).

**Table 1.** Summary of the statistical analyses on workers testing the effects of hibernation conditions (lab and field), type of habitat (city and forest), and treatment (control or cadmium) on the workers mortality rate. T:H:C means treatment–habitat–conditions triple interaction. Bold number indicates significant *p*-value.

	<b>Worker Mortality Rate</b>
T:H:C	$F_{125,119} = 0.87$ $p = 0.52$
Treatment–Habitat	$F_{125,123} = 0.0099$ $p = 0.90$
Treatment–Conditions	$F_{125,123} = 1.98$ $p = 0.14$
Habitat–Conditions	$F_{125,122} = 0.75$ $p = 0.52$
Treatment	<b><math>F_{125,126} = 4.91</math></b> <b><math>p = 0.028</math></b>
Habitat	$F_{125,124} = 0.058$ $P = 0.81$
Conditions	$F_{125,124} = 1.45$ $p = 0.23$
Colony Size–Treatment	<b><math>F_{125,126} = 6.2</math></b> <b><math>p = 0.014</math></b>
Colony Size	NA



**Figure 1.** Worker mortality rate in control (white) or cadmium (gray) treatment for lab (**A**) and field hibernation conditions (**B**). For each box, median and quartiles are shown. Different letters represent significant differences (a, b).

### 3.2. Larvae Mortality Rate

The larvae mortality rate was higher under cadmium treatment (+36%). The effect of cadmium tended to differ between habitat and hibernation conditions (+32%/+51% mortality for city/forest colonies under lab condition, and +31%/28% for city/forest colonies under field condition), with a triple interaction close to the significant level (treatment–habitat–conditions,  $F_{118,119} = 3.28$ ,  $p = 0.072$ ). In order to investigate this trend further, we split our dataset according to the hibernation conditions. When considering only the field hibernation condition, cadmium increased larvae mortality rate (+30%, Table 2 and Figure 2), but there was no differential effect of cadmium treatment depending on the habitat, i.e., city colonies did not resist better to cadmium (no treatment–habitat interaction, Table 2 and Figure 2). Interestingly, when considering only the lab hibernation condition, the decrease in larvae mortality rate under cadmium treatment was less pronounced in urban colonies (+32%) than in forest colonies

(+51%), i.e., urban colonies were less impacted by cadmium (treatment–habitat interaction,  $F_{56,57} = 5.33$   $p = 0.024$ , Table 2 and Figure 2).

**Table 2.** Summary of statistical analyses performed by splitting dataset according to hibernation conditions on larvae mortality rate and size of lab workers. For each model, we tested the effect of habitat (city and forest), treatment (control or cadmium), and colony size. Left side of the column: laboratory hibernation condition (Lab), right side of the column: field hibernation condition. Bold number indicates significant  $p$ -value.

	Larvae Mortality Rate		Size of Lab Workers	
	Lab	Field	Lab	Field
Treatment–Habitat	<b><math>F_{56,57} = 5.33</math></b> $p = 0.024$	$F_{62,60} = 0.18$ $p = 0.83$	$X_1 = 4.06$ $p = 0.043$	$X_1 = 0.50$ $p = 0.47$
Treatment	$Z = -7.59$ $p < 0.001$	$F_{62,63} = 18.41$ $p < 0.0001$	$t_{65} = 6.52$ $p < 0.001$	$X_1 = 50.73$ $p < 0.0001$
Habitat	$Z = -1.30$ $p = 0.19$	$F_{61,62} = 0.20$ $p = 0.65$	$t_{63} = 0.88$ $p = 0.38$	$X_1 = 12.84$ $p = 0.0003$
Colony Size–Treatment	$F_{57,58} = 1.69$ $p = 0.19$	$F_{62,63} = 4.57$ $p = 0.036$	$X_1 = 0.17$ $p = 0.67$	$X_2 = 3.05$ $p = 0.22$
Colony Size	<b><math>F_{58,59} = 3.63</math></b> $p = 0.061$	NA	$X_1 = 4.46$ $p = 0.035$	$X_1 = 2.91$ $p = 0.088$

### 3.3. Larval Paused Development Rate

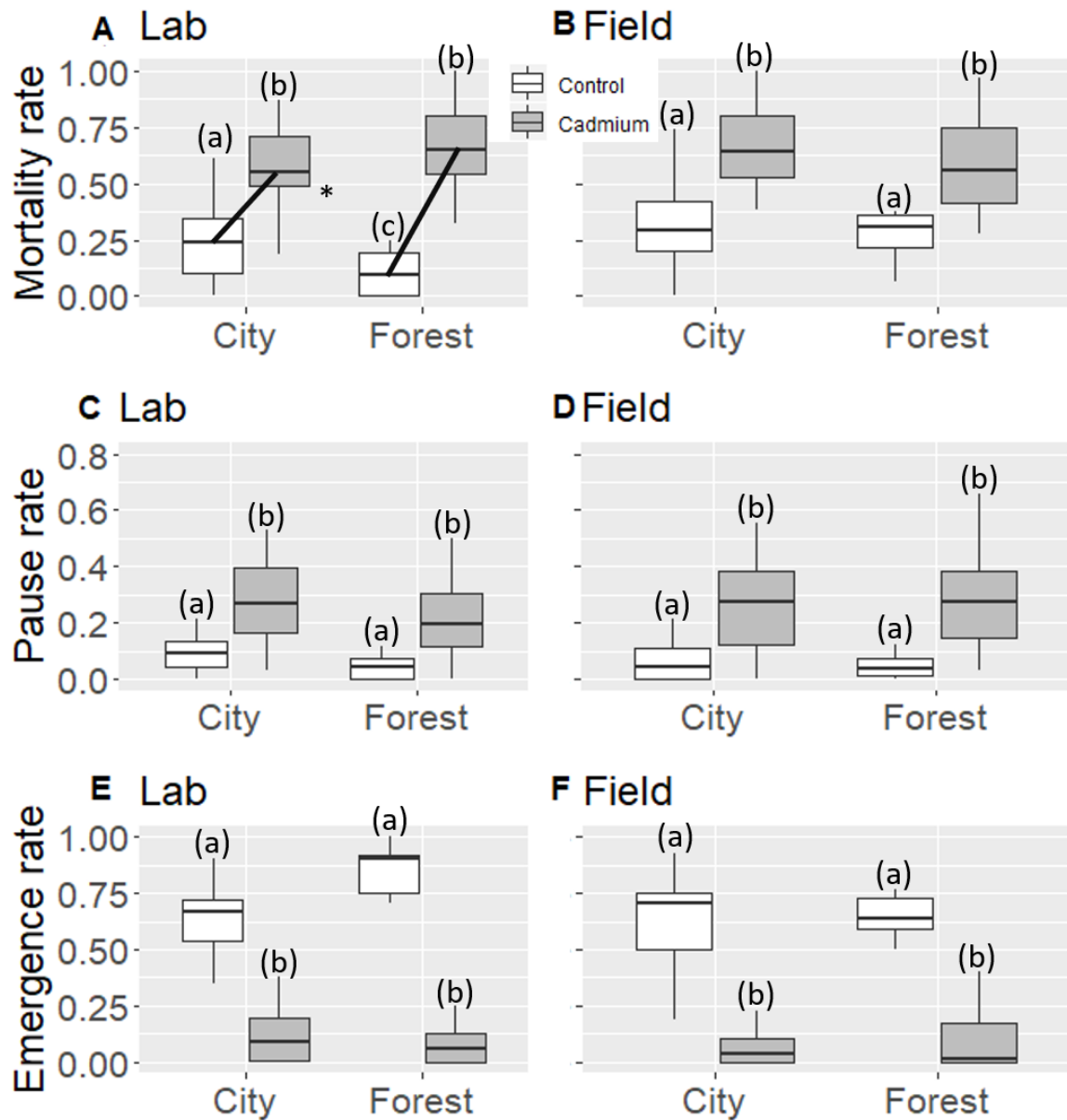
The larval paused development rate was higher under cadmium treatment (+20%, Table 3 and Figure 2). Cadmium treatment affected larval paused development rate to a similar extent for the two habitats (no treatment–habitat interaction, Table 3 and Figure 2), i.e., there was no better cadmium resistance in city colonies. Cadmium treatment also affected larval paused development rate to a similar extent for the two hibernation conditions (no significant treatment–conditions interaction, Table 3 and Figure 2). The other tested factors had no significant effects on the larval paused development rate (Table 3).

**Table 3.** Summary of statistical analyses on larvae testing the effects of hibernation conditions (lab and field), type of habitat (city and forest), and treatment (control or cadmium) for larval pause development rate and emergence rate. T:H:C means treatment–habitat–conditions triple interaction. Bold number indicates significant  $p$ -value.

	Pause Rate	Emergence Rate
T:H:C	$F_{121,127} = 0.78$ $p = 0.58$	$F_{126,121} = 1.29$ $p = 0.26$



Treatment–Habitat	$F_{127,125} = 1.64$ $p = 0.19$	$F_{126,124} = 1.33$ $p = 0.26$
Treatment– Conditions	$F_{127,125} = 0.37$ $p = 0.69$	$F_{126,125} = 0.040$ $p = 0.84$
Habitat–Conditions	$F_{127,124} = 0.87$ $p = 0.46$	$F_{126,124} = 1.09$ $p = 0.33$
Treatment	<b><math>F_{127,128} = 100.14</math></b> <b><math>p &lt; 0.0001</math></b>	<b><math>F_{126,127} = 257.29</math></b> <b><math>p &lt; 0.0001</math></b>
Habitat	$F_{127,126} = 1.69$ $p = 0.19$	$F_{126,125} = 1.82$ $p = 0.17$
Conditions	$F_{127,126} = 0.034$ $p = 0.85$	<b><math>F_{126,127} = 6.64</math></b> <b><math>p = 0.011</math></b>
Colony Size– Treatment	$F_{127,125} = 0.17$ $p = 0.84$	$F_{126,124} = 0.50$ $p = 0.60$
Colony Size	$F_{127,126} = 0.32$ $p = 0.57$	$F_{126,125} = 0.23$ $p = 0.63$



**Figure 2.** Mortality, larval paused development, and emergence rate of larvae for lab (A,C,E) and field hibernation conditions (B,D,F). White: control treatment, gray: cadmium treatment. For each box, median and quartiles are shown. (\*) shows a significant double interaction. Black lines denote the double interaction. Different letters represent significant differences for each lines (a, b).

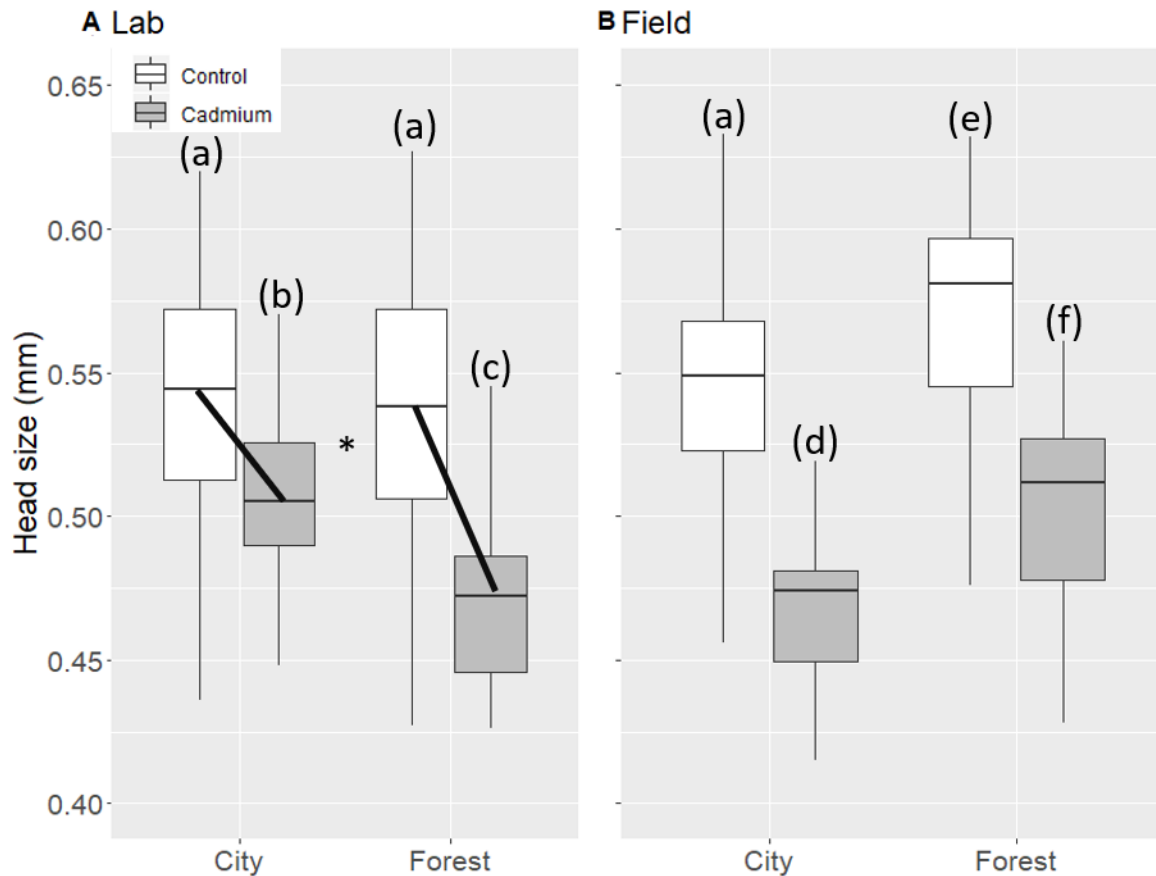
### 3.4. Emergence Rate

The larvae emergence rate was lower under cadmium treatment ( $-57\%$ , Table 3 and Figure 2), with a similar extent for the two habitats and two hibernation conditions (no significant triple and double interactions, Table 3 and Figure 2). The larvae emergence rate was higher for colonies hibernating in the lab than in the field ( $+8\%$ , Table 3).

### 3.5. Size of Lab Workers

Under lab hibernation conditions, we collected 56 city and 32 forest workers in the cadmium treatment, and 203 city and 257 forest workers in the control treatment. Under the field hibernation conditions, we collected 23 city and 36 forest workers in the cadmium treatment, and 207 city and 167 forest workers in the control treatment. The sample size (in terms of number of workers) was therefore unbalanced between cadmium and control treatments.

Cadmium treatment decreased the size of lab workers, but the extent of the effect differed depending on the level of both habitat and hibernation conditions (marginally significant treatment–habitat–conditions interaction,  $X_1 = 4.17$   $p = 0.041$ , Figure 3). Because of the marginally significant triple interaction, we split our dataset according to the hibernation conditions. When considering only the field hibernation condition, the decrease in size of lab workers under cadmium treatment was similar in both types of habitat, i.e., city colonies did not respond better to cadmium than the forest colonies (no significant treatment–habitat interaction, Table 2 and Figure 3). However, when considering only the lab hibernation condition, the decrease in size of lab workers under cadmium treatment was less pronounced in city than in forest habitat (significant treatment–habitat interaction,  $X_1 = 4.06$   $p = 0.043$ , Table 2 and Figure 3). Moreover, forest colonies produced significantly larger workers than city colonies (Table 2 and Figure 3).



**Figure 3.** Head size of lab workers in lab (A) and field hibernation conditions (B). White: control treatment, gray: cadmium treatment. For each box, median and quartiles are shown. (\*) shows a significant double interaction. Black lines denote the double interaction. Different letters represent significant differences (a, b, c, d, e, f).

## Discussion

Populations living in trace-metal contaminated environments such as mining site or cities are often more tolerant to trace metals than populations living in pollution-free environments [12,30,40]. In the ant *T. nylanderi*, Jacquier et al. [28] showed that urban populations are less sensitive to cadmium than their forest counterparts, with a lower larvae mortality rate, a lower decrease in emergence rate, and a lower size reduction of lab workers when exposed to cadmium. We proposed two alternative hypotheses regarding the enhanced cadmium resistance of city colonies: (i) if it is due to a baseline resistance of urban populations, it should still occur following common garden (lab) hibernation; (ii) if it comes from a plastic response caused by hibernation in an urban environment, it should disappear following common garden (lab) hibernation. We found that the lower cadmium sensitivity of urban colonies was

maintained after four months of hibernation under laboratory common garden, suggesting a different baseline level of cadmium resistance that could reflect genetic adaptation. As in Jacquier et al. [28], urban colonies reared under laboratory common garden for several months had a lower larvae mortality rate and a lower size reduction of lab workers relative to forest colonies. However, in contrast with Jacquier et al., we did not find a higher emergence rate in laboratory common garden urban colonies under cadmium. For worker mortality rate and larval paused development rate, the treatment with cadmium had a similar negative impact for the city and forest colonies. Unexpectedly, there was no differential cadmium resistance between city and forest colonies that hibernated in the field, in contrast with previous results in the same localities [30]. Overall, our results suggest that the differential response to cadmium between city and forest colonies is not directly caused by the different hibernation conditions in the field since it was still observed under lab hibernation conditions. However, the unexpected fact that we were unable to detect a differential response in the field in this study suggests that it could be expressed only under specific hibernation conditions as discussed below.

In a previous study [30], the differential response to cadmium between urban and forest colonies was found for one pair of locations in 2017 (Paris), and for three other pairs of locations in 2018 (Paris, Lyon, and Bordeaux). Relative to forest colonies, urban colonies displayed a lower worker size reduction, a higher emergence rate [30], and a lower larvae mortality rate in response to cadmium (only 2018 data available, personal data). The pattern was the same in all four comparisons, suggesting that this differential response was stable. In agreement with this, in our study, we found the same pattern under laboratory hibernation condition but quite surprisingly we did not detect any differential response in the field. The sample size in Jacquier et al. was larger (148 field colonies compared with 66 field colonies in our study), and thus we may have lacked statistical power in the present study. However, graphical tendencies seem to point a lack of effect or a reversed effect of cadmium (with lower larvae mortality rate and lower worker size reduction in forest colonies in response to cadmium), and therefore it is unlikely that the different pattern found in this study originated from a lack of statistical power. Another hypothesis is that the hibernation environment plays a role in the expression of the differential response to cadmium even though it does not directly explain the differential baseline response of city and forest colonies. More specifically, we suggest that cold winter is necessary to induce the differential response observed between forest and city colonies. Insect survival during overwintering is often related to temperature. For example, warmer winter increases mortality rate in insects such as the rose-gallin wasp *Dilopepsis spinosa* [41] or the boreal ant *Formica aquilonia* [26], probably because of higher metabolic rate that depletes fat

reserves. On the other hand, some species seem to perform better under warm temperature, such as the ant *Lasius niger*, for which workers have lower mortality rate at warm temperatures [25]. In our study, mean winter temperature in 2019/2020 was 8.1 °C in Paris city and 6.9 °C in Chantilly forest (infoclimat data, <https://www.infoclimat.fr/stations-meteo/analyses-mensuelles.php>), higher than in 2017/2018 (city 6.2 °C, forest 4.7 °C) and 2018/2019 (city 6.3 °C, forest 5.2 °C) and higher than in the laboratory common garden environment (4 °C for both habitats). Cold winters could favor the strongest individuals, as suggested by Haatenen et al. [25]. Therefore, toxicological assays may have been performed on colonies in poor condition. This, however, was not well-supported by our mortality data, with a similar mortality rate for colonies whatever the hibernation temperature (cold in the lab and warm in the field). In addition, we removed parasitized colonies from our experiment, which were more susceptible to be in poor conditions. Further studies performed on both unparasitized and parasitized colonies could be interesting to investigate ecological interactions between parasites and trace metal resistance. Cold stress triggers secretion of anti-stress proteins that are also involved in trace metal detoxification or resistance. For instance, in the earthworms *Dendrobaena octaedra*, freezing of body fluids triggers the secretion of metallothionein, a well-known protein group involved in trace metal detoxification [42]. In *Ostrinia nubilalis*, the gene expression patterns of cold-acclimated diapausing individuals during diapause initiation, maintenance, and termination differ from those of warm-acclimated diapausing individuals, with higher quantity and stability of mRNA in cold-acclimated individuals at the end of the diapause [16]. Proteins coded by those mRNA are involved in temperature-related stress (heat shock protein, HSP) but interestingly also in antioxidative defenses and metal detoxification (metallothionein, thioredoxin, ferredoxin), which may help individuals to better cope with future trace-metal related stress. We therefore propose that the field hibernation conditions in 2019/2020 were not cold enough to trigger the expression of differential response to cadmium. The absence of differential cadmium response between city and forest for field colonies may therefore have been due to warmer temperature during winter diapause. In contrast, our laboratory hibernating conditions were colder than what was observed in the field (4 °C, i.e., 1–2 °C colder than in winter 2017/2018 and 3–4 °C colder than in winter 2019/2020). This could explain why the differential response was found in the common garden experiment and not in the field in our study. However, in contrast with Jacquier et al. (2020), this study was only conducted on a single urban/forest pair of habitats. Therefore, we cannot conclude whether the importance of hibernation conditions is a general pattern or is specific to the Paris location. However, a genetic study highlighted the very high genetic homogeneity of *T. nylanderi* populations throughout

France. In addition, the differential effect of cadmium between city and forest habitats was found to be similar in three distant locations in France (Jacquier et al. 2020). These two studies support the idea that our results could be generalized to most large cities in France, although further studies with replicated locations would be needed to confirm this.

Interestingly, even though the hibernation temperature in the laboratory common garden was even cooler than the average field temperature, it seemed to be not so stressful, at least for post-hibernation performances, as the worker mortality rate and the size of lab-workers were similar in Jacquier et al. [28] and in our study, whatever the hibernating conditions. This indicates that the threshold under which cold temperature has negative effects on post-hibernation traits had not been reached under our common garden hibernation experiment. This is not surprising given that the temperature of the common garden (4 °C) is the same order of magnitude as the mean winter temperature commonly observed under such latitude (available online: <https://www.infoclimat.fr/climato/>, accessed on 2020-12-04).

The differential response to cadmium found between forest and city colonies under common hibernating laboratory environments supports the idea that this differential response could be at least in part genetically determined. A genetic adaptation to trace metal has already been shown in other insect populations living in ancient mining sites [12], in fish populations living in polluted water [43], and in urban bird populations [40]. A recent genetic analysis found 19 discriminating single-nucleotide polymorphisms (SNPs) between urban and forest populations of *T. nylanderi* [44]. One of these SNPs may be involved in the differential response between urban and forest colonies to cadmium exposure, but this however remains highly speculative. More studies are needed to disentangle the effects of genetic adaptation from maternal or epigenetics effects. In any case, our results suggest that urban populations have higher basal levels of anti-stress gene expression than their forest counterparts, in line with the higher expression of immune response, antioxidative, detoxification, and repair systems genes in urban populations of other species (*Parus major* birds [45], *Anopheles* spp. mosquitoes [46], *Peromyscus leucopus* rodents [47]). Interestingly, those anti-stress genes are involved in trace metal resistance. For instance, detoxification proteins such as metallothionein or cytochrom P450 (CYP450) improve trace metal resistance by binding metals [27,48]. As trace metals also increase oxidative stress, enhancing the expression of antioxidant defenses (e.g., glutathione S transferase or thioredoxin) or DNA repair systems (e.g., EXO1 endonuclease [2]) may help to cope with such pollutants. As those classes of genes are overexpressed in urban populations for quite divergent species, we can hypothesize that urban populations of *T. nylanderi* also overexpressed such types of genes. This hypothesis would merit further attention and could be

tested by quantifying the differential expression of stress-related genes, known to be sensitive to temperature, between urban and forest populations.

## **Conclusion**

In conclusion, this study highlights the interconnection between genetic and environmental components in the trace metal response of urban and forest ant colonies for one specific area (Paris). We hypothesized that the better resistance to cadmium of urban colonies is only found under a specific environmental condition, i.e., cold winter. In the light of future climate change and warmer winter temperatures, it is of particular importance to assess whether adaptation to a specific anthropogenic disturbance is stable or sensitive to other environmental disturbances, possibly impeding adaptation processes.

**Supplementary Materials:** The following are available online at [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1): Table S1: Emergent head width (individual-level data). Table S2: Summary of the colony-level data (worker mortality, larval mortality, larval paused, emergence).

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**Data Availability Statement:** Data are available in the supplementary materials section (Table S1 and Table S2).

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**Disentangling the roles of social and individual effects on cadmium tolerance in the ant *Temnothorax nylanderi*.**

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## **Abstract**

Urbanization brings new pressures for individuals. Among them, trace elements such as cadmium are important stressors. A recent study highlights a weaker negative effect of cadmium on city colonies relative to their forest counterparts in the ant *Temnothorax nylanderi*. Here, we aim to test whether this better tolerance of city colonies in this species results from (1) a better ability of workers to rear larvae despite stressful conditions and/or (2) a better ability of larvae to develop properly despite stressful conditions. We performed a cross-fostering experiment of workers and larvae from city and forest colonies, under common garden in the laboratory. Colonies were fed using cadmium-enriched or cadmium-free food for two months, and we measured four life-history traits. As expected, cadmium had a negative impact on all traits. Unexpectedly, we did not observe a better tolerance of city colonies to cadmium contrary

to our previous study, preventing us from disentangling the respective contributions of workers and larvae to cadmium tolerance. Interestingly, forest colonies seem to be of better quality in our laboratory conditions. Finally, colony size increased adult survival, but only in the absence of cadmium, suggesting that social buffering could collapse under strong external disturbances.

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

## **Introduction**

Cities are natural laboratories for investigating evolutionary changes 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 land cover and increases in temperature (including the ‘urban heat island’ - (Levermore *et al.*, 2018; Diamond & Martin, 2020). Pollution is also a major component of urbanization (be it sound, chemicals, light or others; e.g. Westby and Medley, 2020), especially trace metals which are persistent pollutants that bioaccumulate in the food chain. For example, cadmium is a highly toxic trace metal to various species (e.g. increasing mortality rate, decreasing hatching rate or impeding development, Gomot, 1998 (snails); Järup and Åkesson, 2009 (human); Malakar *et al.*, 2009 (grasshopper)) that can be found in concentrations ten times higher in cities than in rural soils (Foti *et al.*, 2017). These growing pressures lead to changes in various individuals traits (physiology, morphology, phenology, behavior), both plastically (e.g. changing in behavior (Ditchkoff, Saalfeld, & Gibson, 2006) or morphology (Prosser, Hudson, & Thompson, 2006)) and evolutionarily (e.g. changes in phenology (Chick *et al.*, 2019) or physiology (Partecke, Schwabl, & Gwinner, 2006) - see also Johnson and Munshi-South, 2017; reviewed in Alberti *et al.*, 2017). For instance, urban populations 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).

Although many organisms face these pressures alone, individuals living in groups benefit from a filter provided by their society against external disturbances. The social environment is a key component to be considered when studying the response of social species to external pressures, as it may buffer external disturbances (Straub *et al.*, 2015). For example

in social insects, colony size plays a role in buffering the effects of temperature, with larger colonies usually having less mortality or stronger trait stability (cold: Heinze *et al.*, 1996; warm: Molet *et al.*, 2017). This social buffering is found for various pressures, such as exposure to parasites (Scharf *et al.*, 2012), seasonality (Kaspari & Vargo, 1995), starvation (Heinze *et al.*, 1996; Modlmeier, Foitzik, & Scharf, 2013) and pesticides (Crall *et al.*, 2019). In addition to workers, the presence of larvae can also improve colony resistance to stress in harsh periods, such as under starvation (Rueppell & Kirkman, 2005; Modlmeier *et al.*, 2013). Furthermore, the social environment highly modulates brood development in insect societies such as in ants (Linksvayer, 2006; Wills *et al.*, 2018) or bees (Linksvayer *et al.*, 2011), and therefore can influence the phenotypes produced within colonies. Cross-fostering experiments, mixing worker and larval origins, are particularly useful to partition the effects of the social environment, that is assessing the relative contribution of adults and larvae. For example, crossfostering has been used in some ants species to assess the relative contributions of genetic and social effects on individual and colony traits (Linksvayer, 2006), the influence of social structure on brood development (Purcell & Chapuisat, 2012), the effects of matriline and patriline on immune defense (Armitage *et al.*, 2011) and the consequences of the rearing environment on individual and colonial personalities (Keiser *et al.*, 2015). However, as far as we are aware, it has never been used to investigate the relative effect of the social environment and larvae themselves on the ability to tolerate stressful conditions such as pollution.

In this study, we used a cross-fostering experiment while rearing colonies under common laboratory conditions in the ant *Temnothorax nylanderi* in the presence or absence of a stressor (cadmium). A previous study showed that city colonies were less negatively affected by cadmium than forest ones for two traits: the size of produced workers and the survival of larvae (Jacquier *et al.*, 2021a). In the present study, we used the same laboratory rearing setup and we measured the same traits (i.e. body size at emergence and survival) as well as the development duration. Moreover, in contrast with Jacquier *et al.* (2021a) who only assessed worker traits, we also measured sexual production and size of sexuals. Size of sexuals is an important trait linked with fitness in ants as larger sexuals have greater mating success and colony founding success in some species (Abell *et al.*, 1999; Wagner & Gordon, 1999; Wiernasz *et al.*, 2001; Wiernasz & Cole, 2003; Fjerdingstad & Keller, 2004). By performing a cross-fostering, our study allows us to test the relative contribution of the origin of workers (forest or cities) and of the origin of larvae (forest or cities) on the life history traits measured in presence and absence of cadmium.



## 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 (a few dozen workers), and common in urban and forest habitats (Foitzik, Strätz, & Heinze, 2003). We collected colonies at two sites where a differential response to cadmium has been documented (Jacquier *et al.*, 2021a). We collected 103 city colonies in an urban park in Paris (Parc des Buttes-Chaumont, 48°52'47.1"N; 2°23'45.6"E) and 109 forest colonies in a forest located 50km away from Paris (Chantilly forest, 49°10'59.8"N; 2°28'43.6"E). Colonies were harvested in April 2019 and then installed in the laboratory. The artificial nest consisted of two microscope slides (75 x 26 x 1 mm) separated by a 1-mm auto-adhesive layer of 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. We discarded 52 colonies with more than one queen or with no queen (28 colonies in city, 24 in forest), as well as 18 colonies (3 in city, 15 in forest) with cestode-infected workers (that display typical pale-yellow coloration (Scharf *et al.*, 2012)). We finally retained 72 queenright colonies for city habitat and 70 colonies for forest habitat. Workers and larvae in each colony were counted. Colony size ranged from 3 to 256 workers and brood from 8 to 96 larvae. Colonies were acclimated for seven days in the laboratory before the experiment began. Using a microscope, we only kept second instar larvae (i.e. larvae under 2mm long with hairs mainly on the front, Plateaux, 1970), so that all larvae would be exposed to cadmium at the same time and for the majority of their development duration.

### *Experimental design*

Workers and larvae had two different origins (city or forest), resulting in four different crosses: city workers with city larvae from another colony (CC, 36 colonies), forest workers with forest larvae from another colony (FF, 36 colonies), city workers with forest larvae (CF, 35 colonies) and forest workers with city larvae (FC, 35 colonies). Colonies were matched by pairs for brood exchange, depending on the number of workers and larvae. Each colony received all the second-instar brood from its paired colony. Larvae of the two paired colonies were exchanged simultaneously, larvae remained isolated for a maximum of 15 minutes without workers. Such brood exchanges have already been performed in other *Temnothorax* species (Linksvayer, 2006, 2007), and the brood is accepted by workers without discrimination.

Each colony was then exposed to a feeding treatment with two levels: control or cadmium. Colonies were evenly assigned to control or cadmium treatment depending on their

colony size, in order to obtain a similar distribution of colony sizes within treatment levels. 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.*, 2021a). The concentration used in the food corresponds to the LC50 (the concentration that triggers 50% mortality over a given period of time), a physiologically but not ecologically relevant dose. Indeed, this concentration is about fifty times higher than the typical cadmium concentration measured in urban soils. However, in the field, recurrent exposure to pollutants can lead to bioaccumulation and cadmium concentration can reach 102 mg/kg in the ant *Formica polyctena*, for a soil concentration of 2 mg.kg<sup>-1</sup> (Starý & Kubizňáková, 1987). Therefore, the concentration that we used was high but still in the upper range of real field values (Jacquier *et al.*, 2021a). We used 15 “CC”, “FC” and “FF” colonies, and 16 colonies “CF” with control food. We assigned more colonies to cadmium treatment in order to compensate for the potential risk of mortality, so we used 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 22-27°C – 12h : 12h cycle with natural photoperiod for 64 days, so that larvae could emerge as adults (Molet *et al.*, 2017). Workers that emerged in the laboratory (called “laboratory-born 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 every week to prevent the hatching of additional larvae in order to keep the initial number of larvae constant and not to interfere with the experimental larvae that originate from crosses. To do so, we removed the top microscope slide from the nest, took out the eggs, and then let the colony move to a new nest positioned just in front of the old one. This way, we did not need to put a microscope slide back on the nest, which could have crushed some workers.

Adult workers born in the field were removed from the colonies when they died and counted to compute the worker mortality rate. The emergence rate was computed as the ratio between the number of larvae that developed to adulthood over the number of larvae initially given to the colony. For each produced worker, gyne and male, we also measured the development duration (computed as the number of days from the beginning of the experiment to emergence) as well as the head width (see below).

### *Size measurement*

For morphological measurements, 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 and measured with ImageJ 1.8 (Abràmoff, Magalhães, & Ram, 2004). Head width is a standard measurement of body size in ants (Tschinkel, Mikheyev, & Storz, 2003).

### *Statistical analyses*

We investigated the effects of worker origin (forest/city), larval origin (forest/city), treatment (cadmium/control) and the initial colony size on the adult mortality rate, the emergence rate, the development duration of produced individuals (sexuals and workers) and the size of produced individuals. We included a second-order interaction between feeding treatment, larval origin and worker origin, that allowed us to test whether the effect of treatment differed across each crossing combination. We also included the interaction between the feeding treatment and the colony size 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](http://www.r-project.org)). All plots were generated using *ggplot2* (Wickham, 2009). First, we assessed the effect of predictor variables on colony-level dependent variables (adult worker mortality rate, emergence rate, and the head width of produced individuals) using generalized linear models (GLMs). Adult worker mortality and emergence were analyzed using quasibinomial distribution. Finally, we assessed the effect of predictor variables on the development duration and the head width of emerged individuals, two individual-level dependent variables, using linear mixed models (LMMs, package *nlme*, (Pinheiro *et al.*, 2016)), with colony as random factor to take into account pseudo-replication. 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  $\text{Chi}^2$  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). We first tested the significance level of the second-order interaction, then first-order interactions. Normality of the residuals and homogeneity of variances were checked visually following (Pinheiro & Bates, 2000); no transformation of the data was necessary.

### *Data availability statement*

The dataset and R scripts are available on Zenodo (10.5281/zenodo.5105734).

### **Results**

Six queens died during the experiment (one ‘FF’, ‘FC’ and ‘CC’ colony; three ‘CC’ colonies) and their colonies were excluded from data analyses. Our final dataset consisted of 136 queenright colonies. We collected a total of 1 688 workers, 1 092 males, one intercaste (worker phenotype with two ocelli, Okada et al., 2013) and 134 gynes. As we collected 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), we did not include gyne production in our dataset because of a lack of statistical power, with only five gynes under cadmium treatment.

Whatever the variables measured, we did not find a significant second-order interaction (Treatment : Worker origin : Larva origin, Table 1), i.e. there was no differential sensitivity to cadmium depending on the crossing. Moreover, contrary to our expectation, there was no better cadmium tolerance in city colonies: we did not find any interaction between Treatment and Worker origin (at the exception of male development duration, see below) or Treatment : Larval origin interaction (Table 1). Those interactions were therefore removed from the model and in the following results, we only present the main effects of worker origin, larval origin, colony size and treatment, as well as the interaction between colony size and treatment (detailed statistics are presented in Table 1).

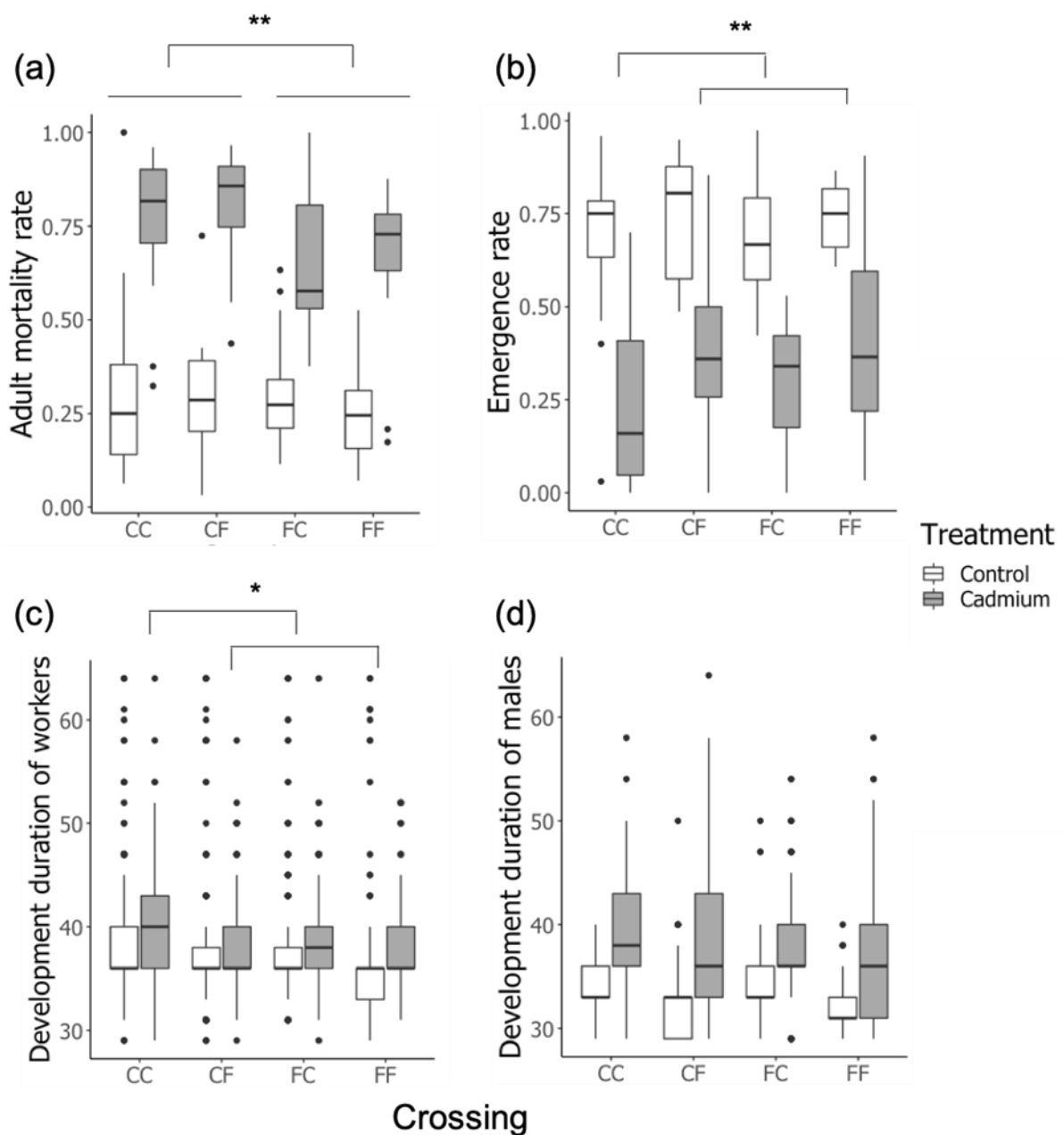
**Table 1:** Models and statistics for the different response variables. “Minimum model” means that the predictor was retained in the minimum model selected. A mixed effect model with colony as random factor was used for the development duration and head width of produced individuals, in order to control for the origin of workers. Significant effects are in bold.

Predictors	Response variables					
	Adult mortality rate	Emergence rate	Worker development duration	Male development duration	Worker head width	Male head width
Treatment	<b>Minimum model</b> <b>F<sub>131:133</sub> = 92.43,</b> <b>P &lt; 0.001</b>	<b>Minimum model</b> <b>F<sub>131:132</sub> = 107.93,</b> <b>P &lt; 0.001</b>	<b>Minimum model</b> <b>χ<sup>2</sup><sub>1</sub> = 7.14,</b> <b>P = 0.0075</b>	<b>Minimum model</b> <b>χ<sup>2</sup><sub>1</sub> = 64.69,</b> <b>P &lt; 0.001</b>	<b>Minimum model</b> <b>χ<sup>2</sup><sub>1</sub> = 157.97,</b> <b>P &lt; 0.001</b>	<b>Minimum model</b> <b>χ<sup>2</sup><sub>1</sub> = 156.24,</b> <b>P &lt; 0.001</b>
Worker origin	<b>Minimum model</b> <b>F<sub>131:132</sub> = 7.72,</b> <b>P = 0.0060</b>	F <sub>130:131</sub> = 0.81, P = 0.37	χ <sup>2</sup> <sub>1</sub> = 0.46, P = 0.50	χ <sup>2</sup> <sub>1</sub> = 0.56, P = 0.45	χ <sup>2</sup> <sub>1</sub> = 0.57, P = 0.45	<b>Minimum model</b> <b>χ<sup>2</sup><sub>1</sub> = 3.57,</b> <b>P = 0.059</b>
Worker origin - Treatment	F <sub>130:131</sub> = 2.96, p = 0.087	F <sub>129:131</sub> = 0.60, P = 0.55	χ <sup>2</sup> <sub>2</sub> = 2.00, P = 0.37	<b>Minimum model</b> <b>χ<sup>2</sup><sub>1</sub> = 3.82,</b> <b>P = 0.05</b>	χ <sup>2</sup> <sub>2</sub> = 0.62, P = 0.73	χ <sup>2</sup> <sub>1</sub> = 0.027, P = 0.87
Colony size	<b>Minimum model</b> <b>F<sub>131:133</sub> = 2.77,</b> <b>P = 0.066</b>	F <sub>130:131</sub> = 1.65, P = 0.20	<b>Minimum model</b> <b>χ<sup>2</sup><sub>1</sub> = 6.22,</b> <b>P = 0.012</b>	<b>Minimum model</b> <b>χ<sup>2</sup><sub>1</sub> = 5.61,</b> <b>P = 0.017</b>	χ <sup>2</sup> <sub>1</sub> = 1.01, P = 0.31	χ <sup>2</sup> <sub>1</sub> = 0.22, P = 0.64

Colony size - Treatment	<b>Minimum model,</b> <b>F<sub>131:132</sub> = 3.67,</b> <b>P = 0.057</b>	F <sub>129:131</sub> = 2.28, P = 0.10	$\chi^2_1 = 0.01,$ P = 0.91	$\chi^2_1 = 0.75,$ P = 0.38	$\chi^2_2 = 3.59,$ P = 0.16	$\chi^2_2 = 0.24,$ P = 0.89
Larva origin	F <sub>131,130</sub> = 0.0054 P = 0.94	<b>Minimum model</b> <b>F<sub>131:132</sub> = 7.54,</b> <b>P = 0.007</b>	<b>Minimum model</b> <b><math>\chi^2_1 = 4.22,</math></b> <b>P = 0.039</b>	$\chi^2_1 = 2.53,$ P = 0.11	$\chi^2_1 = 0.41,$ P = 0.52	$\chi^2_1 = 0.07,$ P = 0.80
Larva origin - Treatment	F <sub>131,129</sub> = 0.14 P = 0.86	F <sub>130:131</sub> = 0.91, P = 0.34	$\chi^2_1 = 1.21,$ P = 0.27	$\chi^2_2 = 2.9,$ P = 0.23	$\chi^2_2 = 2.57,$ P = 0.28	$\chi^2_2 = 0.30,$ P = 0.86
Second-order interaction worker origin - larva origin - Treatment	F <sub>131,126</sub> = 0.82 P = 0.53	F <sub>126:131</sub> = 0.45, P = 0.81	$\chi^2_5 = 3.70,$ P = 0.59	$\chi^2_4 = 4.38,$ P = 0.36	$\chi^2_6 = 6.01,$ P = 0.42	$\chi^2_5 = 2.78,$ P = 0.73

### Adult mortality rate

Cadmium increased adult mortality rate by 148% (Treatment effect,  $F_{131:133} = 92.43$ ,  $P < 0.001$ ; figure 1a). The origin of workers (city or forest) significantly affected the mortality rate ( $F_{131:132} = 7.72$ ,  $P = 0.0060$ ) with city workers ('CC' and 'CF') having a 14% higher mortality rate than forest workers (origin of workers effect,  $F_{131:132} = 7.72$ ,  $P = 0.0060$ ; figure 1a). Not surprisingly, there was no effect of the origin of larvae (Table 1). The mortality rate marginally differed depending on the treatment in interaction with the colony size (Treatment x Colony size interaction,  $F_{131:132} = 3.67$ ,  $P = 0.057$ ). The mortality rate increased with the initial colony size under cadmium, while it decreased with the initial colony size in the control (figure 2).



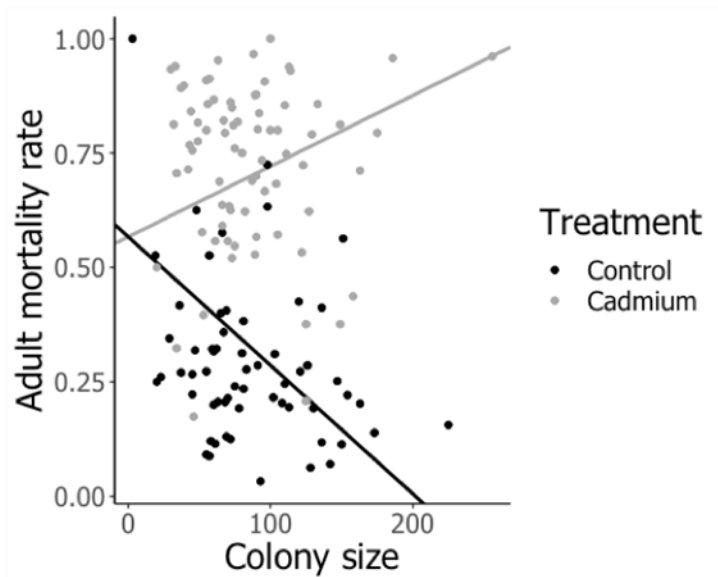
**Figure 1:** Boxplots comparing the effects of the worker and larval origins (crossing) and feeding treatment on (a) the adult worker mortality rate, (b) the larval emergence rate, the development duration of laboratory-born (c) workers and (d) males. Boxes show median, quartiles and extremes. Significance 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.

#### *Emergence rate and development duration*

Cadmium decreased emergence rate by 50% (Treatment effect,  $F_{131:132} = 107.93$ ,  $P < 0.001$ ; figure 1b). The origin of workers had no effect on emergence rate ( $F_{129:131} = 0.55$ ,  $P = 0.60$ ; figure 1b) whereas the origin of larvae significantly affected the emergence rate (origin of larvae effect,  $F_{131:132} = 7.54$ ,  $P = 0.0070$ , figure 1b) with forest larvae having a 17% higher emergence rate than city larvae. There was no effect of colony size on emergence rate (Table 1).

The development of produced individuals was 6% longer under cadmium (Treatment effect, workers:  $\chi^2_1 = 7.14$ ,  $P = 0.007$ ; figure 1c - males:  $\chi^2_1 = 64.69$ ,  $P < 0.001$ ; figure 1d). The origin of workers had no effect on worker development duration whereas the origin of larvae had a significant effect (origin of larvae effect,  $\chi^2_1 = 4.22$ ,  $P = 0.039$ ; Table 1). Forest larvae took 3.1% less time to develop into adult workers than city larvae (figure 1c). We found no effect of colony size on the development duration of larvae (Table 1). Regarding males, we found a marginally significant Treatment : Worker origin interaction ( $\chi^2_1 = 3.82$ ,  $P = 0.051$ ), with longer development under cadmium for males, but not under control. The origin of larvae had no effect on the development duration of males (Table 1). Development duration increased with the initial colony size (Colony size effect, workers:  $\chi^2_1 = 6.22$ ,  $P = 0.012$ ; males:  $\chi^2_1 = 5.07$ ,  $P = 0.024$ ).

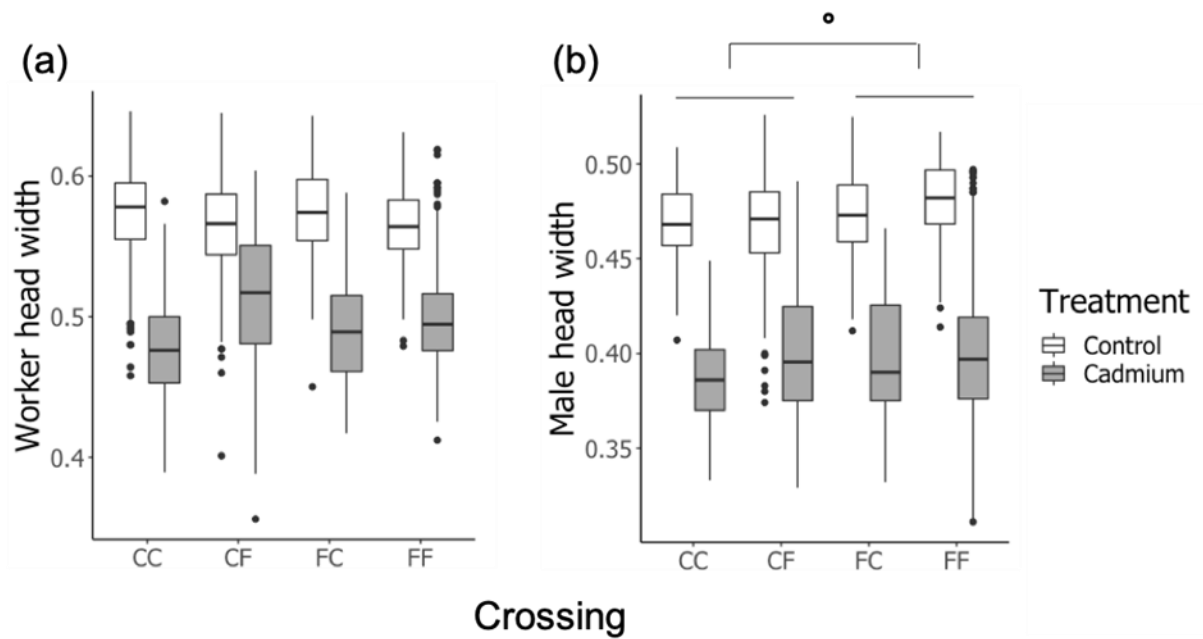




**Figure 2:** Correlation between the initial colony size and the adult worker mortality rate. When the initial colony size increased, the adult mortality increased for cadmium treatment and decreased for control treatment (black line: control, estimate =  $-2.81e^{-3} \pm 0.43e^{-3}$ , grey line: cadmium, estimate =  $1.54e^{-3} \pm 0.42e^{-3}$ ).

#### *Size (head width) of produced individuals*

Cadmium reduced the size of the produced workers and males by 14% and 16% respectively (Treatment effect, workers:  $\chi^2_1 = 157.97$ ,  $P < 0.001$ ; figure 3a - males:  $\chi^2_1 = 156.24$ ,  $P < 0.001$ ; figure 3b). Neither worker origin nor larvae origin had an effect on worker head width (Table 1). Colony size had no effect on worker head width (Table 1). Regarding males, forest workers produced marginally larger males relative to city workers (+2.3%, effect of worker origin,  $\chi^2_1 = 3.57$ ,  $P = 0.059$ ; figure 3b). Larvae origin and colony size had no effect on male head width (Table 1).



**Figure 3:** Boxplots comparing the effects of the worker and larval origins (crossing) and treatment on the head width (mm) of laboratory-born (a) workers and (b) males. Boxes show median, quartiles and extremes. Trend ( $0.05 < p < 0.06$ ) is indicated by °. Treatment had a significant effect on all variables (not shown in the figure for clarity). Statistics are presented in Table 1.

## Discussion

We aimed at disentangling the implications of workers (the social environment) and larvae in the better cadmium tolerance of larvae previously observed in city colonies by Jacquier *et al.* (2021a). Just like other studies (den Besten *et al.*, 1989; Gomot, 1998; Cervera *et al.*, 2004; Demenesku *et al.*, 2014; Jacquier *et al.*, 2021a), we found that cadmium had a negative impact on all life history traits measured, i.e. it increased worker mortality rate (+148%), reduced emergence rate (-50%), extended development duration (+6%) and reduced the size of individuals, for both workers (-14%) and males (-16%). The intensity of the effect on workers was of the same order of magnitude as in Jacquier *et al.* (2021a). However, in contrast with Jacquier *et al.* (2021a), the effect of cadmium was similar in city and forest colonies regarding emergence rate and worker head width, preventing us from disentangling the effect of workers and larvae on the expected differential cadmium tolerance. This absence of differential response between habitats could result from annual random variation and the fact that a comparison between only two sites did not allow us to detect a difference in our study. However, our sample

size in terms of number of colonies was large, excluding a lack of statistical power. In addition, the differential response between the two habitats was observed for five replicated comparisons (Jacquier *et al.*, 2021a,b) making chance an unlikely explanation. Also, as the same persons performed the experiments, with the same laboratory set up (even though in our experiment larvae were manipulated which was not the case in Jacquier *et al.* (2021a) – see below), we think that the observed difference is a true biological phenomenon.

We propose three non-mutually exclusive hypotheses that could explain the unexpected absence of a differential cadmium tolerance between forest and city colonies. A first explanation is a disruptive effect of cross-fostering. This experimental method is commonly used to split the relative contributions of different social aspects on measured traits in ants (Linksvayer, 2006; Armitage *et al.*, 2011; Purcell & Chapuisat, 2012). Therefore, adult workers had to rear larvae from foreign colonies. Because workers are unrelated to these larvae, they may invest less in rearing them. However, Foitzik *et al.*, (2007) showed that in *Temnothorax nylanderii* there was little to no discrimination of non-nestmates, maybe because colonies and populations are weakly differentiated genetically and thus could have similar cuticular hydrocarbons, therefore impeding worker discrimination based on this cue. Besides, as in other successful cross-fostering experiments (Linksvayer, 2006; Armitage *et al.*, 2011; Purcell & Chapuisat, 2012), larvae were largely accepted by workers at the beginning of our experiment (more than 90% of the brood survived after the larval exchange between colonies), so workers are probably not able to detect that larvae originated from another colony. A second hypothesis is a within year variation of cadmium tolerance. We collected colonies in April, whereas (Jacquier *et al.*, 2021a) collected them in February. In some species, trace metals show a seasonal pattern of accumulation, correlated with seasonal variation in detoxification enzymes or proteins (Fialkowski *et al.*, 2003; Gorbi, Baldini, & Regoli, 2005). Recent data suggest that the better cadmium tolerance of city colonies depends on both hibernation temperature and the period of the year at which the experiment was conducted (Jacquier *et al.*, 2021b; Jacquier *et al.*, *under review*). In this study, we collected colonies later (April) than in (Jacquier *et al.*, 2021a), in order to obtain more sexuals. This may have impeded further differential cadmium tolerance between city and forest colonies, as Jacquier *et al.* (*under review*) showed that colonies collected several weeks after hibernation (and therefore with eggs that were laid after the hibernation period, i.e. early June here) do not have differential emergence rates in response to cadmium. Further studies are needed to investigate the possible effect of within-year variation in environmental factors on cadmium tolerance between the two habitats, since urban and forest ants have a slightly shifted phenology (Chick *et al.*, 2019). A last hypothesis stands in inter-

annual variation in cadmium tolerance. In Jacquier et al (2021a), urban colonies tolerated cadmium better than forest colonies, but the level of differential tolerance varied among sites and years. In this study, we only used one study site, therefore we lack replication and the observed results could be due to natural variation in cadmium tolerance at this site for this particular year. Moreover, Jacquier *et al.* (2021b) suggested that cadmium tolerance could also depend on environmental factors such as hibernation temperature. Colonies may have experienced special climatic conditions the year of our experiment, and therefore we found results that contrast with previous findings.

Our study quantified the effect of cadmium on male production, and therefore the potential consequences on the reproductive success of colonies. In *T. nylanderi*, brood production is highly synchronized (Kipyatkov, 1993), but males develop faster than workers (our study and (Jacquier *et al.*, 2021a). As we started our study later (April vs February – March for Jacquier *et al.* 2021a) and winter was milder, colonies were probably more advanced in the process of male production. The relative lack of gyne production is not surprising, given that resource allocation can be redirected towards workers and males after a large worker loss (29% on average for control colonies, 72% for cadmium colonies at the end of the experiment) in this species (Foitzik & Heinze, 2000; Foitzik *et al.*, 2003; Honorio, Doums, & Molet, 2020). Forest workers reared males that were 1.7% larger than those reared by city workers. Thus, when forest workers reared larvae, they produced larger males whatever the origin of larvae and whatever the treatment. Larger males have greater mating success and sperm production in ants (Abell *et al.*, 1999; Wiernasz *et al.*, 2001; Brown & Keller, 2006), therefore forest colonies may have a higher fitness than city colonies whatever the treatment, suggesting a better general condition. As adult city workers unexpectedly faced a higher mortality (in contrast with Jacquier *et al.* (2021a), a hypothesis is that city workers may have undergone higher stress levels, maybe leading to less brood care.

Different studies documented phenotypic divergence between city and rural insect populations. For example, body size is sometimes higher (Weller & Ganzhorn, 2004; y Gomez & Van Dyck, 2012; Lowe, Wilder, & Hochuli, 2014; Merckx *et al.*, 2018; Theodorou *et al.*, 2021) and sometimes lower (Merckx *et al.*, 2018; Eggenberger *et al.*, 2019) in city populations depending on the species. Interestingly, in *Temnothorax* species, previous studies found no differences in worker size across urban and rural populations (Yilmaz *et al.*, 2019; Jacquier *et al.*, 2021a). Emergence rate (grasshoppers: Gomez and Van Dyck, 2012; damselfly: Tüzün et al., 2017) and development duration in the field also differs between urban and rural populations depending on the species (mosquitoes: Li et al., 2014, damselfly: Tüzün et al., 2017). In this

study, we found a limited effect of the origin of larvae, independently of the feeding treatment, and no effect of the social environment, except for male size. Forest larvae had a 17% higher emergence rate and a 3.1% shorter development duration but a 2.3% larger size at emergence when they developed into workers, therefore appearing to be of better quality (even though worker size does not seem to be of primary value for colony fitness in this species (Colin et al., 2017; Honorio et al., 2020, 2021 - but see Modlmeier et al., 2013). Our study does not allow to further substantiate the processes involved, but two potential proximate mechanisms can be proposed. First, city colonies may have had a poor physiological and metabolic state at the beginning of the experiment because they faced multiple stressors related to their urban environment prior to the experiment. Therefore, city queens could lay lower quality eggs, with larvae that develop slower and undergo lower emergence rate. Differences in egg provisioning through maternal or epigenetic effects can generate differences in development and emergence rate between larvae. For instance, juvenile hormone and ecdysteroid are transmitted to brood and influence development and emergence rate in ants (Schwander *et al.*, 2008; Cahan, Graves, & Brent, 2011; Libbrecht *et al.*, 2013) - see also (LeBoeuf *et al.*, 2016, 2018). Second, the differences between city and forest colonies could be genetically determined, as a recent study found a few discriminating loci (Khimoun *et al.*, 2020). However, this study also highlighted a lack of genetic differentiation between urban and forest populations.

Another important social trait is colony size. Stress tolerance increases with colony size in many eusocial species (Naug, 2009; Scharf *et al.*, 2012; Modlmeier *et al.*, 2013; Crall *et al.*, 2019). Here, we found no buffering effect of colony size with the exception of a contrasting effect on worker mortality rate (positive relationship under control treatment, but negative under cadmium) and a positive relationship with the development duration of workers and males. This is surprising, as other studies on *T. nylanderi* showed that larger colonies better resist starvation (Modlmeier *et al.*, 2013), parasites (Scharf *et al.*, 2012) or high temperatures (Molet *et al.*, 2017). (Jacquier *et al.*, 2021a) did not find any buffering effect of colony size on worker mortality, whatever the treatment. This suggests that the buffering effect of colony size in *Temnothorax nylanderi* is quite unstable and may depend on the measured traits and on the time at which the experiment is conducted. The absence of buffering effect of colony size under cadmium in our study could be explained by a higher sensitivity of large colonies to disturbances. Having more individuals could enhance the exposures to various stressors (pathogens, parasites, pollutions, etc.) and their transmission to nestmates. For example, in honey bees, larger colonies could be more sensitive to sublethal effects of pesticides and pathogens and undergo colony collapse disorder (Barron, 2015). When the disturbance is too

high, larger colonies could be more destabilized and collapse. Thus, social organisms could be more exposed and vulnerable to environmental stressors than solitary organisms. Finally, the positive relationship between colony size and the development duration of workers and males is consistent with the loss of per-capita productivity as a function of colony size, found in many ant species (reviewed in Kramer et al., 2014).

To conclude, we did not find any difference in cadmium tolerance between forest and city colonies, so we were not able to assess the relative contributions of social environment and larval intrinsic factors. Replicating this study with colonies collected at the end of winter (the period at which a differential response to cadmium was found between forest and city colonies) would be necessary to assess it. However, interestingly, we found phenotypic differences between forest and city colonies, independently of the treatment, with forest larvae having a higher emergence rate and turning into larger workers. These differences were independent of the social environment, i.e. workers, contrasting with previous studies on other social insect species (Pankiw, Tarpay, & Page, 2002; Linksvayer, 2006, 2007; Linksvayer *et al.*, 2011; Armitage *et al.*, 2011). However, regarding males, we found a slight effect of the social environment, suggesting a higher social control on the phenotype of sexuals than on that of workers. We suggest that more attention should be paid to the role of the social environment in phenotypic divergence. Since our studies only involved two sites and one species, the generalization of our results will require the assessment of additional locations and species.

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### **Author's contributions**

LJ and 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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### **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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# **Chapitre trois : Variation intra-annuelle des traits d’histoire de vie entre populations urbaines et forestières**

**Article 5 : accepté dans *Ecotoxicology***

**Cadmium tolerance in city and forest colonies is higher in spring than at the end of hibernation in the ant *Temnothorax nylanderi***

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## **Conflicts of interest**

The authors declare that they have no conflict of interest.

## **Availability of data and materials**

Data are provided with the manuscript in the supplementary information section.

## **Ethical approval**

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

### **Authors' contribution**

LJ designed the study, collected colonies, reared them, performed the experiments and statistical analyses, and wrote the manuscript. MM co-wrote the manuscript. CD contributed to statistical analyses and co-wrote the manuscript. All authors read and approved the final manuscript.

**Key words: trace metal, colony size, diapause, spring, winter**

### **Abstract**

Cities are usually contaminated with trace metals because of human activities. Depending on their concentration, trace metals can be toxic for organisms. A recent study showed that, in the ant *Temnothorax nylanderi*, city colonies are more tolerant to cadmium than forest colonies. However, because of annual variation in biological factors (e.g. body size, anti-stress protein production or trace metal accumulation rate), trace metal tolerance may vary over the year. We aimed at testing whether tolerance to cadmium (a trace metal) of colonies of *T. nylanderi* shows variation between two different seasons within the same year (winter and spring). We also assessed whether the better cadmium tolerance of city colonies was constant over these two different time points. We collected colonies at the end of their hibernation period (winter colonies) and several weeks after (spring colonies) from two different habitats (forest and city) to assess whether this response was consistent regardless of the environment. We exposed colonies to a cadmium or a control treatment for 61 days. We compared tolerance to cadmium between spring/winter and city/forest colonies by measuring several life history traits. We found that spring colonies tolerates cadmium better than winter colonies, and that city colonies have a higher tolerance to cadmium but only in spring. Although further studies with replicated pairs of city/forest habitats will be necessary to draw a generalizable conclusion, our study suggests that tolerance to trace metals can fluctuate along the yearly cycle.

### **Introduction**

Pollution is one of the main human-induced rapid environmental changes. The nature of pollutants is diverse: physical such as light or noise, or chemical such as plastic and trace metals. While physical pollutants are more or less spatially localized, chemical pollutants are more diffuse and found in all ecosystems, even in those that were once considered as pristine (Lenoir

et al., 2016). Pollution is not evenly distributed throughout the world, and some locations are more contaminated than others. Trace metal pollution is important in active and disused mining or industrial sites and also in cities (Foti et al., 2017; Roelofs et al., 2009). Some trace metals such as zinc are essential for life, but they can also have many deleterious effects on organisms at high concentration. For example, they increase oxidative damages (Sevcikova et al., 2011), impair embryonic development, and increase mortality rates (Gomot, 1998; Hutchinson and Sprague, 1986). Some populations of organisms living in heavily-contaminated sites have evolved better resistance to trace metal pollution (Jacquier et al., 2021a; Mouneyrac et al., 2003; Roelofs et al., 2009), for example through higher expression or turnover of metallothionein (a protein involved in trace-metals detoxification processes, Mouneyrac et al., 2003; Roelofs et al., 2009) or higher quantity of metal-rich granules that store heavy metals (Wallace et al., 1998).

Most of the studies investigating the response to trace metal exposure are carried out at a specific period of the year (Gomot, 1998; Malakar et al., 2009; Vesela and Vijverberg, 2007). However, organisms react differently to environmental changes and stress depending on the season (Bujan et al., 2020; Morgan and Morgan, 1993; Rabitsch, 1997). Especially, trace metal accumulation in organisms can differ among seasons (Cossa et al., 1980; Fialkowski et al., 2003; Morgan and Morgan, 1993; Rabitsch, 1997). For example, by sampling 30-50 workers from three different colonies every month for one year, Rabitsch (1997) showed that concentration of trace metals in the body of the workers of the red wood ant *Formica pratensis* is lower during spring and autumn (when the workers are the heavier), for five different collection sites (at different distances from a smelter site). In a mussel species, *Mytilus edulis*, the accumulation rate of trace metals was higher during spring because of reproductive activities, and larger individuals had lower trace metal concentration, in four different sites (Cossa et al., 1980). Therefore, variation in trace metal concentration can be impacted by the yearly seasonal variation of body mass. Trace metal concentration in the environment is also season-dependent, for example because of yearly seasonal variations in human activities or climate conditions (Kulshrestha et al., 2009; Li and Zhang, 2010). This may impact further trace-metal resistance in organisms, for example by changing their trace metal accumulation rate or by impacting the expression of trace-metal resistance genes. Metallothionein concentration can also vary across seasons independently of trace metal concentration (Gorbi et al., 2005; Oaten et al., 2017). Moreover, physiological processes accompanying the course of seasonal changes trigger the secretion of anti-stress proteins, including chaperones or metallothionein (Denlinger et al., 2001; Robert et al., 2016; Yocum, 2001). For example, winter



diapause triggers the secretion of anti-stress factors, such as heat shock protein (Yocum, 2001), immune protein (Robert et al., 2016), anti-oxidative protein (glutaredoxin, Popović et al., 2015) and/or metallothionein (Popović et al., 2015), which may ultimately impact the way individuals cope with stress related to trace metals. The impacts of variations in trace metal concentrations and in anti-stress factor secretion on the tolerance of organisms to trace metals remain largely untested.

Ants are good candidates to monitor the impacts of trace metals on wildlife (Grześ, 2010a). In ants, foragers are wingless and forage locally, and colonies are long-lived and sedentary, favoring the bioaccumulation of trace metals (Grześ, 2010a). Ants are eusocial organisms: they live in colonies where individuals perform tasks according to their caste (worker or queen), resulting in caste-specific contamination risks (Grześ, 2010a). However, ants seem to be relatively tolerant to trace metals, as they are able to maintain colonies in highly contaminated sites (Grześ, 2010a). The accumulation of trace metals in their body depends on several factors, such as caste, diet or season. For example, foragers accumulate more trace metals than queens or pupae in the field (Markert, 2008) and workers are less sensitive to zinc than larvae in *Myrmica rubra* in the laboratory (Grześ, 2010b). Accumulation also varies greatly among species because of different diets with honeydew-based regimes being much more subject to trace metal intake (Starý and Kubizňáková, 1987). The bioaccumulation of trace metals in some temperate ant species varies across seasons, with lower concentration during spring and autumn (Rabitsch, 1997), which may be due to individual mass variation as workers have more fat tissues during spring and autumn, when they forage actively. However, the simple measure of trace metal contents in individual body does not entirely reflect trace metal tolerance. There is still a lack of studies investigating variations of trace metal tolerance in ants depending on the annual season, especially after winter diapause (but see Rabitsch, 1997). Overwintering can be a particularly important phase of the colony life cycle as winter temperature can induce diapause and affect subsequent larval development (Gill et al., 2017; Kipyatkov et al., 1997).

In this paper, we compared colony tolerance to cadmium between city and forest populations collected just after the hibernation period (winter) and during the growing season (spring) in the ant *Temnothorax nylanderi*. This species is an interesting model because colonies nest in twigs and acorns above ground and are thus directly exposed to winter climatic conditions (Herbers, 1989). Interestingly, a previous study carried out in winter showed that city colonies of this ant are more tolerant to cadmium than their forest counterparts under common garden laboratory conditions (Jacquier et al., 2021a), regarding larval emergence rate, larval mortality rate and body size of emerging workers. Cadmium is a trace metal with toxic effects on

organisms, as it binds to the mitochondria leading to cell proliferation, apoptosis or production of Reactive Oxygen Species (ROS) , and interferes with DNA repair mechanisms, and with other essential metals such as calcium, zinc or iron (Martelli et al., 2006; Rani et al., 2014). Organisms exposed to cadmium undergo decreased hatching rate, extended or stopped development, or reduced body size (Cervera et al., 2004; Gomot, 1998; Malakar et al., 2009). Cadmium is found in high concentrations in active or disused mine sites, but also in cities, where it can be ten times more concentrated than in forests because of traffic and industrial activities (Foti et al., 2017). The better tolerance to cadmium of city colonies in *T. nylanderi* was found in three different locations in France (Jacquier et al., 2021a). However, this study was performed at one specific time of the year, at the end of winter, when colonies were just coming out of winter diapause, and larvae and workers had just finished diapause (Jacquier et al., 2021a). The aim of our study was to test whether (i) the response to cadmium was constant depending on the time point at which the study is conducted and (ii) the response to cadmium over different time points was constant between two different habitats, i.e. city and forest. On one hand, we could expect spring colonies to tolerate cadmium better than winter colonies as hibernation decreases the fat content and overall body mass of workers and larvae in winter colonies. On the other hand, we could expect winter colonies to tolerate cadmium better as winter diapause triggers the secretion of anti-stress proteins (for example, metallothionein or heat shock proteins) that may enhance trace metal tolerance (Denlinger et al., 2001; King and MacRae, 2015). Moreover, because of their chronic exposition to stress (e.g. heat, trace metal, human presence), city populations often have higher anti-stress gene expression (Cassone et al., 2014; Watson et al., 2017) that could be even more expressed after hibernation leading to a better cadmium tolerance than forest colonies in winter (as found in Jacquier et al 2021a). Whatever the underlying mechanism, city colonies are therefore expected to have a higher cadmium tolerance than forest colonies, especially in winter colonies at the end of hibernation. In order to assess colony tolerance to cadmium, we measured the impact of cadmium on different life history traits depending on the season: worker mortality rate, larval mortality rate, larval paused rate and size of laboratory-born workers.

## **Material and Methods**

### ***Colony collection and rearing***

The small acorn ant *Temnothorax nylanderi* is a common species in Europe, found in both city and rural habitats. Colonies consist of few hundreds individuals and nest in pieces of dead wood on the ground (twigs, acorns, chestnuts, etc.), making them easy to collect. Collection

sites were a city park of Paris (Jardins Ecologiques, 48°50'59.684"N 2° 21' 40.385"E) and a forest 50km away from Paris (Chantilly, 49°10'59.8"N 2°28'43.6"E). These two sites have deciduous trees (mainly ashes, hazelnut and oaks), and the ground is covered with ivy and leaf litter. The city park is about 1 hectare, and it is surrounded by unsuitable areas for nesting. The forest is approximately 6300 hectares. The soil of the city wood is more contaminated with trace metals, such as cadmium, than the soil of the forest (Foti et al., 2017).

We quantified the response of city and forest colonies to cadmium at two different times of the year: just after the winter diapause (winter colonies), and several months after colonies had come out of diapause (spring colonies) and we therefore collected colonies at two different times of the year. The first one occurred between 8<sup>th</sup> and 16<sup>th</sup> March 2020 (113 ‘winter colonies’) and the second one between 1<sup>st</sup> and 4<sup>th</sup> June 2020 (129 ‘spring colonies’). Different colonies were collected for March and June. Colonies were brought back to the laboratory and put in 11.5x11.5x5.5cm plastic boxes. Boxes were then kept in a climatic chamber (reference CTS TP10/600) at 22-27°C 12-12 light cycles under 70% hygrometry. The lid of the boxes was pierced for air circulation. Artificial nests were made of two microscope glasses separated by a 2mm thin moss chamber. Artificial nests were covered by a dark plastic sheet to keep colonies in the dark. Colonies were provided ad libitum water in a small tube plugged with cotton. We discarded 54 colonies with no queen, 16 colonies with more than one queen, and 4 colonies with pale-yellow cestode-infected workers. We kept 70 winter colonies (37 city colonies, 33 forest colonies) and 85 spring colonies (46 city colonies, 39 forest colonies). We let colonies acclimatize to the laboratory conditions for about three weeks.

Before the start of the experiments, we removed all eggs and larvae from the colonies except for the second instar larvae, which correspond to an early developmental stage. We counted workers (‘colony size’, ranging from 11 to 225 in winter colonies and from 6 to 150 in spring colonies) and second instar larvae (ranging from 4 to 33 in winter colonies and from 2 to 29 in spring colonies).

### ***Experimental design***

We started the experiment on April 6<sup>th</sup> 2020 for winter colonies, and on June 24<sup>th</sup> 2020 for spring colonies. In the climatic chamber, we exposed colonies to two different treatments for 61 days: control or cadmium. Spring colonies were smaller than winter colonies (48 workers in average against 99 workers in average,  $F_{145,146}=73.21$ ,  $P<10^{-13}$ ). For a given season, colony

size distribution was the same between city and forest habitats ( $F_{143,146}=0.17$ ,  $P=0.68$ ). We evenly assigned colonies of a given season to control or cadmium treatment so that the colony size distribution was the same between the two treatments within each season (anova,  $F_{145,146}=0.26$ ,  $P=0.60$ ). We removed every newly laid egg every week. Sixty-one days correspond to the maximal duration of larvae development from second-instar to a newly emerged worker, at 22-27°C, so any larva that would have escaped the egg removal protocol could not have reached adulthood before the end of the experiment. Colonies were fed every other day with a mixture of yoghurt, dried crickets, diluted honey and vitamins, with 100µg of cadmium per g of food (cadmium treatment) or without cadmium (control treatment). Food was provided in excess. Cadmium concentration was set to this value as Jacquier et al., 2021a found that it led to approximately 50% worker mortality after a 2 months exposure.

### ***Response variables***

We counted dead workers every week and removed corpses from the boxes. We computed the “worker mortality rate” as the ratio between the total number of dead workers and the initial number of workers in each colony. We also counted the remaining number of larvae every other day. The “larval mortality rate” was computed as the ratio between the total number of dead larvae (larvae missing at the end of the experiment, but that had not developed into adult) and the initial number of larvae. The “emergence rate” was computed as the ratio between the total number of emerged adults within a colony (workers, males and gynes) at the end of the experiment and the initial number of larvae. As observed in Jacquier et al (2021a), some larvae were still alive at the end of the experiment but apparently did not grow during the entire course of the experiment and had apparently paused their development. The “larval pause rate” was therefore computed as the ratio between the number of paused larvae and the number of initial larvae.

We measured newly born workers (subsequently called “lab-workers”), which are easily recognizable by their typical pale orange color. We collected lab-workers, froze them, and stored them in 90° ethanol. At the end of the experiment, they were beheaded, and their head were stuck on double-side tape. Heads were photographed using a stereomicroscope (Zeiss Discovery.V12). Head width was measured as the eye-to-eye (posterior-side) length, a good proxy of worker size (Araujo and Tschinkel, 2010). Measures were performed with ImageJ (<https://imagej.nih.gov/ij/>; Abramoff et al., 2004). Some males and gynes emerged during the experiment, but only in winter colonies. Sexualls were probably not produced in spring colonies because they only develop from larvae that underwent winter temperature (Vargo and Passera,

1992). In the present study, we only left young stage 2 larvae in colonies, so the larger and older larvae that had overwintered were removed from spring colonies. Therefore, we chose to not present results regarding the size of males and gynes.

### ***Statistical analyses***

We used R v3.6.2 (Team, 2018) for all subsequent statistical analyses.

Six queens died over the course of the experiment (four for forest winter colonies, and two for forest spring colonies). These colonies were removed from subsequent analyses. We used models assessing the effects of treatment (control or cadmium), habitat (city or forest) and season (winter or spring) and their second and first-order interactions on four response variables (worker mortality rate, larval mortality rate, larval paused rate and head width of lab-workers). We also included colony size as a covariate and the interaction between colony size and treatment, as colony sizes differed between spring and winter colonies. We expected a differential effect of treatment depending on the season and habitat, that is a Treatment : Habitat : Season second-order interaction.

Two distinct models were used for statistical analyses. 1/ Regarding colony-level variables (worker mortality rate, larval mortality rate, emergence rate, larval pause rate), we used generalized linear models (GLMs, <https://CRAN.R-project.org/package=citation>) with quasibinomial distribution. Indeed these variables were based on pairs of counts (e.g. worker mortality rate was input as the number of dead workers and the number of surviving workers). The quasibinomial distribution was used to incorporate over-dispersion. 2/ Regarding the individual-level variable (head width of lab workers), we used mixed models with colony as a random factor to control for pseudoreplication (Pinheiro et al., 2016) as several measurements were made within each colony. In both cases, the complete initial models contained all independent variables (treatment, habitat, season) and their interactions (second-order and first-order), as well as the independent variable colony size and its first-order interaction with treatment. We obtained a Minimum adequate model (MAM) using a backward stepwise procedure. We first tested the second-order interaction, then first-order interactions, and finally simple variables. When the P-value was higher than 0.05, the tested independent variable or interaction was removed from the model, until no variable or interaction could be removed (i.e. the MAM had been obtained). Final P-values were obtained by adding or removing an dependent variable from this MAM and comparing models using a log likelihood ratio test. When a first-order interaction was found, we used the emmeans package (Lenth, 2020) on the MAM to test for the effect of each independent variable separately. Analyses were performed using the lme4 package (Pinheiro et al., 2016).

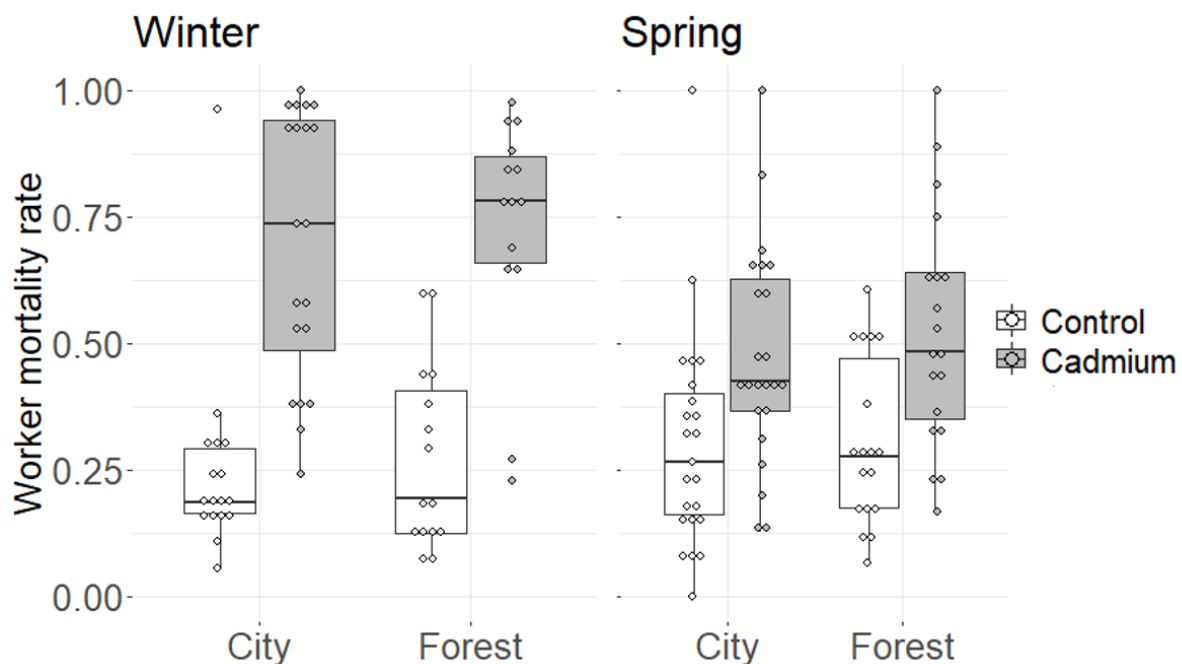
We visually checked for homoscedasticity of data and normal distribution of the model residuals for mixed models using plot functions. No transformation of data was required.

## Results

Out of the 66 winter colonies and 83 remaining spring colonies, we collected a total of 367 workers, one male and zero gyne for spring colonies, and 437 workers, 146 males and 24 gynes for winter colonies. Gynes were produced by control forest colonies only. Due to this lack of males and gynes in spring colonies, we could not perform statistical analyses regarding males and gynes. Details about the numbers of colonies, lab-workers and males for each season, habitat and treatment are given in Table S1.

### Worker mortality rate

As expected, worker mortality significantly increased in colonies treated with cadmium ( $z=-9.58$ ,  $P<0.0001$  Table 1, Fig 1). The effect was stronger for winter colonies than for spring colonies whatever the habitat (no Treatment : Season : Habitat interaction,  $F_{145,141}=0.21$   $P=0.93$ ; significant Treatment : Season interaction  $F_{147,145}=17.82$   $P<10^{-6}$ , Table 1, Fig 1). Worker mortality decreased with colony size in the control, but not under cadmium (Colony size : Treatment interaction  $F_{140,139}=14.31$   $P=0.00022$ , Table 1, Fig 2). Worker mortality rate under cadmium was high whatever the colony size, so that the higher mortality rate of winter colonies under cadmium is not due to their higher mean colony size.



**Fig 1** Comparison of worker mortality rate between spring and winter colonies from city and forest. For each box, median and quartiles are shown, as well as colony-level data points. White: control treatment, grey: cadmium treatment

### *Larval mortality rate*

The effect of cadmium on larval mortality rate significantly differed between seasons and habitats (significant Treatment : Habitat : Season interaction,  $F_{141,140}=5.90$   $P=0.016$ , Table 2). We first tested for a season-specific effect of cadmium within each habitat. Regarding city colonies, cadmium increased the larval mortality rate more in winter than in spring (significant Treatment : Season interaction,  $F_{77,78}=7.20$   $P=0.0089$ , Fig 3). Regarding forest colonies, this effect was reversed, i.e. cadmium increased the larval mortality rate more in spring than in winter (significant Treatment : Season interaction,  $F_{60,61}=5.32$ ,  $P=0.024$ , Fig 3).

We then tested for a habitat-specific effect of cadmium within each season. Regarding winter colonies, cadmium increased larval mortality rate (treatment effect,  $F_{64,65}=38.97$   $P<10^{-7}$ , Table 2, Fig 3) in a similar way in the two habitats (no significant Treatment : Habitat interaction  $F_{64,62}=0.17$ ,  $P=0.84$ , Table 2 Fig 3). Regarding spring colonies, cadmium also increased larval mortality rate (Treatment effect,  $z=4.75$ ,  $P<0.0001$  Table 2, Fig 3), but city colonies tolerated cadmium better than forest colonies (significant Treatment : Habitat interaction,  $F_{79,78}=7.40$   $P=0.0080$ , Table 2, Fig 3). Colony size did not impact larval mortality rate (no significant Treatment : Colony size interaction, no significant Colony size effect, Table 2).

### *Larval pause rate*

Some larvae did not grow during the entire course of the experiment. This allowed us to compute a larval pause rate, that should not be confused with winter diapause. The effect of cadmium on larval pause rate significantly differed between seasons and habitats (significant Treatment : Habitat : Season interaction,  $F_{141,140}=5.61$   $P=0.019$ , Table 2). We tested for a season-specific effect of cadmium within each habitat. City colonies increased their larval pause rate in response to cadmium more in winter than in spring (significant Treatment : Season interaction,  $F_{77,78}=12.80$   $P=0.00060$ ). Forest colonies increased their larval pause rate when treated with cadmium in winter, but they decreased it in spring (significant Treatment : Season interaction,  $F_{60,61}=41.85$   $P<10^{-7}$ ).

We tested for a habitat-specific effect of cadmium within each season. In winter colonies, the larval pause rate was significantly higher under cadmium (Treatment effect,  $F_{64,63}=52.76$   $P<10^{-9}$ , Table 2, Fig 3) in a similar way in city and forest colonies (no Treatment : Habitat interaction,

$F_{62,61}=0.58$   $P=0.45$ , Table 2, Fig 3). Interestingly, in spring colonies, city and forest colonies responded in an opposite direction to cadmium, with higher larval pause rate in city colonies under cadmium, but lower pause rate in forest colonies under cadmium (significant Treatment : Habitat interaction  $F_{79,78}=7.99$   $P=0.0059$  Table 2, Fig 3). Under control, larval pause rate decreased with colony size. Under cadmium, larval pause rate was high whatever the colony size (significant Treatment : Colony size interaction,  $F_{140,139}=4.56$   $P=0.034$ , Table 2, Fig 2).

### Emergence rate

Cadmium significantly decreased the emergence rate of larvae (Treatment effect,  $z=-10.03$   $P<0.0001$ ) with a stronger effect in winter colonies (Treatment : Season interaction,  $F_{143,144}=35.42$   $P<10^{-7}$  Table 2, Fig 3) whatever the habitats (no significant Treatment : Habitat : Season interaction,  $F_{143,140}=0.13$   $P=0.94$ , Table 2, Fig 3). Emergence rate slightly decreased with colony size whatever the treatment (significant colony size effect,  $F_{143,144}=19.17$   $P<10^{-4}$  Table 2).

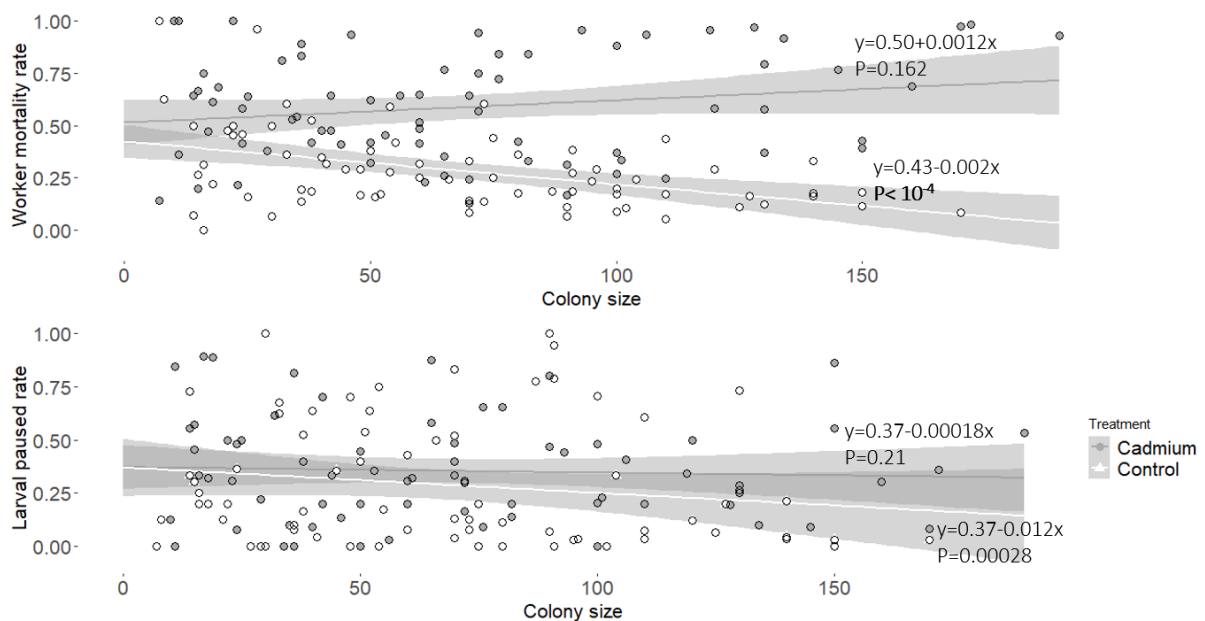
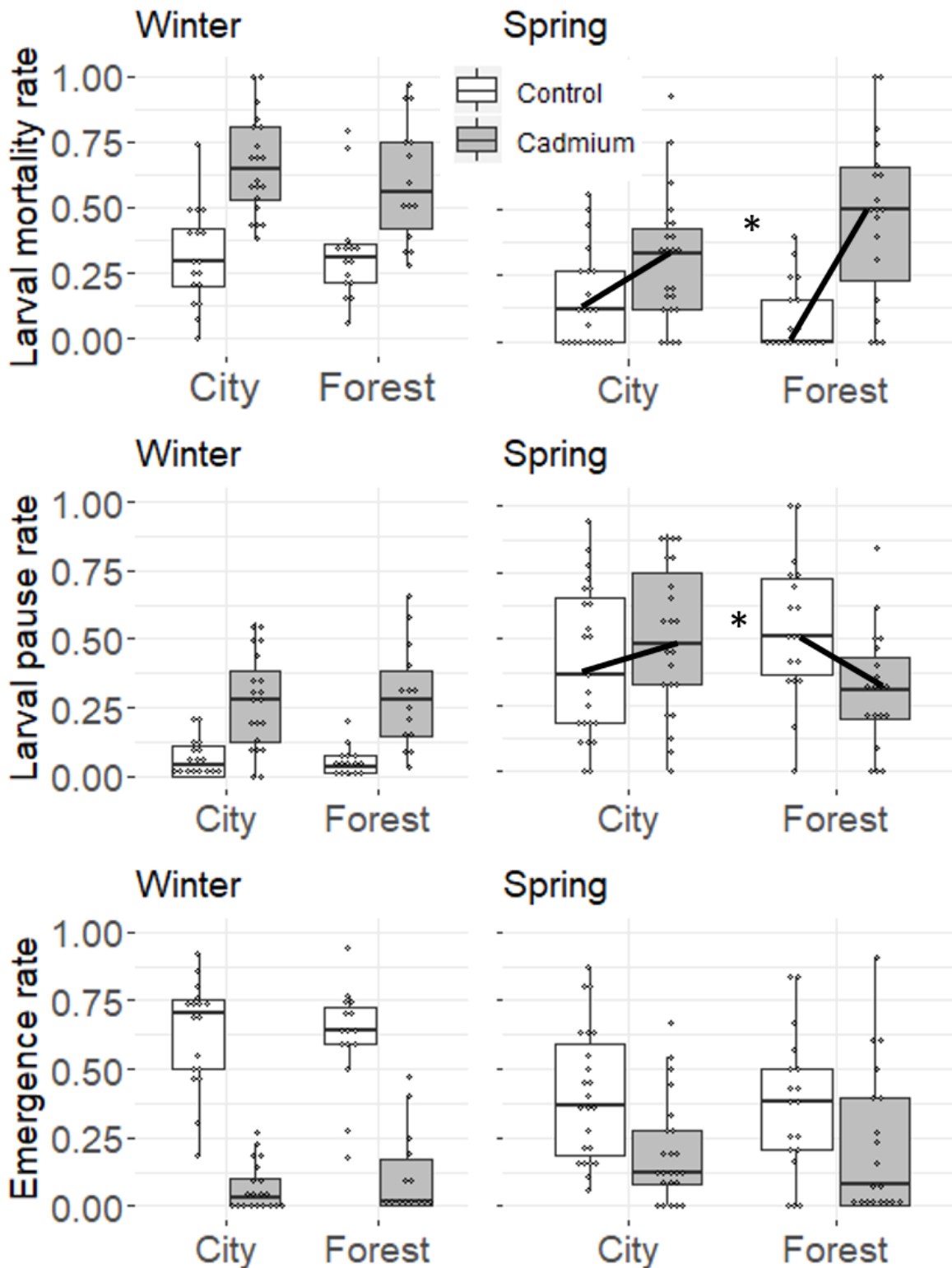


Fig 2: Worker mortality rate and larval pause rate depending on colony size and treatment (grey circles: cadmium, white circles: control). For each graph, line equation is provided as  $y=\text{intercept}+\text{slope}\cdot x$ . Ninety-five percent confidence intervals are shown as grey zones. P-values correspond to the colony size effect within each treatment.



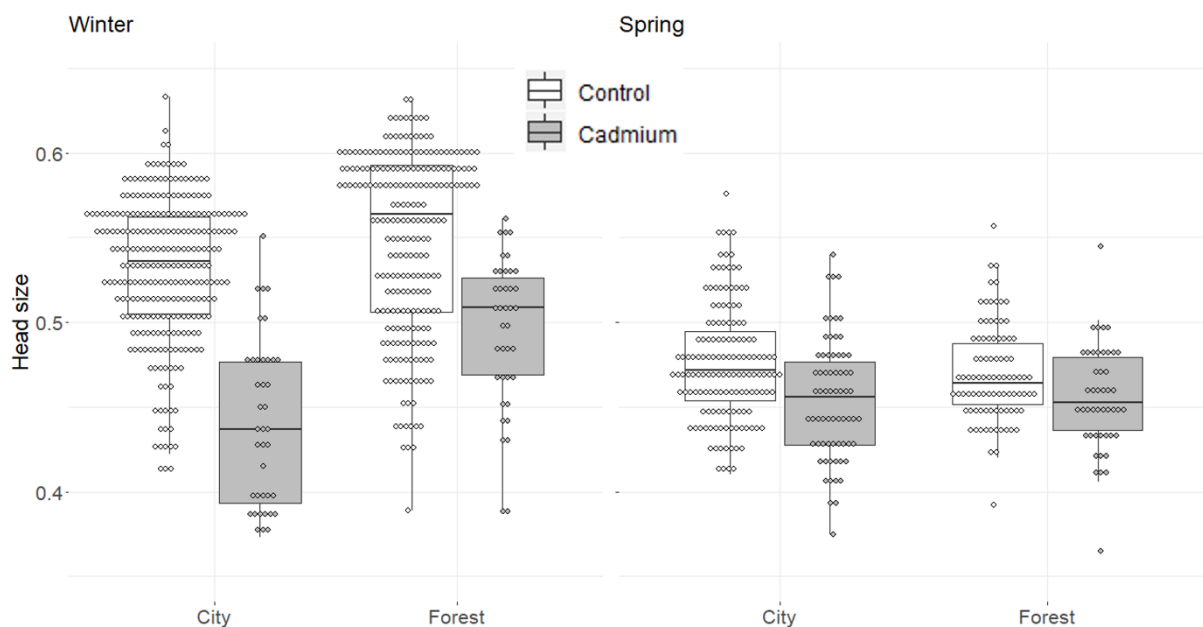


**Fig 3** Comparison of larvae mortality rate, larval paused development rate and emergence rate between spring and winter colonies from city and forest. For each box, median and quartiles are shown, as well as colony-level data points. White: control treatment, grey: cadmium treatment. Significant Treatment : Habitat interactions are shown by \* and depicted by black lines. All variables for which a significant Treatment : Habitat first-order interaction was found

were also subject to a significant Treatment : Habitat : Season second-order interaction (not shown for clarity, but visible by comparing the left 'Winter' and right 'Spring' panels).

#### *Size of lab-workers*

The size of lab-workers decreased under cadmium ( $t_{130}=8.84$   $P<0.0001$ , Table 1, Fig 3) but the effect of cadmium was less pronounced in spring colonies than in winter colonies (significant Treatment : Season,  $\chi_1=24.11$   $P<10^{-6}$ ). Moreover, the effect of cadmium was more pronounced during winter in city colonies than in forest colonies, but this differential effect faded in spring colonies (marginally significant Treatment : Season : Habitat, graphical tendencies, marginally significant Treatment : Habitat interaction,  $\chi_1=3.42$   $P=0.064$ , Table 1, Fig 4). Colony size had no effect on the size of lab-workers ( $\chi_1=2.43$   $P=0.12$ , Table 1).



**Fig 4** Comparison of lab-workers head size (mm) between spring and winter colonies from city and forest. For each box, median and quartiles are shown, as well as individual-level data points. White: control treatment, grey: cadmium treatment

**Table 1.** Effects of the different independent variables (season, treatment, habitat, colony size) and their interactions on the worker-related variables, i.e. worker mortality rate and size of lab-workers. When a first-order interaction was significant, we used the emmeans package to assess the separate effects of the variables involved in the interaction. In that case, the t ratio is presented. NA means that there was no biological meaning in testing the effect because of a significant higher-level interaction (first-order). Significant P-values are shown in bold.

	Worker mortality rate	Size of lab-workers
Treatment : Habitat : Season	F <sub>145,141</sub> =0.21 P=0.93	X <sub>2</sub> =4.81 P=0.090
Treatment : Habitat	F <sub>145,143</sub> =0.35 P=0.70	X <sub>1</sub> =3.42 P=0.064
Habitat : Season	F <sub>145,143</sub> =0.40 P=0.67	<b>X<sub>1</sub>=7.37</b> <b>P=0.0066</b>
Treatment : Season	<b>F<sub>147,145</sub>=17.82</b> <b>P&lt;10<sup>-6</sup></b>	<b>X<sub>1</sub>=24.11</b> <b>P&lt;10<sup>-6</sup></b>
Treatment	<b>z=-9.58</b> <b>P&lt;0.0001</b>	<b>t<sub>130</sub>=8.84</b> <b>P&lt;0.001</b>
Habitat	F <sub>145,144</sub> =0.70 P=0.40	<b>t<sub>100</sub>=2.28</b> <b>P=0.02</b>
Season	<b>z=5.84</b> <b>P&lt;0.0001</b>	<b>t<sub>123</sub>=6.23</b> <b>P&lt;0.001</b>
Colony size : Treatment	<b>F<sub>140,139</sub>=14.31</b> <b>P=0.00022</b>	X <sub>1</sub> =1.83 P=0.17
Colony size	NA	X <sub>1</sub> =2.43 P=0.12

**Table 2.** Effects of the different independent variables (season, treatment, habitat, colony size) as well as their interactions on the larvae-related response variables (larval mortality rate, emergence rate and larval paused rate). When a significant Treatment : Habitat : Season (T : H : S) interaction was found, the subsequent statistical analyses are shown for data splitted by season (left side of the column: winter, right side: spring). When a significant first-order interaction involving a discrete variables (Treatment, Season, Habitat) was found, we used the emmeans package to assess the separate effects of the variables involved in the interaction. In that case, the z ratio is presented. NA means that there was no biological meaning in testing the effect because of a significant higher-level interaction (first-order or second-order). Significant P-values are shown in bold.

	Larval mortality rate		Emergence rate	Larval pause rate	
	Winter	Spring		Winter	Spring
T : H : S	<b>F<sub>141,140</sub>=5.90</b> <b>P=0.016</b>		F <sub>143,140</sub> =0.13 P=0.94	<b>F<sub>141,140</sub>=5.61</b> <b>P=0.019</b>	
Treatment :	F <sub>64,62</sub> =0.17, P=0.84	<b>F<sub>79,78</sub>=7.40</b> <b>P=0.0080</b>	F <sub>143,142</sub> =0.35 P=0.55	F <sub>62,61</sub> =0.58 P=0.45	<b>F<sub>79,78</sub>=7.99</b> <b>P=0.0059</b>
Habitat	NA		F <sub>143,142</sub> =0.011 P=0.91	NA	
Habitat : Season	NA		<b>F<sub>143,144</sub>=35.42</b> <b>P&lt;10<sup>-7</sup></b>	NA	
Treatment :	<b>F<sub>64,65</sub>=38.97,</b> <b>P&lt;10<sup>-7</sup></b>	<b>z=4.75</b> <b>P&lt;0.0001</b>	<b>z=-10.03</b> <b>P&lt;0.0001</b>	<b>F<sub>64,63</sub>=52.76</b> <b>P&lt;10<sup>-9</sup></b>	<b>z=1.87</b> P=0.061
Treatment	F <sub>64,63</sub> =0.072 P=0.78	z=0.24 P=0.81	F <sub>143,144</sub> =1.11 P=0.29	F <sub>63,64</sub> =0.044 P=0.83	<b>z=2.01</b> <b>P=0.040</b>
Habitat	NA		<b>z=-2.38</b> <b>P=0.017</b>	NA	
Season	F <sub>140,139</sub> =1.66 P=0.20	F <sub>140,139</sub> =1.43 P=0.55		<b>F<sub>140,139</sub>=4.56</b> <b>P=0.034</b>	
Colony size :	F <sub>140,141</sub> =0.41 P=0.52	<b>F<sub>143,144</sub>=19.17</b> <b>P&lt;10<sup>-4</sup></b>		NA	
Colony size	NA				

## Discussion

As expected, high cadmium concentration had a negative effect on worker and larval mortality rates, emergence rate of lab-workers and head size of lab-workers, as described in other studies performed in the laboratory on invertebrates (snail: Gomot, 1998; ants: Jacquier et al., 2021a). This negative effect was found for both forest and city habitats, as well as for both winter and spring seasons. First, we showed that spring colonies tolerate cadmium better than winter colonies, with lower increase in worker mortality rate, lower decrease in worker emergence rate and lower reduction of lab-worker size when exposed to cadmium. This suggests that the putative secretion of anti-stress factors during cold winter (e.g. heat shock proteins or detoxification enzymes, see Robert et al., 2016; Yocum, 2001) does not improve robustness against cadmium in winter. Second, in contrast with what we expected based on other studies investigating tolerance to trace metal in city populations (Andrew et al., 2019; Jacquier et al., 2021a; Mireji et al., 2008) we did not find any differential response to cadmium between city and forest colonies in winter colonies. We found a higher tolerance to cadmium of city colonies, but only in spring colonies regarding larval mortality rate. We also found a differential response to cadmium in spring colonies regarding larval pause rate, which increased under cadmium exposure in city colonies but decreased in forest colonies. Spring colonies also had a higher larval pause rate than winter colonies, under both control and cadmium.

Many ecotoxicological studies report seasonal variation in trace metal accumulation in organisms (Cossa et al., 1980; Morgan and Morgan, 1993; Rabitsch, 1997). For example, Cossa et al., 1980 showed that in the mussel *Mytilus edulis*, the trace metal accumulation rate increased in all the assessed sites from spring to summer (estuary and gulf). Morgan and Morgan (1993) monitored cadmium and lead concentrations in two earthworms species every months for 13 months and found that cadmium concentration was at its lowest and the lead concentration at its highest in summer (July), which corresponds to the diapause period of one the two studied species (the other one does not go through diapause). Rabitsch (1997) showed that in worker ants of *Formica pratensis*, trace metal accumulation varied consistently over eight months and in five sampling sites, with the lowest metal concentration found in spring and autumn workers. Such variation can be linked with annual variation in the environmental availability of trace metals. Hence, higher trace metal accumulation in a given period could just reflect a higher trace metal concentration in the environment, but not necessarily a better tolerance to these trace metals. In our study, the lower worker mortality rate in spring could be due to a heavier body mass of workers. Many studies showed a positive link between mean body size and trace metal tolerance because of lower metabolism rate per unit in larger individuals (Durou et al.,

2005; Grześ, 2010b; Vesela and Vijverberg, 2007). Ant body mass varies across seasons, with body mass reaching its maximum during spring or autumn, as workers forage actively and accumulate fat reserves, whereas towards the end of hibernation, workers have depleted fat reserves (Blanchard et al., 2000; Rabitsch, 1997; Tschinkel, 1993). Because of the lower worker mortality rate of workers, more of them are available to rear spring larvae, which could explain the higher emergence rate and larger emerging lab-workers. To our knowledge, our study is the first to address how tolerance to a trace metal differs between two points along the yearly cycle specifically in terms of life history traits, while other studies only assessed bio-accumulation. The better cadmium tolerance of spring colonies could be caused by differences in colony size between spring and winter colonies, as colony size impacts stress buffering in ant (Crall et al., 2019; Kaspari and Vargo, 1995; Naug, 2009). However, we did not find any effect of colony size on larval mortality rate nor on lab-worker size. Emergence rate decreased with colony size in our study, but treatment affected colonies similarly whatever their size. As the emergence rate in winter colonies under control condition was higher than in spring colonies, it is unlikely that lower spring colony size could explain the better tolerance against cadmium in spring. Interestingly, we found that the effect of colony size differed between control and cadmium treatments for worker mortality rate and larval paused rate. Worker mortality rate decreased with colony size under control but increased with colony size under cadmium. This suggests a limit to colony stress-buffering under very stressful conditions. It also suggests that winter colonies had a higher worker mortality rate than spring colonies under cadmium because they were larger. Larval pause rate decreased with colony size under control, but barely varied with colony size under cadmium. Therefore, it is unlikely that the higher larval pause rate under cadmium in spring colonies is due to their lower colony size.

The larval pause rate varied depending on both season and habitat. This rate was much higher in spring colonies than in winter colonies, even in control treatment, but it was also the only measured trait that showed opposite patterns of response to cadmium between city and forest spring colonies. The paused larval development observed in our experiment may correspond to what is called diapause, i.e. “a state of arrested growth or development and prolonged hypometabolism associated with various stressful challenges” (Popović et al., 2015). In some ant species, diapause is controlled by the social environment. In *Myrmica rubra*, worker control over initiation or termination of larval diapause depends on their physiological state: spring workers can trigger diapause termination whereas autumn workers cannot (Brian, 1955). In contrast, in *Leptothorax acervorum* (closer to the *Temnothorax* genus than *Myrmica*, Prebus, 2017), larvae seem to control queen diapause (Kipyatkov et al., 1997). In our case, we can

hypothesize that spring city larvae initiate or maintain their developmental pause in response to stress caused by cadmium, and that spring forest larvae lose this ability because stress is too high. Winter colonies may not have this problem as they just ended winter diapause, and they may therefore keep the ability to pause the development of larvae efficiently in both city and forest habitats. Another hypothesis is that city and forest workers differ in their ability to respond to larval stimuli; because city colonies are exposed to higher cadmium concentration than forest colonies in nature ( $2.45\text{mg}\cdot\text{kg}^{-1}$  in city wood soil vs  $0.30\text{mg}\cdot\text{kg}^{-1}$  in forest soils, see Foti et al 2017), city workers could maintain control over larval pause despite high levels of cadmium, but forest spring workers could become incapacitated by cadmium stress. A cross-fostering experiment would be useful to test these two alternative hypotheses. However, it is important to note that other stressors than trace metals could differ between rural and city sites and be responsible of the observed difference, but we did not specifically test them in this study. Overall, the higher rate of paused larvae in city colonies could be an adaptive response to cadmium; paused larvae avoid feeding on cadmium and they may resume their development once cadmium exposure is over, as shown in other studies (Aránguiz-Acuña and Serra, 2016; Oskina et al., 2019). This may also explain the differential larval mortality rate in spring colonies, with lower mortality in city colonies because of more paused larvae. However, all those hypotheses remained highly speculative and would deserve further investigations to confirm or infirm this.

We expected city colonies to tolerate cadmium better, whatever the season. In spring, we found that city colonies tolerate cadmium better than forest colonies, with a lower larval mortality rate, as found in a previous study (Jacquier et al., 2021a). However, we did not find a better cadmium tolerance of city colonies regarding emergence rate and lab-worker size, in contrast with Jacquier et al. (2021a). We even found that forest colonies were marginally more tolerant than city colonies as their lab-worker size decreased less when exposed to cadmium. We found a differential response to cadmium for the larval pause rate, with more paused larvae in city colonies but less paused larvae in forest colonies when treated with cadmium. Regarding winter colonies, in contrast with Jacquier et al (2021a), we found that forest and city winter colonies did not exhibit any differential response to cadmium whatever the life history traits measured. One possible explanation is that 2019/2020 winter was unusually warm, i.e.  $2^{\circ}\text{C}$  warmer relative to 2017/2018 and 2018/2019 winters (November 2017 to March 2018, <https://www.infoclimat.fr/stations-meteo/analyses-mensuelles.php>). Winter temperature impacts individual and colony survival. For example, in the ant *Lasius niger*, warm winters enhance worker survival (Haatanen et al., 2015), as found in other insect species (Takeda et al.,

2010). On the other hand, warm winters decrease winter survival in the boreal wood ant *Formica aquilonia* and other insect species (Radchuk et al., 2013; Sgolastra et al., 2011; Stuhldreher et al., 2014), maybe because metabolism remains high (Hahn and Denlinger, 2011; Sinclair, 2015). In the *Temnothorax* genus, colonies are directly exposed to weather conditions during winter because they nest above-ground (Herbers, 1989). A warm winter may have disturbed physiological processes in city and/or forest colonies, leading to low cadmium tolerance and a lack of city/forest differential response.

To conclude, our study suggests that the seasonal context of studies is of high importance, even though additional replicates would be needed to generalize our findings. We found that, for one specific area (Paris), spring colonies tolerate cadmium better than winter colonies. We also found that differential tolerance to trace metal between city and forest ant colonies is not detectable during winter, but it is detectable in spring. The absence of differential response to cadmium during winter 2020 could be due to a warmer winter, as Jacquier et al. (2021b, 2021a) found a differential response to cadmium when working on the exact same populations but during colder winters or under cold laboratory conditions (2°C colder in mean from November 2017 to March 2018, Jacquier et al., 2021b, 2021a). Seasonality impacts some life history traits, in our case the response to a trace metal, but more studies are needed as we only used two time points (two different seasons) within the same year. Nevertheless, experiments assessing responses to environmental changes should avoid focusing on a single time point but instead incorporate the whole yearly life-cycle of the model organism. Repeating the experiment at different times of the year could highlight season-dependent response to a given stressor.

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# Conclusion

L'objectif majeur de cette thèse est de déterminer comment une espèce eusociale (la fourmi *Temnothorax nylanderi*) répond aux changements environnementaux, à travers l'exemple de la réponse à l'urbanisation. Le premier chapitre a pour objectif de déterminer s'il existe des traits différenciés entre populations urbaines et forestières. Le deuxième chapitre s'intéresse aux modes de détermination de ces différences entre populations, avec une emphase sur le rôle de l'environnement social dans la détermination du phénotype. Enfin, le dernier chapitre a pour but de déterminer si les variations annuelles dans les dynamiques de colonie ont un impact sur la divergence de traits entre populations urbaines et forestières.

## **1- La divergence des traits d'histoire de vie entre populations urbaines et rurales met en évidence un filtre urbain s'exerçant à l'échelle des populations**

Les études portant sur l'effet de l'urbanisation sur les espèces eusociales à l'échelle populationnelle se sont en grande partie intéressées à l'effet de l'urbanisation sur les traits liés à la réponse à la chaleur, tels que la modification de résistance thermique en milieu urbain ou des changements de phénologie (Angilletta et al., 2007; Chick et al., 2019; Diamond et al., 2017, 2018). Notre premier chapitre a permis de mettre en évidence que d'autres traits sont également impactés. Le premier, la meilleure tolérance aux métaux traces des colonies urbaines à travers l'exemple du cadmium, a été choisi sur la base de données de différences de pollution entre environnements urbains et non-urbains (plus pollué en milieu urbain en général), et avait jusqu'à ce jour été assez peu étudié. L'autre trait étudié, portant sur le comportement (agression et fourragement), a été choisi sur la base des nombreuses études mettant en évidence une différenciation comportementale des populations urbaines par rapport à leurs homologues ruraux, phénomène bien documenté chez les vertébrés mais assez peu chez les insectes sociaux.

### **a- Les populations urbaines tolèrent mieux les métaux traces**

Notre première expérience portant sur la tolérance aux métaux traces a mis en évidence une meilleure tolérance des populations urbaines au cadmium par rapport à leurs homologues

forestières (figure 1) : les larves issues de colonies urbaines et élevées par des ouvrières urbaines ont subi une moins grande baisse de leur taux d'émergence et une moindre diminution de taille adulte que les larves forestières. Le cadmium est un métal trace aux nombreux effets négatifs sur les organismes, dont la concentration est souvent plus importante en ville qu'en forêt (Foti et al., 2017; Meillère et al., 2016). La meilleure tolérance et/ou l'adaptation aux métaux traces de populations d'arthropodes (collemboles, fourmis) vivant en milieu très pollué (anciens sites miniers ou d'industrie lourde par exemple) a déjà été documentée (Grzes, 2010; Posthuma and Van Straalen, 1993; Roelofs et al., 2009). Cependant, ce genre d'étude n'avait jamais été effectué sur des arthropodes des milieux urbains, malgré la multiplicité des polluants y étant présents (cadmium, plomb, cuivre, zinc... Foti et al., 2017; Meillère et al., 2016). La tolérance au cadmium documentée dans notre étude pourrait résulter soit d'une meilleure tolérance au cadmium, soit d'une meilleure tolérance généralisée à tous les métaux traces, appelée co-tolérance (Lucas et al., 2013; Tlili et al., 2011). Une autre hypothèse serait que les populations urbaines seraient plus capables de répondre à des stressseurs multiples de natures différentes. En effet la température d'élevage utilisée ici (22-27°C) est stressante pour cette espèce (15-20°C habituellement, Molet et al., 2017). En conditions contrôle, les colonies forestières comme les urbaines pourraient être capables de résister à ce seul stress, mais pas en conditions de traitement au cadmium où la superposition des stressseurs (température + cadmium) pourrait mener à un effondrement des colonies forestières, incapables de faire face à une multiplication des stressseurs. Des phénomènes de co-tolérance à des stressseurs multiples ont déjà été documentés chez quelques espèces (algues : Bozhkov et al., 2010, bactéries : Díaz-Raviña et al., 1994, tabac : Kumar et al., 2012) , mais restent assez rares. Il serait intéressant de tester si les populations urbaines deviennent effectivement tolérantes à des stressseurs multiples, en variant les types de stressseurs et leur combinaison. Le caractère adaptatif des moins grandes variations dans les traits d'histoire de vie des colonies urbaines en réponse au cadmium reste également à démontrer, la fitness des colonies n'ayant pas été mesurée en laboratoire. Cependant, une meilleure survie des larves est probablement avantageuse car elle conduit à une plus grande production d'individus, qu'ils soient sexués ou non, ce qui augmente la fitness des colonies (la quantité de sexués produits augmente avec le nombre d'ouvrières dans la colonie, Cole, 2009). Le caractère adaptatif de la moins grande perte de taille des ouvrières produites reste beaucoup plus hypothétique, des études ayant montré une absence d'effet de la taille ou de la diversité de taille sur la fitness des colonies de *T. nylanderii* en conditions semi-naturelles (Honorio et al., 2020, 2021). Une hypothèse possible est que des individus plus grands permet de diminuer l'impact du cadmium sur ces individus, les métaux traces ayant en général un

impact plus fort sur les petits individus du fait de leur taux métaboliques plus élevés (Durou et al., 2005; Vesela and Vijverberg, 2007).

La réponse que nous avons mise en évidence est convergente sur les différents sites échantillonnés (Paris, Lyon, Bordeaux). Ceci suggère donc un phénomène global en réponse à l'urbanisation. En revanche, l'amplitude de la réponse variait selon les sites. Ceci pourrait s'expliquer par une variabilité inter-sites dans les taux de pollution, menant à la sélection d'allèles de tolérance différents selon ces sites. Cette hypothèse mériterait d'être explorée par des relevés précis des quantités de polluants sur chaque site, cette thèse ne bénéficiant de relevés globaux qu'à l'échelle de l'Ile de France (Foti et al., 2017). Cependant cette hypothèse n'a pas été corroborée par les études génétiques réalisées sur cette espèce, qui montre une absence de différenciation des populations urbaines-rurales mais aussi une très faible structuration génétique des populations à l'échelle de la France et qui n'est pas expliquée par l'origine géographique des colonies (Khimoun et al., 2020; Pusch et al., 2006). Une autre hypothèse serait alors une plasticité différente selon les sites en fonction des taux de pollution, avec des colonies issues des milieux très pollués moins impactées par l'exposition à des métaux traces du fait de modifications plastiques de leur expression génétique antérieure à l'expérience. Des études de transcriptomique et d'analyse d'expression différentielle de gènes (globale ou ciblée sur des gènes connus de réponse aux métaux traces) entre différentes populations pourraient permettre de valider ou non cette hypothèse.

#### **b- Les colonies urbaines fourragent plus, mais sont moins agressives que les colonies forestières**

Le comportement en milieu urbain est souvent modifié (Ditchkoff et al., 2006; Miranda, 2017; Mueller et al., 2013, 2020). Cependant peu d'études ont été faites sur les espèces eusociales, malgré leur grande plasticité comportementale. Dans cette expérience, nous avons comparé les comportements de fourrage (temps de découverte de la nourriture, nombre moyen et nombre maximum de fourrageuses, nombre de voyages nid-nourriture) et d'agression (temps de morsure) de colonies urbaines et forestières en utilisant 6 sites différents (3 urbains, 3 forestiers). Nous avons mis en évidence que les colonies urbaines fourragent plus (plus de voyages nids-nourriture), mais sont moins agressives face à un conspécifique que les colonies forestières (figure 1). Cependant, il est à noter que concernant le fourrage, un seul des traits sur les quatre testés diverge (le nombre de trajet nid-nourriture), ce qui montre une différence de comportement de fourrage assez limitée entre les deux habitats. Un fourrage plus

actif en ville a déjà été documenté à l'échelle de la population chez quelques espèces abeilles (*Tetragonula carbonaria* et *Bombus terrestris*, Kaluza et al., 2016; Stelzer et al., 2010). A l'échelle de l'espèce, Dáttilo et MacGregor-Fors (2021) ont montré qu'en milieu urbain les espèces de fourmis qui monopolisent le mieux la nourriture sont aussi celles qui ont tendance à fourrager activement et à trouver rapidement la nourriture. Les résultats trouvés ici corroborent partiellement ces observations chez les autres ordre d'insectes sociaux à l'échelle de la population sur le fourragement, mais des études prenant en compte des traits plus divers (distance maximum de fourragement, quantité de nourriture ramenée au nid...) menées sur le terrain pourraient être intéressantes. La moins grande agressivité des colonies urbaines est, en revanche, plutôt à contre-courant de la majorité des études qui ont plutôt tendance à montrer une augmentation de l'agressivité des individus urbains (Miranda, 2017; Scales et al., 2011). Ceci est d'autant plus surprenant qu'une étude a montré que pour la fourmi *Temnothorax rugatulus* les milieux offrant le moins de sites de nidification favorables étaient aussi ceux dans lesquels les colonies tendaient à être plus agressives (Bengston and Dornhaus, 2015). Comme les milieux urbains proposent moins de sites favorables à la nidification de notre espèce, du fait de la forte réduction des espaces disponibles et le ramassage régulier des branches mortes tombées au sol dans les parcs (voir aussi Friedrich and Philpott, 2009), nous nous attendions à trouver des colonies urbaines plus agressives. Les taux de pollution aux métaux traces plus importants en milieu urbain qu'en milieu forestier sur les sites utilisés (Foti et al., 2017) pourrait expliquer cette moins grande agressivité des colonies urbaines. En effet, (Sorvari and Eeva, 2010) ont montré que chez la fourmi *Formica aquilonia*, les colonies provenant d'un ancien site minier très pollué en métaux trace étaient moins agressives que les colonies provenant de sites moins pollués (potentiellement à cause de désordres neuronaux causés par les métaux traces, ou par une perte de reconnaissance des signaux chimiques de la cuticule de leurs homologues). La pollution en milieu urbain pourrait donc amener à une impossibilité pour les colonies urbaines de reconnaître leurs conspécifiques, et donc générer des comportements moins agressifs. Les conséquences sur la fitness de cette perte d'agressivité sont encore à déterminer.

Les études faites ici ont été réalisées en laboratoire, mais sur des colonies ramenées du terrain peu de temps auparavant : les différences observées peuvent donc être aussi bien d'origine génétique que plastique. Les mêmes expériences réalisées sur des colonies maintenues depuis plusieurs mois au laboratoire, avec des ouvrières nées en jardin commun, pourrait permettre de trancher en faveur d'une origine génétique ou plastique de ces différences. **Quelle qu'en soit l'origine, les travaux de cette thèse ont donc permis de mettre en évidence qu'il existe des**



**traits différenciés entre populations urbaines et forestières. Les stressseurs urbains sélectionnant ces traits restent encore hypothétiques, et sont potentiellement multiples.**

## **2- L'origine de ces divergences de traits : rôle limité de l'environnement social ?**

### **a- Le rôle de la génétique et de l'environnement abiotique dans la divergence urbain-forestier de la réponse au cadmium**

Nous avons, par une expérience de jardin commun, cherché à savoir si la meilleure tolérance au cadmium des colonies urbaines était d'origine génétique ou plastique. En effet, l'expérience réalisée au chapitre un a été effectuée sur des colonies sortant juste d'hibernation sur le terrain ; ainsi la réponse différentielle au cadmium aurait pu être due à des différences dans les conditions d'hibernation entre populations urbaines (en milieu plus chaud) et forestières. Pour cela nous avons récolté des colonies avant de les faire hiberner en jardin commun à 4°C pendant quatre mois. A la fin de cette période, nous avons utilisé ces colonies ainsi que des colonies ayant hibernées sur le terrain (comme dans l'expérience du chapitre un) avant de les soumettre à un traitement au cadmium ou en contrôle. Les colonies hibernant en jardin commun montrent une réponse différentielle au cadmium selon leur origine urbaine/forestière, suggérant un effet génétique (figure 1). Cependant, pour les colonies ayant hibernées sur le terrain, cette réponse différentielle n'est plus détectable. L'hiver de l'année de l'expérience a été particulièrement doux (7-8°C en moyenne en forêt/ville contre 5°C les autres années, et 4°C en jardin commun, <https://www.infoclimat.fr/stations-meteo/analyses-mensuelles.php>). Or, les températures basses activent l'expression de facteurs anti-stress tels que les Heat Shock Protein (HSP) ou les métallothionéines (Fisker et al., 2016; King and MacRae, 2015; Popović et al., 2015) qui pourraient être impliquées dans la tolérance au cadmium, et qui auraient donc pu être sécrétées en moins grande quantité cette année. Des analyses protéiques ou de transcriptomique sur ces gènes en fonction de la température pourrait fournir des arguments en faveur de cette hypothèse. **La meilleure tolérance au cadmium des populations urbaines pourrait donc être d'origine génétique, mais ne s'exprimer qu'à des températures froides (interaction génétique et environnement, figure 1)).**

## **b- L'environnement social semble jouer un rôle limité dans la tolérance au stress**

Chez les espèces eusociales, les traits d'histoire de vie d'un individu peuvent dépendre de celui des autres individus du même nid et donc de leur environnement social. Une manière classique d'étudier l'impact potentiel du phénotype des ouvrières ou des larves sur le phénotype des autres individus de la colonie est le cross-fostering. Cette expérience consiste à mélanger des colonies possédant des traits différenciés (par exemple des ouvrières plus grosses, des reines plus agressives...) et à produire ainsi des colonies « chimériques », puis d'étudier les traits d'histoire de vie des individus produits par ces colonies. Ces manipulations ont régulièrement démontré un rôle de l'environnement social dans le phénotype des individus (Linksvayer, 2007; Linksvayer et al., 2011; Purcell and Chapuisat, 2012). L'objectif de notre chapitre deux était donc de déterminer le rôle de l'environnement social dans la réponse différentielle au cadmium. De manière surprenante, nous n'avons pas retrouvé les résultats présentés au premier chapitre : les colonies chimères constituées de larves et d'ouvrières de ville (100% urbain donc) ne présentaient pas de meilleure tolérance au cadmium que les colonies constituées de larves et d'ouvrières forestières ou que les colonies mi-urbaines, mi-forestières, nous empêchant ainsi que tester un effet de l'environnement social sur la tolérance différentielle au cadmium. Plusieurs hypothèses peuvent être avancées : tout d'abord, la réponse différentielle au cadmium pourrait montrer une variabilité inter-année, et donc l'expérience aurait pu être effectuée une année où cette réponse était faible. En effet, nous avons montré que, l'année de l'expérience, les colonies de forêt semblaient globalement être de meilleure qualité que les colonies de ville : les larves se développaient plus vite en adultes (3.1% plus vite), ces adultes étaient plus gros (+2.3%), et les taux d'émergence meilleurs (+17%) dans les colonies forestières par rapport aux colonies urbaines. Ceci suggère donc que les colonies urbaines auraient pu, cette année-ci, être en mauvais état physiologique et métabolique général à cause d'un facteur inconnu lié au milieu urbain. Ceci pourrait avoir mené à la ponte d'œufs de mauvaise qualité, et donc à des larves en mauvais état physiologique dès le début de l'expérience, ce qui pourrait expliquer une absence de réponse différentielle au cadmium cette année-ci. L'hypothèse d'un éventuel effet stressant du croisement (les ouvrières devant élever des larves d'une autre colonie) nous semble peu probable, étant donné le taux d'acceptation élevé des larves par les ouvrières et les faibles taux de mortalité en condition contrôle. Une dernière hypothèse vient du fait que les colonies n'ont pas été testées exactement au même moment de l'année : la réponse au cadmium pourrait montrer une variabilité intra-annuelle, ce que nous verrons au chapitre 3.

Un autre trait important des espèces sociales est la taille de la colonie. Selon la théorie des superorganismes, les colonies qui devraient résister le mieux aux perturbations externes seraient les plus grosses (Straub et al., 2015). De nombreuses études tendent à valider cette théorie, avec les plus grosses colonies qui en général survivent mieux aux perturbations. Cela se trouve à l'échelle des communautés, où les espèces d'abeilles possédant les colonies les plus peuplées sont également celles qui survivent le mieux à l'exposition aux pesticides (Rundlöf et al., 2015). A l'échelle de la population, il a été montré que les colonies les plus grandes résistaient le mieux à la famine (Modlmeier et al., 2013), aux pesticides (Crall et al., 2019), à l'exposition à un parasite (Scharf et al., 2012), au froid (Heinze et al., 2016), à la chaleur (Molet et al., 2017) ou aux variations saisonnières (Kaspari and Vargo, 1995). De manière originale, nous avons montré à plusieurs reprises un effet différentiel de la taille de la colonie selon le type de traitement auquel les colonies étaient exposées (figure 1). Le taux de mortalité des ouvrières diminue avec la taille des colonies en condition contrôle, modérément stressante. En revanche, lorsque les colonies sont exposées au cadmium (et donc un stress important), le taux de mortalité des ouvrières augmente avec la taille de colonie. Comment expliquer cet effet délétère de la taille des colonies à forts niveaux de stress ? Une première hypothèse vient de la structure des réseaux de communication au sein des colonies. (Naug, 2009) a montré, chez une espèce eusociale de guêpe (*Ropalidia marginata*), que l'hétérogénéité des réseaux d'interaction augmente avec la taille de colonie ; c'est-à-dire que plus la colonie est grande, et plus les nœuds de communication seront concentrés sur quelques individus précis, tandis que dans des petites colonies chaque individu est en contact avec tous les autres. La perte d'un individu clé dans une grosse colonie peut donc totalement déstabiliser le réseau de communication, menant à perte d'efficacité de la colonie (Fewell, 2003). En revanche les petites colonies pourraient avoir des réseaux plus résilients à la perte de quelques individus. Dans le cas de nos expériences, les grosses perturbations dues à l'exposition au cadmium pourraient avoir tué quelques-uns de ces individus clés (les taux de mortalité avoisinant les 50%), et donc mené à une désorganisation des colonies. De plus, dans les grandes colonies, les individus tendent aussi à être plus spécialisés dans certaines tâches (Holbrook et al., 2011; Jeanson et al., 2007; Thomas and Elgar, 2003), ce qui augmente l'efficacité de ces individus dans une tâche précise mais peut également s'avérer problématique pour la survie de la colonie quand certaines catégories sont plus touchées que d'autres. Par exemple, chez les abeilles, la perte de fourrageuses induit un changement de comportement chez les individus plus jeunes qui s'occupent normalement des larves : une partie d'entre elles partent alors fourrager pour remplacer les fourrageuses perdues (Perry et al., 2015). Ces jeunes individus étant moins efficaces et sujets à des taux de mortalité

élevé, ceci entraîne à nouveau une vague de mortalité, qui induit à nouveau le passage de nouveaux individus de nurser vers fourrageur ect...., menant ainsi à un effondrement complet et rapide de la colonie entière, phénomène connu sous le nom de colony collapse disorder (Barron, 2015; Perry et al., 2015). Une deuxième hypothèse pour expliquer la mortalité accrue dans les grandes colonies en réponse au cadmium serait que celles-ci sont plus susceptibles de rentrer en contact avec un stresser (polluant, parasite...) du fait de leur plus grand nombre d'individus. Cette exposition accrue à un stresser pourrait augmenter la probabilité de ramener et répandre ce stresser au sein de la colonie, mais cette hypothèse reste à tester (Barron, 2015). Les grandes colonies, avec beaucoup d'individus et des individus plus spécialisés, aux réseaux d'interactions hétérogènes, pourraient donc être plus sensibles aux perturbations environnementales. **La socialité pourrait donc avoir un effet négatif sur la réponse des espèces à des stressers forts, entraînant des effondrements potentiellement rapides des colonies. En revanche, à des niveaux de perturbations limités, la société pourrait permettre de tamponner ces perturbations. Cette thèse souligne donc à nouveau le rôle ambigu de la socialité dans la réponse des espèces sociales aux modifications environnementales, l'urbanisation ici.**

### **3- Des divergences de traits stables dans le temps ? L'impact potentiel de la variation intra-annuelle des dynamiques de colonie sur la divergence urbain-rural**

La variabilité temporelle dans la réponse au stresser est déjà relativement documentée chez les abeilles, avec des colonies par exemple plus sensibles aux parasites au printemps (Traver et al., 2012), ou une sensibilité accrue aux pesticides lorsque les températures sont importantes (Henry et al., 2014). Chez les fourmis, la susceptibilité à un parasite montre également des variations saisonnières (D'Ettorre et al., 2004; Valles et al., 2010), ce qui suggère un effet de la variabilité de l'environnement et/ou de la dynamique coloniale au cours de l'année (par exemple, un changement d'investissement de la colonie dans la réponse aux stressers au cours des saisons). Des variabilités dans les concentrations en métaux traces dans l'environnement ou une variation dans les taux d'accumulation de ces métaux traces dans les organismes ont déjà été documentées, mais aucun lien n'a été fait avec une éventuelle meilleure tolérance sur certaines périodes de l'année (Cossa et al., 1980; Fialkowski et al., 2003; Rabitsch, 1997). A

notre connaissance, aucune étude à ce jour ne s'était intéressée à une éventuelle variation temporelle dans la réponse à des stresseurs urbains chez les fourmis.

En comparant la réponse au cadmium de colonies sortant d'hibernation à celle des colonies en phase de productions de sexuées (début juin), nous avons montré que la divergence des traits entre populations urbaines ou forestière, ainsi que la force de la réponse au stresser au sein d'un même habitat, montraient des variations intra-annuelles. Les colonies de printemps, qu'elles soient d'origine urbaine ou forestière, étaient globalement moins sensibles au cadmium. De plus, la réponse différentielle ville/forêt au cadmium dépendait elle aussi de la période de l'année à laquelle le test a été fait, en interaction avec des facteurs environnementaux. La réponse différentielle n'était pas détectable en hiver de cette année (peut-être à cause des températures douces d'hibernation, voir plus haut), mais détectable au printemps. De manière surprenante, les traits d'histoire de vie différenciellement exprimés au printemps n'étaient pas les mêmes que ceux trouvés dans une précédente étude sur des colonies sortant d'hibernation : les taux d'émergence des larves et la taille des émergentes en hiver, contre taux d'arrêt de développement larvaire et taux de mortalité des larves au printemps. Ainsi, la réponse au cadmium, en plus de montrer une variation inter-annuelle, montre également une variation intra-annuelle (figure 1).

Quelles hypothèses pourraient expliquer ces variations intra-annuelles dans la réponse au cadmium ? Plusieurs études ont mis en évidence un lien entre masse corporelle et tolérance aux métaux traces, les plus gros organismes s'en sortant mieux grâce à leur taux métabolique par unité de masse plus faible (Durou et al., 2005; Grześ, 2010; Vesela and Vijverberg, 2007). Au sein d'un même habitat, la meilleure tolérance des colonies printanières pourrait donc s'expliquer par la masse plus importante des individus à cette période de l'année qu'à la sortie de l'hibernation, pendant laquelle les réserves ont été consommées (Blanchard et al., 2000; Rabitsch, 1997). Cependant des mesures des masses et des quantités de lipide chez les ouvrières seraient nécessaires pour valider cette hypothèse, certaines études rapportant une masse semblable entre ouvrières en sortie d'hibernation et au printemps (Tschinkel, 1993). Ceci souligne l'importance d'effectuer des tests de tolérance à certains stress urbains sur plusieurs points temporels dans l'année. Si l'on compare la réponse au cadmium entre plusieurs habitats cette fois ci, la réponse différentielle ville/forêt montre également des variations. Une première hypothèse tient à des facteurs intrinsèques aux larves. Chez le genre *Temnothorax*, pour qu'une larve ait le potentiel de se transformer en gyne, il faut que celle-ci ait hiberné (Wheeler, 1986). Les larves issues d'œufs pondus au printemps et qui se développent avant l'hiver ne donneront toujours que des ouvrières (ou des mâles). Dans notre expérience, nous avons éliminé toutes les

larves susceptibles de se développer en gynes pour les colonies de printemps, mais pas d'hiver. Il est donc possible que la nature des larves ainsi exposées au cadmium fasse varier les traits d'histoire de vie impactés, mais les mécanismes sous-jacents restent à découvrir. Une autre hypothèse est liée à des variations de l'environnement social et/ou la physiologie des larves. Chez certaines espèces de fourmis, l'initiation, la maintenance ou la terminaison de la diapause larvaire sont sous contrôle des ouvrières (Brian, 1955). Dans notre expérience, au printemps, les larves de ville voient leur taux de pause développementale augmenter en réponse au cadmium, alors que l'inverse est observé chez les larves de forêt. Les ouvrières de villes pourraient initier ou maintenir la pause larvaire en réponse au stress causé par le cadmium, mais les ouvrières de forêt pourraient avoir perdu cette capacité à cause d'un stress trop important, menant à la mort des larves. En sortie d'hiver, les larves étant encore en diapause, le problème de l'initiation de la diapause ne se poserait pas, ce qui expliquerait l'absence de réponse différentielle ville/forêt. Il pourrait également s'agir d'un facteur intrinsèque aux larves, sans lien avec les ouvrières. Les larves d'hiver pourraient être restées en diapause, mais pas celles de printemps qui auraient à initier à nouveau cette diapause en réponse au cadmium. Les larves de ville pourraient y parvenir plus facilement grâce à une tolérance initiale accrue au stress, et les larves de forêt être trop perturbées pour avoir le temps d'initier à nouveau cette diapause avant de mourir. **Ce chapitre suggère donc l'importance d'étudier la réponse des insectes sociaux à un stresser à différents moments de leur cycle de vie colonial, car les réponses à ces stressers peuvent varier en amplitude comme en qualité de réponse.**

#### **4- Perspectives**

Nous avons mis en évidence des traits différenciés entre populations urbaines et forestières de *T. nylanderi* (réponse à un métal trace, comportement). Cependant, ces études ne permettent pas de déterminer avec précision quel(s) stresser(s) en particulier mènent à ces réponses différentielles. Pour la réponse au cadmium, il est probable qu'elle soit effectivement médiée par des concentrations différentes en métal trace entre les deux habitats. Des relevés de concentration en métaux traces ont déjà été effectués en région parisienne (Foti et al., 2017), mais effectuer ces mesures sur les autres sites utilisés ici (Bordeaux, Lyon) permettrait de confirmer cette hypothèse. Les concentrations utilisées dans notre étude sont également assez éloignées des concentrations rapportées dans les sols, au moins pour Paris (100mg/kg de

cadmium dans l'expérience, contre 2.45 mg/kg dans les sols). Bien qu'il existe des phénomènes de bioaccumulation, menant à des concentrations en cadmium dans le corps des individus de certaines espèces de fourmi (*Formica polyctena*) proches de celle utilisée dans notre expérience (Starý and Kubizňáková, 1987), il serait tout de même intéressant de tester des concentrations en cadmium plus faibles, mais sur de plus longues périodes de temps, afin d'être plus proches des conditions réelles de terrain. Il serait également intéressant de mesurer la concentration en métaux traces dans les colonies elles-mêmes : les colonies de ville supportent-elles de plus hauts niveaux de contamination, ou bien excrètent-elles plus efficacement les polluants ? Concernant le comportement, il est difficile de conclure quant à un facteur qui pourrait expliquer les différences observées en termes de fourragement (colonies de ville plus actives) et d'agression (colonies de ville moins actives). D'autres études portant sur plus de villes, avec des relevés des différentes caractéristiques biotiques et abiotiques au sein des sites (par exemple, densité de nids, taux de pollution, disponibilité en ressources nutritives, interaction avec d'autres espèces...), pourrait donner des pistes.

Dans notre chapitre deux, notre expérience d'élevage en jardin commun suggère une origine génétique dans la meilleure tolérance des colonies urbaines au cadmium. Cependant, ce design expérimental ne permet pas d'exclure totalement des effets maternels, ou bien des effets plastiques sur le long terme (par exemple via des modifications épigénétiques). Pour confirmer ou infirmer l'origine génétique de la tolérance au cadmium des populations urbaines, il serait intéressant d'effectuer des élevages sur plusieurs générations en laboratoire avant d'effectuer les tests de réponse au cadmium. Concernant l'étude portant sur le comportement, les tests ont été effectués en laboratoire, mais assez peu de temps après la récolte des colonies. Aussi, nous ne pouvons pas conclure sur une origine génétique ou plastique de cette différence. A nouveau, des élevages sur plusieurs années permettraient de déterminer cette origine. Une autre possibilité serait de faire des expériences de translocation croisée de colonies : des colonies de ville élevées en forêt, et vice-versa, avant d'être récoltées et ramenées en laboratoire pour des tests. (Honorio et al., 2020, 2021) ont déjà montré qu'il était possible d'élever des colonies en milieu semi-naturel (enclos pour les colonies, ce qui permet de retrouver les colonies d'une année sur l'autre). L'avantage de ce type de design expérimental serait qu'il permettrait également de déterminer approximativement la fitness des colonies sur le terrain (en comptant le nombre de sexués produits au moment de la récolte de la colonie par exemple), ce qui donnerait également des arguments en faveur du caractère adaptatif ou non des divergences observées. Enfin, une étude en RAD-Seq effectuée sur les populations de *T. nylanderi* a mis en évidence qu'il existait quelques SNP (Single Nucleotide Polymorphism) différenciés entre

populations urbaines et forestières (18 SNP détectés au total) alors qu'aucune structuration génétique n'a été observée à l'échelle de la France (Khimoun et al., 2020). Dans l'hypothèse que l'origine génétique des divergences de traits d'histoire de vie serait confirmée, il serait intéressant de comparer les profils d'expression des gènes auxquels appartiennent ces SNP, afin de mettre en évidence une éventuelle expression différentielle entre colonies urbaines et forestières. Dans le cas où l'origine génétique serait infirmée, il serait tout de même intéressant d'effectuer une analyse d'expression différentielle de gènes à l'échelle du transcriptome, afin de déterminer quelles grandes familles de gènes sont exprimés de manière plastique en réponse à l'urbanisation.

Le chapitre deux s'intéressait également au rôle de l'environnement social dans la réponse à l'urbanisation. L'expérience de cross-fostering n'a malheureusement pas permis de trancher quant aux rôles respectifs des larves et des ouvrières dans la réponse différentielle au cadmium, à cause d'une absence de réponse différentielle cette année-là. Sur la base des résultats d'un autre article, qui suggère qu'une hibernation en conditions froides serait nécessaire à l'expression de cette réponse différentielle, une solution possible serait donc de faire hiberner en laboratoire des colonies avant de procéder à une nouvelle expérience de cross-fostering. Les différents articles de cette thèse ont aussi mis en évidence un rôle de la taille de la colonie dans la tolérance au stress causé par le cadmium : les plus grosses colonies semblent plus sensibles au cadmium. Il serait intéressant de mesurer les concentrations en cadmium dans les ouvrières et les larves selon la taille des colonies, afin de déterminer si une plus grosse taille de colonie expose plus au cadmium. Une autre hypothèse avancée était celle de la déstabilisation des réseaux d'interactions. Pour tester cette hypothèse, il serait envisageable de déterminer quels sont ces individus clés au sein des colonies par étude des réseaux d'interaction, avant d'en retirer tout ou une partie et d'étudier l'effet de cette perturbation sur les performances de la colonie.

Le dernier chapitre a mis en évidence une variabilité de l'amplitude et de la qualité de la réponse aux stressors (cadmium ici). Cependant cette étude n'a été faite que sur deux points temporels : il serait intéressant de tester un panel plus large de points, par exemple à la fin de l'été, lorsque les sexuées s'appêtent à quitter le nid, ou en début d'automne lors de la phase de préparation à l'hibernation. Les différentes expériences réalisées au cours de cette thèse ont aussi mis en évidence une variabilité interannuelle de la réponse, possiblement liée aux conditions d'hibernation. D'autres mesures de la tolérance au cadmium sur plusieurs années, en lien avec des conditions d'hibernation clairement documentées, ou bien au laboratoire sous conditions



contrôlées, permettraient de déterminer les facteurs externes à l'origine de cette variabilité interannuelle.

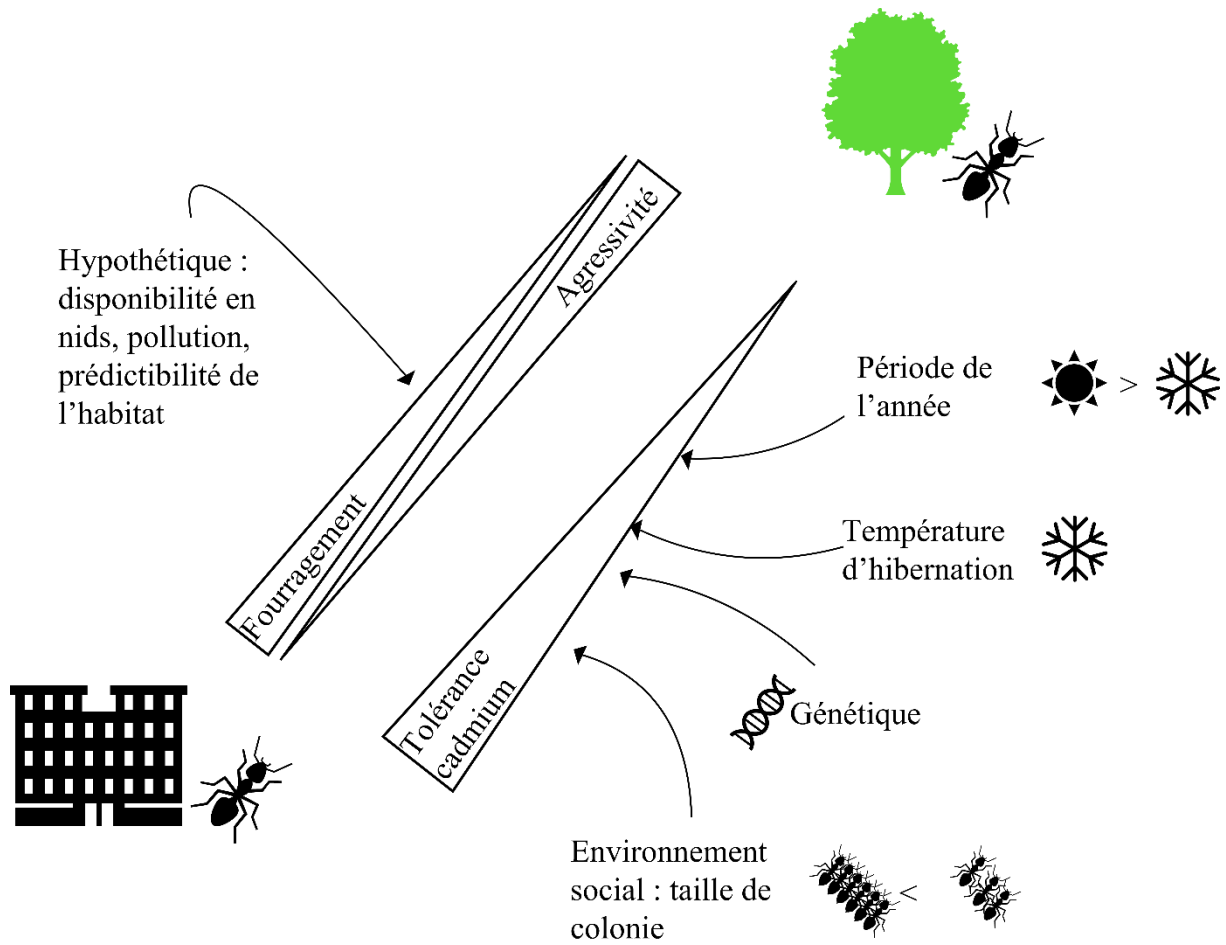


Figure 1 : Résumé des différentes divergence de traits entre colonies urbaines et forestières étudiées lors de cette thèse, ainsi que les facteurs affectant ces traits.

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## Résumé :

L'urbanisation est un processus qui a tendance à s'accroître ces dernières années. Les villes imposent un certain nombre de changements environnementaux aux espèces y vivants : températures élevées, habitat fragmenté, pollué (pollution lumineuse, acoustique et/ou chimique)... Malgré leur importance écologique et économique, peu d'études se sont penchées sur la réponse des espèces eusociales à cette urbanisation. Ces espèces pourraient avoir des capacités de réponses aux changements environnementaux différentes des espèces solitaires, du fait de leur grande plasticité phénotypique et de leur vie en société. L'objectif de cette thèse est donc de déterminer comment l'eusocialité peut influencer sur la réponse à l'urbanisation. Dans une première partie, nous avons montré, à travers une expérience de jardin commun, que les colonies de *Temnothorax nylanderi* toléraient mieux un métal trace (le cadmium) que les colonies de forêt. Nous avons également trouvé une divergence des comportements de fourragement et d'agression chez ces populations. Dans une deuxième partie, nous avons mis en évidence un rôle des facteurs génétiques dans la meilleure tolérance des colonies urbaines au cadmium. Nous avons également effectué une expérience de cross fostering, afin de distinguer les effets des ouvrières de ceux des larves sur la meilleure tolérance au cadmium des colonies urbaines. Une absence de réponse différentielle au cadmium cette année-là nous a empêché de tester cette hypothèse, mais a permis de mettre en évidence un effet tampon social de la taille des colonies ainsi que ses limites. Enfin, le dernier chapitre nous a permis de mettre en évidence des variations intra-annuelles dans la réponse des espèces eusociales aux stressors. Cette thèse souligne l'importance de prendre en compte les traits propres aux espèces sociales dans la réponse de ces espèces aux changements environnementaux majeurs.

Mots clés : [*Temnothorax*, cadmium, métaux traces, comportement, jardin commun, environnement social, hibernation]

## **[Response to urbanization in a eusocial insect: interconnexion of genetic and plastics responses, and the role of the social environment]**

### Abstract:

Urbanization is an ongoing process which tend to accelerate these recent years. Cities are imposing some environmental pressures on species living there: high temperatures, fragmented habitat, pollution (being sound, light, or chemicals). Despite their ecological and economic importance, few studies have investigated so far how eusocial species respond to urbanization. Indeed, those species could have different response abilities to environmental changes, because of their great phenotypic plasticity or their social life. This thesis objective is to determine how eusociality could impact response to urbanization. In a first chapter we demonstrated through a common garden experiment that *Temnothorax nylanderi* colonies better tolerate a trace metal (cadmium) than their forest counterparts. We also reported a behavioral divergence between those two populations (in foraging and aggression). In a second chapter, we highlighted that this differential cadmium tolerance could have a genetic origin. Using a cross fostering experiment, we also aim at disentangling relative contribution of workers and larvae in the better tolerance to cadmium in urban colonies. A lack of differential response the year of the study impede us to conclude on this point, but we highlighted a buffering of colony size and it limits. Finally, in the last chapter we investigated intra-annual variation in eusocial species response to urban stressors. Overall, this thesis emphasizes the importance to take into account social species unique traits to assess those species response to environmental changes.

Keywords: [*Temnothorax*, cadmium, trace metal, behavior, common garden, social environment, hibernation]