Université Paris VI - Pierre et Marie Curie

Thèse

Présentée pour l'obtention du grade de Docteur de l'Université Paris VI

Discipline : Écologie et Biologie Évolutive

Evolution des stratégies de dispersion et de reproduction chez la fourmi *Cataglyphis cursor*

Par

Johanna CLEMENCET

Soutenue le 17 Mai 2006 devant le jury composé de :

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Jürgen HEINZE	Professeur (Universität Regensburg, Allemagne)	Rapporteur
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Claudie DOUMS	Maître de conférences (EPHE, Université de Paris VI, France)	Directrice de Thèse
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Laboratoire Fonctionnement et Évolution des Systèmes Écologiques
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RÉSUMÉ

Chez les insectes sociaux, les stratégies de dispersion et de reproduction ont un rôle majeur dans l'évolution des actes altruistes puisqu'elles déterminent l'apparentement entre individus au sein et entre les colonies. Le but de ce travail est d'explorer les stratégies de dispersion et de reproduction, *a priori* exceptionnelles, de la fourmi *Cataglyphis cursor*.

Notre étude de génétique des populations, à l'aide de marqueurs nucléaires (microsatellites) et mitochondriaux (gène COI), supporte l'idée que les colonies se reproduisent par fission chez cette espèce monogyne et souligne l'influence de l'habitat sur la distribution de la variabilité génétique à fine échelle. Une étude socio-génétique menée dans deux populations issues de différents habitats a confirmé que les reines utilisent la parthénogenèse thélytoque pour produire les nouvelles reines, mais s'accouplent avec plusieurs mâles pour produire les ouvrières. Nous montrons, pour la première fois chez les fourmis, qu'à tailles égales, les colonies les plus diverses génétiquement ont en moyenne des ouvrières de plus grande taille.

Dans plusieurs populations étudiées, la taille des ouvrières est positivement corrélée avec la taille des colonies. Une augmentation de la taille des ouvrières peut représenter un avantage important chez cette espèce thermophile. Elle permettrait aux colonies de fourrager à des températures difficilement supportables pour les petites ouvrières et les autres espèces. Nous avons montré expérimentalement que la résistance à la dessiccation augmente avec la taille des ouvrières. Toutefois, sur le terrain, les fourrageuses n'étaient pas significativement plus grandes aux heures les plus chaudes de la journée.

Dans les colonies orphelines de *C. cursor*, les ouvrières peuvent également produire des oeufs diploïdes (ouvrières et reines) par parthénogenèse thélytoque. Des observations comportementales ont mis en évidence que le potentiel reproducteur des ouvrières augmente avec leur taille. Ceci souligne que chez *C. cursor*, les traits phénotypiques des colonies et des ouvrières sont le résultat de pressions de sélections potentiellement divergentes (sélection individuelle *vs* sélection au niveau de la colonie).

Mots-clefs: Insectes sociaux, *Cataglyphis cursor* (Hymenoptera, Formicidae), dispersion, fondation dépendante, viscosité des populations, parthénogenèse thélytoque, polyandrie, taille de colonie, taille d'ouvrière, fécondité, dessiccation, niveaux de sélection, microsatellites, ADNmt

ABSTRACT

In social insect, understanding the reproductive and dispersal strategies are of major interest as they determine the relatedness between interacting individuals (within and among colonies), a parameter with a major influence on the evolution of altruistic behaviour (division of labour, regulation of reproduction). The aim of this work was to explore the peculiar reproductive and dispersal strategies observed in the ant *Cataglyphis cursor*.

A population genetic study of *C. cursor* populations strongly supported the idea that colonies reproduce by fission in this monogynous species and emphasized the role of habitat in driving the pattern of local population genetic structure. A socio-genetic study conducted in two populations from different habitat types showed that the peculiar reproductive system, previously demonstrated in a single population, appears to be a general situation. Indeed, in both populations, the queens used thelytokous parthenogenesis to produce new queens but mated with several males (5-12) to produce the workers.

We found that the more genetically diverse colonies harboured a greater mean worker size after controlling for colony size. In the two populations studied, worker size was indeed positively correlated with colony size. In this thermophilic species, an increase of worker size could be advantageous to the colony by allowing a foraging temperature that small workers and other species can not tolerate. Effectively, we experimentally found an increase of worker thermal tolerance with size. However, in the field, foragers were not significantly larger than inside nest workers.

In orphaned colonies of *C. cursor,* workers can also produce diploid eggs (gynes and workers) by thelytokous parthenogenesis. Behavioural observations of an orphan colony indicated that size is also linked to the reproductive potential of workers, larger workers being more likely to reproduce than smaller. This underlines that colony and individual phenotypic traits in *C. cursor* are the result of conflicting (individual *vs* colony level selection) selective pressures.

Key words: Social insect, *Cataglyphis cursor* (Hymenoptera, Formicidae), sex-biased dispersal, dependant colony foundation, population viscosity, thelytokous parthenogenesis, polyandry, colony size, worker size, worker reproductive potential, desiccation, level of selection, microsatellites, mtDNA

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INTRODUCTION

Les fourmis ont toujours provoqué l'étonnement et stimulé la curiosité intellectuelle des biologistes. Darwin, le premier, s'intéressa à la caste stérile des ouvrières qu'il perçut comme un sérieux accroc à la théorie de l'évolution par sélection naturelle (1859). En effet, dans le cadre de la théorie darwinienne, comment expliquer l'évolution et le maintien du comportement altruiste des ouvrières qui ne se reproduisent pas et travaillent pour aider d'autres individus ? La théorie de la sélection de parentèle proposée par Hamilton (1964a) apporta une solution élégante à cette énigme évolutive, en postulant que les individus peuvent également propager leurs gènes de façon indirecte, en favorisant la reproduction d'individus apparentés. Dès lors, étudier l'évolution de la diversité des systèmes de reproduction et de dispersion chez les insectes sociaux revêt un intérêt tout particulier. Ils déterminent en effet les degrés d'apparentement entre individus au sein des colonies et entre colonies voisines, éléments fondamentaux pour comprendre l'évolution et le maintien de la socialité et de tout comportement altruiste.

La fourmi *Cataglyphis cursor* présente des caractéristiques remarquables au sein des fourmis, qui en font un modèle unique pour aborder l'évolution des systèmes de reproduction et des traits d'histoires de vie chez les insectes sociaux. La thèse présentée ici se propose de caractériser par une approche génétique les modalités de reproduction et de dispersion *a priori* exceptionnelles de cette espèce. Nous nous intéresserons également à l'évolution de paramètres cruciaux, tels que la taille de colonie et le polymorphisme de taille des ouvrières.

Avant d'aborder notre travail, il convient de rappeler brièvement ce qu'est l'eusocialité. Nous présenterons ensuite, la diversité des modes d'organisations des colonies, les différentes stratégies de reproduction et de dispersion existant chez les insectes sociaux, et leurs conséquences sur les degrés d'apparentement entre individus, au sein et entre les colonies. Nous exposerons les hypothèses généralement proposées pour expliquer l'origine évolutive des différentes stratégies de reproduction.

1. L’eusocialité

Le passage de la vie solitaire au stade le plus avancé de la vie en société, l’eusocialité, est marqué par l’apparition de paramètres de complexité croissante : l’inter-attraction, le comportement parental, l’existence de sites d’élevage commun, la coopération dans les soins aux jeunes, la spécialisation des tâches et l’apparition d’individus spécialisés dans la reproduction. Selon Wilson (1971), le stade eusocial est atteint lorsque trois critères sont remplis :

- le chevauchement d’au moins deux générations d’individus, de telle sorte que les descendants assistent leurs parents pendant au moins une partie de leur vie,
- l’existence d’une coopération dans le soin aux jeunes,
- l’existence d’individus spécialisés dans la reproduction, aboutissant à une division du travail reproducteur entre les **individus féconds** (reproducteurs) et les **individus plus ou moins stériles** (helpers).

Pour Sherman et al. (1995), l’eusocialité peut être considérée comme un *continuum* entre des espèces dites primitivement eusociales, chez qui tous les individus peuvent *a priori* se reproduire et des espèces hautement eusociales, chez lesquelles il existe une division stricte du travail reproducteur.

Chez les espèces primitivement eusociales, les fourmis sans reines (Peeters 1991), certaines guêpes (Reeve 1991), certaines abeilles (Wilson 1971), certains bourdons, mais aussi chez certains mammifères et oiseaux sociaux (Solomon & French 1997 ; Stacey & Koening 1990), les individus sont totipotents à la naissance et peuvent aussi bien devenir reproducteurs qu’assistants (helpers). Les reproducteurs ne sont donc pas morphologiquement différenciés des individus stériles et la division de la reproduction s’effectue par une régulation comportementale entre adultes (Wilson 1971).

Chez les espèces hautement eusociales (les termites, la plupart des fourmis, certaines guêpes et abeilles, les rats-taupes (Jarvis 1981)), les individus reproducteurs et les individus stériles sont morphologiquement distincts (Wheeler 1986 ; Bourke 1999). Cette différenciation morphologique se fait, selon les espèces, à différents stades de la vie des individus. Chez les insectes sociaux, la différenciation intervient au cours du développement larvaire. Les hyménoptères sociaux (guêpes, fourmis, abeilles) ont un développement holométabole : après la métamorphose, aucune transformation d’ouvrière en reine n’est possible, la différenciation morphologique est donc irréversible. En revanche, certains isoptères (termites) ont un développement hétérométabole, une réversibilité est alors toujours possible. Les ouvriers et ouvrières sont des stades larvaires autonomes n'ayant pas encore fini leur développement et qui, dans certaines circonstances, peuvent évoluer vers la caste reproductrice. Chez les rats-taupes glabres (*Heterocephalus glaber*), la reine ne naît pas morphologiquement spécialisée pour la reproduction mais le devient au cours de sa vie. Elle accède d'abord au statut de dominante puis, une fois fertile, grossit suite à l'élargissement de ses vertèbres (O'Riain et al. 2000).

L'évolution et le maintien d'individus stériles et altruistes au sein des sociétés eusociales posèrent une difficulté majeure à Darwin. D'après sa théorie de la sélection naturelle (1859), seul le succès reproducteur des individus permet la transmission des caractères. Pourquoi alors, les ouvrières sont-elles aussi altruistes et n'hésitent pas à sacrifier leur propre reproduction ? Comment ce caractère peut-il se propager d'une génération à l'autre, puisque les ouvrières sont incapables de se reproduire ?

2. La sélection de parentèle

La théorie de la sélection de parentèle ou *kin selection* proposée par Hamilton (1964a) apporte une explication élégante à ce paradoxe évolutif. Elle établit que les individus peuvent transmettre des copies de leurs gènes à la génération suivante, non seulement en se reproduisant, mais également de manière indirecte, en favorisant la reproduction d'individus apparentés. La capacité d'un individu à propager ses gènes est alors appelée la valeur sélective globale ou *inclusive fitness*. La théorie de la sélection de parentèle stipule qu'un gène déterminant un comportement altruiste peut être favorisé par la sélection naturelle et se répandre dans la population lorsque :

$$\boldsymbol{c < b \times r}$$

avec :

- c : le coût infligé à l'acteur du comportement altruiste lié à la diminution de son succès reproducteur, exprimé en terme de nombre de copies de gène non transmises.
- b : le bénéfice que retire le destinataire de l'acte altruiste, ce gain se traduisant par une augmentation de son succès reproducteur, exprimé en terme de nombre de copies de gène transmises.

- r : la corrélation génétique, *relatedness*, entre le donneur et le receveur de l'acte altruiste, autrement dit la probabilité que le receveur du comportement altruiste possède aussi le gène de l'altruisme.

L'altruisme sera favorisé lorsque le coût infligé au gène altruiste sera plus que compensé par l'accroissement de sa fréquence *via* le bénéficiaire de l'acte. Il est alors possible d'expliquer l'apparition d'individus stériles au sein de société animale lorsque le coût de leur stérilité est plus que compensé par l'augmentation du succès reproducteur des bénéficiaires de l'altruisme.

L'évolution de l'altruisme par la sélection de parentèle demande donc, non seulement que les individus interagissant soient apparentés, mais aussi un rapport approprié entre coûts et bénéfices. Si la corrélation génétique r entre les individus joue un rôle capital dans le modèle de l'évolution de l'altruisme de Hamilton, il n'est cependant pas nécessaire que la valeur de r soit très élevée. L'altruisme peut évoluer et se maintenir même lorsque la corrélation génétique est faible entre individus, il suffit simplement que r soit supérieure à zéro. Cette situation peut apparaître, par exemple, lorsque que le coût de l'altruisme est très faible. Dans un environnement contraignant (en présence de prédateurs, de parasites, de compétiteurs, de limitation de l'habitat, de faibles ressources, de faible taux de succès des fondatrices pour initier de nouvelles colonies), les coûts de la stérilité sont largement compensés par les bénéfices de la vie sociale (défenses collectives contre les prédateurs, parasites, compétiteurs, accès facilité aux ressources). D'une manière générale, l'action conjointe de la sélection de parentèle et des contraintes écologiques a joué un rôle important dans l'évolution de l'altruisme de reproduction (Aron & Passera 2000). La règle d'Hamilton implique par définition que les facteurs génétiques et écologiques doivent être considérés conjointement pour comprendre l'évolution de tout comportement altruiste et de l'eusocialité (Bourke & Franks 1995). Si les analyses génétiques permettent d'évaluer la corrélation génétique entre individus, les paramètres c et b, coûts et bénéfices des comportements, sont difficilement évaluables et dépendent fortement de l'environnement des individus.

2.1 Chez les organismes diploïdes

Chez les organismes diploïdes, comme les termites, le roi et la reine transmettent chacun la moitié de leurs gènes à leurs descendants. Un individu stérile (ouvrier ou ouvrière) d'une colonie monogyne (une seule reine) et monoandre (reine accouplée avec un seul mâle) est donc autant apparenté à ses frères et sœurs qu'il le serait à sa propre descendance (*r* = 0,5). Il n'y a donc pas de gain génétique qualitatif à élever ses frères et sœurs plutôt que ses propres descendants. En revanche, dans la mesure où la colonie produit beaucoup plus de sexués que n'en produirait un individu solitaire, il y a un gain génétique quantitatif très fort à vivre en société. Chez les espèces diploïdes eusociales comme les rats-taupes (Jarvis 1981), certaines espèces de pucerons (Benton & Foster 1992), les crevettes du genre *Synalpheus* (Duffy 1996), une espèce de coléoptère xylophage (Kent & Simpson 1992) et les termites, le gain génétique quantitatif que procure la vie en société est probablement un facteur important expliquant l'apparition d'individus stériles dans ces espèces.

2.2 Chez les organismes haplo-diploïdes

La sélection de parentèle constitue une théorie encore plus puissante chez les organismes dont le mode de détermination du sexe est lié au degré de ploïdie des individus[1]. Chez les hyménoptères, les mâles sont haploïdes (n chromosomes) et obtenus à partir du développement d'œufs vierges produits par parthénogenèse arrhénotoque. Les femelles (reines et ouvrières) sont diploïdes (2n chromosomes) et se développent à partir d'œufs fécondés. Ce déterminisme du sexe par haplo-diploïdie induit des asymétries de parentés génétiques entre les individus au sein d'une même société. Dans une colonie monogyne (une seule reine) et monoandre (reine accouplée avec un seul mâle), une ouvrière est apparentée à 75% avec une sœur, 50% avec son éventuelle descendance (fils ou fille) et 25% avec un frère (Figure 1, ci-après). Les ouvrières peuvent donc augmenter leur succès reproducteur global (c'est-à-dire le nombre de gènes transmis à la génération suivante) en élevant préférentiellement des sœurs fortement apparentées (*r* = 0,75) plutôt que de tenter de se reproduire et d'élever leurs propres descendants (*r* = 0,5) (Bourke & Franks 1995). À ce gain génétique qualitatif s'ajoute également un gain génétique quantitatif. Comme chez les espèces

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[1] La détermination du sexe est en réalité plus subtile et dépend du nombre d'allèles distincts à un ou plusieurs loci de détermination du sexe (Crozier & Pamilo 1996). Si les individus possèdent deux allèles distincts pour au moins un des loci, ils deviendront femelles. Dans le cas contraire (un seul allèle), ils deviendront mâles. Ainsi, les œufs diploïdes peuvent *a priori* devenir mâles ou femelles. En revanche, les oeufs non fécondés haploïdes sont hémizygotes aux loci concernés et deviennent toujours des mâles. Ces mâles diploïdes sont généralement stériles (Cook & Crozier 1995 ; Krieger et al. 1999), leur production représente donc un fardeau pour la colonie. Ceci explique sans doute qu'un très grand nombre d'allèles pour les gènes du déterminisme du sexe ait été sélectionné par la sélection naturelle (Beye et al. 2003). En conséquence, les mâles diploïdes sont relativement rares chez les insectes sociaux et généralement issus de croisements consanguins (Pamilo et al. 1994). Nous considérerons donc, pour simplifier, que les individus diploïdes deviennent des femelles.

eusociales diploïdes, les colonies haplo-diploïdes produisent beaucoup plus de sexués que ne pourrait en produire un individu solitaire.

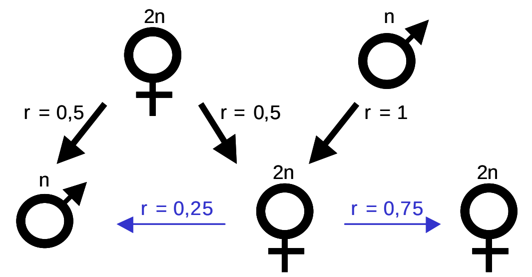

Figure 1. Parenté au sein des sociétés d'hyménoptères.

Le mâle (n) transmet l'intégralité de ses gènes à ses filles, mais ne transmet jamais de gènes aux fils de la colonie. La reine (2n) transmet la moitié de ses gènes à chacun des deux sexes. Les ouvrières ont donc une parenté plus élevée avec leurs sœurs qu'avec leurs frères.

L'eusocialité est ainsi apparue plusieurs fois de façons indépendantes chez les hyménoptères (12 fois au minimum d'après Wilson 1971), mais aussi également chez certaines espèces de thrips, haplo-diploïdes également, où des soldats stériles défendent la colonie (Crespi 1992).

Chez les hyménoptères, le déterminisme du sexe par haplo-diploïdie responsable d'une très forte corrélation génétique entre les femelles a certainement favorisé l'eusocialité par la sélection de parentèle. Cette dernière s'exprime néanmoins dans un contexte où les contraintes écologiques (défense collective contre les parasites, les prédateurs…) et les avantages de la coopération sont très probablement considérables. Soulignons que l'haplo-diploïdie ne conduit pas inévitablement à la vie sociale, de nombreuses espèces haplo-diploïdes (aleurodes, rotifères, certaines guêpes…) ont une vie solitaire (Aron & Passera 2000). Chez les espèces haplo-diploïdes, les seuils écologiques favorables à l'évolution du comportement altruiste sont néanmoins inférieurs à ceux attendus chez les espèces diploïdes.

3. Diversité des modes d’organisation et des stratégies de reproduction

Les nombreuses études empiriques montrent que l'organisation des sociétés s'éloigne très souvent du schéma classique où la colonie ne possède qu'une seule reine accouplée avec un seul mâle (Figure 1 ; Bourke & Franks 1995 ; Heinze & Tsuji 1995 ; Bourke 1999). Chez les fourmis en particulier, l'organisation sociale est extrêmement variable d’une espèce à l’autre et parfois au sein de la même espèce (Hölldobler & Wilson 1990 ; Ross & Shoemaker 1997 ; Herbers & Banschbach 1999 ; Tsutsui & Case 2001). Cette diversité est principalement liée à deux facteurs : (1) le nombre de reines par colonie, monogynie (une reine) *vs* polygynie (plusieurs), et (2) le nombre de mâles avec lesquels chaque reine s’accouple, monoandrie (un mâle) *vs* polyandrie (plusieurs) (Bourke & Franks 1995). La polygynie et la polyandrie tendent à diminuer l’apparentement moyen entre les ouvrières au sein des colonies et ainsi les bénéfices des actes altruistes. Une ouvrière transmettra ses gènes moins efficacement que si elle se reproduisait elle-même. La diminution du succès reproductif indirect des ouvrières peut théoriquement fragiliser la cohésion sociale (Reichardt & Wheeler 1996) et ainsi affaiblir les forces sélectives qui maintiennent l'eusocialité. Le « paradoxe » de la polyandrie et de la polygynie chez les insectes sociaux, soulevé par Hamilton lui-même (1964b), peut néanmoins s’expliquer de la façon suivante : une fois que la socialité a été sélectionnée et maintenue au cours de l’évolution, par un mécanisme hamiltonien, elle a pu ensuite dériver et prendre des formes qui apparaissent contradictoires avec la sélection de parentèle. Dès lors que les individus sont pris dans le piège évolutif de la socialité, la polyandrie et la polygynie ont pu être sélectionnées et maintenues par un mécanisme consécutif de type darwinien, ceci pour avoir apporté un nouvel avantage adaptatif à une vie sociale pré-existante.

3.1 Monogynie / Polygynie

Chez les insectes sociaux, le nombre de reines par colonie est extrêmement variable d’une espèce à l’autre et parfois au sein de la même espèce (Bourke & Franks 1995). Si, chez les espèces/formes faiblement polygynes, les reines sont souvent apparentées et parfois sœurs (Pamilo & Rosengren 1984 ; Seppä 1994), en revanche, chez les espèces fortement polygynes où chaque société compte plusieurs milliers de reines reproductrices peu ou non apparentées

(Passera 1984 ; Passera & Keller 1994 ; Rosengren et al. 1993 ; Ross & Shoemaker 1993), l'apparentement entre ouvrières peut alors descendre jusqu'à des valeurs proches de zéro.

Des contraintes écologiques sur l'efficacité de la dispersion des femelles (coût de la dispersion, fragmentation de l'habitat, climat) et des fortes pressions de sélection locale sur la fondation de nouvelles colonies (limitation des sites de nidifications) sont généralement considérées comme étant à l'origine de l'évolution de la polygynie (Keller 1995 ; Pamilo 1991 ; Bourke & Heinze 1994). Par exemple, si l'habitat d'une espèce est rare, une nouvelle reine aura peu de chance de fonder une nouvelle colonie si elle s'éloigne de sa colonie natale. L'habitat risque, pour cette raison, d'être saturé en colonies. Il sera donc moins risqué pour une reine de s'établir dans une colonie mature. Plusieurs études montrent en effet que les facteurs écologiques déterminent en grande partie l'organisation sociale des colonies (Bourke & Franks 1995 ; Herbers 1986, 1989). Herbers & Banschbach (1999) ont montré que l'organisation sociale de la fourmi *Myrmica punctiventis* était influencée par deux paramètres écologiques, la disponibilité de nourriture et de nids exploitables. Chez *Leptothorax longispinosus,* des études comparatives révèlent également que la proportion de colonies polygynes est plus forte dans les populations à forte densité (Herbers 1989). Chez la fourmi *Solenopsis invicta,* l'organisation sociale des colonies est déterminée génétiquement par le gène G*p*-9 (Ross & Keller 1998 ; Ross & Keller 2002).

3.2 Monoandrie / Polyandrie

L'évolution et le maintien de la polyandrie chez les hyménoptères eusociaux sont des phénomènes énigmatiques (Boomsma & Ratnieks 1996). Comme chez les autres espèces animales, les accouplements multiples des femelles sont probablement coûteux pour les reines elles-mêmes. Ils impliquent non seulement une perte de temps et d'énergie (Thornhill & Alcock 1983) mais également une augmentation des risques de prédation et d'infections parasitaires (Thornhill & Alcock 1983 ; Hurst & Peck 1996 ; Arnqvist & Nilsson 2000). La valeur adaptative de la polyandrie suscite de nombreuses interrogations. Le coût des accouplements multiples pour les reines n'a cependant jamais été estimé.

Par ailleurs, l'accouplement réduit la corrélation génétique moyenne entre individus d'une même colonie pouvant ainsi affecter la stabilité et la structure sociale des colonies (Reichardt & Wheeler 1996). La corrélation génétique moyenne entre ouvrières (r) chute de

0,75 à 0,25 à mesure que le nombre d'accouplements de la reine augmente (Figure 2 ci-dessous; $r = 0{,}25 + (0{,}5/M_p)$ avec M_p le nombre de partenaires différents contribuant équitablement à la production d'ouvrières).

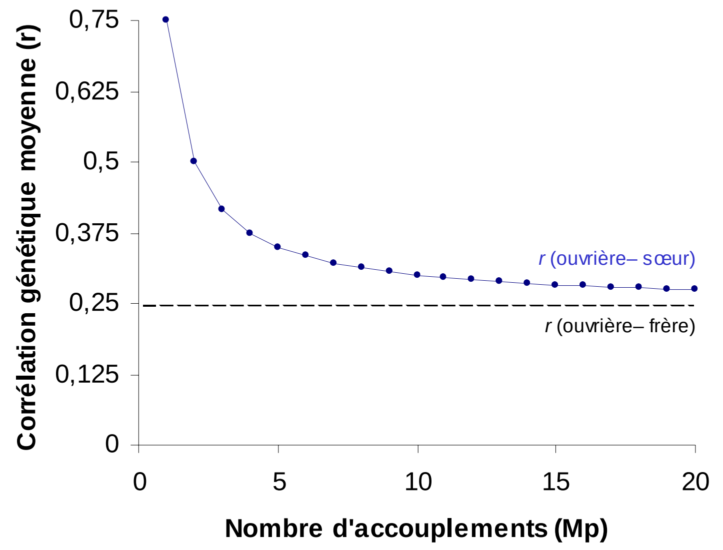

Figure 2. Évolution de la corrélation génétique moyenne (*r*) entre ouvrières en fonction du nombre d'accouplements des reines.

Si la monoandrie semble être la règle chez la majorité des Hyménoptères sociaux (Boomsma & Ratnieks 1996; Chapuisat 1998; Boomsma et al. 1999; Strassmann 2001 pour revue), il existe néanmoins des exceptions notables dans chacun des groupes (abeilles, guêpes et fourmis). De forts taux de polyandrie ont été détectés chez les fourmis des genres *Atta*, (M_e [2] = 3.1 ; Fjerdingstad & Boomsma 1998), *Acromyrmex* (M_e = 3.9-5.3 ; Reichardt & Wheeler 1996 ; Boomsma et al. 1999, Sumner et al. 2004), *Pogonomyrmex* (M_e = 6.8, Cole & Wiernasz 1999a, 1999b ; Gadau et al. 2003; Wiernasz et al. 2004), *Dorylus* (M_e = 17.5 ; Kronauer et al. 2004) et *Eciton* (M_e = 33.4 ; Denny et al. 2004) chez la guêpe *Vespula maculifrons* (M_e = 7.14 ; Ross 1986 *)* et chez les abeilles du genre *Apis* (M_e = 5.6 à 40 selon les espèces ; Palmer & Oldroyd 2000, 2001).

Diverses hypothèses ont été proposées pour expliquer quels pouvaient être les bénéfices susceptibles de compenser les coûts associés à la polyandrie chez les Hyménoptères sociaux (voir Bourke & Franks 1995 ; Chapuisat 1998 ; Boomsma & Ratnieks 1996 ; Crozier & Pamilo 1996 ; Strassmann 2001 ; Crozier & Fjerdingstad 2001 ; pour revues).

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[2] M_e = le nombre d'accouplements effectifs

Hypothèse 1 : La femelle peut accepter de multiples copulations car cela peut représenter un coût plus élevé de résister que de subir (Thornhill & Alcock 1983). Toutefois, diverses observations biologiques semblent indiquer que cette hypothèse est peu probable chez les Hyménoptères sociaux. Chez les abeilles par exemple, les reines effectuent plusieurs vols nuptiaux (Tan et al. 1999). L'accouplement multiple est donc bien un choix des reines, elles ne sont pas contraintes par les mâles. Chez les bourdons, les reines sont d'ailleurs capables de chasser les mâles indésirables ainsi que de fermer leur orifice génital (Duvoisin et al. 1999).

Hypothèse 2 : Une autre hypothèse s'appuie sur des processus de sélection spermatique post copulatoire. Un accouplement multiple peut permettre à la reine d'effectuer une sélection spermatique du meilleur mâle ou du mâle le moins apparenté (Thornhill & Alcock 1983 ; Starr 1984). Selon cette hypothèse, la polyandrie devrait être associée à un biais de représentation des mâles dans la descendance de la reine. Si un tel biais a bien été détecté chez les fourmis *Acromyrmex actospinosus* (Boomsma et al. 1999) et *A. echinatior* (Bekkevold et al. 1999), le sperme est utilisé aléatoirement chez la fourmi *Formica truncorum* (faiblement polyandre) ainsi que chez certaines espèces d'abeilles (Boomsma & Ratnieks 1996 ; Haberl & Tautz 1998 ; Boomsma & Sundström 1998).

Hypothèse 3 : Une hypothèse propose que les femelles s'accouplent avec plusieurs mâles pour constituer une grande réserve de sperme (Cole 1983). Chez *Atta colombica*, la longue vie de la reine (de 10 à 16 ans) associée à une forte reproduction (plusieurs millions d'ouvrières) semble indiquer que la reine a besoin de plus de sperme que ce que lui fournit un seul mâle. L'accouplement multiple lui permet d'augmenter la quantité de sperme disponible et donc sa productivité (Fjerdingstad & Boomsma 1998). En revanche, chez les abeilles, les reines, elles aussi très productives, ne retiennent pas davantage de sperme que ce que peut fournir un seul mâle (Crozier & Page 1985). Cette hypothèse est également peu probable chez des espèces dont les reines ont des vies de courte durée et une production limitée. Par ailleurs, cette hypothèse a été très critiquée dans la mesure où la sélection naturelle devrait favoriser les mâles portant assez de sperme pour correspondre aux besoins des reines (Crozier & Page 1985).

Hypothèse 4 : La polyandrie peut permettre de diminuer le fardeau génétique dû à la production de mâles diploïdes stériles (Pamilo et al. 1994). Chez les hyménoptères, nous avons vu que le sexe est déterminé par le génotype à un ou plusieurs loci spécifiques. Les hétérozygotes à ce locus deviennent des femelles, alors que les homozygotes ou hémizygotes (haploïdes) deviennent des mâles. Les mâles homozygotes diploïdes sont stériles et représentent une charge pour la colonie d'origine. Aussi, une reine qui s'accouple un grand nombre de fois augmente ses chances de ne produire qu'une petite fraction de mâles diploïdes. Si cette hypothèse permet d'expliquer le passage de l'état monoandre à polyandre, elle justifie difficilement l'évolution de taux extrêmes détectés chez certaines espèces (jusqu'à 100 mâles chez *Apis dorsata*, Wattanachaiyingcharoen et al. 2003). L'influence de la charge en mâles diploïdes n'a pas encore été clairement démontrée chez une espèce polyandre.

Hypothèse 5 : L'augmentation de la variabilité génétique peut également réduire les conflits sociaux entre reines et ouvrières. Une reine peut s'accoupler avec plusieurs mâles pour forcer l'intérêt des ouvrières à converger vers le sien concernant la valeur de la sexe-ratio de la descendance sexuée (Starr 1984 ; Pamilo 1991). En situation monoandre, les ouvrières sont trois fois plus apparentées aux filles ($r_{\text{ouvrière – fille sexuée de la reine}} = 0.75$) qu'aux fils ($r_{\text{ouvrière - fils de la reine}} = 0.25$) de la reine. Elles devraient donc favoriser la production des sexuées femelles au détriment de celle des mâles (sexe-ratio attendue de 3 femelles pour 1 mâle). En revanche, la reine est autant apparentée à ses fils qu'à ses filles, et devrait favoriser une sexe-ratio équilibrée. À mesure que le nombre d'accouplements augmente, l'apparentement des ouvrières aux femelles décroît (Figue 2, p. 17) diminuant ainsi l'intensité du conflit quant à la sexe-ratio de la descendance sexuée (Figure 3 ci-après). Chez *Formica truncorum*, les ouvrières des rares colonies polyandres (jusqu'à trois mâles) produisent davantage de mâles que les ouvrières des colonies monoandres (Sundström 1994). En revanche, chez *Lasuis niger*, aucune différence n'a été observée entre les colonies monoandres et les colonies polyandres (jusqu'à quatre mâles) (Fjerdingstad et al. 2002). La faiblesse de cette hypothèse est qu'il semble peu probable que les ouvrières puissent détecter le nombre exact de mâles avec lesquels les femelles se sont accouplées, et donc ensuite ajuster avec précision la sexe-ratio (Palmer & Oldroyd 2000).

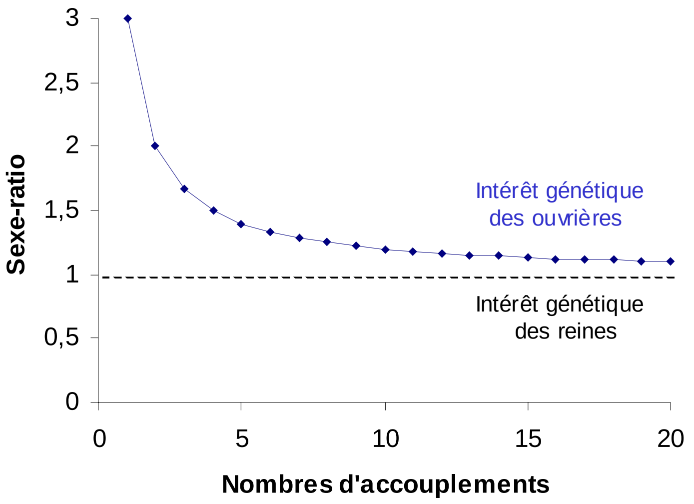

Figure 3. Evolution de la sexe–ratio optimale des ouvrières en fonction du nombre d'accouplements des reines.

Hypothèse 6 : Une plus forte variabilité génétique des ouvrières à l'intérieur des colonies augmenterait la performance des colonies (Crozier & Consul 1976) par une meilleure résistance aux parasites ou aux pathogènes (Hamilton 1987 ; Shermann et al. 1988 ; Schmid-Hempel ; 1994, 1998), par une meilleure distribution des tâches (Fewell & Page 1993) ou encore par une meilleure capacité d'adaptation à des modifications environnementales (Williams 1966). Ceci suppose donc l'existence d'une base génétique à la résistance aux parasites ou pathogènes et un déterminisme génétique des performances des ouvrières dans les différentes tâches au sein des colonies. Chez la fourmi *Pogonomyrmex occidentalis*, les colonies où les ouvrières sont les moins apparentées ont effectivement le plus fort taux de croissance (Cole & Wiernasz 1999a, 1999b), la raison exacte n'est cependant pas encore déterminée. Des études expérimentales sur les bourdons ont montré que les colonies caractérisées par une plus grande variabilité génétique étaient avantagées en terme de production de sexués et étaient moins infestées par différentes espèces de parasites (Shykoff & Schmid-Hempel 1991 ; Baer & Schmid-Hempel 1999). En revanche, chez différentes espèces d'abeilles (Woyciechowski et al. 1994), le taux d'infestation à un parasite particulier n'est pas différent entre des colonies polyandres et monoandres. Neumann et Moritz (2000) n'ont trouvé aucune association entre la fréquence d'accouplement, la taille des colonies, la productivité ou même la résistance aux parasites chez *Apis mellifera*. Cependant, il a été montré que les colonies ayant de plus hauts niveaux de variabilité génétique s'adaptent mieux à des changements environnementaux pendant le fourragement (Fewell & Page 1993). Par ailleurs, la redistribution de la variabilité génétique diminuerait les pressions de sélection au niveau des colonies puisque la variance génétique entre les colonies est diminuée (Page & Metcalf 1982).

Finalement, les études empiriques qui ont été menées afin de tester ces différentes hypothèses sont peu nombreuses et souvent contradictoires. Dans la mesure où ces hypothèses ne sont pas mutuellement exclusives, l'influence relative des différentes pressions de sélection est difficilement estimable et reste largement méconnue.

Bien que le taux de polyandrie ait été estimé chez différentes espèces (Boomsma & Ratnieks 1996 ; Strassmann 2001 ; pour revue), peu d'études ont visé à tester la variation de ce taux entre les populations. Pourtant, une variation des taux de polyandrie entre populations pourrait permettre de tester les hypothèses émises concernant l'évolution de la polyandrie. À ce jour, seuls quelques exemples de variation intra-spécifique sont disponibles. Chez la fourmi monogyne *Lasius niger*, Boomsma et Van der Have (1988) ont détecté des différences de taux de polyandrie entre populations et un plus fort niveau de polyandrie a été observé dans la population où la compétition était la plus forte et les ressources limitées. Chez *Bombus terrestris*, les reines des populations du nord de l'Europe s'accouplent plusieurs fois alors que dans le reste de l'Europe, la monoandrie est la règle (Estoup et al. 1995). Chez Apis *mellifera canica*, le nombre de partenaires des reines varie d'une île à l'autre (M_e = 13±1.1 *vs* M_e = 18±1.1 ; Neumann et al. 1999)

3.3 Reproduction sexuée et asexuée

Nous avons vu précédemment que les hyménoptères sociaux utilisent la reproduction sexuée pour produire des femelles (issues d'œufs fécondés) et la parthénogenèse arrhénotoque pour produire des mâles (issus de gamètes non fécondés). Chez quelques rares espèces, les femelles utilisent également la reproduction asexuée pour produire les femelles. Ces femelles sont issues d'œufs diploïdes produits par parthénogenèse thélytoque. Ce type de reproduction n'a été mis en évidence que chez sept espèces issues de familles très différentes :

1 : *Pristomyrmex pungens,* Myrmicinae (Itow et al.1984 ; Tsuji 1988),

2 : *Cerapachys biori*, Cerapachyinae (Tsuji & Yamauchi 1995),

3 : *Platythyrea punctata*, Ponerinae (Heinze & Hölldobler 1995 ; Hartmann et al. 2005),

4 : *Messor capitatus*, Myrmicinae (Grasso et al. 2000),

5 : *Cataglyphis cursor*, Formicinae (Cagniant 1979 ; Pearcy et al. 2004),

6 : *Wasmannia auropunctata*, Myrmicinae (Fournier et al. 2005),

7 : *Apis mellifera capensis* (Greff 1996).

D'une espèce à l'autre, la parthénogenèse thélytoque peut être facultative (5,6), prédominante (3) ou obligatoire (1,2). Elle est l'apanage des reines (6), des ouvrières (7, 2, 3), ou des deux (5). Les mécanismes cytologiques mis en jeu sont également différents d'une espèce à l'autre (Schilder et al. 1999 ; Pearcy et al. 2004 ; Fournier et al. 2005). En effet, la parthénogenèse peut être de type automictique (5) ou apomictique (3, 6), respectivement avec ou sans méiose. Dans le premier cas, une femelle hétérozygote pourra produire par parthénogenèse des génotypes différents (homozygote et hétérozygote). Dans le second cas, la femelle transmet de façon clonale son génome.

La reproduction parthénogénétique des femelles est donc vraisemblablement apparue à plusieurs reprises de façon indépendante au cours de l'évolution. L'origine évolutive de la reproduction parthénogénétique des femelles chez les insectes sociaux a reçu relativement peu d'attention. Pour des raisons économiques, la parthénogenèse thélytoque a été davantage étudiée chez l'abeille du Cap. Une étude récente montre que la thélytoquie est sous le contrôle d'un seul gène (*th*) et déterminée par un allèle récessif à un seul locus donné (Lattorff et al. 2005).

L'utilisation stricte de la parthénogenèse conduit inévitablement à des structures clonales des colonies et parfois des populations (*Platythyrea punctata* ; Schilder et al. 1999) alors que son utilisation facultative permettrait, en théorie, d'obtenir toute une gamme de diversité génétique au sein des colonies.

4. Diversité des stratégies de dispersion et de reproduction des colonies

Au même titre que les stratégies de reproduction des individus au sein des colonies, les stratégies de dispersion des sexués et de reproduction des colonies déterminent la façon dont la diversité génétique se repartit au sein et entre colonies voisines. Connaître les relations génétiques entre nids adjacents et la viscosité des populations est un pré-requis à une analyse correcte de l'organisation sociale (Ross et al. 1999).

Chez les fourmis, un consensus général a établi que les modalités de reproduction et de dispersion sont fortement associées (Bourke & Franks 1995). L’organisation sociale des colonies, en particulier le nombre de reines par colonie, est considérée comme étant un déterminant majeur des modalités de reproduction et de dispersion. Chez les espèces/formes monogynes, les sexués s’accouplent généralement lors de vols nuptiaux durant lesquels ils se dispersent sur de plus ou moins longues distances. Les reines ailées sont physiologiquement capables d’établir de nouveau nid sans l’aide d’ouvrières, on parle alors de fondation indépendante des colonies. En revanche, chez les espèces/formes polygynes, les femelles s’accouplent en général près de leur nid natal et sont incapables d’établir seules de nouvelles colonies. Elles débutent leur reproduction dans des nids déjà existants. La reproduction des colonies se fait par fission (fondation dépendante), un processus au cours duquel une ou plusieurs reines accompagnées par un groupe d’ouvrières partent établir une nouvelle colonie à proximité du nid maternel. Cette association entre monogynie/fondation indépendante et polygynie/fondation dépendante est aujourd’hui largement remise en question (Peeters & Ito 2001). Chez certaines espèces monogynes, la fondation de nouvelles colonies se fait également de façon dépendante. C’est le cas des fourmis mercenaires, *Eciton* (Franks 1989), des fourmis sans reine du genre *Diacamma* (Doums et al. 2002) et de la fourmi *Aphaenogaster senilis* (T. Monnin, com. pers.). Ceci pourrait être également le cas chez de nombreuses espèces tropicales qui, à ce jour, restent très peu étudiées par rapport aux espèces des milieux tempérés (*Formica*, *Lasius*). Par ailleurs, chez de nombreuses espèces/formes polygynes, les colonies présentent des stratégies mixtes, elles peuvent émettre des reines ailées capables de fonder seules des nouvelles colonies et également fonder de nouvelles colonies par bourgeonnement de la colonie mère (Cherix et al. 1991).

À l'échelle de la population, les modalités de dispersion et de reproduction vont fortement affecter la distribution de la variabilité génétique. La fondation dépendante des colonies peut générer une agrégation spatiale des fréquences alléliques et induire une forte viscosité à l’intérieur des populations, c’est à dire que les colonies sont d'autant plus proches génétiquement qu'elles le sont géographiquement (Hamilton 1964). Plusieurs études ont effectivement montré une forte viscosité des populations chez les espèces polygynes (Chapuisat & al 1997 ; Seppä & Pamilo 1995 ; Tsutsui & Case 2001). De nombreuses études comparatives, chez *Solenopsis invicta* (Shoemaker & Ross 1996 ; Ross et al. 1997) ou entre espèces très proches chez les genres *Formica* (Pamilo & Rosengren 1984 ; Sundström 1994) et *Myrmica* (Seppä & Pamilo 1995), ont mis en évidence l'existence d'une structure génétique locale chez les populations polygynes et son absence chez les populations monogynes à fondation indépendante des colonies (pas de viscosité).

À une plus grande échelle, les capacités de dispersion sont également souvent corrélées à la structuration génétique entre les populations (Bohonak 1999). La structuration génétique entre les populations est souvent d'autant plus forte que les capacités de dispersion des espèces sont réduites (Bohonak 1999). Chez les fourmis, la fondation dépendante des colonies, si elle est associée à une dispersion limitée des mâles à grande échelle, peut induire une forte structuration génétique entre les populations. Connaître cette structuration est essentiel pour comprendre les processus évolutifs en général, puisqu'elle conditionne les potentialités d'adaptation locale. Des divergences génétiques entre populations pourraient par ailleurs induire des différences comportementales, en particulier concernant les modalités de reproduction, et à terme conduire à des processus de spéciation (Ward 1989). Le nombre d'études de génétique des populations à grande échelle chez les fourmis reste cependant faible par rapport à celui des études concernant l'organisation sociogénétique et la viscosité des populations.

5. La fourmi *Cataglyphis cursor*

Parmi les espèces de fourmis connues à ce jour, l'espèce méditerranéenne *Cataglyphis cursor* (voir Figure 4 ci-après), présente un ensemble de caractéristiques tout à fait originales et pertinentes pour étudier l'évolution des systèmes de reproduction et des traits d'histoire de vie en lien avec l'évolution des paramètres sociaux, comme la régulation de la reproduction ou la division du travail.

5.1 Des stratégies de reproduction exceptionnelles

Avant d'entreprendre ce travail de thèse, nous connaissions de *C. cursor*, deux caractéristiques remarquables :

1. Chez cette espèce monogyne, les ouvrières sont capables de produire, en l'absence de la reine, des femelles (reines et ouvrières) par parthénogenèse thélytoque, et des mâles par parthénogenèse arrhénotoque (Cagniant 1973). La production de femelles parthénogénétiques est, nous l'avons vu, exceptionnelle et n'a été reportée que chez cinq autres espèces de fourmis. D'après Lenoir (1986), la parthénogenèse des ouvrières ne serait qu'un mécanisme « de secours » en cas de disparition accidentelle de la reine mère. À ce jour, l'utilisation de la parthénogenèse par les ouvrières en milieu naturel reste néanmoins à démontrer.

2. Les observations de terrain, indiquent que les reines s'accouplent successivement avec plusieurs mâles (Lenoir et al. 1988). Toutefois nous ignorions si ces accouplements multiples s'accompagnent de transferts efficaces de sperme. Seules des analyses génétiques permettent d'estimer le taux de polyandrie des reines. Les accouplements multiples (non anecdotiques) des reines sont également extrêmement rares chez les fourmis (voir 3.2).

C. cursor offrait d'ores et déjà un modèle unique pour étudier la diversité des stratégies de reproduction et leur impact sur la structure des colonies, puisque la polyandrie des reines augmente la diversité génétique au sein des colonies alors que la parthénogenèse des ouvrières la diminue potentiellement.

Au cours de ce travail de thèse, les travaux de Morgan Pearcy (Pearcy et al. 2004) ont mis en évidence d'autres caractéristiques remarquables, jusqu'alors jamais observées chez les fourmis.

1. Dans l'unique population étudiée, des analyses génétiques ont confirmé les transferts

efficaces de sperme lors des nombreux accouplements. Le nombre de partenaires par femelles varie de quatre à huit (5.6±1.3 en moyenne, n = 12).

2. Le génotypage de gynes (reines vierges) et d'ouvrières a révélé que les reines étaient, elles aussi, capables d'utiliser la parthénogenèse thélytoque pour produire des femelles. Plus surprenant encore, elles sont capables d'utiliser la reproduction sexuée et asexuée de manière sélective. Les nouvelles reines (gynes) sont produites en majorité par parthénogenèse thélytoque automictique[1] alors que les ouvrières sont produites par reproduction sexuée. Cette stratégie, inédite chez les fourmis, permet donc à la reine de maximiser la proportion de gènes transmis à la génération suivante tout en conservant une diversité génétique élevée au sein des colonies, tirant ainsi les bénéfices de chacun des types de reproduction (Pearcy et al. 2004). Il conviendra de vérifier si l'utilisation de la parthénogenèse et de la polyandrie est exceptionnelle à la population étudiée ou une caractéristique générale de l'espèce.

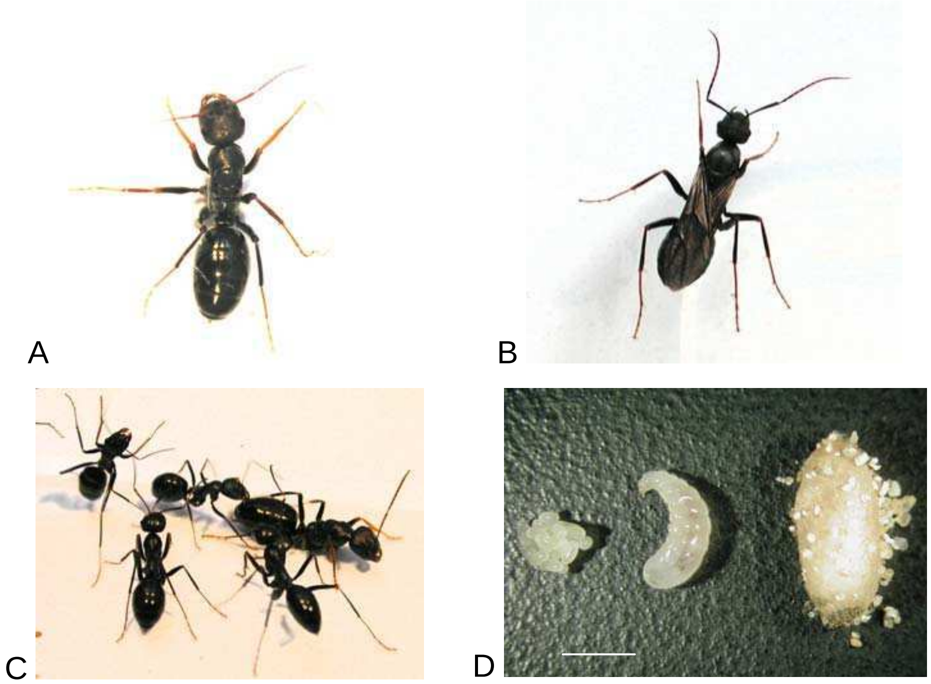

Figure 4. L'espèce *Cataglyphis cursor*

A- Reine ; B- Mâle ; C- Reine et Ouvrières ; D- Couvain : œufs, larve et cocons

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[1] La parthénogenèse utilisée est de type automictique. La cellule mère subit une division de méiose (des recombinaisons peuvent avoir lieu) puis les noyaux polaires contenant chacun une moitié différente du génome fusionnent et forment le zygote. Une femelle hétérozygote pourra donc produire par ce type de parthénogenèse deux génotypes différents, un homozygote pour les allèles maternels et un hétérozygote.

5.2 Dispersion des sexués et reproduction des colonies

Contrairement à la majorité des espèces monogynes connues, chez *C. cursor,* les reines semblent incapables de voler et la fondation des nouvelles colonies se fait de façon dépendante (avec l'aide d'ouvrières). Bien qu'elles soient ailées, les reines de *C. cursor* ne s'engagent pas dans un vol nuptial mais s'accouplent au sol, à proximité de leur nid natal. Des études d'anatomie comparée indiquent que les réserves énergétiques des reines sont très faibles et vraisemblablement insuffisantes pour permettre le vol (Keller & Passera 1989 ; Passera & Keller 1990). Par ailleurs, la fondation indépendante de nouvelles colonies par les reines semble très improbable, ceci pour plusieurs raisons : (i) aucune fondatrice isolée n'a été observée en milieu naturel (Lenoir & Cagniant 1980 ; Lenoir et al. 1988 ; obs. pers.), (ii) en laboratoire, les femelles inséminées meurent rapidement lorsqu'elles sont isolées ou même accompagnées de quelques ouvrières (Lenoir & Cagniant 1980), (iii) à deux reprises, des fissions ont été observées en milieu naturel (Lenoir et al. 1988), des jeunes reines fécondées accompagnées d'un groupe d'ouvrières quittaient le nid natal pour établir un nouveau nid. La dispersion par voie femelle est donc probablement limitée à de courtes distances (distance de marche des ouvrières). Les mâles ailés, quant à eux, peuvent voler et donc disperser leurs gènes sur de plus longues distances. Un tel biais des capacités de dispersion entre les sexes est rare chez les espèces monogynes (voir Article 1).

5.3 Cycle de vie des colonies

Les colonies de *C. cursor* suivent un cycle annuel, sans couvain hivernant. En automne, les colonies ne contiennent que la reine et les ouvrières qui se regroupent dans le fond du nid pour se préparer à hiverner. En hiver l'activité cesse totalement, l'entrée des nids est obstruée et les chambres superficielles s'effondrent sous l'action des pluies (Cagniant 1976b). Après la diapause hivernale et la reprise d'activité (mars), les sexués ailés émergent les premiers au printemps (mai-juin), suivis par les ouvrières jusqu'à la fin de l'été. La reproduction a lieu dès la sortie des sexués, les mâles s'envolent rapidement et parcourent au moins plusieurs dizaines de mètres à la recherche d'autres nids. Les femelles ailées sortant du nid ne s'envolent pas et restent à l'entrée du nid où les mâles viennent les rejoindre. L'accouplement peut avoir lieu avec plusieurs mâles lors d'une course nuptiale. Après l'accouplement, les femelles sont réadoptées dans leur nid d'origine où elles vont perdre leurs ailes. Les reines aptères peuvent ressortir du nid et s'accoupler à nouveau. Dans les jours suivants, un groupe d'ouvrières pourrait quitter le nid avec une femelle inséminée pour fonder une nouvelle colonie (ceci n'a été observé que deux fois ; Lenoir et al. 1988). Si la fission n'a pas lieu, les reines surnuméraires sont alors supprimées ou rejetées de la colonie et la monogynie est rétablie. Au cours des mois suivants les jeunes ouvrières, reconnaissables à leur tégument encore pâle, émergent en nombre. La production diminue en août et se termine début septembre au plus tard.

5.4 Traits d'histoire de vie

Comme l'ensemble des espèces du genre C*ataglyphis*, *C. cursor* est thermophile, strictement diurne et fourrage aux heures les plus chaudes de la journée (Cerdá et al.1989). Elle est principalement nécrophage et se nourrit essentiellement d'arthropodes morts. L'espèce n'est pas territoriale et les aires de récoltes des colonies se recouvrent souvent. Les ouvrières présentent un polymorphisme de taille assez important, de 3 à 11 mm, sans caste différenciée (Cagniant 1983 ; obs. pers.). Les colonies de *C. cursor* sont de tailles relativement petites (675 ± 440 ; Lenoir et al. 1988). Les effectifs sont néanmoins très variables, les plus petites colonies récoltées (probablement des jeunes colonies) comptant une centaine d'ouvrières et les plus grosses jusqu'à 3000 individus (Cagniant 1976b ; Retana & Cerdá 1990 ; obs. pers.). Dans les chemins de vignes et dans le sable en bord de mer, les nids sont nombreux et régulièrement répartis au sol avec une distance moyenne de 3,5 m entre eux (obs. pers.). La densité des nids est relativement forte et peut atteindre jusqu'à un nid par m^2 dans les sentiers sableux du littoral (Lenoir 1986, obs. pers.).

Les nids de *C. cursor* sont étroits et profonds (Bernard 1968) et présentent systématiquement la même architecture (Cagniant 1976a ; obs. pers.). Ils s'ouvrent directement sur l'extérieur par un discret orifice de 0,5 à 1 cm de diamètre (Figure 5). Il n'y a pas de cratère de déblais autour de l'entrée, les ouvrières déposant la terre qu'elles remontent assez loin de l'ouverture, contrairement aux espèces nord africaines de *Cataglyphis* (Cagniant 1973). L'entrée débouche sur un tunnel vertical qui s'ouvre d'abord sur quelques chambres superficielles pouvant s'étendre sur un rayon de 30 cm. Les cocons et les larves âgées y sont entreposés au printemps et en été pendant la journée. La galerie, plus ou moins verticale, rejoint ensuite des chambres plus profondes où se tiennent la reine, les œufs, le jeune couvain et la majorité des ouvrières. La profondeur des nids varie selon les stations et la nature du substrat (Cagniant 1976a ; Lenoir et al. 1988 ; voir Article 4). Sur le littoral, les nids construits dans un sol sableux sont peu profonds en raison du fort taux d'humidité lié à la présence de la nappe phréatique à environ 60 cm sous la surface du sol. Sur terrain calcaire, les nids peuvent atteindre jusqu'à 120 cm de profondeur (voir Article 4), les nids descendant à travers la terre et les cailloux jusqu'à la limite de la roche mère.

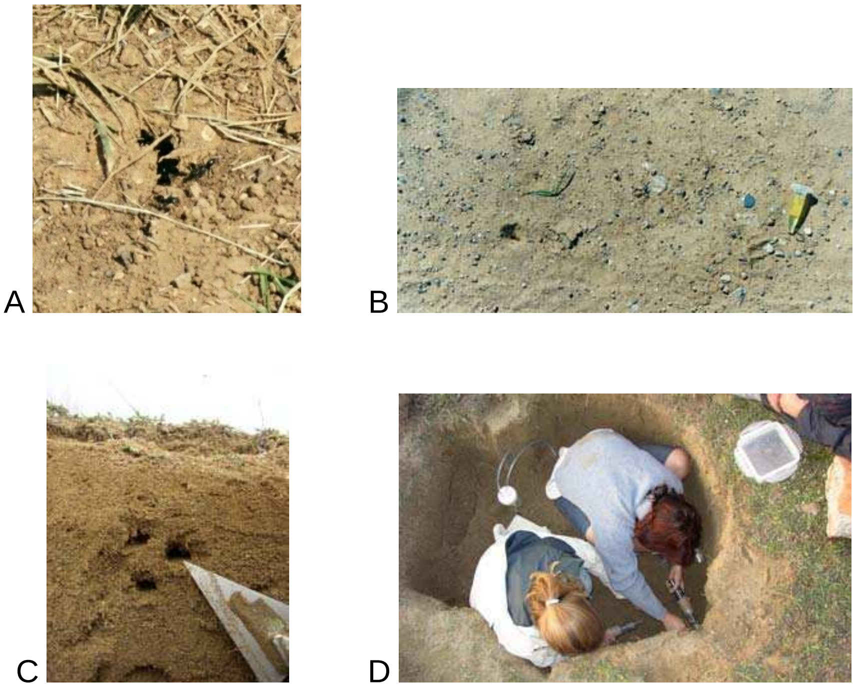

Figure 5. Nid de *Cataglyphis cursor*
A- B- Entrée de nid de *C. cursor*, respectivement en sol calcaire (à Boutenac) et sableux (à Canet) ; C- Chambres superficielles d'un nid en sol sableux ; D- Récolte de colonie à Canet (sol sableux).

5.5 Des habitats *a priori* très différents

L'espèce *Cataglyphis cursor* est distribuée dans toute la partie nord du bassin méditerranéen (Cagniant 1976a). *C. cursor* colonise des sites ouverts, plats, arides et pauvres en végétation. Au sein de son aire de répartition française (Figure 6), on distingue deux types d'habitats : le bord de mer et l'intérieur des terres (les Corbières). En bord de mer, les nids sont construits dans un sol sableux (potentiellement instables), ils sont proches du niveau de la mer et sont peu profonds (environ 60 cm). À l'intérieur des terres, dans les Corbières, les nids sont construits dans un sol calcaire et peuvent être beaucoup plus profonds (Cagniant 1976a ; Lenoir et al. 1988). La distribution des colonies est plus ou moins continue en bord de mer, mais apparaît beaucoup plus irrégulière à l'intérieur des terres. Dans les Corbières, les sites sont souvent assez isolés les uns des autres, et parfois séparés par des espaces forestiers non colonisés par *C. cursor*.

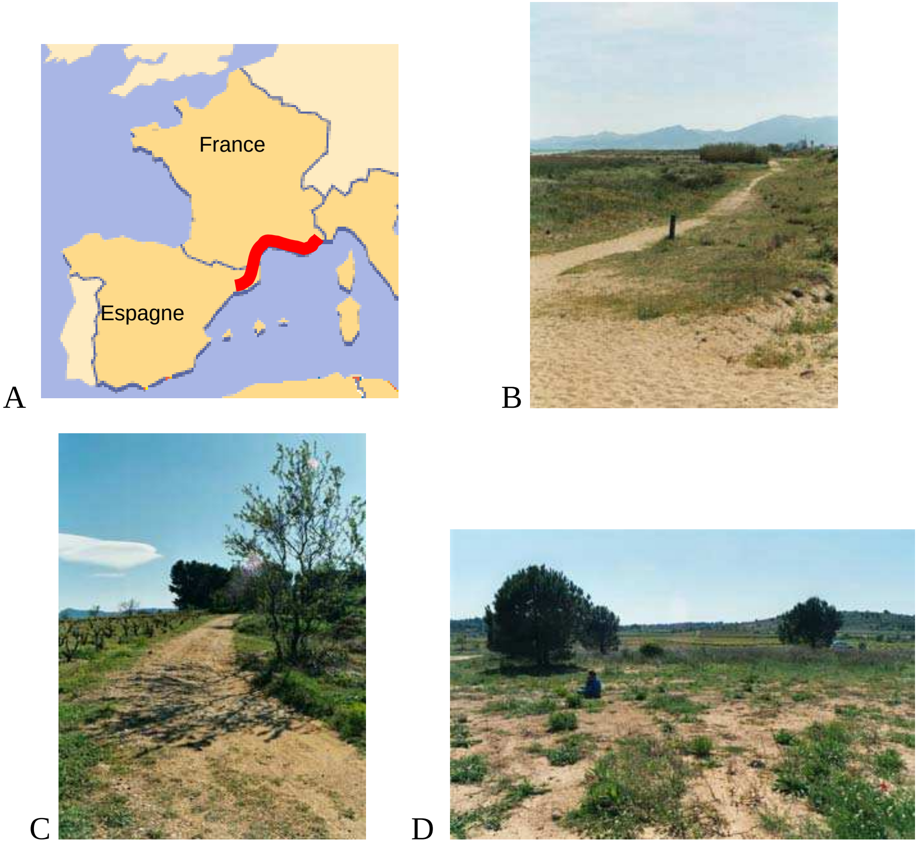

Figure 6. Distribution géographique et illustration des différents types d'habitats colonisés par *C. cursor*

A- Distribution de l'espèce *C. cursor* en France (en rouge); B- Bord de mer (St-Cyprien) ; C- Chemin de vigne (Ste-Marie, Corbières) ; D- Friches (Ferrals, Corbières)

6. Problématique

La fourmi *Cataglyphis cursor* offre un modèle exceptionnel pour étudier l'évolution des stratégies de reproduction chez les insectes sociaux. Aucune étude génétique n'avait été menée chez *C. cursor*.

Dans un premier temps, nous avons donc conduit une approche de génétique des populations à différentes échelles spatiales. Ce type d'étude est essentiel pour définir l'échelle populationnelle et caractériser la différenciation génétique entre les populations, paramètre déterminant pour connaître les potentialités d'adaptation locale et donc comprendre tout processus évolutif. Chez les organismes sociaux, cette approche revêt un intérêt supplémentaire car identifier les limites de la population est indispensable pour estimer correctement l'apparentement entre les membres des sociétés. Cet apparentement est, en effet, fonction des fréquences alléliques au sein des populations. Une connaissance de l'apparentement des individus au sein des sociétés est, comme nous l'avons vu en introduction, un paramètre fondamental lorsque l'on s'intéresse à l'évolution des paramètres sociaux. Nous avons donc déterminé l'organisation sociale des colonies, à savoir les niveaux de polyandrie et de parthénogenèse.

Dans un deuxième temps, nous nous sommes intéressés au polymorphisme de taille des ouvrières, qui nous semble être un trait d'histoire de vie crucial pour les colonies, car il peut avoir des conséquences sur les stratégies de reproduction. Chez cette espèce thermophile, la taille des ouvrières pourrait être liée à leur résistance à la température. Seules les ouvrières de grande taille tolèreraient de fortes températures. Une grande taille d'ouvrière pourrait ainsi représenter un avantage pour la colonie, permettant aux ouvrières de rechercher la nourriture à des heures très chaudes de la journée pendant lesquelles la compétition inter-spécifique est extrêmement réduite. Toutefois, au niveau énergétique, les grandes ouvrières sont plus coûteuses à produire et à maintenir que les petites ouvrières. Les colonies sont probablement confrontées à un compromis : investir dans des ouvrières de grande taille ou investir dans un grand nombre d'ouvrières. Seules les colonies les plus performantes (les plus grandes ou même les plus diverses génétiquement) pourraient alors investir dans des grandes ouvrières. Cette espèce *a priori* polyandre offre un modèle pertinent pour tester l'hypothèse de division du travail qui est déjà bien étudiée chez les abeilles, mais rarement considérée chez les fourmis. S'il existe un déterminisme, au moins en partie, génétique de la taille des ouvrières, alors une plus forte diversité génétique au sein des colonies permettrait une plus large gamme de tailles des ouvrières et pourrait permettre une meilleure division du travail (en particulier dans l'activité de récolte).

Pour mener ce travail, nous avons essayé, dans la mesure du possible, de considérer la diversité des habitats colonisés par *C. cursor* (bord de mer *vs* Corbières). Nous avons donc travaillé sur des populations représentatives des deux types de milieux afin de détecter d'éventuelles variations intra-spécifiques concernant la distribution de la variabilité génétique et l'organisation socio-génétique des colonies. Les facteurs écologiques sont en effet susceptibles d'exercer de fortes pressions de sélection sur l'évolution des stratégies de reproduction et des traits d'histoire de vie, mais leurs variations sont rarement prises en compte chez les insectes sociaux. Chez *C. cursor*, un seul facteur écologique (la nappe phréatique) est susceptible d'exercer une forte contrainte sur la profondeur des nids. La taille des colonies, paramètre crucial pour l'évolution de la socialité et de divers paramètres sociaux (Bourke 1999), pourrait donc varier considérablement d'un habitat à l'autre, entraînant probablement des répercussions sur d'autres traits d'histoire de vie des colonies, comme l'étendue du polymorphisme de taille des ouvrières, ou encore les modalités de fission des colonies.

7. Objectifs de notre étude

Pour répondre à nos questions, des marqueurs génétiques nucléaires (huit loci microsatellites polymorphes) et mitochondriaux (gène cytochrome oxydase COI) ont été développés pour *Cataglyphis cursor* (Article 1 et 2). Les marqueurs microsatellites sont des marqueurs co-dominants, hautement polymorphes, généralement considérés comme neutres et à hérédité mendélienne (Jarne & Lagoda 1996). Ils sont particulièrement pertinents pour les analyses de parenté et l'étude de la structuration intra- et inter-populations de la diversité neutre (Queller et al. 1993). Les marqueurs mitochondriaux sont, quant à eux, des marqueurs à hérédité maternelle et permettent donc de suivre exclusivement les flux géniques femelles. Les modalités de reproduction et de dispersion, souvent difficiles à évaluer par des moyens directs, peuvent être obtenues à partir des empreintes qu'elles laissent sur la distribution de la variabilité génétique à différentes échelles spatiales (Avise 1994).

Afin de caractériser les capacités de dispersion de *C. cursor*, dans des habitats plus ou moins fragmentés, nous avons évalué la diversité génétique au sein d'une vingtaine de populations de *C. cursor* et étudié comment elle se structure à différentes échelles spatiales de la région Languedoc-Roussillon (Article 2). L'utilisation des deux types de marqueurs (nucléaire et mitochondrial) est indispensable pour caractériser la structure génétique et la viscosité des populations, en particulier lorsque les capacités de dispersion des deux sexes sont asymétriques. La dispersion réduite des reines, due au mode de fondation par fission, devrait induire une forte structuration génétique nucléaire entre les populations ainsi qu'une viscosité à fine échelle si les flux géniques mâles sont insuffisants pour homogénéiser la distribution de la variabilité génétique nucléaire. La différence de fragmentation de l'habitat à l'échelle de quelques kilomètres, pourrait contraindre les potentialités de dispersion et ainsi affecter la distribution spatiale de la variabilité génétique. De plus, en raison du caractère *a priori* instable du sol et de la submersion probable des colonies du bord de mer, l'histoire démographique de ces populations pourrait être ponctuée par des goulots d'étranglement diminuant la diversité génétique.

Nous avons ensuite caractérisé les niveaux de polyandrie des reines et vérifié l'utilisation de la parthénogenèse thélytoque pour la production des gynes dans deux populations provenant de chacun des types d'habitats (Article 3). Nous avons testé si une plus forte diversité génétique au sein des colonies était associée à une plus grande variance de taille des ouvrières et une meilleure performance des colonies. Nous avons considéré la moyenne de taille des ouvrières et sa variance dans la mesure où elles pourraient être indirectement associées à la productivité des colonies. Plusieurs études montrent, en effet, qu'une augmentation de la taille moyenne des ouvrières ou de leur variance, accroît le succès des colonies *via* une meilleure exploitation de leur environnement extérieur (Wilson 1953, 1980 ; Billick 2002).

Nous nous sommes alors intéressés plus précisément à ce polymorphisme de taille. Chez cette espèce thermophile, nous avons vérifié expérimentalement si la résistance à la température des ouvrières augmente avec leur taille. Sur le terrain, nous avons testé si la taille des ouvrières affectait leur activité de fourragement. Si les grandes ouvrières sont plus résistantes à la température que les petites, on s'attend donc à observer des ouvrières de plus grande taille sortir chercher de la nourriture aux heures les plus chaudes de la journée. Nous avons donc également regardé si la taille moyenne des fourrageuses variait en fonction des heures de la journée, comme cela a été observé chez une espèce proche de *Cataglyphis* (Article 4).

Par une approche écologique dans plusieurs populations, nous avons étudié la taille moyenne des ouvrières. On s'attend à ce qu'elle augmente linéairement avec la taille de la colonie, confirmant l’hypothèse que seules les colonies suffisamment grandes peuvent investir dans des ouvrières de grande taille (Article 5).

Enfin, dans la mesure où les ouvrières ont gardé la possibilité de se reproduire, de fortes pressions de sélection peuvent également s’exercer au niveau individuel sur les traits phénotypiques des ouvrières, en particulier sur leur taille. Au sein d’une colonie orpheline, une étude comportementale associée à des analyses génétiques et morphométriques a été réalisée afin de déterminer si le potentiel reproducteur des ouvrières est lié à leur taille et/ou diffère selon leur lignée paternelle (Article 6).

ARTICLE 1

Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*

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Développement de marqueurs génétiques microsatellites pour la fourmi *Cataglyphis cursor*

Nous avons développé huit paires d'amorces de loci microsatellites à partir de l'ADN génomique de *Cataglyphis cursor*. Ces huit marqueurs se sont révélés hautement polymorphes. Cinq à dix allèles par locus et de forts taux d'hétérozygotie (de 0,77 à 0,85) ont été détectés sur seulement 19 ouvrières issues d'une même population. Des amplifications ont également été réalisées avec succès sur cinq autres espèces appartenant au genre *Cataglyphis* (*C. fortis, C. velox, C mauritanicus*, *C. bicolor* et *C. nodus*). Ces marqueurs microsatellites, destinés à l'étude de la structure génétique des populations et de l'analyse socio-génétique des colonies de *C. cursor,* pourront donc également servir pour d'autres espèces de *Cataglyphis*.

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PRIMER NOTE

Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*

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Abstract

The ant *Cataglyphis cursor* is exceptional in that unmated workers are potentially able to lay both male and female eggs. We characterized eight pairs of primers for microsatellite loci, developed from genomic DNA for this species. Variability was tested with DNA from 19 workers and all eight loci were highly polymorphic, displaying 5–10 alleles and a high level of heterozygosity. Cross-species amplifications indicate that these microsatellites might be useful in genetic studies of other species belonging to the genus *Cataglyphis*.

Keywords: ant, Cataglyphis, cross-species amplifications, microsatellite, thelytoky

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Kin selection (Hamilton 1964) explains the evolution of a sterile worker caste among social insects in a context of natural selection. Altruistic individuals do not reproduce but still can transmit copies of their genes to the next generation by favouring reproduction of kin, such as parents and siblings. Ants are a well known example of societies where workers sacrifice their own reproduction to participate in cooperative tasks, such as foraging, nest building or rearing offspring nestmates (Hölldobler & Wilson 1990). In the ant *Cataglyphis cursor*, workers have kept functional ovaries and can lay eggs that will develop into males, by arrhenotokous parthenogenesis, but also into females, by thelytokous parthenogenesis (Cagniant 1979). Worker reproduction is rare in the Formicidae and thelytokous parthenogenesis has been shown in only four other species: *Pristomyrmex pungens* (Itow *et al.* 1984), *Cerapachys biroi* (Tsuji & Yamauchi 1995), *Platythyrea punctata* (Heinze & Hölldobler 1995) and *Messor capitatus* (Grasso *et al.* 2000). Despite its peculiar reproductive biology, population and colony genetic structure in *C. cursor* remains unknown and no genetic markers are available for the genus.

Here, we characterize eight highly polymorphic microsatellite markers from *Cataglyphis cursor* nuclear DNA. We also report the results of cross-amplification of these primers on five other *Cataglyphis* species belonging to four different groups of the genus: *C. bicolor* (*bicolor* group), *C. fortis* (*fortis* group), *C. mauritanicus* and *C. velox* (*altisquamis* group) and *C. nodus* (*niger* group). In addition to these five species, we also tested these primers on workers belonging to a remote *C. cursor* population from Mongolia.

Genomic DNA was extracted from three cocoons of *C. cursor* collected at Port Leucate (France) using a high salt procedure (Sambrook *et al.* 1989). RNA strands were removed by incubating the sample for 2 h at 37 °C with 1% RNAase. Genomic DNA was then partially digested with the enzyme *Sau*3A and a fraction ranging from 400 to 800 base pairs was isolated after electrophoresis in low melting agarose gel (*NuSieve*®). The resulting fragments were purified with GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences), ligated into a phagemid vector (*pbluescript®II SK*(+), Stratagene), and cloned in *Escherichia coli* XL-1 Blue competent cells (Stratagene). Synthetic oligonucleotides $(TC)_{10}$ and $(TG)_{10}$, labelled with the DIG system (Boehringer Mannheim) were used to screen about 3150 recombinant colonies. Out of the 73 positives clones that were successfully sequenced (single strand) by Genome Express (France), 65 contained repeated patterns. Twenty-seven sequences contained mononucleotide sequences: 8 polyA and 21 polyT. Thirty-eight sequences contained dinucleotide sequences. From these sequences, 20 pairs of primers were designed using the online software PRIMER 3 (Rozen & Skaletsky 2000; URL: http://www.molbiol.ox.ac.uk/cgi-bin/primer3_www.cgi/). After a prescreening using

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Table 1 Characteristics of microsatellite loci in the ant *Cataglyphis cursor*. The size range, the number of alleles (N_a), the observed (H_O) and expected (H_E) heterozygosities and the frequency of the most common allele [Freq (A)] are based on 19 workers collected in St Hyppolite (France) in 2001

Locus	AN	Repeat motif	Size (bp)	N_a	H_O	H_E	Freq (A)	Primers (5′–3′)
Ccur11	AY590645	$[GA]_{15}$	239–279	10	0.851	0.827	0.237	F: GATTGGCTTGGCGGTATC* R: GGTCGCAAGAGTCACGAG
Ccur26	AY590646	$[GA]_{14}$	102–114	5	0.781	0.780	0.333	F: TGACTCGTGATCTGCTCGTG* R: ATGGGCGTGAATCTCTCTTTC
Ccur46	AY590647	$[TC]_{17}$	151–181	10	0.750	0.733	0.474	F: TGACAACTACCTGCAATAGACG* R: CCAACCAACGAGATTCGAC
Ccur58	AY590651	$[TG]_{15}$	134–182	9	0.833	0.810	0.316	F: TAGTTGGCGTGCTCGAATG* R: TGTGCCTGTCGTTGAATCTC
Ccur63b	AY590654	$[GA]_{19}$	180–214	9	0.787	0.765	0.421	F: GTGTATTAACTCTCCGAAGCAAG* R: GTACCTCACCTGGCATACCG
Ccur76	AY590656	$[TG]_{19}$	190–212	9	0.799	0.800	0.389	F: TGCCTCCCGTTGAGTATG R: TGCTTGGAAGGTAAATGAGC*
Ccur89	AY590658	$[AG]_{22}$	126–144	9	0.729	0.780	0.500	F: CTGAAGTCCTCCGACATATAGC R: GAACGAGAACCAGGCAAGG*
Ccur99	AY590662	$[CA]_8$	101–137	10	0.840	0.840	0.306	F: GCGGAACATTACACGCATAC* R: GTTGAATGACCAGTAACACACG

*Labelled primer.

Table 2 Cross-species amplifications of four polymorphic microsatellite loci. The localization of the sampling is indicated for each species, and the number of individuals (workers) screened is given in brackets. The size range of the amplification product is given for each locus together with the number of alleles found among the sample (in brackets)

Locus	*C. fortis* (3) Tunisia	*C. velox* (2) Spain	*C. mauritanicus* (5) Tunisia	*C. bicolor* (6) Tunisia	*C. nodus* (3) Greece	*C. cursor* (2) Mongolia
Ccur11	243 (1)	245–257 (2)	242–250 (3)	258–267 (3)	—	239–243 (2)
Ccur26	106–126 (2)	104–121 (2)	100–106 (4)	109–119 (3)	94 (1)	94–112 (2)
Ccur46	137 (1)	—	—	—	—	137 (1)
Ccur58	138 (1)	175–193 (2)	138–201 (4)	154 (1)	—	145–154 (2)
Ccur63b	171 (1)	164–185 (2)	171–185 (6)	171 (1)	—	179–192 (2)
Ccur76	—	176–184 (3)	184–194 (4)	—	194–198 (2)	196–206 (3)
Ccur89	118–124 (2)	113–121 (2)	112–114 (2)	118–123 (3)	118 (1)	112–118 (2)
Ccur99	—	122–132 (2)	90–94 (2)	100–117 (2)	102 (1)	103–117 (2)

radioactive dATP, eight markers out of the nine that successfully amplified were selected on the basis of the quality of amplification and potential variability. Their variability was then assessed further on a sample of 19 workers originating from 19 different colonies (one worker per colony) collected in a population close to St Hyppolite (France) in 2001.

Amplifications were carried out in a 10 µL volume containing about 20 ng of genomic DNA, 0.19 mM of each dNTP, 0.25 µM of each primer, 1X *Taq* Buffer (containing 15 mM $MgCl_2$) and 0.03 U *Taq* DNA polymerase (Qiagen). Amplifications were performed in a GeneAmp PCR system 2700 (Applied Biosystems) thermal cycler with the following parameters: after an initial denaturing step of 2 min at 94 °C, the PCR consisted of 10 cycles of 15 s at 94 °C, 15 s at 52 °C (the annealing temperature) and 30 s at 72 °C, followed by 20 cycles of 15 s at 89 °C (to preserve the fluorescent markers), 15 s at 52 °C and 30 s at 72 °C, with 10 more minutes at 72 °C for the final extension. Amplified fluorescent fragments were visualized using the automated Applied Biosystems Prism 310 sequencer (Perkin-Elmer, USA). The size of the different alleles was determined using the ROX-400HD internal size standard and the GENESCAN version 3.2.1 analysis software (Applied Biosystems).

All eight loci were highly polymorphic (Table 1). The expected heterozygosity (H_E) ranged from 0.73 to 0.83 and we found five to 10 alleles within the study population. The probability of deviation from Hardy–Weinberg equilibrium or linkage disequilibrium between pair of loci were both calculated using GENEPOP version 3.4 (Raymond & Rousset 1995), and alpha was adjusted using sequential Bonferroni correction for multiple tests. No significant deviation from Hardy–Weinberg equilibrium were detected for any locus, and all pairwise tests of linkage disequilibrium were nonsignificant.

Results of the cross-species amplifications are given in Table 2. Seven of the eight loci amplified successfully on most other *Cataglyphis* species. The locus Ccur46 successfully amplified only in samples of *C. fortis* and *C. cursor* from Mongolia. The occurrence of different alleles indicate that these primers are potentially useful for genetic studies in other species of this genus.

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ARTICLE 2

Hierarchical analysis of population genetic structure in the monogynous ant *Cataglyphis cursor* using microsatellite and mithochondrial markers

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Molecular Ecology (2005) **14**: 3735-3744

Analyse hiérarchique de la structure génétique des populations de la fourmi monogyne *Cataglyphis cursor* à l'aide de marqueurs génétiques nucléaires et mitochondriaux

Chez *C. cursor*, les reines, pourtant ailées, sont incapables d'établir seules de nouvelles colonies, la fondation de nouvelles colonies se fait donc par fission. La dispersion par voie femelle est donc probablement très restreinte puisqu'elle se limite à la distance de marche des ouvrières.

Afin d'évaluer l'étendue des flux géniques femelle et nucléaire, dans des habitats plus ou moins fragmentés (bord de mer et Corbières), nous avons étudiée la distribution de la variabilité génétique à l'aide d'un marqueur génétique mitochondrial et de huit marqueurs microsatellites. Nous avons caractérisé la diversité génétique au sein d'une vingtaine de populations et étudié la façon dont elle se structure à différentes échelles spatiales de la région Languedoc-Roussillon. La distribution de la variabilité génétique a été étudiée à une grande échelle spatiale (16 sites séparés de 2,5 à 120 Km) ainsi qu'à une échelle plus fine (le long de deux transects de 4,5 Km dans chacun des types d'habitat).

La forte structuration génétique mitochondriale détectée à fine échelle indique que la dispersion efficace des femelles est très restreinte. Par ailleurs, la distribution des haplotypes à grande et fine échelle suggère que les nouveaux sites sont colonisés par des sites voisins, confirmant les faibles capacités de dispersion par voie femelle. Une forte structuration génétique nucléaire a également été détectée à toutes les échelles spatiales étudiées. Les flux géniques mâles sont donc insuffisants pour homogénéiser la structuration induite par la dispersion limitée des femelles, ceci même à l'échelle de quelques centaines de mètres. Néanmoins, les flux de gènes mâles entre populations sont vraisemblablement plus importants que les flux géniques femelles puisque la structuration observée avec des marqueurs nucléaires est 15 fois inférieure à celle observée au niveau mitochondrial.

Malgré les capacités de dispersion limitées de *C. cursor* et l'utilisation potentielle de la parthénogenèse thélytoque pour produire de nouvelles reines, nous ne détectons aucun signe de perte de diversité génique importante dans les populations étudiées.

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Hierarchical analysis of population genetic structure in the monogynous ant *Cataglyphis cursor* using microsatellite and mitochondrial DNA markers

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Abstract

Despite having winged queens, female dispersal in the monogynous ant *Cataglyphis cursor* is likely to be restricted because colonies reproduce by fission. We investigated the pattern of population genetic structure of this species using eight microsatellite markers and a mitochondrial DNA (mtDNA) sequence, in order to examine the extent of female and nuclear gene flow in two types of habitat. Sampling was carried out at a large spatial scale (16 sites from 2.5 to 120 km apart) as well as at a fine spatial scale (two 4.5-km transects, one in each habitat type). The strong spatial clustering of mtDNA observed at the fine spatial scale strongly supported a restricted effective female dispersal. In agreement, patterns of the mtDNA haplotypes observed at large and fine spatial scales suggested that new sites are colonized by nearby sites. Isolation by distance and significant nuclear genetic structure have been detected at all the spatial scales investigated. The level of local genetic differentiation for mitochondrial marker was 15 times higher than for the nuclear markers, suggesting differences in dispersal pattern between the two sexes. However, male gene flow was not sufficient to prevent significant nuclear genetic differentiation even at short distances (500 m). Isolation-by-distance patterns differed between the two habitat types, with a linear decrease of genetic similarities with distance observed only in the more continuous of the two habitats. Finally, despite these low dispersal capacities and the potential use of parthenogenesis to produce new queens, no signs of reduction of nuclear genetic diversity was detected in *C. cursor* populations.

Keywords: *Cataglyphis cursor*, dispersal, fragmented habitat, microsatellites, mtDNA, population viscosity

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Introduction

The ability of individuals to disperse is a fundamental life history trait shaping the distribution of genetic variability within and between populations and, is sometimes crucial to ensure population survival (Clobert *et al.* 2001). In a fragmented habitat, the restricted migration among populations as well as the potentially lower population size is supposed to lead to high genetic differentiation between populations. Even in a continuous habitat where no obvious fragmentation prevents the movements of individuals, other mechanisms can also lead to some genetic differentiation (Ehrich & Stenseth 2001). For instance, a decrease of dispersal efficiency with geographical distance, associated with local genetic drift, can create a pattern of 'isolation by distance' (IBD; an increase of genetic differentiation with geographical distances) (Wright 1943). Most natural habitats are, however, not truly continuous or fragmented and the spatial scale considered can largely affect the level of fragmentation observed. Even at a fine scale, an IBD process could be observed in species with highly restricted dispersal, leading to some population viscosity, i.e. an increase in genetic similarity between potentially interacting neighbours (Hamilton 1964; Rousset 2000). The biology of a species, especially its dispersal behaviour, is one of the main factors (with the level of fragmentation) influencing the scale at which genetic differentiation takes place. The sampling scale is therefore a crucial parameter affecting the observed pattern of population genetic structure. Even though the

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species' life history can give some obvious cues for choosing the relevant sampling scale, a clear picture of the population genetic structure may often require the study of different sampling scales, especially in species with sex-biased dispersal. In social species, the fine scale is of particular interest as the level of population viscosity affects the relatedness between interacting individuals and therefore the evolution of altruistic behaviours (Kelly 1992; Queller 1992).

In this study, we investigated at different spatial scales the population genetic structure of the ant *Cataglyphis cursor*, living in more or less fragmented habitats and in which female dispersal is likely to be restricted. Indeed, even though queens are winged, they seem unable to fly (Lenoir *et al*. 1988; Keller & Passera 1989; Passera & Keller 1990) and the foundation of new colonies by fission (a new colony is established with the help of workers) has been observed in the field (Lenoir *et al*. 1988). This mode of colony foundation clearly restricts female dispersal to ant walking distance and should lead to high mitochondrial DNA (mtDNA) genetic differentiation whatever the spatial scale considered. In contrast, the winged males can potentially disperse their genes over longer distances with male gene flow being likely dependent on the geographical distance. This may translate into different patterns of nuclear genetic structure according to the spatial scale considered. We therefore investigated two main spatial scales, a large spatial scale (populations from 2.5 to 120 km apart) at which male gene flow could be clearly restricted by geographical distance, as well as a fine spatial scale (from 1 to 4500 m) at which no clear prediction on male gene flow could be made.

At a local scale (< 5 km), the habitat could also affect the pattern of population genetic structure. *C. cursor* inhabits dry and open areas and colonies are found both on the seaside in a sandy soil and in vineyards in a stony soil. In the region studied, these two habitats differ not only by the hardness and the stability of their soil but also by their level of fragmentation. The colonies are more or less continuously distributed on the seaside but patchily distributed in the vineyard. If female, but also male gene flow is limited at this scale, the pattern of IBD could differ according to the habitat considered. A pattern of IBD could be expected in the continuous habitat and not in the fragmented one.

Restricted dispersal and genetic drift may decrease local genetic variability, especially within fragmented populations in the vineyard where less than 30 colonies can sometimes be found within a given site. This process can be reinforced by the peculiar reproductive system recently described in a population of this species. In this monogynous species (one single queen per colony), gynes (unfertilized young queens) are produced by thelytokous parthenogenesis whereas the queen mates multiply and uses sexual reproduction to produce workers (Pearcy *et al*. 2004a). The thelytokous parthenogenesis with central fusion observed in *C. cursor* should lead to an increasing level of queen's homozygosity over time (Pearcy *et al*. 2004a). In this particular context, male gene flow appears as a crucial parameter for maintaining worker genetic diversity within colonies and populations. Potential variations in reproductive systems among populations as well as the difference between habitats in the level of fragmentation could induce variations in genetic diversity among populations.

The aim of this study was to address the following questions. First, does restricted female dispersal translate into a male-biased gene flow at the two main spatial scales considered? Second, does restricted dispersal lead to a pattern of IBD and is this pattern affected by the level of habitat fragmentation at a local scale? Third, do the restricted dispersal and the peculiar reproductive system of this species leave footprints on the level of genetic diversity within populations?

Materials and methods

Samples

In order to investigate the pattern of population genetic structure at different spatial scales, two different samplings were performed. First, a large-scale sampling was conducted in July 2001 to assess the distribution of genetic variability between sites separated by 2.5–120 km. More than 300 colonies were sampled in 16 sites (100 × 150 m areas) distributed in six subregions (6–9 km diameter), themselves included in three regions (20–50 km diameter) in Languedoc-Roussillon, France (see Fig. 1). Such sampling allowed to investigate different hierarchical levels of genetic differentiation: (i) between sites within subregion, (ii) between subregions within regions and (iii) between regions. Both seaside and vineyard sites were sampled in order to cover the different types of habitats colonized by *Cataglyphis cursor*. The characteristics and number of colonies sampled in each site are given in Table 1.

Second, a fine spatial scale sampling was conducted in May 2002 by sampling and mapping 82 colonies along two transects of about 4.5 km. One transect was located on the seaside near Argelès (Tr_S) whereas the other transect was sampled in an inland area with vineyard and bushes near Lézignan (Tr_V) (Fig. 1). These two transects clearly differed by the level of habitat fragmentation. In the seaside transect, the colonies are more or less regularly found along the beach whereas in the vineyard transect, colonies are irregularly found, due to the rarity of colonies within the vineyard and their absence in the bushes. For both transects, colonies were sampled within five and six patches more or less regularly located along vineyard and seaside transects, respectively (see Results, Fig. 4a, b). This sampling schema of colonies was chosen because in the vineyard; the habitat discontinuities did not allow to perform a regular sampling

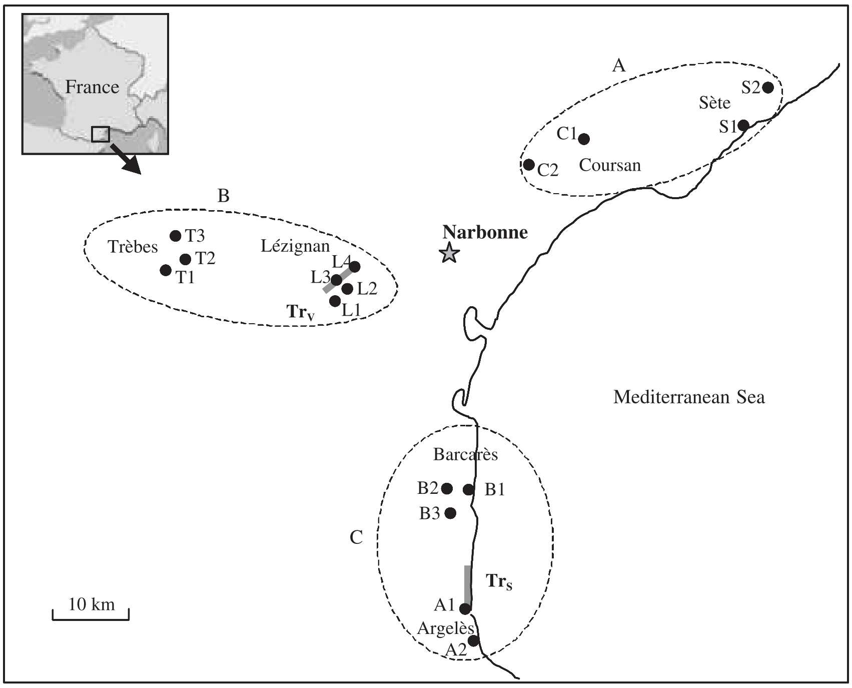

Fig. 1 Geographical location of *Cataglyphis cursor* sites (black points) and transects (grey lines) in Languedoc-Roussillon, France. Three regions (ovals A, B, C), two subregions within each region (characterized by the name of the closest village) and 16 sites (characterized by the first letter of their subregion and a number) were studied. The transect was 3.2 km long on the seaside (Tr_S) and 4.2 km long in the vineyard (Tr_V).

Table 1 Sites and transects description. The number of colonies sampled (*N*), the habitat type, Nei's estimator of gene diversity (H_S), allelic richness (*A*), and mtDNA haplotypes are given for each site. mtDNA sequences were deposited in GenBank (Accession nos from DQ105530 to DQ105559)

Region	Subregion	Site	*N*	Habitat type	H_S	*A*	Haplotypes
A	Sète	S1	19	seaside	0.75	5.45	H_1
		S2	17	vineyard	0.81	5.92	H_2, H_3
	Coursan	C1	12	vineyard	0.75	4.81	H_4
		C2	15	vineyard	0.79	5.56	H_5
B	Trèbes	T1	20	vineyard	0.65	4.12	H_6, H_7
		T2	18	vineyard	0.66	4.41	H_8, H_9
		T3	21	vineyard	0.60	3.78	H_7, H_{10}
	Lézignan	L1	28	vineyard	0.80	5.9	H_{11}
		L2	26	vineyard	0.82	6.22	H_{11}, H_{12}
		L3	20	vineyard	0.71	4.85	H_7
		L4	22	vineyard	0.74	5.34	H_{13}
C	Barcarès	B1	27	seaside	0.79	5.24	H_{14}
		B2	19	pondside*	0.80	5.66	H_{15}
		B3	16	vineyard	0.82	6.22	H_{16}
	Argelès	A1	19	seaside	0.73	4.74	H_{17}
		A2	18	seaside	0.85	6.66	H_{18}
Tr_S			59	seaside	0.75	1.76	H_{19}, H_{20}, H_{21}, H_{22}
Tr_V			65	vineyard	0.72	1.70	H_{23}, H_{24}, H_{25}, H_{26}, H_{27}, H_{28}, H_{29}, H_{30}

*B2 is located on the sandy side of a pond.

and for comparative purpose the same sampling was also chosen on the seaside. For each transect, a map of the colonies was made using a compass and a measuring tape within a patch and using a GPS for locating patches. For the vineyard transect, we used the colonies sampled and mapped in July 2001 in the sites L3 and L4. As the mating season is very short and occurs usually before the end of June, the parental generation has not changed and therefore the allelic frequencies of workers should not vary between the two sampling dates.

For each colony sampled, workers were collected at the nest entrance and immediately placed in 95% ethanol. For both types of markers, we determined the genotype of only one individual per colony to avoid the nonindependence of genotypes attributable to family structure. For the microsatellite markers, all colonies sampled were analysed (i.e. 317 individuals for the large scale and 82 others for the fine-scale sampling, i.e. a total of 399 individuals). Concerning the mtDNA marker, for the large-scale sampling, only two individuals from the most distant colonies were sequenced in each site (i.e. 32 individuals). For the fine-scale sampling, we sequenced 42 and 40 individuals in Tr_S and Tr_V, respectively.

Genetic analysis

DNA was extracted using two different methods, first following a classic high salt protocol, and then using a QIAGEN DNeasy kit, which provided better results. Extracted DNA was resuspended in 100 μL elution buffer and stored at −20 °C.

Eight microsatellites developed for *C. cursor* were used to study nuclear polymorphism (Pearcy *et al.* 2004b). Polymerase chain reactions (PCR) were carried out as described in Pearcy *et al.* (2004b), except that two sets of loci were co-amplified (first set: Ccur26, Ccur89, Ccur 46, Ccur 63b, second set: Ccur 99, Ccur 58, Ccur 76, Ccur 11). Each PCR was run in a 10-μL volume containing 1 μL of DNA solution, 200 μM of each dNTP, 0.15 μM of each primer, 1 μL Buffer 10× and 0.1 unit of *Taq* polymerase (QIAGEN). The amplified fluorescent fragments were visualized using an automated ABI PRISM 310 Sequencer (Applied Biosystems) and allele sizes were estimated using the GENESCAN software.

Mitochondrial DNA variation was assayed following the amplification of a region of the mtDNA cytochrome oxidase subunit 1 region (COI). To develop specific primers for *C. cursor*, we sequenced 6 kb around the cytochrome *c* region using the insect's universal primers Jerry and Barbara, in the middle of COI and in COII, respectively (Simon *et al.* 1994). This sequence was used to design two specific primers: CC-COI (L), 5′-AGGAGCTGTATTTGCTATTATTG-3′ and CC-COII (R), 5′-TTTCAATTAGATCTTGA-3′. Each PCR was run in a 35-μL volume containing 1 μL of DNA solution, 10 mM of each dNTP, 0.15 μM of each primer, 1× *Taq* buffer and 1.25 units of *Taq* polymerase (QIAGEN) using a PCR-100 thermal cycler (MJ Research). The thermal cycle profile was as follows: an initial denaturation of 2 min at 94 °C; 30 amplification cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 50 °C and extension for 45 s at 72 °C; and a final extension for 3 min at 72 °C. Purified template DNA was sequenced with an ABI 310 automatic sequencer (PerkinElmer). For the large-scale sampling, PROSEQ 2.9.1 software (Filatov 2002) was used to analyse the 408 pb sequences. For the fine-scale sampling, the amplified products obtained were sequenced by Genomexpress, which permitted to obtain 600 bp sequences.

Data analysis

Large-scale sampling. The analysis of microsatellite data was carried out using GENEPOP 3.3 (Raymond & Rousset 1995) and FSTAT 2.9.3 (Goudet 1995) programs. Linkage disequilibrium between each pair of loci and deviation from Hardy–Weinberg equilibrium at each locus were examined in all sites by exact tests using GENEPOP. None of the linkage disequilibrium test performed for each locus pair across all sites was significant (all $P > 0.5$). Hence, independence among loci was assumed in the subsequent analyses. Allele frequencies, allelic richness (A) and expected frequencies of heterozygotes (H_S) in each site were estimated from worker genotypes using FSTAT. Permutation tests conducted by FSTAT permitted to determine whether genetic diversity (A and H_S) significantly differed between regions and subregions as well as between habitats.

The significance of the genetic differentiation between sites was examined by conducting permutations tests of allele frequency differentiation (GENEPOP). The joint probabilities of differentiation over all microsatellite loci were obtained using Fisher's combined probability tests (Sokal & Rohlf 1995). The Wright's fixation index, F_{ST}, was used to describe the amount of genetic differentiation between sites, subregions or regions. F_{ST} was estimated using the method of Weir & Cockerham (1984), which corrects for unequal sample size (FSTAT). Standard errors of the estimates were obtained by jackknifing over sites and loci (FSTAT), and probabilities that F_{ST} were significantly different from zero were assessed using permutations tests (GENEPOP). As described above for A and H_S, permutation tests were conducted by FSTAT to determine whether the amount of genetic differentiation observed between sites among regions and subregions significantly differed.

A pattern of isolation by distance was tested by plotting modified F_{ST} [i.e. $F_{ST}/(1 - F_{ST})$] coefficients between pairs of sites against the logarithm of geographical distances (Slatkin 1993; Rousset 1997). The significance of Spearman rank correlation coefficient between genetic differentiation and geographical distance was assessed using a Mantel test with 10 000 permutations (GENEPOP).

We further investigated the importance of the scale on spatial genetic structuring by performing a hierarchical F analysis, allowing the estimate of the amount of genetic variation found at each hierarchical level. A nested four-level analysis of molecular variance (AMOVA; Weir 1996) was performed by partitioning the total sum of squares into components representing variation among regions, among subregions within regions, among sites within subregions and among individuals within sites using the GDA software (Lewis & Zaykin 2001).

The analysis of mitochondrial data was carried out using the software ARLEQUIN 2.00 (Schneider *et al.* 2000). The genetic relationship between all mitochondrial haplotypes was investigated by constructing the minimum-spanning network of the haplotypes using the pairwise differences with ARLEQUIN.

Fine-scale sampling (100–4500 m). As described above for the large spatial scale, A and H_S were estimated and compared for both transects. The significance of the genetic differentiation between patches was examined and tested by conducting permutations tests of allele frequency differentiation (GENEPOP), and the F_{ST} index was used to describe the overall amount of nuclear genetic differentiation among patches. The distribution of nuclear genetic diversity along the transects was first investigated using the individual-based method of Rousset (2000). The estimator a_r of genetic differentiation between individuals, analogous to $F_{ST}/(1 - F_{ST})$, was calculated using GENEPOP and the significance of the correlation between a_r and the logarithm of geographical distance was tested using a Mantel test as described above. As viscosity can be restricted to small distances, we also studied the distribution of alleles within each transect by spatial autocorrelation analysis (Sokal & Oden 1978) using the program SPAGEDI 3.0 (Hardy & Vekemans 2002). Spatial autocorrelation has the advantage of providing results on the shape of the relationship between genetic and geographical divergences (Stow *et al.* 2001). Moran's I statistics for diploid multilocus genotypes were computed for five (Tr_V) and six (Tr_S) geographical distance classes, which were defined such that there was approximately equal number of pairwise comparisons in each class. To test the significance of each Moran's I, they were compared to the distribution of the statistics under the null hypothesis of no spatial structure generated using 10 000 resamplings of the data, permuting spatial location among distance groups.

Mitochondrial genetic structure was examined for each transect by a classical analysis of variance calculating haplotype frequency-based F_{ST}. The probability that the fixation indices were significantly positive (indicating differentiation) was determined by permutation analyses using 1000 randomly permuted data sets with SPAGEDI. To study mtDNA viscosity, spatial autocorrelation analyses were also conducted for each transect. Moran's I statistics were tested as described above for the nuclear markers. Finally, minimum-spanning networks of the haplotypes were constructed with ARLEQUIN using the pairwise distance for both seaside and vineyard transects.

Results

Large-scale sampling

Genetic diversity. Only seven of the 128 probability tests for Hardy–Weinberg equilibrium were significant at $P < 0.05$. Moreover, all these tests were not significant after a Bonferroni correction (Sokal & Rohlf 1995), suggesting a general lack of inbreeding in workers of *Cataglyphis cursor*. The eight microsatellite loci displayed fairly high and quite similar degree of variability in the 16 sites studied (Table 1). Allele frequencies for each locus and site are available upon request. The total number of alleles detected per locus in all *C. cursor* samples ranged from 11 (locus Ccur26) to 28 (locus Ccur76), allelic richness (A) per site ranged from 3.78 (T3) to 6.66 (A2) and genetic diversity (H_S) from 0.6 (T3) to 0.85 (A2) (see Table 1). Interestingly, the three lowest values of gene diversity and allelic richness were obtained for the three sites of Trèbes subregion located in a fragmented landscape at the limit of the repartition area of *C. cursor*. The mean values obtained for Trèbes subregion ($A_{\text{Trèbes}} = 4.1$, $H_{S\,\text{Trèbes}} = 0.64$) significantly differed from those obtained when considering all others subregions together ($A_{\text{other}} = 5.58$, two-side P value after a 10 000 permutations test: $P_A = 0.0012$ and $H_{S\,\text{other}} = 0.78$, $P_{Hs} = 0.0014$). However, no significant difference was detected when comparing between the two types of habitats ($A_{\text{Seaside}} = 5.23$, $A_{\text{Vineyard}} = 5.52$, $P_A = 0.53$ and $H_{\text{Seaside}} = 0.74$, $H_{\text{Vineyard}} = 0.78$, $P_{Hs} = 0.44$).

Over the 32 individuals sequenced, 18 different mtDNA haplotypes were detected with 48 variables sites out of 408 bp (percentage of polymorphic sites = 11.8%). The overall nucleotide diversity, i.e. average number of nucleotide differences per site between two sequences (Nei 1987), was 2.3% (± 1.2 SD). In all sites, the two individuals sequenced share the same haplotype except for the three sites of Trèbes subregions (T1, T2, T3) and the sites L2 and S2 (Table 1).

Genetic differentiation among sites. Exact test of genic differentiation computed across all pairs of sites were highly significant at each microsatellite locus, as well as over all loci ($P < 10^{-5}$), except for the pair T1–T2, which are 3 km distant ($P = 0.089$). In agreement with this high genetic heterogeneity, the overall F_{ST} value was relatively high 0.139 ± 0.021(± SE) compared to its maximum value ($F_{ST\,\max} = 0.245$) deduced from the average within-locality homozygosity (Hedrick 1999). The values of F_{ST} decreased with decreasing the hierarchical level considered (Table 2), though they always stayed highly significantly different from zero (all $P < 10^{-5}$). In agreement with these results, a significant pattern of isolation by distance was detected ($R = 0.69$, $P < 10^{-5}$, Fig. 2). The F_{ST} values estimated between sites within regions did not significantly differ among the three regions ($P = 0.705$). Similarly, the F_{ST} values estimated between sites within subregions did not significantly differed among the six subregions studied ($P = 0.994$). The hierarchical AMOVA (Table 3) revealed that some nuclear variation was found at each hierarchical level (varying between 4% and 8% of the total variation). Fixations indices at each level were all significant (Table 3) and confirmed the general trend that values of F_{ST} decreased with decreasing the hierarchical level considered.

Table 2 Allelic richness (A), expected heterozygosity (H_S, Nei's estimation) and estimates of genetic differentiation between sites (F_{ST}) with jackknifed standard errors are given for the different spatial scales investigated

Spatial scale	H_S	A	F_{ST}
Over all sites			0.139 ± 0.021
Within region:			
A	0.77	5.43	0.125 ± 0.023
B	0.72	4.95	0.089 ± 0.019
C	0.79	5.70	0.092 ± 0.015
Within subregion:			
Sète	0.78	5.69	0.035 ± 0.022
Coursan	0.76	5.18	0.050 ± 0.016
Trèbes	0.64	4.11	0.039 ± 0.016
Lézignan	0.77	5.58	0.039 ± 0.011
Barcarès	0.80	5.71	0.075 ± 0.014
Argelès	0.79	5.70	0.067 ± 0.020

Sixteen out of 18 mtDNA haplotypes were specific to a given site (Table 1). Haplotypes from the same subregion were in general more similar than haplotypes from different subregions as illustrated in the phenogram (Fig. 3). However, there are two exceptions to this general pattern, the two haplotypes found in S2 (H_2, H_3) are highly divergent from all the other haplotypes including those from the same subregion (H_1). Similarly, the haplotypes found in B2 and B3 (H_{15}, H_{16}) were highly divergent from the others, especially the one observed in the other site of this subregion (H_{14}). Interestingly, these two subregions with high mtDNA divergences are the only ones including sites from both seaside and vineyard habitats and in both cases the vineyard sites were the most divergent. Note that these divergences are not reflected in the F_{ST} values estimated with the nuclear markers.

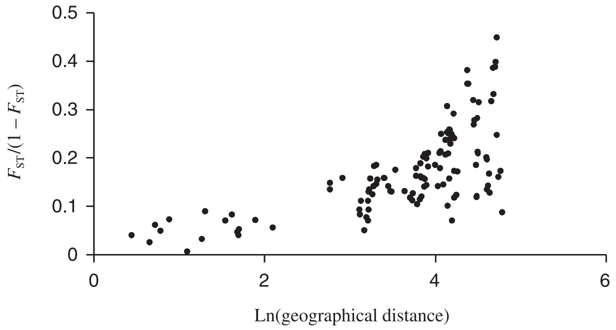

Fig. 2 Relationship between logarithm of geographical distance and nuclear genetic differentiation between sites, estimated as $F_{ST}/(1 - F_{ST})$. The correlation is high and significant ($R = 0.69$, $P < 10^{-5}$).

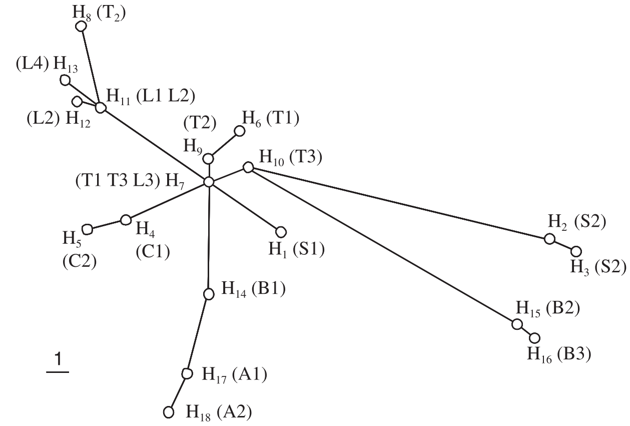

Fig. 3 Minimum-spanning network depicting relationships between the mtDNA haplotypes (408 pb) detected in the 16 sites. The tree was constructed using the pairwise distance. For each haplotype, the site in which it was detected is indicated in parentheses.

Fine-scale sampling

No difference in nuclear genetic diversity was detected between the two transects both for allelic richness ($A_{Seaside} = 1.726$, $A_{Vineyard} = 1.708$, $P = 0.4$) and gene diversity ($H_{S\,Seaside} = 0.758$, $H_{S\,Vineyard} = 0.726$, $P = 0.48$). However, the number of mitochondrial haplotypes was twice higher in the vineyards transect (8) than in the seaside transect (4), but this is not significantly different (Fisher's exact test; $P = 0.356$).

For both transects, nuclear genetic differentiation between pairs of nests, measured as pairwised a_r, was significantly positively correlated with the logarithm of geographical distance (Mantel test $P_{Seaside} < 0.001$ and $P_{Vineyard} = 0.004$), which shows that nuclear gene flow is also restricted by

	F statistics	Percentage variation	d.f.
Among regions	0.161 (0.120; 0.208)	4.59	2
Among subregions within regions	0.115 (0.074; 0.162)	7.79	3
Among sites within subregions	0.038 (0.015; 0.064)	3.76	10
Within sites		83.86	303

Table 3 Four-level hierarchical analyses of nuclear molecular variance (AMOVA). Hierarchical fixation indices and the percentage of genetic variance explained by each hierarchical level are given. The significance of F statistic estimates were obtained by bootstrapping over loci (1000 replicates), 95% confidence intervals are given in parentheses

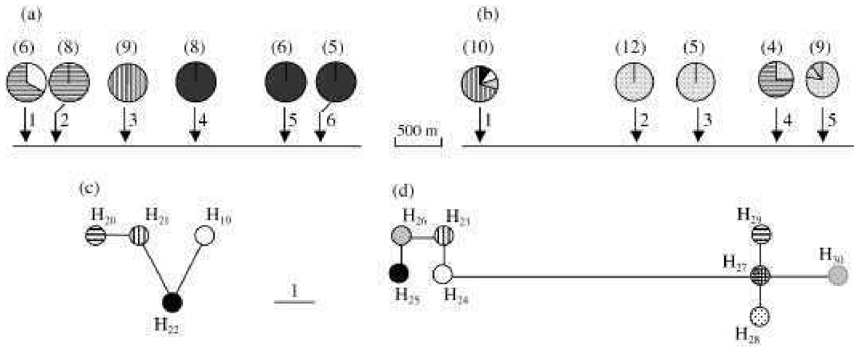

Fig. 4 Distribution of sampled patches and haplotype frequencies within patches along both seaside (a) and vineyard (b) transects. Along each transect, patches are represented by a circle labelled by a number and the number of workers successfully sequenced is given into parentheses. Within a transect, each colour is characteristic of one single mtDNA haplotype. For both seaside (c) and vineyard (d) transects, the relationship between mtDNA haplotypes (600 pb) are depicted by minimum-spanning network constructed using pairwise distance.

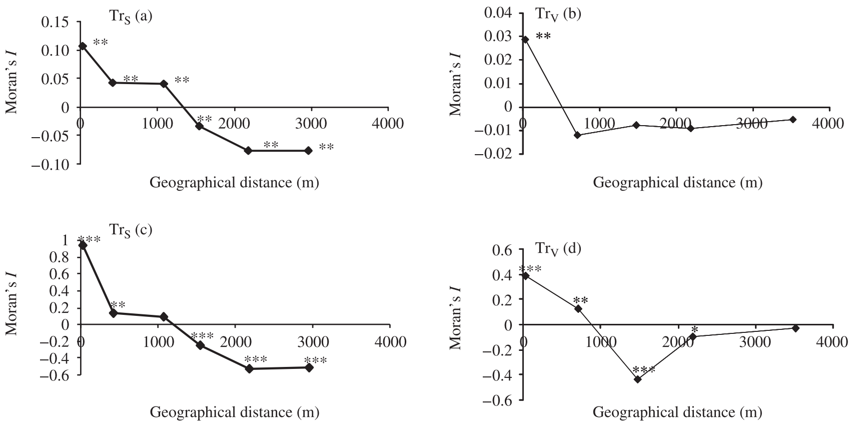

Fig. 5 Spatial autocorrelograms estimated from multilocus microsatelitte genotypes for seaside (a) and vineyard (b) transects and from mtDNA haplotypes, for seaside (c) and vineyard (d) transects. Significance levels of Moran's *I* are indicated by stars (* for $P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

distance at this spatial scale (< 5 km). However, the slope of the regression was significantly higher in the continuous habitat than in the fragmented habitat ($b_{\text{Seaside}} = 0.014$, $b_{\text{Vineyard}} = 0.005$, *t*-test = 12.63, $P < 0.001$). A difference between the two transects was also found in the pattern of spatial autocorrelation. For the seaside transect, the Moran's index decreases regularly with the distance classes, with all indices being significantly different from zero (Fig. 5a). This indicates a linear decrease of genetic similarities with distance in the habitat with the continuous distribution of colonies. For the vineyard transect, the pattern is not linearly decreasing with distances given that only the first Moran's *I* was positive and significantly different from zero (Fig. 5b). This indicates that nests less than 54 m apart (upper limit of the first distance class) were more similar than two nests taken at random on the transect. The habitat discontinuities might preclude a regular pattern of IBD along this transect. Spatial autocorrelograms pattern of mtDNA were qualitatively similar to the one obtained for the nuclear marker, with a linear decrease of Moran's indexes only on the seaside transect (Fig. 5c, d). Note though that the values of Moran's *I* are about 10 times higher for the mtDNA reflecting the stronger genetic structure. The geographical distribution of haplotypes along both transects reveals a strong spatial clustering (Fig. 4a, b). Networks depicting the genetic relationships between mtDNA haplotypes matched quite well the geographical distance relationships, confirming the strong genetic viscosity (Fig. 4c, d). Interestingly, the four haplotypes (H_{23}, H_{24}, H_{25} and H_{26}) found in a single patch were all closely related but quite distant from those detected in the other transect's patches (see Fig. 4).

For both transects, the overall level of nuclear genetic differentiation was significantly different from zero ($F_{\text{ST Seaside}} = 0.058 \pm 0.012$ and $F_{\text{ST Vineyard}} = 0.041 \pm 0.006$) but did not differ significantly between the two transects ($P = 0.09$). Concerning the mtDNA, a very high and significant levels of genetic differentiation over all patches were detected in both transects ($F_{\text{ST Seaside}} = 0.90 \pm 0.06$ and $F_{\text{ST Vineyard}} = 0.59 \pm 0.05$).

Discussion

Mitochondrial genetic structure and females' dispersal abilities

Our results revealed an extremely high level of mtDNA genetic differentiation among patches separated by 300–4500 m, suggesting that at this fine spatial scale, effective queen dispersal is very restricted. This strongly supports the hypothesis that despite having wings, new queens do not fly and found new colonies by fission, as suggested by Lenoir *et al*. (1988). Active female dispersal is thus restricted to ants' walking distances. Moreover, the low content of fat and more specially of carbohydrates (energy needed for flight) in queens, compared to flying males or to queens of other species with known nuptial flights (Keller & Passera 1989; Passera & Keller 1990), points out that flight should be very restricted or even not possible in *Cataglyphis cursor* queens. In agreement with this restricted dispersal, the patterns of the mtDNA haplotypes at both large and fine spatial scales suggest that new sites are colonized by nearby sites.

Our finding of restricted female dispersal in the monogynous ant *C. cursor* is interesting as it stands in contrasts with the traditional view that monogyny would be associated with nuptial flights and high rates of female dispersal whereas polygyny would be associated with colony budding and low dispersal rates (Bourke & Franks 1995). In the later case, ecological constraint on female dispersal, such as cost of dispersal, habitat patchiness or availability of nest sites, may have promoted dependent colony foundation, and potentially selected for polygyny (e.g. Keller 1995). At a fine spatial scale, some population genetic studies have indeed revealed no mtDNA structure in monogynous ants (Shoemaker & Ross 1996; Ross *et al*. 1997, 1999; Seppa *et al*. 2004) and a significant genetic differentiation of mtDNA in polygynous ones (Ross & Shoemaker 1997; Goodisman & Ross 1998; Liautard & Keller 2001; Ruppell *et al*. 2003). However, other recent studies tend to show that monogyny is not necessarily associated with high rates of female dispersal. First, in ant species with no queen caste or with apterous or short-winged (brachypterous) queens, female dispersal should be clearly limited whatever the number of queens per colony. This was confirmed by population genetic studies in the monogynous queenless ant, *Diacamma cyaneiventre* (Doums *et al*. 2002), as well as in the monogynous ant with brachypterous queens, *Nothomyrmecia macrops* (Sanetra & Crozier 2003). Second, even in monogynous species with fully developed wings, a philopatric behaviour of queens can limit female dispersal, as suggested by the strong spatial structure of mtDNA haplotypes detected in some inbred populations of *Formica exsecta* (Sundström *et al*. 2003) and in the slavemaking ant, *Protomognathus americanus* (Foitzik & Herbers 2001). Limited dispersal in ants is therefore not systematically associated with polygyny, and female dispersal behaviour as well as the mode of colony foundation appear to be crucial to determine female dispersal abilities.

Nuclear genetic structure at a fine-scale and male-biased dispersal

At the fine spatial scale (less than a few kilometres), the population genetic structure displayed by the two genomes were very contrasted. The level of genetic differentiation for mitochondrial markers was 15.5 and 14.4 times higher than for the nuclear ones for the seaside and the vineyard transects, respectively. Even if part of the differences can be explained by the smaller effective population size and the larger susceptibility to genetic drift of the mithochondrial markers (Chesser & Baker 1996), the strong discrepancy of genetic structure between the two markers probably reflects differences in dispersal pattern between the two sexes. Such extreme male-biased dispersal in a monogynous ant has been detected in only two other monogynous species: *N. macrops* (Sanetra & Crozier 2003) and *D. cyaneiventre* (Doums *et al*. 2002).

However, even at a fine scale (500 m), male dispersal appears insufficient to homogenize the nuclear genetic structure induced by the restricted female dispersal. As in most ant species, no information is available on male dispersal capacity in *C. cursor*. Male dispersal has rarely been observed directly but this apparently limited dispersal is in accordance with the few observations in nature (personal observation). Males were observed flying clumsily at a very low altitude (less than 1 m). Moreover, male dispersal does not necessarily imply gene flow. The success of dispersers probably decreases with increasing distances, even at a fine spatial scale, because the cost of flight is likely to increase while the probability of encountering other patches of nests, with females ready to mate is likely to decrease with geographical distances. Such process would lead to a pattern of population viscosity as observed at a fine spatial scale (see below).

Patterns of IBD

Isolation by distance and significant nuclear genetic structure have been detected at all the spatial scales investigated. At the large spatial scale, IBD is shown both by the decrease of F_{ST} estimates with the decrease of the geographical scale considered and by the significant correlation between genetic and geographical distances between sites. The pattern of IBD seems to loose its linearity after 65 km with a higher amount of scatter around the regression line (Fig. 2). This larger variance of F_{ST} at longer distances indicates that at this large scale, the influence of genetic drift is strong relative to gene flow (Hutchison & Templeton 1999) and that problems of homoplasy could be more important (Jarne & Lagoda 1996). It is therefore likely that the observed pattern of IBD would probably not hold over the entire home range of *C. cursor*.

At a local scale, even if significant population viscosity was detected in both transects, the pattern of decreasing genetic similarities with distance was different between the two habitats, as highlighted by the spatial autocorrelation analysis. In the seaside transect, where no major obstacle prevents the individual movements, the pattern of isolation by distance was continuous for both markers. On the other hand, in the vineyard transect, only nests sampled within a single patch were more genetically similar than any other pair of nests. This pattern could result from different constrains on gene flow between the two habitats. In the vineyard, the habitat discontinuities and the rarity of patches of nests may force the males to engage in longer flights than at the seaside to find nests. Moreover, in the vineyard transect male can fly in all directions to find new nests whereas at the seaside transect, the direction of male flight is constrained by the sea on one side and by an unsuitable habitat on the other side. Alternatively, the different pattern of IBD may also result from the fact that populations in the vineyard have not yet reached a drift–migration equilibrium, due to either recent colonization of some patches or to mixing events (Slatkin 1993).

Genetic diversity, habitat fragmentation and reproductive strategies

In spite of its restricted dispersal and its potential peculiar reproductive strategies, *C. cursor* does not show any sign of an important reduction of nuclear genetic diversity in workers in all the sites studied, the overall average expected heterozygosity being of 75.5%. Habitat fragmentation could potentially reduce local genetic variability by restricting gene flow and decreasing local effective population size (Ehrich & Stenseth 2001). However, the amount of genetic variability was globally not significantly lower in the vineyard than in the seaside, when comparing between sites as well as between transects. A lower genetic diversity was detected in Trèbes subregion. This lower genetic diversity could result from different processes. These populations could have a lower population size and be more isolated due to their peripheral situation (Durka 1999) in the species range. *C. cursor* cannot be found further north (personal observation; Cagniant 1976). This could also result from different reproductive strategies, such as a lower level of polyandry or/and a higher level of parthenogenesis. The use of parthenogenesis for queens' production and a lower level of polyandry could also decrease local genetic diversity by decreasing female and male effective population size, respectively. Further studies on the reproductive systems of *C. cursor*, in different populations, are needed to have a better understanding of its potential effects on the population genetic structure.

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This work is a part of Johanna Clémencet's doctoral thesis on the population genetic structure, evolution of polyandry and levels of selection in the ant *Cataglyphis cursor*. Claudie Doums is an evolutionary biologist who integrates molecular genetic tools to address questions about social organization and evolution of mating systems with a particular interest in social insects. Barbara Viginier is a laboratory engineer who developed the mitochondrial markers and helped with the screening of individuals.

ARTICLE 3

High level of polyandry in the parthenogenetic ant *Cataglyphis cursor*

Clémencet J, Doums C

Soumis à *Molecular Ecology*

Forts taux de polyandrie chez la fourmi parthénogénétique *Cataglyphis cursor*

Chez les hyménoptères sociaux, l'accouplement multiple des femelles est non seulement coûteux pour les reines qui s'exposent à des risques accrus de prédation et de transmission parasitaire, mais également coûteux à l'échelle de la colonie, puisqu'il diminue l'appartement moyen entre ouvrières et ainsi les bénéfices des actes altruistes. La fourmi *Cataglyphis cursor* est un modèle particulièrement pertinent pour tester quels pourraient être les avantages susceptibles de compenser les coûts de la polyandrie. En effet, dans l'unique population étudiée à ce jour, les reines utilisent la parthénogenèse thélytoque pour produire les nouvelles reines, mais s'accouplent avec plusieurs mâles et utilisent la reproduction sexuée pour produire les ouvrières. Ceci suggère donc que la diversité des ouvrières au sein des colonies doit être avantageuse.

Dans un premier temps, nous avons étudié les stratégies de reproduction de *C. cursor* dans deux populations issues d'habitats très différents (bord de mer et Corbières), afin de détecter d'éventuelles variations intra-spécifiques. Nous avons ensuite testé si la diversité génétique au sein des colonies pouvait être corrélée avec certaines composantes de la valeur sélective des colonies.

Dans les deux populations étudiées, nous confirmons l'utilisation de la parthénogenèse pour la production des gynes (à 70%) et la reproduction sexuée pour celle des ouvrières. Les taux de polyandrie détectés sont très élevés (Me = 8, n = 14) et ne sont pas significativement différents entre les deux habitats. Il n'y a pas de relation positive entre la diversité génétique des colonies et les paramètres classiquement utilisés pour estimer la valeur sélective des colonies (taille de colonie et production de sexués). En revanche, à taille égale, les colonies les plus diverses génétiquement ont en moyenne des ouvrières de plus grande taille. Ce dernier résultat, à ce jour inédit chez les fourmis, suggère qu'à taille égale, les colonies disposant d'une plus forte diversité génétique sont capables d'obtenir davantage de ressources.

Chez cette espèce thermophile, une augmentation de la taille des ouvrières au sein des colonies pourrait constituer un avantage non négligeable dans l'exploitation de l'environnement extérieur.

Genetic diversity and worker size in the highly polyandrous and partially parthenogenetic ant *Cataglyphis cursor*

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Running title: level of polyandry in the ant *C. cursor*

Abstract

In social hymenoptera, multiple mating by females can be costly both at the queen and colony level. The monogynous ant *Cataglyphis cursor* provides an excellent model to investigate benefits potentially outweighing theses costs and thus promoting the maintenance of queen multiple mating. In the single population studied so far, *C. cursor* had an extraordinary reproductive system. Queens produce gynes (virgin queen) by thelytokous parthenogenesis but mate with several males and use sexual reproduction to produce the workers. This suggests that intra-colony genetic diversity should be advantageous in this species. Here, we examined the reproductive strategies of *C. cursor* colonies in two populations from habitat types known to differ in ecological factors and life history traits (colony size and worker size). We confirmed the use of parthenogenesis for the production of gynes but also found that the use of sexual reproduction for the production of gynes is possible and not negligible (almost one third). Workers were sexually produced but our estimates of polyandry in both populations was higher than reported previously (11 males per queen on average). There was no relationship between genetic diversity and colony parameters, such as colony size or sexual production. However, we found, for the first time in ants, that for a given colony size, the mean worker size was larger in colonies with higher level of genetic diversity. This suggests that for a given colony size, colonies with higher genetic diversity can acquire larger amounts of resources.

Introduction

The evolution and maintenance of female multiple mating (polyandry) in social Hymenoptera has long puzzled evolutionary biologists. As in other animal species, this behaviour may impose costs on reproductive females, by increasing time and energy expenditure, risk of predation and risk of acquiring sexually transmitted disease (see Moritz 1985; Sherman *et al.* 1988; Arnqvist & Nilsson 2000). In social Hymenoptera, multiple mating imposes additional costs by reducing relatedness among nestmates and therefore decreasing the advantage of reproductive altruism (Hamilton 1964). Although in many social insect species, a greater fraction of queens mate only once (see Boomsma & Ratnieks 1996; Crozier & Pamilo 1996; Strassmann 2001), in few species from several of the major groups (*i.e.*, honey bees, wasps, ants), queens mate a moderate to a large number of times. High levels of polyandry, with queens commonly mating with more than 10 males, have been documented in honey bees (see Palmer & Oldroyd 2000; Palmer *at al.* 2001), and ants (Kronauer *et al.* 2004; Denny *et al.* 2004). However lower polyandry levels are more often found in social insects (Wasps: Ross 1986; *Atta* and *Acromyrmex* leaf-cutting ants: Boomsma *et al.* 1999; Fjerdingstad & Boomsma 2000; *Pogonomyrmex* seed harvester ants : Gadau *et al.* 2003; Wiernasz *et al.* 2004; Rheindt *et al.* 2004; *Lasius niger* : Fjerdingstad *et al.* 2002). Polyandry level has also been shown to vary among populations within species (Estoup *et al.* 1995; Boomsma & Van der Have 1998; Fjerdingstad *et al.* 1998; Neumann *et al.* 1999). Investigating potential variation within a species is a crucial step to shed light on the factors that drive the evolution of the number of female mating patterns.

Several hypotheses have been proposed to explain the evolution and maintenance of polyandry (see Boomsma & Ratnieks 1996; Crozier & Fjerdingstad 2001; Strassmann 2001 for recent reviews). The ‘front running’ ones, called the genetic variance (GV) hypothesis (Keller & Reeve 1994), holds that increased intra-colonial genetic variability benefits the colony by reducing the cost of diploid, sterile male production (Page 1980; Crozier & Page 1985; Tarpy & Page 2002), by increasing the efficiency of division of labour (Page *et al.* 1989; 1995; Crozier & Page 1985; Fuchs & Moritz 1999), by increasing colony-level resistance to diseases (Hamilton 1987; Sherman *et al.* 1988; 1998; Schmid-Hempel 1994; 1998) or by reducing worker-queen conflict over sex-allocation ratio and male parentage (Starr 1984; Moritz 1985; Ratnieks & Boomsma 1995; Crozier & Page 1985). These potential mechanisms are not mutually exclusive and have in common that they predict an increased fitness of colonies with greater genetic variance thus leading to a higher fitness of multiply mated queens. Indeed, in some species, genetic diversity was positively correlated with colony fitness parameters, such as colony growth or colony resistance to parasites (Fuchs & Schade 1994; Schmid-Hempel 1998; Baer & Schmid-Hempel 1999, 2001; Cole & Wiernasz 1999; Tarpy 2003; Wiernasz *et al.* 2004).

The monogynous ant *Cataglyphis cursor* is an interesting model system to investigate the evolution of reproductive strategies. This species has a unique reproductive strategies known in ants. Most of the queens used asexual reproduction (automictic thelytokous parthenogenesis) to produce new queens while they mated with several males (up to 8) and used sexual reproduction to produce the workers (Pearcy *et al.* 2004a). The fact that queens do not use the less costly asexual production for workers, implies a benefit associated with the genetic diversity of workers.

So far only a single population of *C. cursor* has been studied and nothing is known about the natural variation in the reproductive strategies. In fact *C. cursor* lives in contrasted habitats, ranging from beaches on the seaside to vineyard pathways in the inland. Ecological factors such as soil composition and depth of the water table often vary profoundly between the habitats (Cagniant 1976a), and this can affect major life history traits such as colony size and worker size (Clémencet & Doums, submitted).

In this study, we examined the reproductive strategies of *C. cursor* colonies in two populations from the extreme ends of its habitat spectrum (seaside *vs* vineyard). First, we determined the level of parthenogenesis and polyandry. Then, we investigated whether genetic diversity was correlated with potential components of colony fitness (sexual production, colony size). In particular, we considered mean worker size and its variance, as they could be indirectly associated to colony productivity (Wilson 1953; 1980; Billick 2002).

Materiel & Methods

Study species and sampling

C. cursor is a Mediterranean formicinae ant, living in arid and open habitats. Colonies are monogynous (one queen per colony) and present a clear seasonal pattern of brood development. There is no over wintering brood and reproduction occurs in spring and summer. Sexuals are produced first and typically emerge on mid- May (1:3.2 female to male investment ratio, Pearcy & Aron 2006) , whereas workers are produced till the end of August. Mating occurs in early June and can be followed by colony fission, 1 to 3 weeks later (Lenoir *et al.* 1988). Nests of *C. cursor* are built in the soil, with a single entrance and one vertical burrow leading to chambers located up to 1 m deep (but never deeper than the water level, Cagniant 1976a)

In order to estimate the levels of polyandry, 14 entire colonies were collected in two populations from contrasting habitats in the region of Languedoc-Roussillon, France. Seven colonies were sampled in the village of Luc-sur-Orbieu (Luc) in the inland near the city of Lézignan, the seven others on the beach of Argelès, e. 80 km S-E of Luc. The two locations differed by the hardness of the soil (silty in the inland *vs* sandy on the beach), and by the depth of the water level (60 cm depth on the beach *vs* 150cm in the inland). By sampling in mid-May (13-17 May), we collected sexuals at the adult or cocoon stage, which and allowed us to investigate the mode of production of gynes. Although nest excavation was easy because of its predictable structure, extra care was taken not to miss any room or gallery that might contain individuals or brood. Adults ant (queens and workers) as well as brood at various stage (eggs, larvae and pupae) were collected. Only one colony in the inland and three on the seaside contained pupae. The large size of the pupae indicated that they were all destined to develop into sexuals (Cagniant 1979). However at this early stage of development it was impossible to determine their sex visually.

In the laboratory, the number of workers in each colony was counted before transferring colonies to the nest boxes, where they were reared at 24°C under 12-h light/ 12-h dark cycle and were fed *ad libitum* with mealworm, *Tenebrio molitor*, and sugar water. Queen mating frequencies were estimated on eggs rather than workers to avoid potential overestimation that could be due to the occurrence of sequential polygyny by clonal queens in colonies. During 60 days, some eggs were removed in 10 days intervals to avoid disturbing the colony and stored in 95 % alcohol until genetic analysis. A total of 412 eggs (15 to 47 per colony, from at least three sampling dates) were collected for genetic analysis. We further genotype pupae collected in the field (n = 32) and gynes produced in the laboratory (n = 10).

Genetic Analysis

Six DNA microsatellites developed for *C. cursor* (Pearcy *et al.* 2004b), were used to determine genotypes. DNA extractions from queens, gynes and pupae were carried out using a QIAgen DNAeasy kit (Valencia, CA), with extracted DNA resuspended in 100 µl elution buffer. DNAs from eggs were obtained by classical digestion. Each egg was squashed in a 20µl solution with 10mM Tris, 1mM EDTA, 25mM NaCl and 0.2 µl of Proteinase Kinase (20mg/ml), digestion lasted overnight at 37°C and ended by a final 2min denaturation step at 90°C. The polymerase chain reactions (PCR) were carried out as described in Pearcy *et al.* (2004b), except that two sets of loci were coamplified (first set: Ccur26, Ccur46, Ccur76, second set: Ccur89, Ccur63, Ccur11). Each PCR was run in a 10 µl volume containing 1 µl of DNA solution, 400 µM of each dNTPs, 0.15 units of Taq polymerase (QIAgen), 1 µl Buffer 10X and 0.1 µM of Ccur26, Ccur76 and 0.3 µM of Ccur46 primers or 0.1 µM of Ccur89, 0.2 µM of Ccur63 and Ccur11 primers. The amplified fluorescent fragments were visualized using an automated ABI Prism 310 Sequencer (Applied Biosystems) and allele sizes were estimated using the GENESCAN 2.0.1^{TM} (Perkin Elmer/ABI) software.

Pupae and sexual type determination

In colonies of *Cataglyphis cursor*, female brood is potentially produced by sexual reproduction or by thelytokous parthenogenesis, and male brood by arrhenotokous parthenogenesis. For eggs and pupae for which the sex could not be determined, we used the following criteria to discriminate among these modes of reproduction. Brood was considered as sexually produced females when an allele not present in the queen was detected for at least one locus. When the brood harboured only maternal alleles with at least one heterozygous locus, we considered it a female produced by thelytokous parthenogenesis. The probability that such a female was produced by sexual reproduction with a father harbouring no distinct alleles from the mother at any loci, varied from 0.004 to 3.10^{-6} depending on the colony and was thus considered negligible. When the brood had only maternal alleles at the homozygous state, we considered them as males only if the probability to be a female produced by thelytokous parthenogenesis was lower than 5%. This probability was estimated for each queen using the recombination rate available for four of the loci used in our study (Pearcy *et al.* 2004a). If this probability was higher than 5% we considered the brood as undetermined.

Mating frequency estimated from eggs

The observed number of mates per queen (K_{obs}) was calculated by reconstructing paternal genotypes on the basis of offspring and queens genotypes across the six loci. MATESOFT software vs 1. (Moilanen *et al.* 2004) was used to confirm the genotypes of putative fathers and estimate the sum of squared paternity contributions.

Then, queen mating frequency statistics (i.e the number of equally contributing fathers that would have produced the same average relatedness among offspring) were estimated following Nielsen *et al.* (2003) correcting for sample-size:

$Me = (n{-}1)^2 \Big/ \sum_{i=n}^{k} pi^2 (n{-}1)(n{-}2)+3{-}n$; where n is the sample size, k is the number of patrilines observed and pi is the proportional contribution to the brood of the ith mate.

However, underestimation of mating frequency estimates may arise from limited variation of genetic markers. By chance, two males may bear the same alleles at the 6 loci studied ($P_{\text{non-detection}}$), we therefore estimated this non-detection error in both populations according to Boomsma & Ratnieks (1996): $P_{\text{non-detection}} = \prod_{j=1}^{k} (\sum_{i=1}^{a_j} f_{ij}^2)$; where fij denotes the frequencies of a_j alleles (j =1 to k) at each of k loci. With 8 to 15 alleles per locus and an average heterozygosity (H_e) of 0.82 and 0.76 detected in Luc and Argelès population, respectively, the microsatellites loci were sufficiently variable to adequately differentiate paternal genotypes within the colonies studied. The probabilities of failing to detect a fathering male were very low in both populations ($P_{\text{non-detection}} < 10^{-3}$).

Genetic relatedness

Regression relatedness among diploid eggs of each queen were assessed following Queller & Goodnight (1989) using the program RELATEDNESS 5.0.7 (Goodnight & Queller 1998). Colonies were weighted equally and standard errors were obtained by jackknifing over colonies. To account for differences in allele frequencies between the two sampling sites the relatedness values were estimated from the allele frequencies of eggs of each site using the 'deme' function of the RELATEDNESS program. We controlled using the program FSTAT 2.9.3

(Goudet 1995) that the eggs' inbreeding coefficient (F_{is} of Weir & Cockerham (1984)) was not significantly different from zero ($F_{is} = 0.010 \pm 0.016$) and therefore could not biased relatedness estimates.

Measurements

Thirty randomly chosen workers per colony were measured under a binocular microscope connected to a Leica XC-ST70 video camera module. Matrox Inspector software was used to analyse the digital picture. Given that the traits typically measured in ants are highly significantly correlated (Clémencet & Doums, submitted, Cagniant 1983), only the most repeatable one was recorded: the tibia length.

Data analysis

In both populations, we tested whether relatedness estimates (r) correlated with colony size, number of sexual produced, or mean and variance of worker size. This parameter, r, was chosen as it integrates both the estimated effective mating frequency (M_e) and the level of queen homozygoty. These two parameters explained a significant part of the variation in relatedness estimates among colonies (Multiple Regression, M_e : P=0.011, Queen Homozygoty, P=0.001). Note however that the estimator M_e gave the same qualitative results as r. As in both populations, variation in colony size explained 62 % of the variation in mean worker size (Clémencet et al, submitted), we removed the effect of colony on worker size by using the residuals of the Standard Major Axe methods (type II regression like, Sokal & Rolf, 1995).

Results

Sexual production

In the field, 32 pupae were collected from three colonies and 29 were successfully genotyped (see Table 1). All pupae harboured only maternal alleles. The probability that theses pupae were sexually produced with a father having no distinct alleles from the queen was very low in all colonies ($P_{1031} = 0.0078$, $P_{1059} = 0.0051$, $P_{1077} < 10^{-6}$). From these 29 pupae, 13 had heterozygous loci and were therefore females produced by thelytokous parthenogenesis. The size of these pupae indicated that they were destined to become gynes. The 16 other pupae had a single allele at all loci and were thus likely to be males produced by arrhenotokous parthenogenesis. Given the rate of recombination of the four loci used (Ccur11, Ccur46, Ccur58, Ccur63b) estimated by Pearcy *et al.* (2004a) and according to the queen genotype, the probability that these pupae were diploid gynes produced by thelytokous parthenogenesis was low ($P_{1059} = 0.017$, $P_{1077} = 0.003$).

In the laboratory, five of 14 colonies produced a total of 15 males and 10 gynes (see Table 1). Pedigree analysis revealed that the seven gynes from Col 1073 harboured, at several loci, alleles not present in the mother queen and were thus produced by sexual reproduction. At least four fathering males are needed to explain the observed gyne genotypes. The paternal genotypes, inferred from gyne and queen genotypes, were not different from those deduced from egg's genotypes; hence no additional mates were detected. The other three gynes had only maternal alleles and were thus considered to originate from thelytokous parthenogenesis. The probabilities of mating with a male harbouring no different allele at any loci were very low for both colonies ($P_{1046}= 0.0048$, $P_{1077} < 10^{-6}$). The overall rate of sexual production was 30%, but note that all the sexually produced gynes originated from a single colony (Col 1073, Table 1).

Level of polyandry and relatedness

On average, 26 (range: 10-33) diploid eggs were successfully genotyped per colony. All these diploid eggs had an allele not present in the queen and were therefore sexually produced.

Overall, the observed numbers of matings per queen (K_{obs}) ranged from 5 to 13 (Table 1), and did not significantly differ between the two populations with on average ten males per female (see Table 1). Patriline frequencies among queens'eggs are represented in Fig. 1. Sample-size corrected estimations of effective queen mating frequencies (*Me)* were on average lower (3.4 to 14.5), but still very high for an ant species (see Table 1). *Me* also did not differ between the two sites (Table 1).

As expected for colonies with highly polyandrous queens (Bourke & Franks 1995), the average intra-colonial relatedness estimate was significantly lower than 0.75 (one- tail *t*-test, $P < 10^{-5}$), but still significantly higher than would be expected under monogyny with an infinite number of fathers (one- tail *t*-test, $P = 0.007$). The average population estimates of within-colony genetic relatedness did not significantly differed between the two populations (Table 1, $r_{\text{Luc}} = 0.3 \pm 0.03$; $r_{\text{Argelès}} = 0.35 \pm 0.05$, $P = 0.65$; $Z = 0.45$).

Genetic diversity and components of colony fitness

Colonies producing sexuals were significantly larger than the others (*Mann and Whitney U test*, Z=-2.05, $P = 0.04$). The genetic diversity was similar for colonies that produced sexuals compared to those that did not (*Mann and Whitney U test*, Z = -1.425, $P = 0.142$). Thus, there was no significant correlation between genetic diversity (*r*) and colony size (Fig. 2.a, Spearman rank correlation, $P = 0.68$). Genetic relatedness was not significantly correlated with the variance in worker size (Spearman rank correlation, $P = 0.87$). However, after controlling for the effect of colony size on worker size, a significant negative relationship was detected between relatedness and residual worker size (Fig. 2.c, Spearman rank correlation, $P = 0.033$). For a given colony size, the mean worker size was larger in colonies with the higher genetic diversity (*i.e.* lower relatedness).

Discussion

High level of polyandry was found in two populations differing by their habitat type (seaside *vs* vineyard) and major life history traits (colony size and worker size, Clémencet & Doums, submitted). We found no evidence of single mating and instead queens mated with on average, 10 males in both populations. *C. cursor* represents the fourth independent evolutionary origin of obligate multiple queen-mating in ants and the first case known within the subfamily of the Formicinae. As observed in the population studied by Pearcy *et al.* (2004a), most of the gynes were produced by thelytokous parthenogenesis. However, the proportion of gynes sexually produced was not negligible in our study (7/21 *vs.* 2/56, *Fisher exact test*; $p < 0.01$). The minimum number of males in our study was similar to that in the population studied by Pearcy *et al.* (2004a). In contrast, the mean number of males was almost twice as high (Pearcy: n = 12, range 4-8, mean = 5.56 males), leading to higher estimates of queen mating frequency ($M_e = 7.7\text{-}8.4$ *vs.* $M_e = 4.63$) and lower relatedness estimates ($r = 0.3\text{-}0.35$ *vs.* $r = 0.42$). Given that our estimates were based on egg genotypes and not on freshly eclosed workers (as in Pearcy *et al.*), the difference observed between the two studies might result from variation in patriline survival. Ecological factors can differ between sites or years and this can lead to variation in reproductive characteristics among populations (Estoup *et al.* 1995; Boomsma & Van der Have 1998; Neumann *et al.* 1999). These variation are however subtle and our results showed that the peculiar mode of reproduction of *C. cursor* is not an artefact found in a single population but is likely to be a characteristic of this species.

Our results, together with the knowledge of *C. cursor* biology, allow to discriminate between certain alternatives hypothesis of multiple mating. Field observations indicate that young gynes leave their nest several times to mate with surrounding males (Lenoir *et al.* 1988), suggesting that the hypothesis of "mating by convenience" (Thornhill & Alcock 1983) does not hold in *C. cursor.* It is also unlikely that multiple mating has been selected for to increase the life-time supply of sperm (Cole 1983; Fjerdingstad & Boomsma 1998). Colonies of *C. cursor* are relatively small and hardly exceed 2500 workers. Moreover, the amount of sperm a male can provide does not appear to be a limiting factor for the queen's egg production (Pearcy, PhD thesis). Further, the cost of producing diploid males is unlikely to select for multiple mating in *C. cursor* since diploid males have never been detected (Pearcy, PhD thesis; pers. data) and high degrees of genetic diversity have been reported in *C. cursor* populations (Clémencet *et al.*, 2005).

Of the hypotheses based on the selective advantages of increased within–colony genetic variance, the most likely to apply to *C. cursor* are the “facilitation polyethism” and “parasite resistance” hypothesis (Crozier & Page 1985;, Hamilton 1987; Shermann *et al.* 1988; Schmid-Hempel 1994; 1998; see also Keller & Reeve 1994). Both predict higher colony productivity in the genetically more diverse colonies. We did not find a difference in relatedness between colonies producing sexuals and the others. However, it should be noted that only few colonies produced sexuals in the field. These colonies had a larger number of workers than the others, consistent with previous observations by Lenoir *et al.* (1988). However, because the age of colonies was not known, colony size may be a poor indicator of colony fitness, especially in species with dependent colony foundation. This may be one explanation for the absence of a correlation between colony size and genetic diversity. Interestingly, after controlling for colony size, mean worker size significantly increased with the level of genetic diversity, even though the variance in worker size did not. This is the first empirical evidence that increased genetic diversity could positively affect mean worker size. Other studies on the relationship between worker polymorphism and genetic diversity did not detect a positive relation between genetic diversity and mean or variation in worker size (Bargum *et al.* 2004; Schwander *et al.* 2005). However these studies did not consider the potentially confounding effect of colony size. Producing large workers could be advantageous for this thermophilic ant as larger workers have a better thermal tolerance (Clémencet, Cerda, unpublish data). This may allow the colonies to send foragers at temperature that smaller workers and other species cannot tolerate and therefore to decrease both intra- and inter-specific competition. Moreover, since large workers are more expensive to manufacture and maintain than small workers (Hölldobler and Wilson 1990), the relationship observed here could indirectly suggest that for a given colony size, colonies with higher genetic diversity can acquire more resources. This hypothesis merits to be tested by field investigations.

We should however keep in mind that the advantage of multiple mating need to be evaluated against the cost of mating. In *C. cursor,* queens do not take part in mating flights and mate close to their natal colony surrounded by worker (Lenoir *et al.* 1988), and thus mating costs (energetic or predation) should be low. Interestingly, in the other highly polyandrous ant species(army ants: Kronauer *et al.* 2004; Denny *et al.* 2004; and *Pogonomyrmex* harvester ants: Rheindt *et al.* 2004), queens also mate on the ground, which imposes little cost of mating. Very high levels of multiple mating, in species such as *C. cursor* or army ants, might therefore have no adaptative significance (Tarpy & Page 2000; Denny *et al.* 2004; Kronauer *et al.* 2004). Note that dependent colony foundation is generally associated with polygyny (several queens per nest; Bourke & Franks 1995), and the few monogynous ant species (except the queenless ant species, Peeters 1991) where colonies reproduce by fission have in common with *C. cursor* that they exhibit very high levels of multiple mating (Strassmann 2001; Denny *et al.* 2004; Kronauer *et al.* 2004). In species with dependent colony foundation, the entire colony experiences the strong selective pressures associated to the founding stage whereas in species with independent colony foundation, it is the queen and not the colony that experiences these selective pressures. The difference in the level of selection (queen *vs.* colony) may have two major consequences. First, in monogynous species with dependent colony foundation, colonies could benefit from increased genetic diversity and this might favour the evolution of polyandry. Second, lower selection pressures at the queen level might favour the evolution of parthenogenetic gyne production, because this would decrease its potential costs (e.g. increased of queen homozygosity (Pearcy *et al.* 2004a)). A similar situation of conditional use of sex for worker production, but not for gynes has been reported in the ant *Wasmannia auropunctata*, which also found the colonies with the help of workers (Fournier *et al*. 2005). The mode of colony foundation, by changing the hierarchical level at which selection applies, may therefore be an important determinant of the evolution of mating system.

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Table and figures

Site	Colony label	Colony size	N eggs	Mating frequencies		Relatedness among ♀ eggs	Sexual production		
				Observed K_{obs}	Effective M_e		B	Pupae	Adult
Luc	1030	-	26	5	3.4	0.5 ± 0.16	0	-	-
	1031	1520	28	9	8.1	0.35 ± 0.06	**2**	10♀	6♂
	1032	260	29	13	14.5	0.25 ± 0.05	0	-	
	1035	600	30	10	7.9	0.15 ± 0.12	0	-	-
	1044	1036	22	10	10.5	0.35 ± 0.09	1	-	-
	1045	280	26	12	12.1	0.21 ± 0.09	0	-	
	1046	1252	10	7	9	0.38 ± 0.12	1	-	1♂,*2*♀
	Mean	825		9.4	7.7	0.30			-
	± *SE*	± 214		± 2.8	± 3.54	± 0.03			
Argelès	1052	968	33	12	10.8	0.24 ± 0.05	1	-	-
	1055	208	33	8	5.8	0.46 ± 0.07	0	-	-
	1056	477	27	10	8.4	0.22 ± 0.08	1	-	-
	1058	343	28	11	10.2	0.31 ± 0.12	1	-	
	1059	940	15	7	7.6	0.34 ± 0.07	**2**	11♂	8♂
	1073	811	25	11	9.7	0.43 ± 0.13	1	-	7♀*
	1077	628	22	10	8.9	0.42 ± 0.06	**2**	5♂, 3♀	1♀
	Mean	625		9.9	8.4	0.35			
	± *SE*	± 111		± *1.8*	± 1.7	± 0.05			
	P value	0.47		0.74	0.65	0.65			

* indicated that it was sexually produced

Table I. Colony size, mating frequency, genetic diversity and sexual production are given for each colonies in the ant *C. cursor*. The total number of diploid eggs successfully genotyped (N eggs) is indicated for each colony. K_{obs} is the minimum total number of queen matings (observed number of patrilines), M_{e} the sample-size corrected estimate of the effective queen mating frequency according to Nielsen *et al.* (2003); (harmonic mean ± SE from jackknifing over queens). For each colony is given the estimate of genetic relatedness among diploid eggs. Brood production at colony collection (B) was defined in 3 class, 0: no brood, 1: only few small larvae ; 2: cocoons and larvae. The number and the sex of pupae in the field and sexuals produced in the lab are indicated. The mean (± SE) colony size and mating frequencies parameters are given and compared between the two populations using *Mann and Whitney U tests*, for which the *P* values are given.

Figure 1: Ranked numbers and frequency distribution of observed patrilines among queen' eggs of *Cataglyphis cursor*. Patrilines are represented by alternate shading patterns with their total number (minimum number of detected fathers) given above the bars and the number of unambiguously assigned offspring in parenthesis.

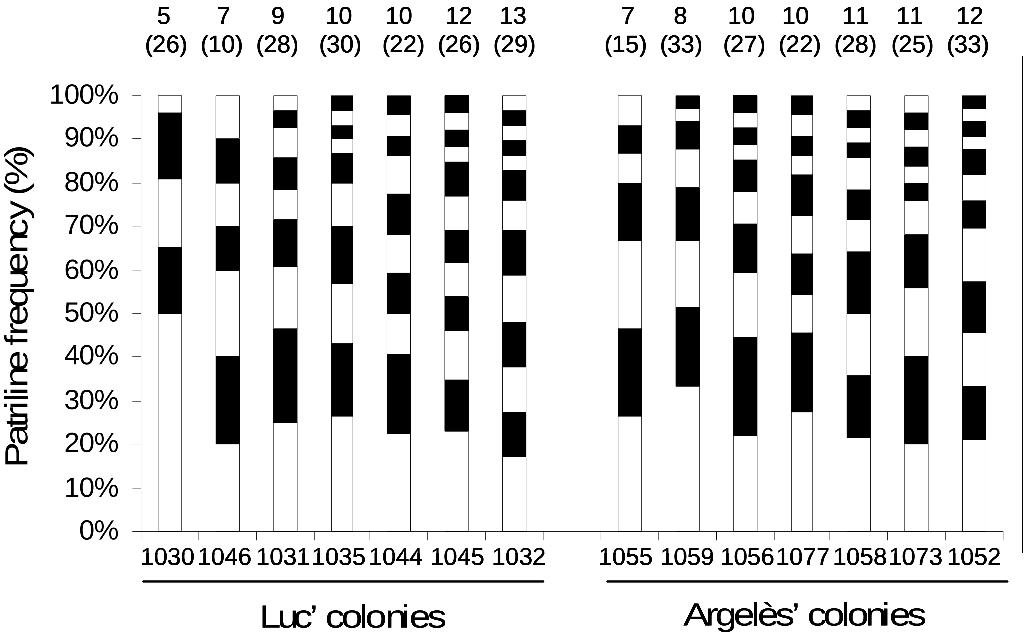
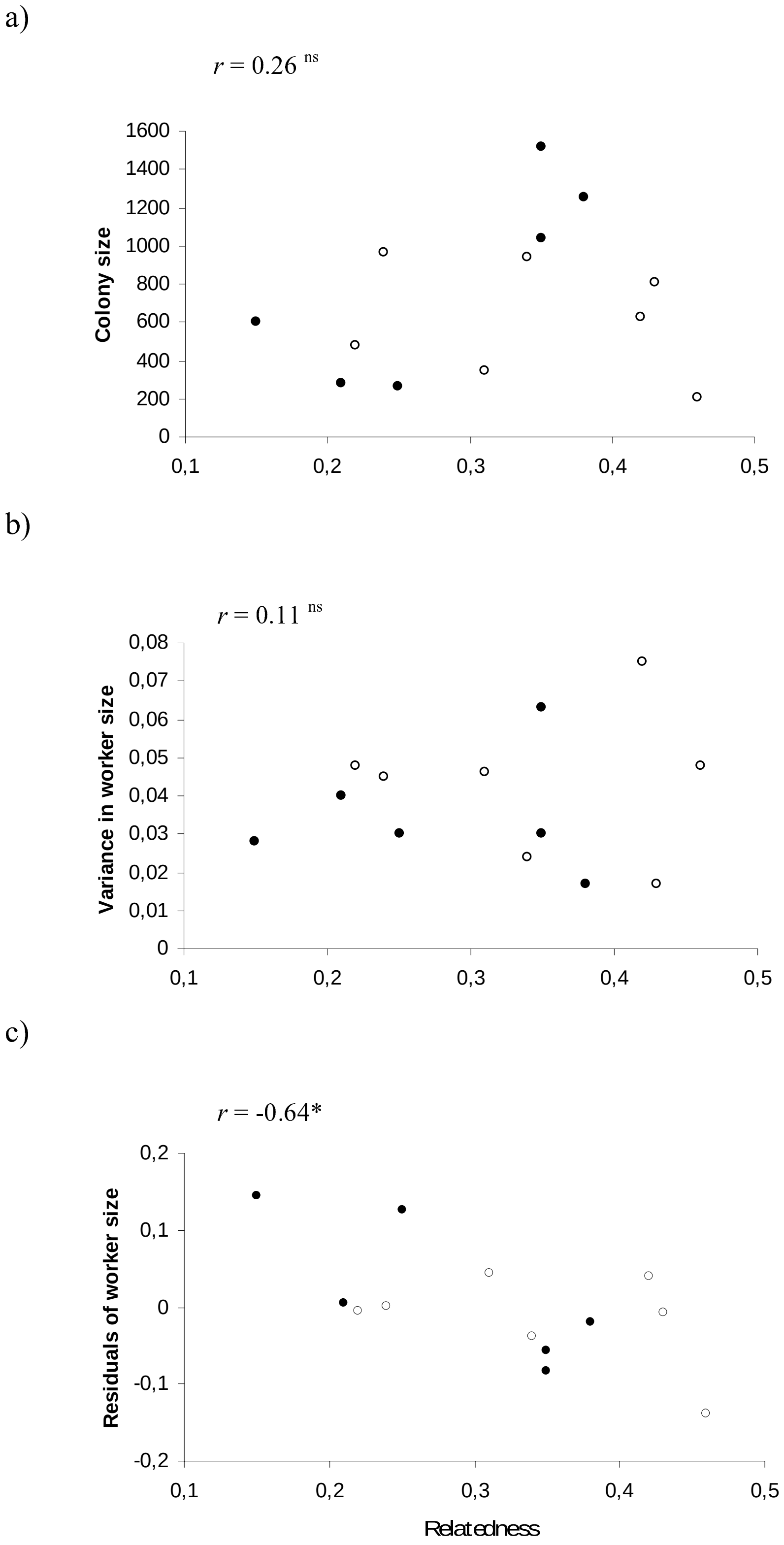


Figure 2: Regression of colony size (a), variance in worker size (b), and residuals of the regression: mean worker size / colony size (c), on genetic relatedness. White circles are colonies sampled in the population on the seaside and black circles are colonies sampled in the population in the vineyard. All correlations were non significant (*P*>0.05), except for (c) *P* = 0.033.



ARTICLE 4

Worker size, thermal tolerance and foraging activity in the thermophilic ant *Cataglyphis cursor* (Hymenoptera; Formicidae)

Clémencet J & Doums C

En préparation

Taille des ouvrières, résistance à la température et activité de récolte chez la fourmi thermophile *Cataglyphis cursor*

Chez les espèces de fourmis, le polymorphisme de taille des ouvrières est généralement étroitement associé à la division du travail au sein des colonies. Chez les espèces thermophiles, ce polymorphisme de taille peut être particulièrement utile pour résister aux fortes températures pendant l'activité de récolte à l'extérieur du nid. Au sein d'une même espèce, la résistance des individus à la dessiccation est souvent liée à leur taille. Les grandes ouvrières résistent mieux au stress thermique que les petites. Ainsi, la présence de grandes ouvrières au sein des colonies peut représenter un avantage non négligeable pour exploiter le milieu extérieur.

Nous avons étudié chez la fourmi méditerranéenne *C. cursor*, le lien entre la résistance à la température des ouvrières et leur taille (de 3 à 11 mm). Nous avons testé en milieu naturel si les ouvrières qui sortent pour fourrager sont plus grandes que les autres, et si leur taille varie en fonction des heures de la journée.

En laboratoire, nous montrons expérimentalement que la résistance des ouvrières à la température augmente significativement avec leur taille. En milieu naturel, nous ne détectons cependant pas de différence significative de taille entre les ouvrières sortant pour fourrager et les ouvrières présentes à l'intérieur du nid. La taille des fourrageuses est la même quelle que soit l'heure de la journée. Néanmoins, nous observons une corrélation positive entre la taille des colonies et la taille moyenne des ouvrières, ce qui laisse supposer que seules les colonies disposant de suffisamment de ressources peuvent investir dans la production d'ouvrières de grande taille. Ces dernières doivent donc être avantageuses pour les colonies qui préfèrent investir dans leur production coûteuse plutôt que dans un plus grand nombre de petites ouvrières.

Worker size, thermal tolerance and foraging activity in the thermophilic ant *Cataglyphis cursor* (Hymenoptera; Formicidae)

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Abbreviated title: thermal tolerance in *C.cursor*

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Abstract

In thermophilic ant species, worker polymorphism, usually thought to be linked to the division of labour, could be of special interest to face high temperature during foraging activity. Within a species, desiccation resistance may vary among individuals according to their size, larger worker resisting better thermal stress than smaller.

We investigated in the thermophilic ant *Cataglyphis cursor* whether worker thermal tolerance is related to their size and further tested in the field whether foraging workers were significantly larger than others. Patterns of thermal tolerance were investigated in colonies from different habitat types (seaside vs vineyard) to detect any variation that could be attributable to different adaptation to diverse environmental conditions. In the laboratory, we found that large workers resist better to high temperature than small workers. No effect of habitat type but a strong effect of colony was detected on worker thermal tolerance. However, in the field, foraging workers were not significantly larger than inside nest workers and the size of foragers did not vary according to the hour of the day as observed in a closely related species.

Introduction

The abiotic environment exerts strong selective pressures on the mechanism of thermal tolerance of terrestrial invertebrates. Survival strategies adopted by ecotherms to adapt to thermal stress have been abundantly documented (Heinrich 1993). Insects may respond differentially to exposure to extreme temperature: avoiding it by escaping, or adapting to it by changes in morphology, life history or physiology (Chen & Kang 2005 for a review). Ant community of Mediterranean regions perfectly illustrates how species vary in their strategies to escape or face thermal stress. In Mediterranean regions, ant colonies have to cope with seasonal variations in environmental temperature, as well as with the naturally occurring daily temperature fluctuation. During the summer, the temperature outside the nest may be considered as stressful and foraging activity of ants in such xeric areas may be limited by desiccation stresses. The role of temperature on Mediterranean ant community structure and activity pattern has been well studied (Cerdá & Retana 1997, 2000; Cerdá et al. 1997, 1998a, 1998b). Among sympatric ant species, temporal differences in foraging rhythms are common (Cros et al. 1997). Indeed, subordinate species are active during the day, when conditions are more severe and forage at high temperature (maximum activity 40-50°C), very close to their physiological thermal limits. These ant species run a high mortality risk by foraging near lethal temperature (*Cataglyphis*: Ghering & Wehner 1995; *Ocymyrmex*: Marsh 1985; *Melophorus*: Christian & Morton 1992). The risk of mortality during foraging can represent an important cost for colonies and different life history traits have evolved to adapt such stressful environment conditions.

Increased worker body size is one of the most obvious mechanisms to face hot environment. It has been abundantly reported that desiccation resistance (Hood & Tschinkel 1990; Kaspari 1993), running speed (Bartholomew et al. 1998; Cerdá & Retana 2000), maximum foraging time and temperature (Lighton & Feener 1989; Porter & Tschinkel 1987) vary among workers according to their size. Specific physiological (Bartholomew & Lighton 1988; Lighton & Feener 1989; Lingthon et al. 1993) and biochemical changes (Gilby 1980; Gerhing & Wehner 1995) have also evolved to get better resistance to high temperatures and to reduce evaporation of the body water. Ant species can also exhibits behavioural mechanisms which decease the thermal stress, such as making frequent pauses at thermal refuges (Marsh 1985; Christian and Morton 1992; Wehner et al. 1992; Cerdá & Retana 2000) or raising their abdomen to protect the vital organs from high temperature (Cerdá & Retana 2000).

Interestingly, within one of the most thermotolerant ant genus *Cataglyphis* (Gehring & Wehner 1995), adaptation to extreme heat conditions followed different evolutionary pathways among species (Cerdá & Retana 2000). In the ant *Cataglyphis velox* (4.5-12 mm), thermal tolerance as well as foraging at high surface temperature is clearly dependant on worker size. Larger workers withstand temperatures that small workers can not tolerate and therefore forage at different time of the day. At the colony level, worker polymorphism generated by the production of large workers, could have evolved to increase the daily activity period and ultimately the benefit in prey collection. In contrast, in the ant *C. rosenhaueri,* which exhibits a lower worker size variation (4.5-7.5mm), thermal tolerance is not related to size, small workers can cope with extreme heat condition by raising their abdomen to protect the vital organs and probably by achieving physiological adaptation (Cerdá 2001).

The thermophilic ant *Cataglyphis cursor* presents interesting features to investigate adaptation mechanism to high temperature, especially to test whether worker thermal tolerance is related to their size and affects pattern of foraging activity. Workers of *C. cursor* do not raise their abdomen to protect the vital organs, as observed in *C. rosenhaueri,* and present an intermediate size variation (range: 3.5-11mm; Cagniant 1983; pers. obs.). The large workers are still more than twice larger that the smallest workers and body weight ranges from 2 to 15 mg (Cagniant 1983). We found in two populations of *C. cursor* that mean worker size increased with colony size, suggesting that only colonies that have sufficient resource invest in the production of large workers (Clémencet & Doums, submitted). Producing these large workers could benefit the colonies by allowing a better exploitation of external environment, as observed in *C. velox*.

In this study we address two main questions. First, we tested in the thermophilic ant *C. cursor* whether worker thermal tolerance is related to their size and whether this relation varies among colonies and populations. Then, we conducted field observations to investigate whether larger workers are more likely to forage than others and whether the size of foragers varies according to the hour of the day. If larger workers are more thermotolerant, we would expect to find them foraging at the hotest hours of the day.

Materials and methods

Species

Among all other species co-occurring in the Mediterranean ant community the position of this subordinated species is extreme. Its greatest activity takes place when open ground surface temperature is the highest (45-50C°) and this species forages very close to its thermal capacities (CTM = 50°C). In the ant community, other species can not withstand such high temperatures, and at the hottest time of the day, *C. cursor* is the only ant species found on the ground surface (see Cerdá and al 1989). Like other species of the genus, *C. cursor* displays an individual foraging strategy. *C. cursor* is a zoonecrophagous scavenger ant that collects mainly corps of arthropods (Cagniant 1976; Cerdá et al. 1989) and occasionally nectar on fennel plants (*Foeniculum vulgare*) (Cerdá et al. 1989; pers. obs.).

Laboratory experiment

To test for a positive relationship between size and thermal resistance of workers, ten colonies of *C. cursor* were collected in two sites from contrasted habitats in Languedoc-Roussillon (France) in May 2004. Five colonies were sampled in the village of Luc-sur-Orbieu (Luc) in the inland, and five other colonies on the beach of Argelès, approximately 80 km SE of Luc.

In the laboratory, thermal tolerance was monitored by means of an electric hot plate (Salvis 30-120°C, AG. Emmenbrücke, Luzern). Ants were exposed to two temperatures: 48°C, the maximal activity temperature of foraging and 52°C, the maximum surface temperature for foraging in the field (Cerdá et al. 1998a,b). For each temperature and each colony, 30 individuals were placed on the surface of the hot plate and the time each ant died or lost muscular coordination was noted. To cover the entire worker size range of each colony, the 30 workers analysed were selected among the smallest (10), the intermediate (10), and the tallest (10) workers of each colony (*i.e.* 30 workers per temperature and colonies).

The 600 desiccated workers were then measured using a binocular microscope associated to a Sony XCD-SX910CR video camera module. National Instruments Vision Assistant vs7. software was used to analyse the digital pictures. Since in *C. cursor* all the classical traits measured in ants are highly significantly correlated (Cagniant 1983; pers. data.), only the most repeatable one was recorded, i.e. the tibia length (Clémencet & Doums, submitted).

Moreover, in the context of our study, tibia length is the most pertinent trait as it directly determines the elevation of the worker body from the hot substrate.

To test for an effect of size, temperature, population and colony treatment on thermal tolerance, a generalized linear model was performed. Populations were considered as a fixed factor given that they were not sampled at random but were chosen to differ in some ecological factors (see above). In contrast, colonies among site were sampled at random and were therefore considered as a random factor nested within population. Time to death was log10-transformed to fulfil the assumptions of the general linear model. The analyses were performed using Proc GLM of SAS 8.0 for Windows (SAS Institute, 1990) with type III sums of square.

Field experiment

A field study was performed on the beach of Canet-Plage (Languedoc-Roussillon, southern France) in Mid June 2003. This site is characteristic of dry and arid habitats of *C. cursor*. It consists of an open area having sparse vegetation and exposed to the sun all day long. Nests of *C. cursor* are quite abundant and can only be scout out by their small entrance on the ground (1cm large).

Foraging activity was studied in 12 colonies distributed within an area of 8x30 meters. Field study was carried out from 11h00 to 16h00, a time period during which more than 90% of the activity of *C.cursor* is recorded (Ruano et al. 2000). Foraging activity was monitored at the nest entrance by capturing the workers leaving the nest during four sessions of 20 min (at 11h15, 12h15, 14h45 and 15h45). During each capture session, the ground surface temperature was measured. The 12 colonies were excaved at the end of the day. Although nest excavation was easy because of its predictable structure, extra care was taken not to miss any chamber or gallery that might contain individuals. Experiments were conducted on four different days, three to four colonies being studied per day. In each colony, a sample of 30 individuals taken at random were measured later in the laboratory. All the workers collected at the nest entrance (n = 632), as well as within colonies (n = 360) were measured as described above (n total = 962, see Table 1).

To test for an effect of activity (foragers *vs* inside workers) and colony treatment on worker size, a generalized linear model (GLM) was performed. We also used a GLM procedure to test for an effect of hour on worker size. The analyses were performed using Proc GLM of

SAS 8.0 for Windows (SAS Institute, 1990) with type III sums of square. Colony was considered as random factor.

Results

Laboratory experiment

Time to death ranged from 31 s to 7382 s at 48°C and from 14 to 405 s at 52°C. Tibia length had a highly significant positive effect on time-to-death (see Figure 1, Table 2). As predicted, temperature had also a significant influence on time-to-death, workers surviving longer at 48°C than at 52°C (Figure 1). Interestingly, we detected a significant effect of colony but no effect of population on time-to-death. We therefore investigated whether the variation among colonies could be explained by differences in colony parameters, such as colony size or genetic diversity. Indeed these parameters could be positively correlated with colony performance. To do so, we used the residuals of the regression of thermal tolerance on worker size, estimated separately for each temperature. The level of genetic diversity was obtained from eight colonies already analysed (Clémencet & Doums, submitted). We first confirmed that the residuals significantly differed among colonies at both temperatures (48°C: $F_{9,290} = 7.98$, $p = 10^{-6}$; 52°C: $F_{9,290} = 4.64$, $p = 10^{-5}$). However, the mean value of the residuals per colony, did not significantly vary neither with colony size (Spearman-rank correlations, n = 9, 48°C: r = -0.49, p = 0.14; 52°C: r = -0.59, p = 0.48), nor with the level of genetic diversity (Spearman-rank correlations, n = 8, 48°C: r = 0.09, p = 0.99; 52°C: r = -0.25, p = 0.31).

Field experiment

As observed in two other populations of *C. cursor* (Clémencet and Doums, submitted), mean worker size (inside the nest) was positively correlated with colony size (Spearman's rank correlation test, n = 12, r = 0.61, p = 0.043). This effect of colony size was also reflected in the mean size of foragers which significantly increased with increasing colony size (r = 0.65, p = 0.029; see Figure 2). No difference in worker size was observed between inside nest workers and foragers (Table 3). However, a significant interaction between colony and activity (foragers and inside workers) was detected. We therefore tested for each colony whether the size of foragers and inside workers differed by using *t*-test comparisons. Over the 12 comparisons, two remained highly significant and one was closed to significantly after correcting for Bonferonni multiple comparisons (Sokal & Rolf 1995). Foragers were significantly larger than inside workers in the smaller colony (982: t_{96}= -4.87; $p < 10^{-4}$), whereas the reverse was observed in the largest colony (953: t_{96}= -4.26; $p < 10^{-4}$). Similarly, no difference in the size of foragers was detected between the different hour of the day, and no interaction between colony and hour was detected (Table 4).

Discussion

In *Cataglyphis cursor*, resistance to temperature is clearly dependent on size, large workers resist longer than small workers both at 48°C and 52°C. These differences could result from different additive mechanisms. Long legs keep the body highest above the ground, thereby decreasing temperatures experienced by large workers compared to small workers. It has been shown that at a height of 4 mm above the ground, the temperature may be 10°C lower than on the soil surface (Gehring & Wehner 1995). For instance, in *C. velox* a height increase of 1-2 mm permits to decrease the temperature experienced by the ant of 6-7°C (Cerdá & Retana 2000; Cerdá 2001). Differences could also result from a better physiological adaptation of large workers. Since surface area is negatively proportional to mass for organisms of a given shape, small organisms tend to desiccate faster than larger (Hood & Tschinkel 1990; Kaspari 1993). Some studies have even reported that large workers had larger body water reserve after adjusting for body mass and therefore loose the water more slowly than small workers, even after adjusting for cuticular surface area (Kaspari 1993; Lighton et al.1994; Cerdá 2001).

Variation in worker size, even if highly significant, explained only a small part of the variation in thermal tolerance. Workers having the same size could differ in their age and physical condition. Younger workers could have a better physical condition with more water reserve allowing longer resistance to high temperature. This variation could also have a genetic origin. Since queens mate multiply (with up to ten males, Pearcy et al. 2004; Clémencet & Doums, submitted), workers of the same colony are genetically diverse and may exhibit different resistance to desiccation. In insects, there is a growing evidence that variation among individuals in thermal tolerance may depend on genetic differentiation in key physiological and biochemical characters (Huey et al. 1991; Krebs & Feder 1997; Krebs & Bettencourt 1999; Neargarder et al. 2003). In polygynous colonies of the ant *Solenopsis invicta,* worker thermal tolerance was not related to size but a genetic variation in time-to-death and a significant heritability of resistance to desiccation have been reported (Li and Heinz 1998). Genetic variation in time-to-death and size among worker merits to be tested in *C. cursor*.

Colony explained also a significant part of the variation in thermal tolerance. However, after controlling for the size of workers, we could not associate this variation neither to colony size, nor to colony genetic diversity. Larger colonies and the more genetically diverse colonies did not tend to have better thermal tolerance than others. However, given that the mean worker size increased with colony size as previously observed in two other populations (Clémencet & Doums, submitted), larger colonies had a greater potential to resist high temperature. Similarly, even if there was no significant difference in pattern of desiccation between the two habitat types colonies from the vineyard populations had indirectly a higher potentiality to resist high temperatures since their workers were on average larger (Clémencet & Doums, submitted)

The better resistance to temperature of large workers could translate into difference in size between foragers and inside workers. In the field, we failed at detecting any size difference between foragers and workers taken at random inside the nest. In addition, contrary to what has been observed in other ant species (*Formica neorufibarbis*; *Solenopsis invicta*: Porter & Tschinkel 1987; *Cataglyphis velox*: Cerdá & Retana 1997), the size of foragers did not significantly differ according to the hour of the day at which they had been captured. Note however that our study has been performed on only few, and sometimes cloudy days. The soil temperature at which the experiment has been conducted in the field (31°C to 49°C) may simply be not sufficiently high to select for large workers only. Our field study did not provide evidence that larger workers are more involved in foraging activities or foraged when temperature is higher as it was observed in the closely related *C. velox*. However, larger workers could still performed longer foraging trip, with a greater running speed because of their longer legs. They could also spend less time at thermal refuge and therefore be more efficient at collecting prey. Small workers are also more sensible to low temperature than large workers, leading to a higher mortality rate during the winter (Cagniant 1983). In this species, a larger worker size may therefore help surviving hibernation and should then be favoured both at the colony and the individual level. Given that *C. cursor* is forced to reduce its midday activity because of very high temperatures on some days, or to accept a high forager mortality risk (Cerdá et al. 1989). Selective pressures on resistance to temperature should be strong. Both physiological adaptations and worker polymorphism might have been developped by colonies to expand their daily activity period and avoid inter-specific competition by foraging mainly during the hottest hours of the day. More experiments are needed to estimate the benefits a colony can obtain by producing these large workers.

In orphaned colonies, workers of *C. cursor* can produce both males and females (gynes and workers) by arrhenotokous and thelytokous parthenogenesis, respectively. Given that their reproductive potential increases with their size (Cagniant 1983; pers. data.), selection at the individual level may have also favored the evolution of larger workers.

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Tables and figures

Table 1. For each colony studied in the field, are given the colony size, the number of foragers collected during the four sessions (N_f) as well as the number of workers collected inside the nest (N_{nf}).

Colony label	Colony size	N_f	N_{nf}
953	1424	68	30
954	559	79	30
955	454	44	30
981	749	61	30
982	138	68	30
983	525	36	30
984	1332	59	30
988	315	45	30
989	771	41	30
991	260	34	30
992	1357	51	30
994	1261	46	30

Table 2. General linear model for thermal tolerance in *C. cursor* workers. Temperature and site were considered as fixed effects and colony as random effect nested within site. Significant effects are indicated in bold.

Source	d.f.	MS	*F*	*P*
Tibia length	1	12.56	103.05	**$< 10^{-4}$**
Temperature	1	1.06	8.69	**0.003**
Site	1	0.19	0.69	0.43
Colony within Site	8	0.28	2.3	**0.020**
Tibia length x Temperature	1	0.41	3.44	0.064
Tibia length x Site	1	0.09	0.76	0.382
Tibia length x Colony	8	0.17	1.43	0.179
Temperature x Site	1	0.002	0.02	0.896
Error	577	70.85		

Table 3. Effect of activity (inside nest workers I *vs* foragers F) and colony on worker size.

Source	d.f	SS	*F*	*P*
Activity (I vs F)	1	0.08	1.64	0.20
Colony	11	5.72	10.06	< 0.0001
Activity*Colony	11	2.42	4.26	< 0.0001
Error	968	50.27		

Table 4. Influence of colony and hour on the size of foragers.

Source	*df*	MS	*F*	*p*
Colony	11	2.59	4.42	$<10^{-4}$
Hour	3	0.21	1.32	0.26
Colony*Hour	32	1.56	0.91	0.61
Error	585	31.2		

Figure 1. Relationships between worker size (tibia length, mm) and thermal tolerance (log10 time to death, s) at 48°C (●) and 52°C (○).

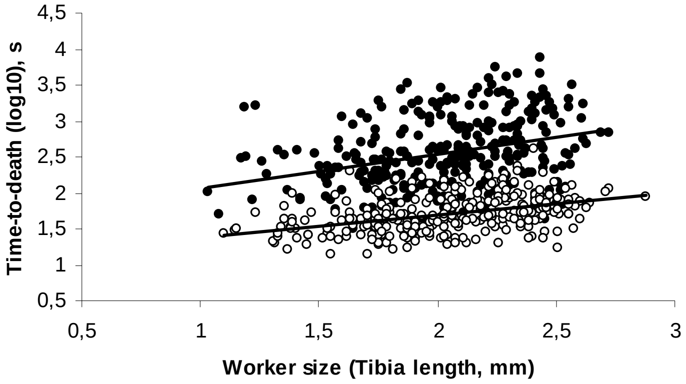
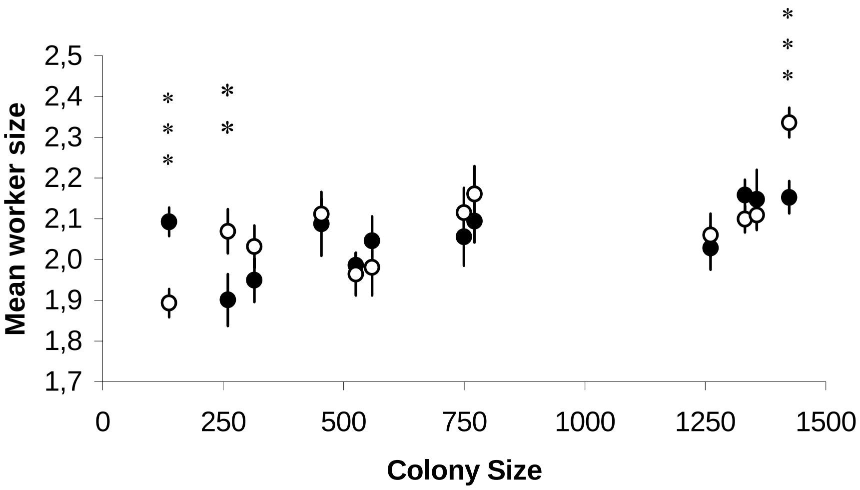


Figure 2. Relationships between colony size and mean (±SE) tibia length (mm) of workers sampled inside (○) or outside the nest (foragers: ●). Both correlations are significant (○, r = 0.61, *p* = 0.043; ●: r = 0.65, *p*= 0.029). Stars indicated when significant differences between foragers and non foragers were detected (*t*-test) within a colony.



ARTICLE 5

Habitat-related microgeographic variation of worker size and colony size in the ant Cataglyphis cursor

Clémencet J, Doums C

Soumis à *Oecologia*

Influence de l’habitat sur les variations de taille de colonies et de taille des ouvrières chez la fourmi *Cataglyphis cursor*

Chez les insectes sociaux, la taille de colonie est un paramètre crucial pour l’évolution et la complexification de la socialité (Bourke 1999). Elle est considérée comme un élément clé de l’évolution du polymorphisme de taille des ouvrières. Les facteurs écologiques susceptibles d’influencer la taille de colonie et d’expliquer les variations observées entre populations sont néanmoins peu étudiés et restent aujourd’hui largement méconnus.

Nous avons étudié, par une approche écologique, les variations de taille de colonies et taille des ouvrières de la fourmi *C. cursor*, dans deux types d’habitats (bord de mer, intérieur des terres). En bord de mer, les nids, construits dans un sol sableux, sont limités en profondeur par la nappe phréatique (environ 60 cm), alors qu’ils peuvent atteindre plus d’un mètre de profondeur à l’intérieur des terres. Si la profondeur du nid influence la taille de colonies, et si la taille de colonies influence la taille moyenne des ouvrières, on peut alors s’attendre à observer des tailles de colonies et des tailles d’ouvrières plus faibles en bord de mer. Dans deux populations (bord de mer et intérieur des terres), les deux prédictions sont vérifiées : la taille de colonie est positivement corrélée à la profondeur des nids, et la taille moyenne des ouvrières à celle de la colonie. Ainsi la taille moyenne des colonies et celle des ouvrières sont significativement plus faibles dans la population en bord de mer. Une étude à grande échelle, dans 16 autres populations issues des deux types d’habitats, nous permet de vérifier que les ouvrières sont en moyenne plus petites dans les populations du bord de mer.

Nos résultats suggèrent qu’un seul facteur écologique proximal peut avoir des conséquences considérables sur l’évolution des traits d’histoire de vie.

Habitat-related microgeographic variation of worker size and colony size in the ant *Cataglyphis cursor*

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Running title: Worker size variation in the ant *C. cursor*

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Abstract

In social insects, colony size is a crucial life history trait thought to have major implication in the evolution of social complexity especially in relation to worker size polymorphism. Yet, little is known about how ecological factors can affect and constrain colony. Here, we explored the pattern of colony size and worker size variation in the Mediterranean ant *Cataglyphis cursor*, in relation with the type of habitats colonized (seaside *vs* vineyard). The high level of the water table in the seaside habitat (60 cm against 150 in the vineyard) could constrain the depth of *C. cursor* underground nests and directly its colony size. If worker size increases with colony size, as observed in others ant species, larger colony size and larger workers should be found in the vineyard populations. By comparing worker size among 16 populations, we verified that workers are significantly larger in the vineyard populations. We further controlled that the morphological similarities detected among populations from the same habitat type were not due to geographic or genetic proximity. In two populations from each habitat type, the depth of nests were positively correlated with colony size and colony size with worker size. Using a type II regression approach, we further showed that the difference in the depth of nest between the two populations was sufficient to explain the difference in colony size and similarly that variation in colony size was sufficient to explain variation in worker size. Our results suggest that a single proximate ecological factor could lead to significant variation in major life history parameters.

Keywords: worker size, colony size, nest structure, dependent colony foundation, social insect.

Introduction

Individual size in insects, as in other organisms, is often considered as an important life-history trait which correlates with major fitness parameters such as fecundity, dispersal, mating success or survival (Stearns 1992). In holometabolous insects, the absence of growth during the adult stage prevents any size adjustment after metamorphosis (Nijhout 2003). Adult size is then a fixed parameter determined by genetic and environmental factors acting during the post-embryologic development of the insect and is tightly linked to the development time and growth rate (Nylin and Gotthard 1998). Natural variation in body sizes has often been investigated at a macro-geographical scale, often in relation to the latitude, with the aim of testing the Bergmann’s rule (Blanckenhorn and Demont 2004 for review). In social insects, variation of individual size has received a lot of attention as it is linked to a key social parameter, the division of labour among colony members (Oster and Wilson 1978; Hölldobler and Wilson 1990). The reproductive individual(s), the queen(s), can exhibit thorax volume up to 8000 times larger than the workers (*Carebara vidua*, M. Mollet, pers. com.).
Even though the workers usually do not reproduce, their body size is still an important life-history trait that can affect the ability of colony to rear offspring and therefore their indirect fitness. For various ant species, larger workers perform better than smaller workers to carry out particular tasks linked to foraging and nest defence (Cerda and Retana 1997; Reyes-Lopez and Fernandez-Haeger 2001; Braendle et al 2003; Nowbahari et al 1999; 2000). Such pattern has also been observed at the inter-specific level (Davidson et al 2004; Ness et al 2004). Larger workers also tend to survive better (Porter and Tschinkel 1985, Calabi and Porter 1989) and be more resistant to starvation (Heinze et al 2003). Producing larger workers could therefore be advantageous for the colony. Large workers are however more energetically expensive to manufacture and maintain than small workers, and colonies have to face the traditional trade-off between worker number and size (Bourke and Franks 1995).

Considering the extra dimensional level of the colony is therefore necessary for understanding the proximate and ultimate factors determining worker size. Colony can be considered as a super-organism with modular growth such as certain plants or corals, the modules being the different members composing the colony (Hölldobler and Wilson 1990, Kaspari 2005). Colony size is then determined by the rate of birth and death of its modules. As worker size, colony size can then be considered as a crucial life history trait of the super-organism. Colony size is generally positively correlated to colony reproductive success (Oster and Wilson 1978;

Tschinkel 1993; Sorvari and Hakkaraien 2005), only large colonies being able to obtain the resources needed to rear the sexuals (Oster and Wilson 1978; Hölldobler and Wilson 1990; Bourke and Franks 1995). Interestingly, a positive relationship between colony size and worker size has been frequently documented (see Brian 1957; Elmes 1974; Wood & Tschinkel 1981; Porter and Tschinkel 1985; Gibson 1989; Tschinkel 1988; 1993; 1998; Wetterer 1994; Kaspari and Byrne 1995) suggesting that only large colonies can also afford the production of large workers. Even though worker size and colony size are two major life history traits, few studies have investigated their pattern of natural variation among colonies and populations. This is however a crucial step not only for understanding life-history traits evolution in social insects, an area still largely unexplored (Bourke & Frank 1995), but also for understanding the evolution of social complexity such as the evolution of worker castes (Hölldobler and Wilson 1990) or the reproductive division of labour (Bourke 1999).

In ants, the size of the nesting cavity, the nature and the availability of the nest-building materials are known to potentially constrain colony growth and size (Wilson 1959, Hansell 1987). The Mediterranean ant, *Cataglyphis cursor*, is an interesting species to investigate how a simple proximate ecological factor, the level of the water table, can constrain colony size and indirectly worker size. *C. cursor* nests are underground and possess a single entrance open on a single vertical well leading to chambers located up to 1m deep. Horizontal galleries have never been observed and the volume of nest can only be increased by increasing nest depth (Cagniant 1976; Lenoir et al 1988; pers. obs). The depth of the water table is a simple ecological factor that clearly limit the depth of nests (Cagniant 1976; Lenoir et al 1988). Interestingly, *C. cursor* colonizes two main types of habitat that clearly differ by the depth of their water table : from 60cm on the seaside (sandy soil) to 1m20 in the vineyard (chalky soil; Lenoir et al 1988).

In this study, we explored the pattern of worker size and colony size variation in *C. cursor*, in relation with the type of habitats. *C. cursor* is a monogynous species with dependant colony foundation, the queen found a new colony with the help of workers (Lenoir et al 1988). Even if the colonies therefore never pass through the critical phase of small incipient colonies, large variation in colony size can still be observed in the field (from 150 to 2500; Lenoir et al 1988;, pers obs.). If the two hypotheses about the positive correlations between nest depth and colony size and between colony size and worker size are verified in *C. cursor*, we could then predict larger colony size and larger workers in the vineyard populations. This prediction was verified by comparing the mean worker size between populations from both types of habitats. Moreover, in two populations (seaside and vineyard), the depth of nests were positively correlated with colony size and colony size with worker size. Using a type II regression approach, we further showed that the difference in the depth of nest between the two populations was sufficient to explain the difference in colony size and similarly that variation in colony size was sufficient to explain variation in worker size. This suggests that a single proximate ecological factor could lead to significant variation in major life history parameters.

Materials and methods

Variation of worker size among populations

We used workers sampled for a previous population genetic structure study (Clémencet et al 2005) for which the two types of habitats (five seaside and 11 vineyard populations) were represented. In July 2001, a total of 317 colonies were sampled in these 16 populations (300x150m areas), in Languedoc-Roussillon, France (see Clémencet et al 2005, for the map of populations). As for the genetic study, only one randomly chosen individual per colony was measured (see sample size in Fig 1). The genetic data obtained using eight microsatellites markers by Clémencet et al (2005) were used to compare the genetic and morphological differentiation.

In the laboratory, workers were removed from alcohol, dissected, dried at room temperature and digitally photographed using a Leica XC-ST70 video camera module. Five morphological traits were measured using Matrox Inspector software (to the nearest 0.015 mm): **B**ody **L**ength from the beginning of the clypeus in top view to the end of the abdomen (BL); **T**ibia **L**ength of the right hind leg (TL); **S**cape **L**ength, i.e. straight-line distance from base to apex of the scape (SL); **H**ead **L**ength from the beginning of the clypeus in top view to the end of the head capsule (HL) and **H**ead **W**idth at the interocular line (HW).

The effect of habitat type (seaside vs vineyard) on the mean size was tested using an analysis of variance with populations nested within habitat type for each morphological trait. The analysis was performed using the MIXED procedure for nested analysis of variance in SAS 7 for Windows (SAS Institute 1996) with habitat type defined as a fixed factor and population as a random factor. Morphological divergences between populations were estimated by Mahalanobis D^2 distances using Proc Candisc in SAS (SAS Institute 1996). Compared to Euclidean or Pythagorean distances, Mahalanobis distances have the advantage of incorporating the effects of correlation between morphological variables (Campbell and Atchley 1981). Degrees of genetic divergences among populations were estimated by F_{ST} pairwise coefficients. Levels of association between the matrices of morphological distances (D^2), genetic distances (F_{ST}) and geographical distances (Km) were examined by Mantel's tests (1967). Significance levels were obtained by comparing the distribution of observed values to 10000 values obtained by random permutation of row and column elements in the independent matrices using XLSTAT-PRO 7.5.

Variation of worker size among colonies

In May 2004, a new sampling was performed to investigate the relationship between nest depth, colony size and worker size. We collected colonies from two populations, one in each type of habitat. Thirteen colonies were sampled in the population L4 in the inland while eleven other colonies were sampled in the population A2 on the seaside. These two populations were included in the previous study and were shown to differ in worker size for the five morphological traits considered (Post hoc test, all $P < 10^{-3}$). Nest excavation was easy because of its predictable structure (see introduction), but extra care was taken not to miss any room or gallery that might contain ants. The depth of the nest was recorded when we attained the deepest chamber, in which the queen was systematically found. In the laboratory, the number of workers in each colony was counted and a sample of 30 randomly chosen workers per colony was measured as described above (n = 720). Given that the five previous morphological measures were all highly correlated, only the one with the lowest measurement error (1.3 %) was taken, i.e the tibia length. Measurement error (ME) was assessed by repeated measurements and was quantified as % ME following Bailey and Byrnes (1990) for a set of 10 *C. cursor* individuals encompassing the entire size range.

We first examine if workers from the two populations differed in size using an analysis of variance model, with colonies nested within populations. The analysis was performed using the procedure MIXED of SAS, with population as a fixed factor and colonies within population as a random factor. We examined whether the depth of nest and colony size differed between the two populations and explored the relationship between colony size and depth of nest by standardized major axis (SMA) methods (type II regression like; Sokal and Rolf 1995). SMA slope fitting technique is appropriate when the purpose is to estimate and compare the line-of-best fit relating two variables each having a random variation. SMAs were fitted for each population individually. Then tests for homogeneity of slopes between populations and calculation of a common slope were conducted following Warton and Weber (2002). When a common slope could be fitted (test of homogeneity, $\boldsymbol{P} > 0.05$), ANCOVA-like comparisons were conducted to test for difference in elevation (intercept) of slopes (i.e significant difference on the Y-axis between populations) and separation of the populations along the common slope (i.e. significant difference on the X-axis between populations) using (S)MATR 1. software (Falster et al 2003). The same SMA procedure was conducted to examine relationships between mean as well as variance in worker size and colony size in both populations.

Results

Variation of worker size among populations

We confirmed that in *C. cursor*, worker size is normally distributed with uni-modal distribution and with the five measures taken being highly correlated (correlation coefficient ranging from 0.76 to 0.92, all significant at $P < 10^{-4}$). Overall size amplitude and coefficient of variation, ranging from 10.7 to 16% for the scape length and the head width, respectively, were very close to those reported by Cagniant (1983). Mean (±SE) of the five morphological traits for the 16 populations are given in Fig.1. The analysis of variance on each of the five morphological traits measured showed that workers from seaside populations were significantly 15% (for BL) to 19% (for HW) smaller than workers from vineyard populations ($P < 0.01$ for the five measurements, Fig.1). All the vineyard populations except one had a mean worker size larger than the seaside populations (Fig 1). Within a given habitat, workers did not significantly vary in size among populations. The genetic distances (F_{ST}) among populations ranged from 0.006 (T1-T2) to 0.309 (S1-T3), while the Mahalanobis D^2 ranged from 0.065 (T2-L4) to 4.96 (T2-A2). The matrix of morphological distances was neither significantly correlated with the genetic distance ($r = 0.002$; $P = 0.27$) nor with the geographical distances matrices ($r = 0.05$; $P = 0.11$).

Variation of worker size among colonies

As predicted from the habitat type, the mean depth of nests was significantly different between the two populations (*Mann-Whitney test*, $Z = 3.048$, $P = 0.002$), with nests being on average almost twice deeper in the vineyard (mean ±SE; $m_{L4} = 62.4 \pm 22$cm) than in the seaside population ($m_{A2} = 38.3 \pm 9.3$cm). As expected from the difference in nest structure, mean colony size was almost twice higher in the vineyard population (mean ±SE; $m_{L4} = 1107.8 \pm 485.8$; $\text{range}_{L4} = 260\text{-}1714$) than in the seaside population ($m_{A2} = 577.7 \pm 275.85$; $\text{range}_{A2} = 208\text{-}968$; *Mann-Whitney test*, $Z = 2.636$, $P = 0.008$). Finally, as detected in the previous part of this study by sampling one worker per colony, mean worker size was significantly higher in colonies from the vineyard than from the seaside population ($F_{1,22} = 14.18$; $P = 0.0011$). Colonies explained a non-negligible part of the random variance component (16%, $P = 0.0024$), indicating that, within a population, mean worker size varied among colonies.

In both populations, the depth of nest significantly increased with colony size (see Fig. 2.a; $P_{L4} = 0.009$; $P_{A2} = 0.049$) and slopes did not differ between populations ($P = 0.404$). In agreement with the difference in colony size between the two populations, a significant separation along the common slope, i.e. along the X-axe (colony size), was detected between the two populations ($F_{1,23} = 13.03$, $P = 0.002$). No significant difference of the intercept value was detected ($F_{1,23} = 0.274$, $P = 0.6$), indicating that for a given depth of nest, colony size should be the same in both populations. The regression of mean worker size on colony size was significantly positive in both populations studied (see Fig 2. b; $P_{L4} = 0.0066$; $P_{A2} = 0.017$) with the two slopes being not significantly different between populations ($P = 0.081$). A significant separation along the common slope, i.e. along the X-axe (colony size), was also detected between the two populations ($F_{1,23} = 14.39$, $P = 0.001$) reflecting the difference of colony size detected between the two populations. Interestingly, no significant difference of the intercept value was detected between the two populations ($F_{1,23} = 0.23$, $P = 0.63$), signifying that for a given colony size, mean worker size should be the same in both populations. In contrast, the variance in worker size was not related to colony size in both populations (see Fig 2.c, $P_{L4} = 0.94$; $P_{A2} = 0.19$). In addition, slopes were not significantly different between the two populations ($P = 0.073$). As observed above, a significant separation along the common slope was detected between the two populations ($F_{1,23} = 9.357$, $P = 0.006$), while no significant difference of the intercept value was detected ($F_{1,23} = 2.367$, $P = 0.138$), indicating that, on average, variance values are the same in both populations.

Discussion

C. cursor colonizes two main types of habitats, seaside and vineyard, that differ by an ecological factor, the depth of the water table, which is known to clearly constrain the depth of nest in this species (Cagniant 1976). The water table in the vineyard populations was shown to be deeper than in the seaside populations (Cagniant 1976; our obs.). We found that the mean worker size was up to 27% smaller (for head width) in the seaside populations than in the vineyard. Such result was predicted assuming a positive relationship between worker size and colony size as well as between colony size and the depth of nests. These two positive relationships were verified in both a vineyard and a seaside population of *C. cursor*. Moreover, the standardized major axis (SMA) methods (type II regression) showed that the regression parameters (slope and intercept) were the same in both populations and that the two populations were significantly separated along this common regression line. This indicated that the difference in the depth of nests between the two populations was sufficient to explain the difference in colony size and that the difference in colony size was sufficient to explain the difference in mean worker size. The deepest nests were twice higher in the vineyard population than in the seaside and corresponded approximately to the appearance of humid soils (Cagniant 1976; pers. obs.). Interestingly, the maximal colony size was about twice higher in the vineyard than in the seaside population even though the minimal colony size was the same (see Fig. 2). It is therefore tempting to conclude that differences of a simple proximate ecological factor lead to a drastic variation in colony size and mean worker size between populations, two major parameters tightly linked to colony productivity.

It could be argued that other differences, of genetic and/or environmental origins, could also shape the pattern observed between populations. First, given that the seaside populations were sampled in a smaller geographical area, genetic similarity or geographical proximity of seaside populations might lead to morphological similarity. However, no significant correlation between morphological and genetic or geographical distances was detected. Second, differences in mating/ breeding system may be linked with worker size variation (Oster and Wilson 1978; Frumhoff and Ward 1992; Fjerdingstad and Crozier 2006). For instance, the number of queen per colony has been shown to affect the body size of workers in *Leptothorax acervorum* (Heinze et al 1995), *Solenopsis invicta* (Goodisman and Ross 1996) and *Formica selysi* (Schwander et al 2005). However, the two populations studied exhibit the same colony organization with a single queen per colony and a high level of polyandry (on average 11.1 ±3.6 males in the vineyard population and 10.8 ±2 in the seaside (Clémencet et al, submitted). Finally, we cannot rule out that other ecological factors could also differ between the populations studied even though the constraint due to the water table appears to be the most likely. The variation in the depth of nest explained a remarkably high part (60%) of the variation of colony size, a parameter generally supposed to be affected by various ecological and social factors (Bourke 1999). Experimental manipulations are however needed to clearly conclude on the causal link of the relationships observed here.

As generally observed in ants (see Brian 1957; Elmes 1974; Wood & Tschinkel 1981; Porter and Tschinkel 1985; Gibson 1989; Tschinkel 1988; 1993; 1998; Wetterer 1994; Kaspari and Byrne 1995), worker size was found to be positively associated with colony size. This pattern is often due to the occurrence of nanitic workers in the small recently founded colonies in species with independent colony foundation (Wilson 1971). It is also commonly observed in ants with clearly polymorphic worker castes. In such case, the largest worker caste is only produced once the colony reaches a sufficient size and the increase in mean worker size with colony size is associated with an increase of the variance in worker size (Brian 1957; Gibson 1989). In *C. cursor*, none of these explanations hold since the species founds the colony dependently and has no distinct worker caste (Cagniant 1983). The minimal colony size observed in our populations as well as in populations studied by Lenoir et al (1988) was around 200. This gives an idea of the minimal propagules size during a fission event, and agrees with the observations of Lenoir et al (1988) estimating around 250 workers the size of a propagule. Basically, a colony of 200 workers can not be as energetically constrained as a queen founding alone its colony. Two hypotheses can explain our correlation between colony size and worker size. First, a small colony might not afford the production of large workers because these workers could specialize to particular tasks and that physical specialization could be costly for small colonies by decreasing flexibility in response to environmental variability (Wheeler 1991). This explanation based on the division of labour, would imply that large workers are produced to increase the variance in worker size and thus increase the efficiency of colonies. The absence of correlation between variance in worker size and colony size goes against this hypothesis. Second, the level of resources a colony can obtain could linearly increase with its size in the colony size range observed in our study. This would mean that colonies never reached the point at which the curve of the resource gained as a function of colony size becomes asymptotic. Note that species that have a possibility to fission when this critical point is attained should be favoured to do it (Tsuji 1995).

From an evolutionary perspective, if there is a trade-off between the number and size of workers, the increase of worker size with colony size suggests that investing in larger worker size is advantageous over investing in a higher number of workers. In thermophilic ants, worker size is a major parameter affecting their thermal tolerance (Cerda and Retana 1997). A higher resistance to temperature of larger workers has also been found in *C. cursor* (Clémencet et al, in prep). A larger worker size could therefore allow colonies to expand their daily activity period and avoid inter-specific competition by foraging mainly during the hottest hours of the day. At the colony level, selection should then favour an increase in worker body size since it would enhance the colony's survival or reproduction (Crozier and Consul 1976). However, at the individual level, if the reproductive potential of workers is linked to their size, as observed in different species (Tsuji 1995; Heinze et al 1999; Heinze and Oberstadt 1999; Dietemann et al 2002; Hartmann and Heinze 2003; Gobin and Ito 2003; Ravary and Jaisson 2004), individual-level selection should favour the evolution of an optimal size for worker reproduction. This optimum might diverge from the one favoured at the colony-level. Such conflicting selective pressures potentially occur in *C. cursor*. In this species, although being unmated, workers have been shown to produce both males and females (gynes and workers) by arrenothokous and thelytokous parthenogenesis, respectively, in the absence of the queen (Cagniant 1983). Moreover, workers of intermediate size (between 6.3 and 7.4 mm) appear to produce more eggs than small or large workers (Cagniant 1983). The optimal size for the worker might therefore be different than the one for the colony. The evolution of worker size in this species probably results from many, and probably conflicting selective pressures and is far from being elucidated.

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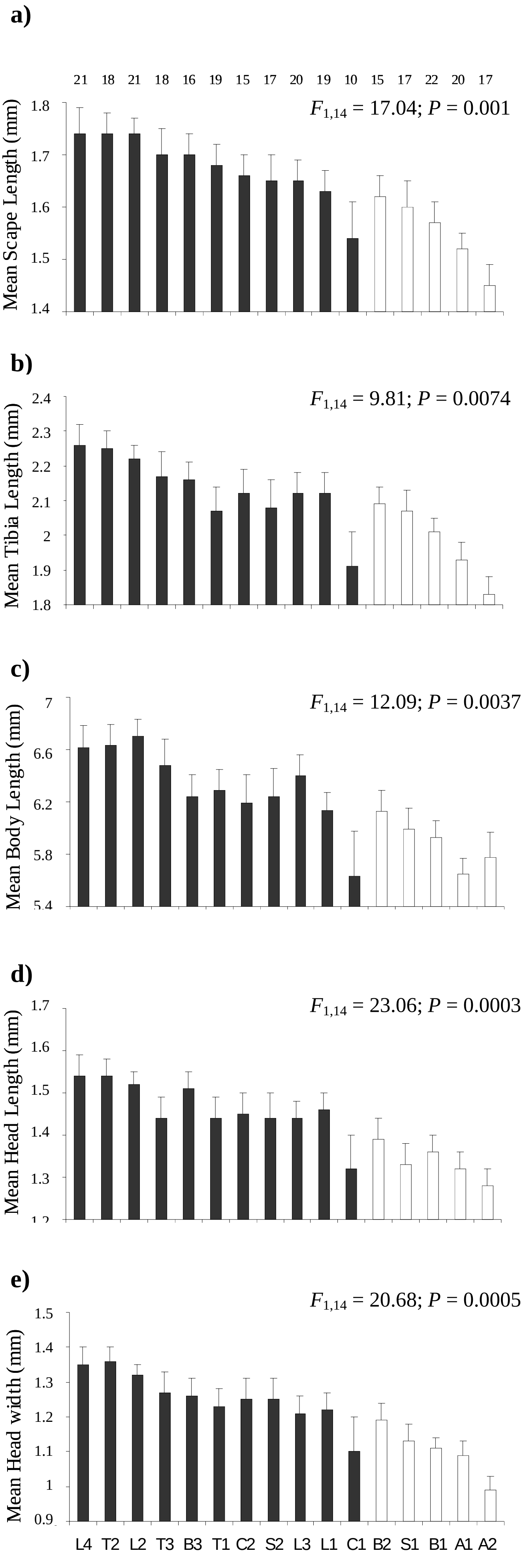
Figure legends

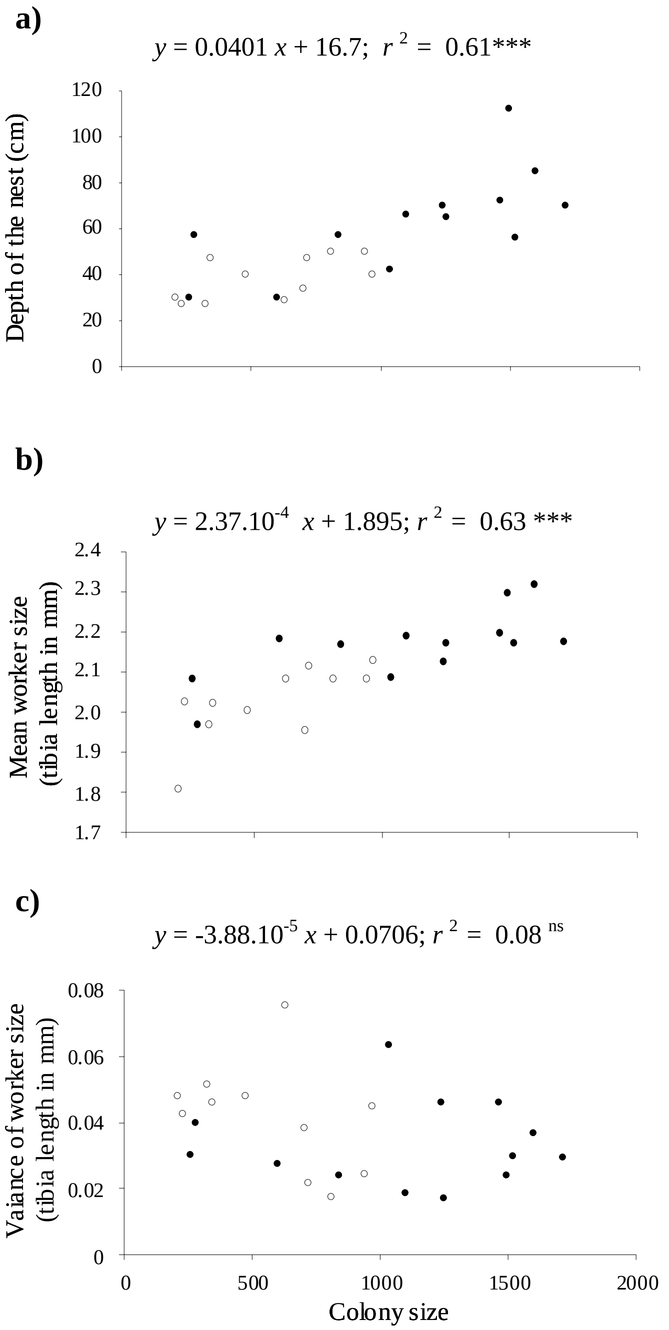
Figure 1. Worker size variation among populations

Morphological measurements [mean ± SE (mm)]: scape (a), tibia (b), body (c) and head length (d) as well as head width (e) are given for each of the 16 populations studied. The number of workers measured in each population is given on the top of each bar in (a). Seaside populations are represented with white bars and vineyard populations with black bar. The results of the analysis of variance testing differences in worker size between habitat types (populations being nested within habitat type) are given for each morphological traits. The percentage of the random variance explained by population ranged from 1 to 7.6% and never significant.

Figure 2.

Regression of depth of the nest (**a**), mean worker size (**b**) and variance of worker size (**c**) on colony size. White circles are colonies sampled in the population A2 on the seaside and black circles are colonies sampled in the population L4 in the vineyard. Significance (* for P <0.05; ** P <0.01 and *** P <0.001) and r^2 values are given for each regressions, equations for the common regression lines are also given.


a)
21 18 21 18 16 19 15 17 20 19 10 15 17 22 20 17
Mean Scape Length (mm)
1.8
1.7
1.6
1.5
1.4
$F_{1,14} = 17.04$; $P = 0.001$
b)
Mean Tibia Length (mm)
2.4
2.3
2.2
2.1
2
1.9
1.8
$F_{1,14} = 9.81$; $P = 0.0074$
c)
Mean Body Length (mm)
7
6.6
6.2
5.8
5.4
$F_{1,14} = 12.09$; $P = 0.0037$
d)
Mean Head Length (mm)
1.7
1.6
1.5
1.4
1.3
1.2
$F_{1,14} = 23.06$; $P = 0.0003$
e)
Mean Head width (mm)
1.5
1.4
1.3
1.2
1.1
1
0.9
$F_{1,14} = 20.68$; $P = 0.0005$
L4 T2 L2 T3 B3 T1 C2 S2 L3 L1 C1 B2 S1 B1 A1 A2


a)
y = 0.0401 x + 16.7; r 2 = 0.61***
Depth of the nest (cm)
0
20
40
60
80
100
120
b)
y = 2.37.10-4 x + 1.895; r 2 = 0.63 ***
Mean worker size (tibia length in mm)
1.7
1.8
1.9
2.0
2.1
2.2
2.3
2.4
c)
y = -3.88.10-5 x + 0.0706; r 2 = 0.08 ns
Vaiance of worker size (tibia length in mm)
0
0.02
0.04
0.06
0.08
0
500
1000
1500
2000
Colony size

ARTICLE 6

Size related fecundity of parthenogenetic workers in an orphaned colony of the polyandrous ant *Cataglyphis cursor*

Clémencet J, Rome Q, Fédérici P, Doums C

En préparation

Lien entre fécondité et taille des ouvrières chez la fourmi parthénogénétique *Cataglyphis cursor*

Chez les hyménoptères sociaux, la division du travail reproducteur est souvent étroitement liée à la différence de taille entre les individus. Au sein des colonies de la fourmi *Cataglyphis cursor,* la reproduction est monopolisée par la reine accouplée à plusieurs mâles. En l'absence de celle-ci, les ouvrières sont capables de produire des gynes (reines non fécondées) et des ouvrières par parthénogenèse thélytoque, ainsi que des mâles par parthénogenèse arrhénotoque. Nous avons testé, chez cette fourmi facultativement parthénogénétique, si la taille des ouvrières (de 3 à 11 mm) influence leur potentiel reproducteur.

Nous avons suivi les comportements de pontes et d'agressions des ouvrières au sein d'une colonie orpheline (n=357, 212h pendant 3 mois). Les ouvrières ont ensuite été mesurées et génotypées à l'aide de six marqueurs microsatellites afin de contrôler si leur taille et leur fécondité diffèrent selon leur lignée paternelle.

Nous montrons que les ouvrières de grande taille survivent mieux, ont une meilleure fécondité et donc probablement un meilleur succès reproducteur. Les ouvrières issues des différentes lignées paternelles ne présentent pas de différences significatives en matière de fécondité et de taille. Nous découvrons, pour la première fois chez cette espèce, l'existence de nombreuses interactions agressives. Les ouvrières reproductrices sont significativement plus agressées que les autres, ce qui suggère une reconnaissance des individus reproducteurs chez *C. cursor*. En revanche, nous montrons que les agressions ne sont pas préférentiellement dirigées contre les ouvrières des autres lignées paternelles.

Notre étude révèle qu'une grande taille peut être également avantageuse au niveau individuel.

Size related fecundity of parthenogenetic workers in an orphaned colony of the polyandrous ant *Cataglyphis cursor*

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Running title: Size related fecundity in the ant *C. cursor*
Keywords: worker size, fecundity, parthenogenesis, ant, *Cataglyphis cursor*

Abstract

In social hymenoptera, the reproductive division of labor is often linked to differences in individual body size with the reproductive caste (the queen) being larger than the workers. Even if less marked, the reproductive potential can also vary with size within the worker caste and could affect the evolution of worker size in social insects. In this paper, such relationship was tested in the facultative parthenogenetic ant *Cataglyphis cursor*. This species has an extraordinary social system for which worker reproductive potential could be of major importance. Colonies are headed by a multiply mated queen but workers can produce gynes (virgin queens) and workers by thelytokous parthenogenesis after the queen death. To determine whether reproductive potential of workers was related to their size, the behaviour of workers (n=357) was observed until the production of gynes (212h over 3 months) in an orphaned colony. The size of workers was measured and their paternal lineage determined using six microsatellite markers, to control for an effect of patriline. Larger workers had a higher probability to reproduce and to lay more eggs indicating that individual level selection could take place. However, the paternal lineage had no effect on the reproductive potential and worker size. From the behavioural and genetic data, we also provided, for the first time in this species, evidence of aggressive interactions among workers as well as a first indication that nepotism could occur in orphaned colonies since the five gynes produced belonged to a single paternal lineage.

Introduction

In many species, individual body size is a key parameter affecting reproductive success (Stearns 1992). In social hymenoptera, the reproductive division of labour often leads to high specialisation of the queen caste which harbours a larger body size than the workers (Hölldobler and Wilson 1990; Michener 2000). In a large number of social insects, the worker caste retains functional ovaries and can produce unfertilised haploid eggs by arrhenotokous parthenogenesis, these eggs turning into viable normal males (Bourke 1988). So, as observed for the queen caste (Wiernasz and Cole 2003), even within the worker caste a larger size may confer reproductive advantages (Frumhoff and Ward 1992). Several studies have reported that large workers exhibited bigger ovarian structure (with more ovariols) and/or have a higher fecundity (reproductive potential) than small workers (Dietemann et al. 2002; Heinze et al. 1999; Gobin and Ito 2003). Similarly, in two obligatory parthenogenetic ant species with no queen caste, the reproductive potential of workers was linked to their size (*Cerapachys biroi*: Ravary and Jaisson 2004; *Pristomyrmex pungens*: Tsuji 1995). Such a relationship may have major implications in the evolution of worker size in ants, that has mainly be considered from a social perspective in relation to division of labour (Oster and Wilson 1978; Hölldobler and Wilson 1990). Indeed, individual-level selection should favour the evolution of an optimal size for worker reproduction which might diverge from the optimal size which could be favoured at the colony-level, especially with respect to division of labour (Frumhoff and Ward 1992).

The ant *Cataglyphis cursor* is an interesting species to test whether the reproductive potential of workers is related to their size. Indeed, in addition to present a large continuous workers size variation (range: 3.5-11mm, Cagniant 1983; pers. obs.), this ant exhibits an extraordinary reproductive system. Colonies are usually headed by a multiply mated queen which monopolizes the reproduction (Pearcy et al. 2004a). Queens usually use thelytokous parthenogenesis to produce gynes (virgin queens) but use sexual reproduction to produce the workers leading to highly genetically diverse colonies (Pearcy et al. 2004a, Clémencet and Doums submitted). In addition, although being unmated, workers have retained functional ovaries and can also produce both haploid males by arrhenotokous parthenogenesis and diploid females (queens and workers) by thelytokous parthenogenesis in the absence of the queen (Cagniant 1983). In orphaned colonies reared in the laboratory, workers rapidly lay diploid eggs, which first develop into gynes to replace the queen. Several gynes are produced

(4-8) but only one survives and heads the colony (Cagniant 1983). Monogyny is therefore rapidly restored and worker reproduction in *C. cursor* is probably just a temporary situation. Producing the new queen confers a strong selective advantage to the worker since its genotype will be transmitted to all the future gynes produced by this new queen. Hence, a phenotypic trait, such as a large size, which increases the probability to produce the gynes, should be favoured by individual level selection. As colonies of *C. cursor* are highly polyandrous (Pearcy et al. 2004a; Clémencet and Doums, submitted), conflicts could occur among patrilines over the access to gyne production as well as over the selection of the gyne that will inherit the nest.

Previous studies suggested that, in orphaned colonies of *C. cursor,* all workers can reproduce and lay more eggs than a colony can rear (Cagniant 1983). No aggression had been documented and no regulation of reproduction had been reported. However, Cagniant (1983) noted that the size of workers may affect their reproductive success in several ways. The number of ovarioles is correlated with worker size, and larger workers activate faster their ovary and are therefore the first to produce eggs. This is important because the first eggs laid are the ones which are the most likely to become gynes.

The primary goal of our study was to determine whether the body size of workers affected their reproductive success. We also observed behavioural interactions among workers to determine whether regulation of reproduction through attacks directed towards reproducing workers occurred in orphaned colonies of *C. cursor*. As a first step, all the workers (n=357) of an orphaned colony were observed during more than 200h over 3 months. We explored the possible associations between body size and some components of the reproductive success by testing whether larger workers i) had a lower mortality rate, ii) were more likely to reproduce or iii) engaged in aggressive interactions towards reproductive workers to potentially ensure their own reproduction. To control for an effect of genotype on fecundity components in this polyandrous ant, all the workers were genotyped using six microsatellite markers.

Methods

Samples and laboratory conditions

In July 2003, a colony of *Cataglyphis cursor* was completely excavated from Canet-Plage beach (Languedoc-Roussillon, France). In the laboratory, the colony was reared in a plaster nest consisting of 4 chambers (approximately 35 cm^2 each) covered by a glass sheet allowing observations, and connected to a foraging arena (19 x 19 cm) where mealworm, *Tenebrio molitor*, and sugar water were provided *ad libitum*. At the end of September the queen was removed and kept at –20°C for subsequent genetic analysis. Afterward, the colony was artificially placed into hibernation by decreasing progressively the temperature to 10°C and kept in obscurity. In Mars 2004, we progressively stopped the hibernation period by increasing the temperature by 2°C every 3 days. The colony was then maintained at 22°C with a 12:12 light/dark cycle. The surviving workers (n=357) were all individually marked on the thorax and on the abdomen with a colour code using Unipaint marker px-20.

Behavioural observations

Behavioural observations were carried out from the exit of the hibernation phase on the 10th of March (day 1) to the end of the gynes' phase elimination, *i.e.* when only one queen remained alive in the colony (1st of June: day 84). Out of these 84 days, observations were performed on 53 days. Workers involved in aggressive interactions and ovipositions were identified during eight daily sessions of 30 minutes, which gives a total observation time of 212h. Observations were conducted between 10am and 4pm, which is the period of maximum activity of *C. cursor* colonies (Cerdá et al. 1989). Aggressions directed towards workers or gynes lasted from 10 seconds to a few minutes and consisted of violent biting of legs or antennae often followed by immobilization and/or displacement of the victim. Each morning, dead workers and/or gynes were removed from the nest and kept at –20°C. On the 8th of June, all the labelled workers and the last remaining gyne were collected and stored at –20°C for subsequent measurements and genetic analysis.

Size measurements

Previous biometric studies on *C. cursor* indicated that all classical measures of worker size are highly correlated (Cagniant 1983) and the highest precision of measurement was obtained for the tibia length (Clémencet et Doums, submitted). Tibia length was therefore chosen as the size index in our study. Right hind leg of each workers (n=357) and the five gynes produced were digitally photographed using a Leica XC-ST70 video camera module at 50x magnification and Matrox Inspector software was used to record the set of tibia's measurements.

Genetic analyses

Six DNA microsatellites developed for *C. cursor* (Pearcy et al. 2004b) were used to determine queen, gynes genotypes as well as the paternity of workers. For each individual, DNA was extracted using a QIAgen DNAeasy kit (Valencia, CA) and resuspended in 50µl of elution buffer. The polymerase chain reactions (PCR) were carried out as described in Clémencet and Doums (submitted). The amplified fluorescent fragments were visualized using an automated ABI Prism 310 Sequencer (Applied Biosystems) and allele sizes were estimated using GENESCAN software. All workers had an allele in common with the queen, confirming monogyny. At each locus, the paternal allele for each worker was deduced by elimination of the known maternal allele. Workers' patriline was then determined using each worker's composite genotype from all six loci. The probability of non-detection of a patriline (Boomsma and Ratnieks 1996) estimated from allelic frequencies obtained in the same site in a previous population genetic study (Clémencet et al. 2005) was very low (0.0033) indicating that the error of non-detection of a second father occurs only 0.3% of the time and could here be neglected.

Statistical analyses

To test for an effect of patrilines and size on workers life history components, we performed an analysis of deviance using the Proc Genmod function in the SAS/STAT v.8 ® software for windows[1]. For binary dependant variables, that is survival (whether or not a worker survived until the end of the experiment) and egg-laying (whether or not a worker was seen laying at least one egg), we assumed a logit link function and a binomial probability distribution (logistic regression). For the number of eggs laid, we assumed a log link function and a Poisson probability distribution (log linear model). Only workers that reproduced were

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included in this log linear model. For analysing egg-laying and the number of eggs laid, only the workers that survived until the end of the experiment were used. Only the patrilines that included at least 10 workers were considered in the statistical models. The number of workers and patrilines therefore varied according to the model considered. The models were never overdispersed and the statistical significance of each effect was therefore classically tested by comparing the change in deviance associated to the removal of this effect against a Chi-square distribution (McCullagh and Nelder 1989). The adequacy of each model was checked by visually inspecting the residuals.

Results

Tibia length was normally distributed and ranged from 1.32 to 2.82 mm (mean ±SE=2.22 ±0.01) with a coefficient of variation of 10.4 %. Nine patrilines were detected within the colony but were not equitably represented (χ^2 *test*=111.5, *df*=8, $p < 10^{-15}$). Forty three percent of the workers belonged to a single patriline (A). The first egg was produced 6 days after the total exit of hibernation (on day 1) and five gynes emerged from day 49 to day 55 (see Fig 1.a). A total of 358 ovipositions, involving 145 workers (i.e. 40.7 % of the workers), were observed during the 212 hours of observation. After an increase of the number of ovipositions during the first 10 days, the rate of ovipositions stayed fairly constant during all the experiment with an average of 6.75±3.15 (±SE) ovipositions observed daily (Fig 1.a). A single worker has never been observed laying more than one egg per day, and there was no monopoly of the egg production by one or two workers. A total of 465 aggressive acts were performed by 74 workers (20.7 % of the workers) towards 164 workers (45.9 %). Aggressive interactions started from day 1, increased rapidly, attained a pick on day 16 (after the emergence of larvae), then progressively decreased and almost stopped after the five gynes emerged (see Fig 1.b). As expected, aggressions were preferentially performed by egg-laying workers (χ^2=26.72, p<0.0001), and preferentially directed at egg-laying workers (χ^2=53.7, p<0.0001), strongly supporting their involvement in competition over reproduction. The observed frequency of aggressions involving half-sister workers was not significantly different from the one expected based on the proportion of egg laying workers in each patriline (χ^2=0.737, p=0.39). Similarly, the observed frequency of aggressions involving half-sister workers was not significantly different from the one expected based on the proportion of workers being aggressed in each patriline (χ^2=1.64, p=0.2). This suggests that workers did not preferentially attack workers from different patrilines as would be expected if nepotism occurred.

Both patrilines and worker size had a significant effect on the probability that a worker survived until the end of the experiment (Table 1). The probability of survival predicted by the model increased with worker size, and this effect was similar for each patriline since no significant interaction between patriline and worker size was detected (Table 1, Fig 2). Considering only the surviving workers, patrilines and worker size had no significant effect on the probability of being an egg-layer and no interaction was detected (Table 1). However, when considering the full data set by including the genetically undetermined individuals, the egg-laying workers were found to be significantly larger (mean=2.31±0.019, *n*=115) than the non egg-laying workers (2.23±0.022, *n*=104; $F_{1,\ 217}$=7.22, *p*=0.008). Considering only the egg-laying workers, the number of eggs laid by workers significantly increased with worker size (Fig 3, Table 1) and no effect of patrilines or interaction between patrilines and worker size was found. Note that the mean worker size did not significantly varied among patrilines (ANOVA, $F_{8,\ 277}$=1.37, *p*=0.21).

The five gynes produced had genotypes compatible with the patriline A only and at least 3 different egg-laying workers are needed to explain the five genotypes observed. To estimate the probability of observing five gynes of patriline 1 by random, we used the proportion of eggs laid by patriline 1 (p_1) during the first 10 days of the experiment (15 over 36 eggs, p_1=0.41). Only eggs laid at the beginning of the experiment could indeed produce the gynes given the development time from eggs to adult in this species (about 40 days). Note that the proportion of eggs laid by patriline 1 during all the experiment was similar (136 over 358 eggs, 38%). From the binomial distribution and p_1, the probability of getting five gynes from patriline 1 appears to be very low (0.012). The numerical superiority of eggs laid by patriline 1 can therefore not explain by itself that all the gynes belong to patriline 1. The aggressions performed by workers towards the gynes (*n*=117) started when all the gynes had emerged from cocoons and progressively decreased until a single gyne remained (Fig 1.b). Except gyne G3 who died rapidly, all gynes received attacks (see Table 2) and had the same proportion of homozygote loci. The selection of the gyne remains unclear. The surviving gyne was neither the largest nor the less attacked (Table 2).

Discussion

The behavioural and genetic studies of an orphaned colony of the ant *Cataglyphis cursor* showed that the probability that workers survive as well as the number of eggs they laid increased with worker size. Two non exclusive hypotheses could explain the higher survival of large workers. First, they may simply have greater fat reserves and a superior physical condition. Second, larger workers could survive better just because they are younger. This could not be tested as the age of workers was unknown. When considering only workers that survived all the experiment, larger workers were more likely to lay eggs than smaller workers and to lay a larger number of eggs. This is consistent with the fact that, in *C. cursor,* the number of ovarioles correlates positively with worker size (from 2 to 8 ovarioles; Cagniant 1983). Our results provide an additional evidence that worker size plays a significant role in the differential reproductive success of workers in orphaned colonies (Dietemann et al. 1992; Gobin and Ito 2003; Heinze et al. 1999). The higher survival rate and reproductive potential of large workers could give them a selective advantage at the individual level. Given that larger workers have also been shown to resist better to high temperature (Clémencet et al., in prep.) and to hibernation (Cagniant 1983), large workers could then also be favoured at the colony level. Resistance to temperature is known to be a crucial parameter in thermophilic ants of the *Cataglyphis* genera (Cerdà and Retana 1997; Cerdà 2001). Selection at both the individual and the colony level could therefore leads to an increase of worker size.

We reported, for the first time in this species, the occurrence of numerous aggressive behaviours among workers. Aggressions were preferentially performed by egg-laying workers. They were also preferentially directed towards egg-laying workers, irrespectively of worker patrilines. This suggests that workers of *C. cursor* can distinguish between reproductive and non-reproductive individuals, as in many other ant species (e.g. Peeters 1987; Hartmann et al. 2005). Even though many aggressive interactions have been observed, there was no hierarchy with a single egg-laying worker as observed in the parthenogenetic ant *Platythyrea punctata* (Heinze and Hölldobler 1995). In orphaned colonies of the ant *C. cursor,* many workers reproduce (at least 40%) but only few (here at least 3) produce the gynes. On the contrary to *P. punctata,* worker reproduction in *C. cursor* is probably just a transitory situation and its frequency in the field is far from clear. Pearcy et al. (1996) argued that about 60 % of queens should be produced by workers in the natural population they studied. This value was obtained using a model linking the observed homozygosity of queens to the proportion of queens produced by workers assuming no sexual production of gynes by the queens and automitic parthenogenesis with central fusion. This mode of parthenogenesis was clearly demonstrated (Pearcy et al. 1996). However, the absence of sexual production of gynes by the queens is far from clear. First, in another population, one queen out of four was shown to produce all gynes sexually (n=7) (Clemencet et al. submitted). Second, in the laboratory, workers have never been showed had activated ovaries in the presence of the queen (Cagniant 1983) and queenless colonies have never been observed in the field over about 300 colonies collected so far (Cagniant 1976; Lenoir et al. 1988; Pearcy et al. 2004a; Clemencet J. umpublished data). Finally, even if the queen dies, workers are able to change the caste fate of the young larvae (Cagniant 1981). The new gynes originating from these larvae, intended to become workers, are therefore likely to be produced sexually. More studies are therefore required to clarify the role of worker reproduction in the field.

If worker reproduction is frequent enough for selection to act, nepotistic behaviours could be selected for increasing the probability for a worker to pass on its genes by helping other workers from the same patrilines to produce the gynes. Our data showed the absence of nepotistic aggressive behaviours. Workers preferentially attacked egg-laying workers, but they did not discriminate between those belonging to the same patrilines versus others. Nevertheless, the probability that the five gynes produced originated from patriline 1, estimated from the rate of egg-laying of each patriline, was very low (1.2 %). This suggests that more subtle nepotistic behaviours could occur in *C. cursor*. For instance, workers could rear preferentially eggs belonging to their own patriline and feed more the larvae. The main patriline would then be advantaged since such discrimination could result in a greater success of its eggs and higher probability of becoming gynes. If nepotism is expected from kin selection theory (Hamilton 1987), intra-colony nepotism has been demonstrated only once in the polygynous ant *Formica fusca* in which workers favour their own close kin when rearing eggs and larvae (Hannonen and Sundström 2003 but see Holzer et al. 2006), while other empirical studies failed to demonstrate the occurrence of nepotistic behaviour in ants (Carlin et al. 1993; DeHeer and Ross 1997). Our result obtained from a single colony merits to be verified in other colonies of *C. cursor*.

Whatever the underlying mechanism allowing the most-represented patriline to produce all the gynes, this suggests that the male that fathers the largest proportion of workers should be favoured by natural selection. Indeed, if queens produce gynes mainly by parthenogenesis, worker reproduction is the main opportunity for the males to pass on their genes to the next generations. By mating first, by transferring the largest amount of sperm or by having the most competitive sperm, a male could increase its rate of genes transmission (Boomsma et al. 2005). The high paternal reproductive skew observed in our colony is a first indication that sperm competition could occur. Given that only few of the gynes produced will inherit a colony and finally reproduce, males could face the dilemma to transfer all their sperm to a single gyne or to share their stocks between several gynes. In the field, males of *C. cursor* have been observed mating successively with several gynes (Lenoir 1986). Additional works are therefore required to obtain a clearer understanding of the reproductive strategies of males in *C. cursor.* Such research have up to now received little or no consideration in ants (see Crozier 1985; Wiernasz 1995; Wiernasz et al. 2001; Fjerdingstad and Boomsma 1997; Schrempf et al. 2005; Boomsma et al. 2005). Our preliminary results, even if based on a single colony, suggest that nepotism and male mating strategies might be two important components to consider for understanding the evolution of female reproductive strategies in *C. cursor*.

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Figure Legend

Figure 1 Evolution of the number of ovipositions (a) and number of attacks (b) directed towards workers (●) and gynes (○) observed daily (total observation time: 212h). The date of apparition of the first larvae (1st L), cocoon (1st C) and worker (1st W) are indicated as well as the dates of emergence and death (†) of each of the five queens (Q) produced.

Figure 2 Survival probability predicted by the logistic model including the effect of worker size (abscise) and the effect of patrilines (different symbols). The survival rate of each patriline is given between parentheses in the legend. The curve of the different patrilines are similar in shape since the model did not include the interaction term which was not significant (see Table 1).

Figure 3 Number of eggs laid by workers in relation to their size (mean tibia length in mm) for the different patrilines (symbols). The mean number of eggs laid (±SE) per patriline is given between parenthesis in the legend. The curve corresponds to the probability of the number of eggs laid predicted by the log-linear model. Given that the patrilines had no effect on the number of eggs laid, the predicted curve is the same for all patrilines (see Table 1).

Fig. 1

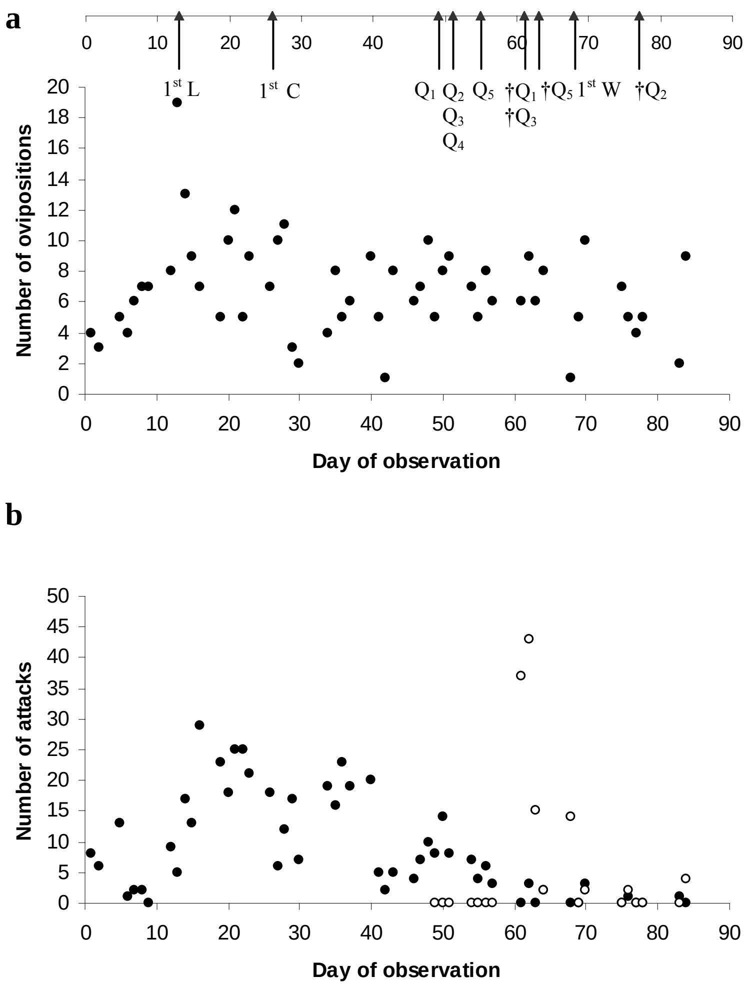
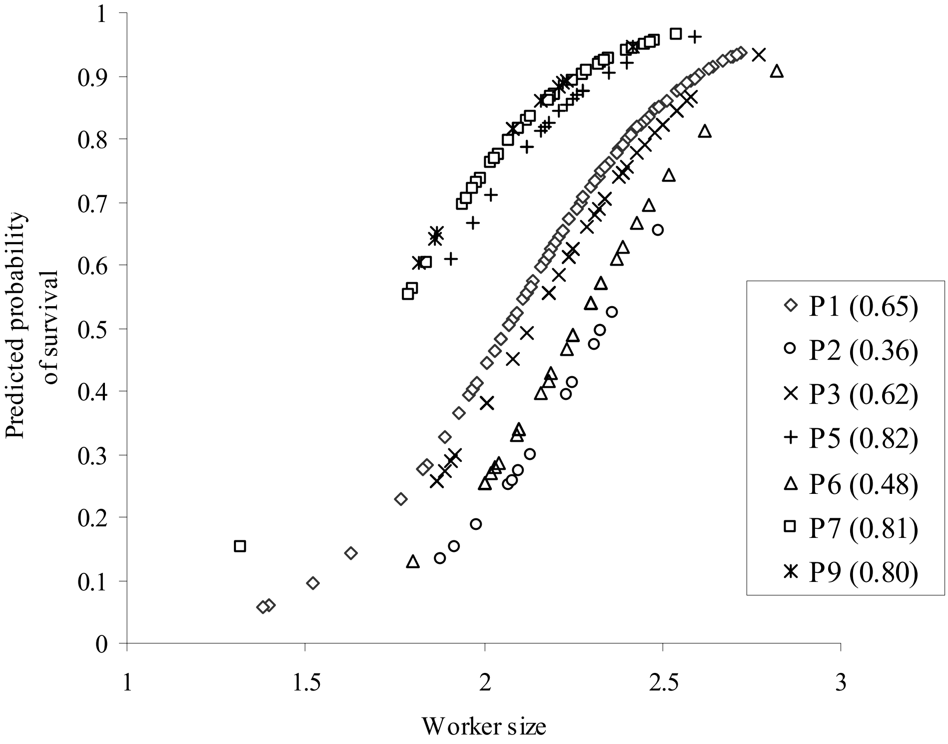

a
0
10
20
30
40
50
60
70
80
90
1st L
1st C
Q1
Q2
Q3
Q4
Q5
†Q1
†Q3
†Q5
1st W
†Q2
Number of ovipositions
0
2
4
6
8
10
12
14
16
18
20
Day of observation
b
Number of attacks
0
5
10
15
20
25
30
35
40
45
50
Day of observation

Fig. 2



1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Predicted probability of survival
1
1.5
2
2.5
3
Worker size
◇ P1 (0.65)
○ P2 (0.36)
× P3 (0.62)
+ P5 (0.82)
△ P6 (0.48)
□ P7 (0.81)
✱ P9 (0.80)

Fig. 3

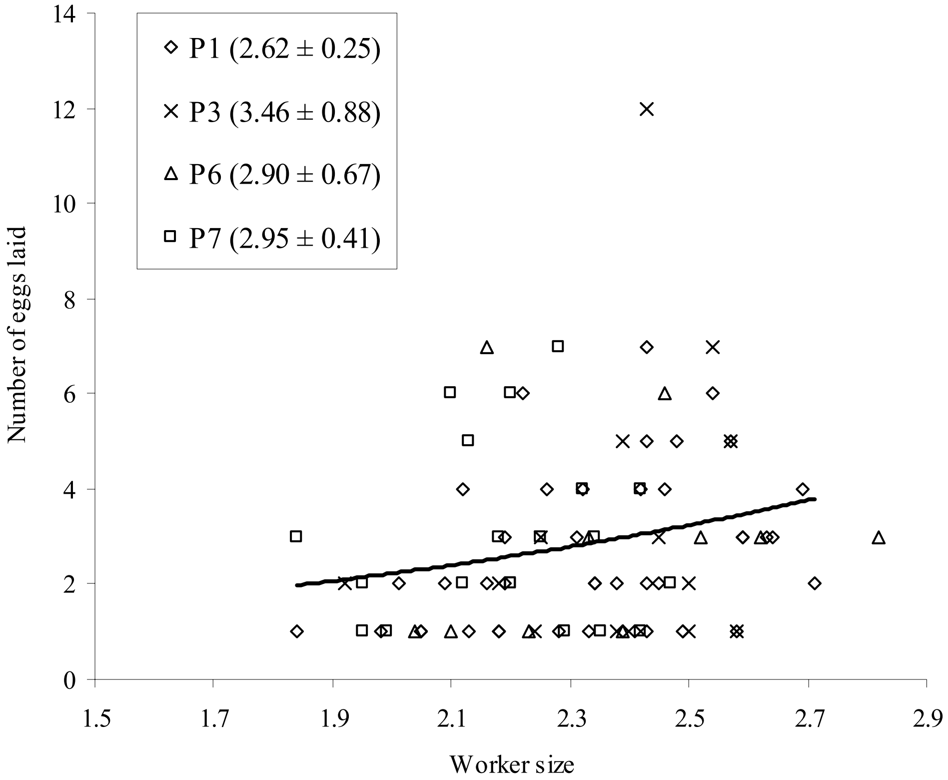

◇ P1 (2.62 ± 0.25)
× P3 (3.46 ± 0.88)
△ P6 (2.90 ± 0.67)
□ P7 (2.95 ± 0.41)
Number of eggs laid
Worker size
0
2
4
6
8
10
12
14
1.5
1.7
1.9
2.1
2.3
2.5
2.7
2.9

Table 1 Analysis of deviance to test for the effect of worker size and patriline on survival (whether or not a worker survived the experiment), egg-laying (whether or not a surviving worker has laid at least one egg) and on the number of eggs (total number of eggs laid by egg-laying workers during all the experiments). For each dependant variable, the change in deviance observed by removing the effect of interest from the model is given with the associated change in the degree of freedom (*df*) and the corresponding *p* value of the χ^2 test (see M&M for more details on the statistical analysis). The *df* and deviance of the full model, corresponding to the unexplained variation, is also given. For the three dependant variables, the interaction terms between size and patriline were not significant and removed from the model (survival : $\chi^2=9.11$, *df*=6, *p*=0.17; egg-laying : $\chi^2=0.64$, *df*=4, *p*=0.95; number of eggs laid : $\chi^2=1.39$, *df*=4, *p*=0.71).

		df	Changed in deviance (χ^2)	*p* value
Survival	Size	1	40.67	< 0.001
	Patriline	6	25.70	< 0.001
	Full model	264	291.29 (deviance)	
Egg- Laying	Size	1	2.71	0.10
	Patriline	4	4.92	0.30
	Full model	160	221.06 (deviance)	
Number of eggs laid	Size	1	6.52	0.01
	Patriline	3	3.17	0.37
	Full model	80	97.28 (deviance)	

Table 2 Number of aggressions received and size measurements for the five gynes, all being produced by workers from patriline 1. The number of aggression received (N_A) and the percentage of aggressions given by workers from patriline 1 ($\%_{AR}$) are given for each gynes. N_{AW} is the number of aggressive workers and $\%_{AW}$ is the percentage of aggressive workers belonging to patriline 1. The size measurements are provided for six morphological traits (TL, BL, HL, HW, THL, AL being measurement in mm of tibia length, body length, head width, thorax length and abdomen lengths, respectively). The only gyne that survived is indicated in bold.

Gynes	Aggressions				Size measurements					
	N_A	$\%_{AR}$	N_{AW}	$\%_{AW}$	TL	BL	HL	HW	THL	AL
G1	12	60	9	58.3	2.31	9.04	1.74	1.68	3.76	3.33
G2	53	36.8	18	43.6	2.14	8.42	1.64	1.58	3.74	3.74
G3	0	-	0	-	2.08	8.58	1.87	1.62	3.65	3.65
G4	**37**	**50**	**14**	**45.9**	**2.03**	**8.70**	**1.60**	**1.53**	**3.72**	**3.72**
G5	15	75	9	50	1.81	8.26	1.68	1.51	3.29	3.29
Total	117	45.2	31	45.4						

Résultats et discussion

SYNTHESE DES RESULTATS

L’ensemble de ce travail de thèse, couplant approches génétique et écologique, apporte des éléments nouveaux sur les stratégies de reproduction chez la fourmi *Cataglyphis cursor*. Nous rappellerons brièvement les résultats principaux et dégagerons les pistes de recherche à privilégier pour améliorer notre connaissance de ce système relativement complexe.

1. Flux de gènes limités entre populations : forte potentialité d’adaptation locale

L’étude de la structure génétique des populations, combinant marqueurs nucléaires et microsatellites, nous a permis d’évaluer les conséquences d’une dispersion femelle limitée liée au mode de fondation dépendante des colonies. En partant fonder de nouvelles colonies avec les ouvrières, les reines limitent donc la distance de dispersion par voie femelle à la distance de marche possible par un groupe de fourmis. À l’échelle locale, cela peut entraîner une importante viscosité des populations (Seppä & Pamilo 1995), c'est-à-dire une diminution de l'apparentement génétique entre colonies en fonction de leur éloignement géographique, et avoir des conséquences sur l’évolution de traits liés à la socialité (Kelly 1992, 1994). Une faible dispersion femelle a été vérifiée par une forte structuration mitochondriale à toutes les échelles spatiales considérées. Dans deux populations, une viscosité importante des reines a été détectée, confirmant la très faible dispersion des femelles à l’échelle de quelques dizaines de mètres (Annexe 1, p 172). À l’échelle inter-populationnelle, une très forte structuration génétique des populations a été observée pour les deux types de marqueurs indiquant un très faible flux de gènes entre les populations et donc de fortes potentialités d’adaptation locale chez *C. cursor*.

2. Utilisation conditionnelle de la reproduction sexuée et asexuée

L’étude des stratégies de reproduction de *C. cursor* dans deux populations issues d’habitats différents nous permet d’établir que la capacité des reines à utiliser conditionnellement la reproduction sexuée ou asexuée semble caractéristique de l’espèce et non une stratégie anecdotique d’une population isolée. Nous montrons que les gynes sont produites par parthénogenèse thélytoque (à 70%) alors que les ouvrières sont produites par reproduction sexuée de la reine. Nous confirmons également que les reines de *C. cursor* s'accouplent avec un nombre de partenaires très élevé (en moyenne 11 mâles par reine), plaçant cette espèce parmi les fourmis les plus polyandres connues à ce jour.

3. Polymorphisme de taille des ouvrières et diversité génétique

Malgré le fort taux de polyandrie observé pour toutes les colonies étudiées provenant de deux populations différentes, une corrélation positive entre la taille moyenne des ouvrières dans la colonie et la diversité génétique est observée, une fois la taille de la colonie prise en compte. En effet, des tailles plus grandes d'ouvrières ne semblent possibles que dans les colonies assez grandes pour pouvoir assurer leur production plus coûteuse. Ainsi, à nombre d'ouvrières égal, les colonies à plus forte diversité génétique produisent, en moyenne, des ouvrières plus grandes. Ceci suggère que ces colonies pourraient être capables d'obtenir davantage de ressources et seraient donc plus performantes.

En effet, nous avons montré que des ouvrières de grande taille pourraient représenter un avantage chez cette espèce thermophile car elle confère une meilleure résistance à la dessiccation. Ceci permettrait aux ouvrières de rechercher la nourriture à des heures très chaudes de la journée pendant lesquelles la compétition interspécifique est extrêmement réduite. Toutefois, contrairement à ce qui a été observé chez d'autres espèces de *Cataglyphis* en milieu naturel (Cerdá & Retana 1997), la taille moyenne des fourrageuses ne semble pas varier en fonction de l'heure de la journée et ne diffère pas de celle des autres ouvrières.

Une grande taille est également avantageuse au niveau individuel. Nous avons mis en évidence dans une colonie orpheline, que les ouvrières de grande taille ont une meilleure survie et une fécondité plus élevée. Les traits phénotypiques qui augmentent la valeur sélective des individus, comme une grande taille, devraient être sélectionnés au niveau individuel.

DISCUSSIONS ET PERSPECTIVES

D’après nos résultats et discussions présentés dans nos différents articles, certains aspects du système de reproduction de la fourmi *Cataglyphis cursor* nous apparaissent surprenants. Nous nous interrogerons sur trois paradoxes majeurs de ce système de reproduction inédit chez les insectes sociaux.

1. Pourquoi s’accoupler avec un si grand nombre de mâles ?

Bien que les reines de *C. cursor* soient capables de produire des femelles diploïdes, elles utilisent la reproduction sexuée et s’accouplent avec de nombreux mâles ($M_e = 8$) pour produire les ouvrières. Ceci suggère donc que la diversité génétique des ouvrières doit être avantageuse pour la colonie. Compte tenu de nos résultats et de la biologie de *C. cursor*, il nous est possible d’écarter, ou de privilégier, certaines des hypothèses présentées en introduction pour expliquer l’évolution et le maintien de la polyandrie chez cette espèce.

1.1 Les hypothèses classiques

Hypothèse 1: « Accouplements forcés ».

Les observations de Lenoir et al. (1988) indiquent que des gynes déjà accouplées ressortent du nid natal et s’accouplent à nouveau avec d’autres mâles. Il semble donc évident que l’accouplement multiple des reines soit bien un choix et non une contrainte.

Hypothèse 2 : « Limitation spermatique ».

Chez *C. cursor*, la taille des colonies est assez petite (maximum 3000 individus) et la durée de vie des reines est probablement relativement courte (deux ans au laboratoire ; S. Chameron, com. pers.) en comparaison des espèces pour lesquelles cette hypothèse à été proposée initialement (Cole 1983). De plus, des comptages spermatiques récemment réalisés sur les vésicules séminales des mâles et des spermathèques de reines révèlent que la quantité de sperme fourni par un mâle n’est pas un facteur limitant pour la femelle (M. Pearcy, com. pers.). Cette hypothèse peut donc être écartée chez *C. cursor*.

Hypothèse 3 : « Sélection spermatique ».

L'analyse de la représentation des lignées paternelles dans les colonies étudiées par Pearcy et al. (2004) montre que dans 75 % des colonies, il n'y a pas de biais. Les lignées sont équitablement représentées. Dans la seule colonie que nous avons étudiée, le fort biais dans la représentation des lignées paternelles pourrait être dû à une mortalité différentielle lors de l'hibernation au laboratoire et est donc difficilement interprétable. Soulignons que pour estimer correctement ce biais, il est nécessaire de comparer la proportion d'oeufs issus de chaque lignée à la quantité de sperme fourni par chacun des mâles.[1] D'après les observations de Lenoir (1986), un mâle peut s'accoupler successivement avec plusieurs femelles (jusqu'à huit). On peut donc s'attendre à ce que d'un mâle à l'autre, la quantité de sperme fournie à une femelle varie en fonction du nombre d'accouplements précédents. L'utilisation du sperme par les femelles n'a reçu que très peu d'attention chez les insectes sociaux (Ross 1986). Pourtant, comme nous le verrons ultérieurement, cette stratégie femelle pourrait avoir des implications sur la structure génétique des colonies et les conflits sociaux.

Hypothèse 4 : « Coût des mâles diploïdes ».

Une très forte diversité génétique a été décelée au sein de chacune des populations étudiées et aucun mâle diploïde n'a jamais été détecté (obs. pers. ; M. Pearcy, com. pers.). Il est donc peu vraisemblable que la polyandrie ait pu être sélectionnée pour diminuer les coûts associés à la production des mâles diploïdes.

Hypothèse 5 : « Diminution du conflit reines *vs* ouvrières sur la sexe-ratio ».

Cette hypothèse pourrait éventuellement expliquer l'apparition de la polyandrie, la parthénogenèse ne serait alors apparue qu'après. En revanche, elle ne justifie pas son maintien aujourd'hui, puisque, quel que soit le nombre d'accouplements des reines, les degrés d'apparentement entre les ouvrières et les fils (r = 0.25) et filles (r = 0.5) parthénogénétiques de la reine restent inchangés. Chez *C. cursor*, l'influence des contraintes écologiques sur la sexe-ratio est probablement beaucoup plus importante que celle exercée par les asymétries de parenté. La dispersion limitée par fondation dépendante des colonies induit une forte compétition entre les femelles apparentées pour l'accès aux ressources (*Local ressource competition*) et peut réduire leur valeur sélective (Clark 1978 ; Frank 1987). Les reines et les

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[1] Nous verrons plus loin qu'il pourrait être avantageux pour la reine d'utiliser de manière sélective le sperme fourni, de telle sorte que les lignées paternelles soient équitablement représentées.

ouvrières ont donc toutes deux intérêt à biaiser la sexe-ratio en faveur des mâles. Chez *C. cursor*, les colonies ont effectivement une sexe-ratio fortement biaisée en faveur des mâles (obs. pers. ; M. Pearcy, com. pers.). Cette hypothèse peut donc être écartée.

Hypothèse 6 : « Variabilité génétique des ouvrières ».

Cette hypothèse selon laquelle une plus grande variabilité génétique des ouvrières augmenterait la performance des colonies par une meilleure division du travail ou une meilleure résistance aux parasites nous semble pertinente (Crozier & Consul 1976). Nos résultats suggèrent, en effet, que les colonies présentant une plus forte diversité génétique seraient capables de récolter davantage de ressources.

Chez *C. cursor*, l'hypothèse classique selon laquelle une plus forte diversité génétique permet une plus grande variance de taille des ouvrières n'est cependant pas vérifiée (Crozier & Consul 1976). Seule la taille moyenne des ouvrières augmente avec la diversité génétique. Ceci semble cohérent dans la mesure où les colonies bénéficieraient davantage d'une augmentation de la taille moyenne des ouvrières plutôt que de sa variance. L'avantage des petites ouvrières sur les grandes pour certaines tâches reste effectivement à démontrer chez *C. cursor*, ainsi que chez les espèces présentant un polymorphisme continu de taille (sans caste différenciée). S'il existe un déterminisme génétique à la résistance aux fortes variations de température, comme cela a été observé chez la fourmi polygyne *Solenopsis invicta buren* (Li & Heinz 1995) ou chez d'autres espèces d'insectes (Neargarder et al. 2003 ; Krebs & Feder 1997 ; Krebs & Bettencourt 1999), alors, une plus forte diversité génétique au sein des colonies permettrait d'augmenter la performance globale des colonies, d'une part en augmentant la température maximale à laquelle les ouvrières peuvent fourrager, et d'autre part, en réduisant la mortalité durant l'hibernation (Cagniant 1983). L'héritabilité de la résistance à la température des ouvrières mériterait d'être testée chez *C. cursor*. L'hypothèse selon laquelle une plus forte diversité génétique augmenterait la résistance globale de la colonie face aux parasites et aux pathogènes est également pertinente puisque *C. cursor* se nourrit d'insectes morts sur lesquels se développent parasites et pathogènes.

Chez *C. cursor*, la diversité génétique des ouvrières est vraisemblablement un paramètre important pour le succès des colonies, puisque la reine choisit de s'accoupler avec de nombreux mâles alors qu'elle a la possibilité de se reproduire seule.

Hypothèse 7 : « Très faible coût à l'accouplement multiple et sollicitations des mâles ». Chez *C. cursor,* le coût des accouplements multiples est probablement très faible puisque les reines s'accouplent à terre et ne font pas de vol nuptial. Par ailleurs, en raison de la sexe-ratio biaisée en faveur des mâles, le nombre de partenaires n'est pas un facteur limitant pour la femelle. Une fois que la femelle est sortie du nid et qu'elle a fait un appel sexuel pour attirer les mâles d'autres colonies (Lenoir et al. 1988), le coût de chaque accouplement supplémentaire devrait être minime. Toutes les espèces de fourmis fortement polyandres connues à ce jour, ont en commun avec *C. cursor* de s'accoupler au sol et de présenter une sexe-ratio fortement biaisée en faveur des mâles (*Eciton burchellii* (Denny et al. 2004), *Dorylus molestus* (Kronauer et al. 2004), *Pogonomyrmex badius* (Rheindt et al. 2004)). De telles conditions ont probablement favorisé, chez ces espèces, l'évolution vers des taux de polyandrie très élevés.

De plus, les femelles de *C. cursor* sont probablement très sollicitées par les mâles. Comme très peu de gynes vont finalement accéder au statut de reines, les mâles ont peut-être intérêt à s'accoupler avec plusieurs femelles plutôt qu'une seule afin d'assurer leur paternité. Nous reviendrons ultérieurement sur les stratégies reproductives des mâles.

Si l'avantage que confère la diversité génétique des ouvrières a pu être à l'origine de l'accouplement multiple des femelles, le faible coût des accouplements successifs a sans doute pu favoriser l'évolution d'un nombre aussi élevé de partenaires.

1.2 Hypothèses liées à la parthénogenèse

Compte tenu de l'utilisation de la parthénogenèse thélytoque chez les femelles de *C. cursor*, deux autres hypothèses peuvent être envisagées pour expliquer le maintien de la polyandrie chez cette espèce.

La première est dérivée d'une hypothèse souvent avancée pour expliquer l'évolution de la polyandrie chez les espèces où les ouvrières peuvent produire des mâles par parthénogenèse arrhénotoque. Cette hypothèse classique propose que l'accouplement multiple des reines permette de diminuer le conflit « reines *vs* ouvrières » quant à la production des mâles (Starr 1984). Chez *C. cursor*, le conflit entre reines et ouvrières s'élargit à la production des femelles. La polyandrie pourrait alors avoir été sélectionnée pour limiter les coûts sociaux entre reines et ouvrières quant à la production des sexués. En effet, les ouvrières sont toujours beaucoup plus apparentées à leurs propres descendants (mâles et femelles) qu'aux sexués produits par la reine (Figure 7 ci-après). Elles pourraient donc augmenter leur valeur sélective globale en favorisant leur propre reproduction. Lorsque la reine utilise la reproduction asexuée, le degré d'apparentement entre une ouvrière et les descendants de la reine ne varie pas. En revanche, les degrés d'apparentement entre une ouvrière et ses neveux ou nièces (les mâles et les femelles issus d'ouvrières) diminuent à mesure que le nombre d'accouplements augmente. En théorie, à partir d'une fréquence d'accouplement supérieure à deux, chaque ouvrière est alors en moyenne plus apparentée à ses frères et sœurs (les sexués produits de façon parthénogénétique par la reine) qu'à ses neveux et nièces (les sexués produits par les autres ouvrières). Ceci devrait conduire à l'évolution d'un comportement de « police » des ouvrières selon lequel ces dernières éliminent tout œuf pondu par une congénère (Foster & Ratnieks 2000, 2001), ou d'auto-restreinte (Ratnieks 1988). Soulignons que si cette hypothèse permet d'expliquer le passage d'une fréquence de un à deux accouplements, elle explique en théorie plus difficilement l'évolution d'un nombre aussi élevé de partenaires. Ceci pourrait s'expliquer par le fait que les reines ont un taux d'homozygotie très élevé et ont besoin de plus de partenaires pour baisser l'apparentement moyen entre ouvrières. Nous montrons effectivement que le taux d'homozygotie des reines explique une part significative de la variation du taux d'apparentement moyen des ouvrières entre colonies (Article 3). En situation extrême de consanguinité, lorsque la reine est accouplée avec dix mâles, l'apparentement entre ouvrières est de $r = 0{,}55$!

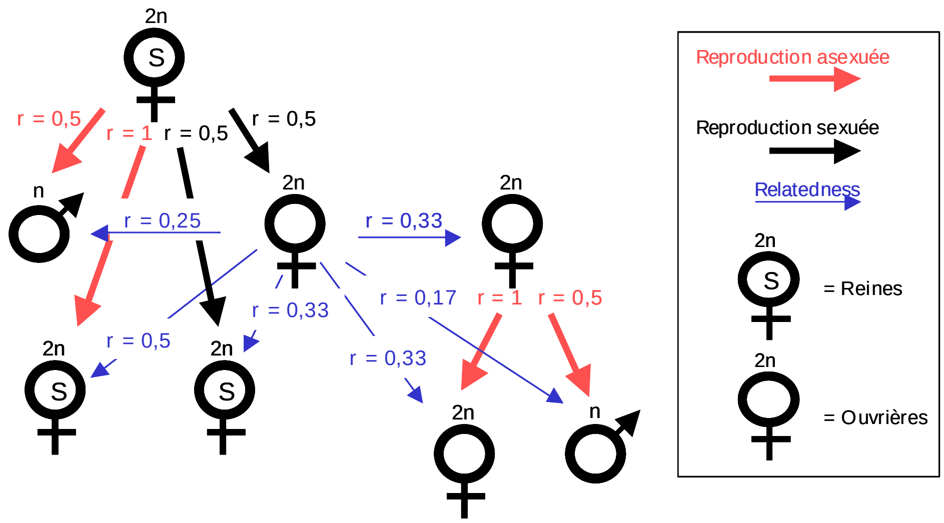

Figure 7. Parenté au sein des colonies polyandres de *Cataglyphis cursor* lorsque les gynes sont produites de façon sexuée ou asexuée. Pour simplifier nous avons négligé les recombinaisons possibles lors de la méiose en supposant que chaque femelle transmet l'intégralité de ses gènes à ses filles (*r* = 1). Nous faisons également l'hypothèse que les reines ne sont pas consanguines, la corrélation génétique moyenne entre une ouvrière et les filles et les fils produits de façon asexuée par la reine sont respectivement *r* = 0.5 et *r* = 0.25 (ces valeurs sont plus fortes en cas de consanguinité des reines). Le degré d'apparentement moyen d'une ouvrière à une femelle produite par reproduction sexuée est estimé d'après nos résultats (*r* = 0.33). On peut en déduire le degré d'apparentement d'une ouvrière à un neveu (*r* = 0.17).

Ceci nous conduit à considérer une deuxième hypothèse : celle d'une stratégie encore plus subtile de la part de la reine qui s'accouplerait avec plusieurs mâles au lieu d'un seul pour « contraindre » les ouvrières à élever ses œufs diploïdes produits par parthénogenèse en reine, plutôt que ses œufs produits de façon sexuée. En situation monoandre, les ouvrières devraient favoriser l'élevage de reines produites de façon sexuée, auxquelles elles sont plus apparentées (*r* = 0.75). À mesure que le nombre d'accouplements augmente, l'apparentement moyen entre une ouvrière et un œuf diploïde produit de façon sexuée par la reine diminue. Il est alors plus favorable à une ouvrière d'élever les œufs diploïdes parthénogénétiques de la reine (*r* = 0.5). La polyandrie serait une stratégie de la reine pour augmenter sa production de femelles sexuées produites par parthénogenèse. Cette hypothèse prédit que les colonies produisant des reines de façon sexuée devraient avoir un apparentement moyen entre ouvrières au moins supérieur à *r* = 0,5. Un tel système conduirait, de façon ultime, au remplacement systématique des reines « trop » consanguines par des reines issues de reproduction sexuée. Un large échantillonnage sera nécessaire pour vérifier nos prédictions dans la mesure où les colonies produisant des femelles sexuées semblent rares. Ceci nous éclairerait sur le contrôle de la reproduction sexuée et asexuée. Qui décide ? Les reines en produisant séquentiellement des oeufs diploïdes de façon asexuée au printemps (période d'accouplement) puis des œufs de façon sexuée le reste de la saison ? Ou les ouvrières en choisissant d'élever préférentiellement l'un ou l'autre ?

Le système de reproduction de *C. cursor* offre, par sa complexité, un modèle unique pour étudier les conflits au sein des colonies d'insectes sociaux et tester les hypothèses concernant l'évolution des stratégies de reproduction de ses membres.

2. Comment la parthénogenèse des reines peut-elle être associée à une si forte diversité génétique ?

Au sein des deux populations de *C. cursor* étudiées, nous trouvons très peu de clones potentiels parmi les reines[1] et une très forte diversité génétique est systématiquement détectée (Article 2). Ceci est également observé dans la population étudiée par M. Pearcy (com. pers.). Cette structure est assez inattendue compte tenu du nombre élevé de gynes produites par parthénogenèse (70% dans notre étude et 96.4% dans celle de Pearcy et al. 2004) et des modalités de reproduction des colonies qui limitent la dispersion femelle. A titre comparatif, au sein des populations de la fourmi polygyne *Wasmannia auropunctata* (Fournier et al. 2005), qui utilise également de façon conditionnelle la reproduction asexuée pour les reines et sexuée pour les ouvrières, les reines ont en général toutes le même génotype. La différence entre les deux espèces est frappante. Plusieurs hypothèses peuvent être avancées pour expliquer le peu de clones au sein des populations de *C. cursor* :

1. Les colonies déménageraient fréquemment et les « clones » sortiraient du périmètre d'étude. Cette hypothèse peut être écartée puisqu'à l'échelle de quelques dizaines de mètres nous observons une forte viscosité au niveau des reines (Annexe 1).

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[1] L'analyse des génotypes de 16 reines, récoltées de façon exhaustive sur le site de Luc sur Orbieu, révèle que seul 1 couple de reines n'a pas plus de deux allèles à chacun des six loci analysés, et peut donc potentiellement être apparenté comme sœurs ou mére-fille issues de parthénogenèse thélytoque. Le chiffre s'élève à 3 couples (en réalité un trio) sur 11 reines étudiées sur le site d'Argelès.

2. Les reines seraient en partie produites par les ouvrières. Contrairement à la fourmi *W. auropunctata,* les ouvrières *de C. cursor* ne sont pas stériles. Les ouvrières ne s'accouplent pas, mais sont capables de produire des femelles par parthénogenèse thélytoque, pouvant présenter le même génotype que des gynes produites de façon sexuée par la reine. Les observations de Cagniant (1980), réalisées en laboratoire, indiquent cependant que les ouvrières n'activent pas leurs ovaires en présence de la reine. Lenoir et al. (1988) ont ainsi proposé que la reproduction des ouvrières chez *C. cursor* ne serait qu'un simple mécanisme « de secours » lorsque la reine viendrait à disparaître. Compte tenu du nombre très faible de clones au sein des populations étudiées, ce mécanisme devrait donc être très fréquent dans la nature. Cette hypothèse nous semble cependant peu probable. La production de nouvelles gynes prend au moins 40 jours (en laboratoire), mais aucune colonie sans reine n'a été observée en milieu naturel, ceci quelle que soit la période de l'année (*n* > 300 ; Cagniant 1976a ; Lenoir et al. 1988 ; Pearcy et al. 2004 ; obs. pers.). Si les ouvrières ont la possibilité d'anticiper la mort de la reine (ce que suggèrent des observations comportementales que nous avons faites de façon anecdotique en laboratoire), de gros cocons (sexués) devraient être observés pendant la saison de reproduction (de juin à septembre), ce qui n'a jamais été le cas. Au laboratoire, les nids orphelins ne produisent de sexués qu'à la sortie de l'hibernation ; l'été, le couvain diploïde se développe en ouvrières, comme dans les sociétés qui possèdent une reine (Cagniant 1979). La reproduction des ouvrières, si elle a lieu, se déroulerait nécessairement à la sortie d'hibernation, soit en remplaçant la reine qui viendrait juste de disparaître (ce qui est peu probable en raison de l'absence de colonie orpheline), soit en développant leurs ovaires en présence de la reine. À ce jour, la reproduction des ouvrières en milieu naturel reste à démontrer. La première étape sera de vérifier si les ouvrières activent ou non leurs ovaires en présence de la reine en conditions naturelles.

3. Les reines sont également produites de façon sexuée. Contrairement à *W. auropunctata*, l'utilisation de la parthénogenèse pour la production des reines n'est pas obligatoire chez *C. cursor.* Dans certaines colonies, les reines sont produites par reproduction sexuée. Ce taux pourrait tout simplement varier d'une année à l'autre et d'une population à l'autre.

4. Les reines issues de reproduction sexuée disperseraient plus efficacement. Chez *C. cursor*, la parthénogenèse n'est pas de type apomictique comme chez *W. auropunctata,* les filles des reines ne sont pas des vrais clones. Le système chez *C. cursor* n'est peut-être pas

aussi parfait qu'il y paraît au premier abord. La parthénogenèse thélytoque de type automictique amène au fil des générations à une perte d'hétérozygotie. Cette augmentation de la consanguinité permet l'expression d'allèles récessifs délétères (Charlesworth & Charlesworth 1999 ; Keller & Waller 2002) et pourrait donc diminuer les chances de survie et le succès reproducteur des reines ainsi produites. Les reines issues de reproduction sexuée, non touchées par la dépression de consanguinité, pourraient avoir de plus forte probabilité de survivre et d'établir avec succès de nouvelles colonies. On pourrait également imaginer que les reines parthénogénétiques ne soient produites que pour remplacer la reine d'une année sur l'autre. Les fissions n'impliqueraient que des reines issues de reproduction sexuée. Ceci pourrait, non seulement expliquer le fait qu'au sein des populations, nous ne retrouvons que très peu de clones potentiels parmi les reines, mais aussi que la reproduction sexuée pour la production des gynes soit maintenue. Soulignons néanmoins que l'impact de la dépression de consanguinité sur le succès reproducteur des reines de *C. cursor* devrait être limité puisque les reines ne fondent pas seules de nouvelles colonies et sont constamment protégées à l'intérieur du nid.

3. Comment les mâles transmettent-ils leurs gènes ?

Chez *C. cursor* et *W. auropunctata*, les reines utilisent de façon conditionnelle la parthénogenèse pour les sexuées et la reproduction sexuée et donc le sperme des mâles pour les ouvrières. Une question se pose alors, comment les mâles transmettent-ils leurs gènes ? Chez W. *auropunctata,* un système exceptionnel permet aux mâles de se reproduire de façon clonale (Fournier et al. 2005). Dans certaines cellules diploïdes issues de fécondation, il y a exclusion du patrimoine génétique maternel. Ainsi, ces cellules devenues « haploïdes » se développent en mâles et portent toujours le génome du père. Les reines ne produisent pas de mâles de façon classique si bien que les gènes mâles et femelles ne s'associent finalement qu'au sein des ouvrières stériles.

Chez *C. cursor*, les mâles peuvent transmettre leurs gènes par deux voies :

1. Par la voie classique, si la reine produit des gynes par reproduction sexuée. Mais nous avons vu que, dans les trois populations étudiées, ce mode de reproduction n'était pas très important chez *C. cursor* puisque la reine produit principalement ses gynes par parthénogenèse et donc sans utiliser le sperme des mâles.

2. Par la voie des ouvrières, lorsque les ouvrières produisent des gynes par parthénogenèse dans les colonies orphelines. Cette situation est probablement rare également. Les mâles ne transmettraient donc que rarement leurs gènes aux générations suivantes mais ils sont produits en grand nombre par les colonies, ceci semble paradoxal.

Dans la première situation, les mâles auraient intérêt à faire en sorte que les ouvrières élèvent des gynes issues de reproduction sexuée. Une possibilité serait d'être le père du plus grand nombre d'ouvrières possible afin de passer la « barre des r = 0,5 entre ouvrières » (si notre hypothèse précédente est vérifiée). La méthode la plus simple pourrait être de transférer le plus de sperme, ou le sperme le plus compétitif afin de s'assurer de sa paternité. Les reines, quant à elles, pourraient contrer ce biais en utilisant le sperme de telle sorte que les ouvrières des différentes lignées soient en proportions égales dans la colonie et, comme on l'a vu, en essayant de s'accoupler avec le plus de mâles possible.

Dans la seconde situation, un mâle aurait tout intérêt à faire en sorte que ce soit les ouvrières de sa lignée qui produisent les gynes, sinon ses gènes ne seront pas transmis. Se pose alors la question de la régulation de la reproduction dans les colonies orphelines. Y a-t-il un biais de parenté des différentes lignées paternelles qui pourrait amener à la production de gynes par la lignée paternelle la plus représentée ? Nos résultats préliminaires à la sortie d'hibernation semblent indiquer que c'est le cas (Article 6). Ce biais peut-il s'expliquer par des survies différentielles des ouvrières ? On peut également rechercher des signes de compétition spermatique en se posant les questions suivantes. Est-ce que les caractères phénotypiques des mâles (taille, poids …) affectent leur taux de paternité ? Est-ce que la quantité de sperme, l'ordre d'accouplement, la taille des spermatozoïdes affectent la compétition spermatique ? Un choix cryptique du sperme par les femelles ou des effets de dépression de consanguinité peuvent être des facteurs confondants qu'il faudra donc considérer. Certaines questions ne pourront être abordées que si l'accouplement peut être contrôlé en laboratoire. Pour le moment, il n'a été possible d'accoupler des gynes produites en laboratoire qu'avec des mâles capturés pendant la saison de reproduction sur le terrain (A. Lenoir, com. pers.).

Les stratégies de reproduction des mâles chez les hyménoptères sociaux n'ont reçu que peu d'attention (Crozier 1985 ; Wiernasz 1995 ; Boomsma et al. 2005). Des études récentes chez des espèces monoandres ont montré que les mâles pouvaient s'assurer de leur paternité en déposant des bouchons copulatoires ou en laissant une partie de leurs pièces génitales fixée sur l'abdomen de la femelle (Duvoisin et al. 1999 ; Sauter et al. 2001 ; Baer et al. 2001).

Cependant, les effets du comportement des mâles et de la compétition spermatique sur la distribution des paternités dans la colonie chez des espèces polyandres restent méconnus. Or pour les mâles, assurer un fort taux de paternité sur la production des reines est le moyen principal de transmettre leurs gènes dans les espèces classiquement étudiées. Chez *C. cursor,* la situation est inédite puisque la sélection naturelle devrait favorisée les reines qui produisent les meilleurs mâles, mais également celles qui empêchent les mâles, avec lesquels elles s'accouplent, de devenir le père de leurs filles sexuées !

Pour conclure, afin d'émettre des hypothèses sur les scénarii évolutifs des stratégies de reproduction chez *Cataglyphis cursor,* il est essentiel de replacer l'espèce et ses modalités de reproduction au sein du genre *Cataglyphis*. L'étude phylogénétique du groupe *Cataglyphis* et la connaissance approfondie des modalités de reproduction et de dispersion des autres espèces, à ce jour très peu documentées, fourniraient des éléments solides pour retracer l'évolution de ces stratégies au cours de l'évolution. Déterminer qui de la parthénogenèse ou de la polyandrie est apparue en premier, est fondamental pour comprendre les pressions de sélections responsables de telles évolutions. Ainsi, des travaux dans cette voie doivent être réalisés pour apporter des réponses aux énigmes que les insectes sociaux posent aux biologistes.

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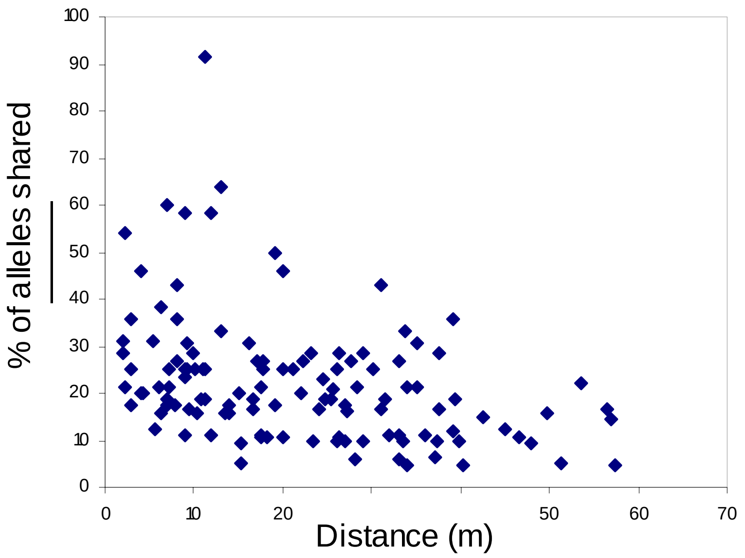
Annexes

ANNEXE 1

Pattern of queen viscosity using microsatellites markers

a)



b)

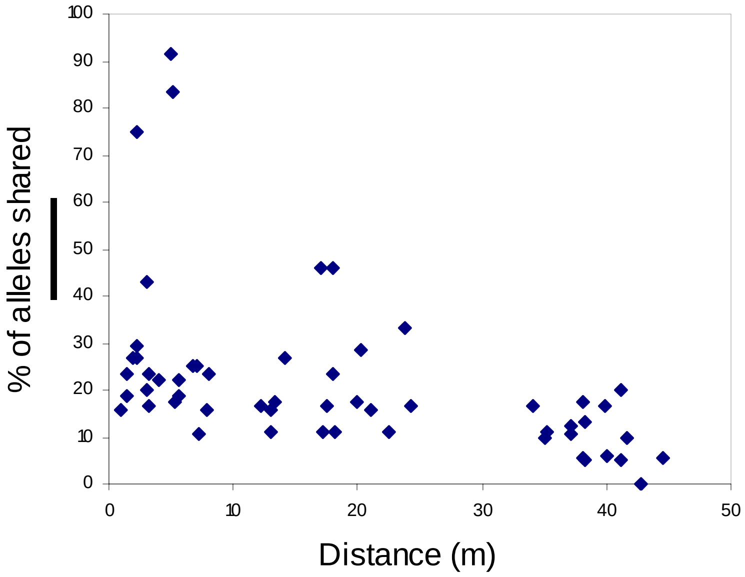


Figure 1. Relationship between genetic similarities, estimated as the percentage of alleles shared, and geographical distance between pairs of queens in Luc sur Orbieu (a) and Argelès (b) populations. Genetic similarities decreases significantly with increasing distances in both Luc sur Orbieu (r_{Luc}= -0.382, P_{Luc}= 0.001) and Argelès ($r_{\text{Argelès}}$= -0.471, $P_{\text{Argelès}} < 10^{-3}$) populations.